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Postprandial Levels of the Natural Vitamin E Tocotrienol in Human Circulation

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ABSTRACT

Compared to tocopherols, tocotrienols are poorly understood. The postabsorptive fate of tocotrienol isomers and their association with lipoprotein subfractions was examined. Normocholesterolemic women were subjected to an oral fat challenge supplemented with vitamin E (capsule containing 77 mg α-tocotrienol, 96 mg δ-tocotrienol, 3 mg γ-tocotrienol, 62 mg α-tocopherol, and 96 mg γ-tocopherol). Plasma samples were collected at every 2 h intervals for up to 8 h following a one-time supplementation. Lipoproteins were measured by NMR spectroscopy, and subfractions of lipoproteins were isolated by density gradient ultracentrifugation. The maximal α-tocotrienol concentrations in supplemented individuals averaged approximately 3 μ*M* in blood plasma, 1.7 μ*M* in LDL, 0.9 μ*M* in triglyceride-rich lipoprotein, and 0.5 μ*M* in HDL. The peak plasma level corresponded to 12- to 30-fold more than the concentration of α-tocotrienol required to completely prevent stroke-related neurodegeneration. Tocotrienols were detected in the blood plasma and all lipoprotein subfractions studied postprandially. *Antioxid. Redox Signal.* 8, 1059–1068.

INTRODUCTION

Vitamin E is a generic term for tocopherols (TCP) and tocotrienols (TCT), which qualitatively exhibit the biological activity of α-tocopherol (6). Both tocopherols and tocotrienols possess a chromanol ring, and the eight known isomers are differentiated as α, β, γ, and δ, according to the presence of methyl groups at positions 5, 7, and 8, respectively. Tocopherols are characterized by a saturated side chain, whereas tocotrienols possess double bonds at carbons 3, 7, and 11 (33–35). Compared to tocopherols, tocotrienols have been poorly studied (34, 46, 47). Vitamin E is often incorrectly used as a synonym for tocopherol disregarding the existence of tocotrienols (18). However, recent interest in the biological properties of tocotrienol has sharply risen because of the unique characteristics of this form of natural vitamin E not shared by the better known tocopherols (33, 35). *In vitro*, α-TCT has been shown to have higher antioxidant activity compared to α-TCP, and it has been hypothesized that the observed differences may be attributable to the unsaturated isoprenoid tail (37). The possible effects on antioxidant activity that may be due to this structural difference have been summarized (27) and include increased distribution of tocotrienols in the membrane bilayer and greater ability to collide with radicals.

The vast majority (>98%) of the current vitamin E literature examines the function of tocopherols (35). The unique physiological functions of the members of the natural vitamin E family has renewed interest in vitamin E research with emphasis on the specific study of tocotrienols (35). Tocotrienol, but not tocopherol, has antiangiogenic properties (23). At concentrations 25–50 μ*M*, α-TCP is known to regulate signal transduction pathways by mechanisms that are not shared by α-TCT (1). Micromolar concentrations of tocotrienol, not to-

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copherol, suppress the activity of hydroxy-3-methylglutaryl coenzyme A reductase (29, 30). The unsaturated side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the liver and brain (41). Both tocopherols and tocotrienols inhibit tumor growth. However, α-TCT have 70-times the cellular uptake potential of α-TCP (32). Tocotrienol suppressed the proliferation of breast cancer cells (25) and hepatocarcinoma cells *in vitro* (48). While the precise mechanism by which this occurs remains unknown, tocotrienols have been shown to interrupt the cell cycle, resulting in apoptosis (50). Compared to α-TCP, tocotrienols display more profound antiinflammatory effects including suppression of inducible adhesion molecule expression and monocytic cell adherence (24). Our studies have shown that both *in vitro* as well as *in vivo* nanomolar concentrations of α-TCT potently prevent stroke-related neurodegeneration while α-TCP does not share that property (19, 20, 36).

From the limited literature available, it is understood that dietary tocotrienols become incorporated into circulating human lipoproteins where they react with peroxyl radicals at least as efficiently as the corresponding tocopherol isomers (38, 39). Indeed tocotrienol supplementation beneficially influenced the course of carotid atherosclerosis in humans (43). Following supplementation to humans, the level of α-TCT in the plasma has been reported to be 1 μ*M* (26). In a recent study of rodents, we noted more efficient tissue delivery of oral TCT in females compared to males (18). In this study, we sought to examine the postabsorptive fate of tocotrienol isomers and their association with lipoprotein subfractions in postprandial Caucasian women.

MATERIALS AND METHODS

Human subjects

The subjects of this study were four young healthy Caucasian women, recruited from within the Department of Nutrition and Food Science at Wayne State University. Written informed consent was in line with the guidelines and approval of Wayne State University's Human Investigation Committee. Subjects were normocholesterolemic and had normal BMI values (mean ± SD: age 23.5 ± 2.2 years; body weight 58 ± 7.5 kg; body mass index 21.5 ± 2.2 kg/m^2; total plasma cholesterol 170 ± 19 mg/dl; HDL-C 57 ± 12 mg/dl; fasting glucose 91 ± 11 mg/dl). None was on any medication and none were taking any vitamin supplements. Normocholesterolemic subjects were used as it has been documented that hypercholesterolemic subjects have decreased absorption of vitamin E (12).

Experimental protocol

Volunteers reported fasting on the morning of the study. Following collection of a fasting blood sample, all subjects were supplemented (one-time) with 400 mg of vitamin E (Tocovid50 SupraBio, Carotech Incorporated, NJ). HPLC analysis of the vitamin E content of the supplement indicated the following composition: 77 mg α-TCT, 96 mg δ-TCT, 3 mg γ-TCT, 62 mg α-TCP and 96 mg γ-TCP. α-TCT represented 33% of total tocotrienols, and α-TCP represented 95% of total tocopherols. α-TCT and α-TCP represented 26% and 21% of total vitamin E, respectively. The subjects consumed the vitamin supplement with a fat-loaded meal comprised of 240 g nonfat milk, 24 g sugar, 140 g frozen strawberries, and 60 g soybean oil. As the level of fat has been reported to differentially affect vitamin E absorption (16), we chose a high-fat challenge for the current study to maximize absorption of the vitamin E isomers. The level of fat employed in the current study (60 g) is comparable to the level used (52 g) previously (7, 8) . The test meal consisted of 9.4 g protein, 60 g fat, and 64.6 g carbohydrate and provided 820 kcal. This meal composition compares favorably to the one previously reported (1143 kcal, 43% carbohydrate, 17% protein, and 41% fat). Meal composition was ascertained using a diet analysis program (Nutritional Analysis Tools and System Version 2.0) (7, 8). The ingredients were blended together and presented to the subjects as a strawberry-flavored smoothie. All subjects consumed the meal and vitamin E supplement within 10 min. Subsequently, additional blood samples were collected from the antecubital vein of the arm at 2, 4, 6, and 8 h after supplementation with vitamin E. During this time period, subjects were only allowed to consume water.

Analyses

Blood was collected by venipuncture into EDTA-coated vacutainers kept on ice. Plasma was separated by centrifugation at 16,000 *g* for 15 min and a cocktail mix containing sodium azide, gentamycin sulfate, benzamidine, and EDTA was added. The blood plasma sample was divided into two aliquots. One aliquot was frozen in liquid nitrogen while the other was kept refrigerated and within 24 h of blood collection, shipped overnight to LipoScience Inc. (Raleigh, NC) for measurement of lipoproteins by NMR proton spectroscopy (2, 40). In brief, this method involves measurement of lipoprotein subfractions based on the diameters of their phospholipids shell. From the resulting amplitude of each particles' signal, the lipoprotein subclass concentration was determined, allowing for simultaneous quantification of numerous subclasses. The latter measurements provided information on TC, HDL-C, and LDL-C, as well as the TG content of the triglyceride-rich lipoproteins (TRL). In addition, particle concentrations of the TRL, LDL, and HDL were available from NMR analyses. For LDL, these included measurements of small and large LDL and HDL. In all, five different HDL subfractions were measured. Finally, a composite measure of the diameter of the TRL, LDL, and HDL was obtained.

Lipoprotein fractions were isolated by discontinuous density gradient ultracentrifugation as described (15). Briefly, plasma density was adjusted to 1.1 g/ml. Four ml of this solution were overlayered with solutions of density 1.053, 1.02, and 1.006 g/ml. Following ultracentrifugation in a Beckman SW40 rotor at 35,000 rpm at 15°C for 15 min, the top 1 ml (Sf > 400 fraction) was collected. The tube was refilled with 1 ml of d 1.006 g/ml solution and centrifuged for 3 h and 40 min to obtain the top 1 ml (Sf 60–400 fraction). The tube was refilled with 1 ml of d 1.006 g/ml and centrifuged for 16 h and the top 1 ml (Sf 20–60 fraction) was collected. Sequential

1 ml fractions were also collected. From these fractions, those corresponding to LDL and HDL were pooled. Lipoprotein and plasma aliquots were used for vitamin E analyses. For such analyses, the three chylomicron fractions (Sf >400, Sf 60–400, and Sf 20–60) were pooled.

Vitamin E extraction and analyses

Vitamin E extraction was performed as described previously (31). Vitamin E analysis was performed using a HPLC-coulometric electrode array detector (CoulArray Detector Model 5600 with 12 channels; ESA Inc., Chelmsford, MA). This system uses multiple channels with multiple redox-potentials. α-TCP was detected on a channel set at 200 mV. α-TCT was detected on a channel set at 600 and 700 mV as described previously (18–20, 31, 36).

Statistical analyses

All statistical analyses were performed using a Power Macintosh 6100 computer (Apple Systems, Cupertino, CA) with the StatView 512+ (Brain Power, Calabasca, CA) statistical package. Difference between means as a function of time after supplementation were tested by ANOVA, with $p < 0.05$ considered significant.

RESULTS

HPLC analyses of the vitamin E capsules showed that the supplement provided 77 mg α-TCT, 96 mg δ-TCT, 3 mg γ-TCT, 62 mg α-TCP, and 96 mg α-TCP. α-TCT represented 33% of total tocotrienols and α-TCP represented 95% of total tocopherols. α-TCT and α-TCP represented 26% and 21% of total vitamin E, respectively.

Consumption of the fat-loaded meal and vitamin E supplement did not influence the concentration of total cholesterol in the blood plasma (Table 1). In contrast, TG increased significantly over the first 2 h and remained elevated at 4 h in comparison to the values observed at 0 h ($p < 0.01$). After 8 h of supplementation, TG had returned to baseline values. Plasma lipoprotein diameters, as measured by NMR, did not reveal any changes for LDL and HDL particle sizes over the 8 h examined. For VLDL, a significant increase in particle size of ~33% was seen in the first 2 h ($p < 0.0001$) and this change sustained for the 8 h studied (Table 1).

The observed changes in plasma TG in response to supplementation were reflected as corresponding changes in the TG content of the VLDL and CM fractions (Table 2). The largest increase occurred in the fraction comprising the chylomicrons, in which an almost sevenfold significant increase was noted after 2 h ($p < 0.003$). The value remained elevated at 4 h and then declined significantly (by ~50%) at 8 h after supplementation. The TG content of the medium-sized VLDL fraction showed a similar, but less pronounced trend than the VLDL/CM fraction, while the small VLDL showed no change. Further analyses of the TG content in the VLDL and chylomicron subfraction varying in mean diameters from 30 to >150 nm revealed changes in TG content of particles of diameter >50 nm (i.e., V4, V5, and V6). The largest quantitative changes in TG were noted in particles with mean diameters of ~70 nm (V4), in which the TG content at 2 h was 30-fold higher than the baseline 0 h value. The TG content of the smaller particles (mean diameter 29–38 nm) was essentially unaffected over the 8 h studied (Table 2).

The observed changes in the TG content in response to supplementation were also paralleled by changes in the respective particle concentrations. The supplementation caused no differences in HDL or LDL cholesterol, and HDL or LDL particle concentrations. In contrast to the total TG changes in the VLDL and CM fractions (Table 2), particle concentrations (reflecting particle numbers) remained unchanged. Despite the lack of change in total particle concentrations, subfraction analyses revealed that as with TG concentrations, changes in particle concentrations were largely confined to the V6 and V5 subfractions. Quantitatively, as with TG content of the V4 subfraction, particle concentrations of the V4 subfraction increased 37-fold from 0 h to 2 h. There were no significant effects on LDL and HDL cholesterol concentrations or LDL and HDL particle concentrations (Table 3) over the 8 h studied.

Blood plasma vitamin E concentrations are shown in Table 4. All three tocotrienol isomers were characterized by an increase in the first 4 h, followed by a subsequent decline over the next 4 h. For α-TCP, there was no significant change in the absolute values measured over the course of the study. Unlike the tocotrienols, which declined from 4 to 8 h, γ-TCP increased steadily up to 4 h and remained elevated at 8 h. In the fasting blood sample, α-TCP accounted for ~77% of total vitamin E, while this value was ~ 45%, 40%, 52%, and 56%, respectively for the 2, 4, 6, and 8 h postprandial samples. α-

TABLE 1. PLASMA LIPID CONCENTRATIONS (MG/DL PLASMA) AND LIPOPROTEIN PARTICLE DIAMETERS (NM)

	0 h	*2 h*	*4 h*	*6 h*	*8 h*
Total cholesterol	149 ± 5	150 ± 6	156 ± 8	156 ± 7	161 ± 9
Triglyceride	66 ± 11[a]	120 ± 12[a,c]	115 ± 21[b,d]	81 ± 16	62 ± 12[c,d]
VLDL	48.3 ± 5.6[e,f,g]	64.0 ± 5.0[e]	60.1 ± 2.2	61.8 ± 2.5[f]	83.3 ± 8.6[g]
LDL	21.2 ± 0.46	21.6 ± 0.35	21.2 ± 0.38	21.6 ± 0.25	21.2 ± 0.31
HDL	9.15 ± 0.27	9.23 ± 0.25	9.25 ± 0.28	9.33 ± .23	9.28 ± 0.24

In a given row, values sharing a common superscript were significantly different from each other by ANOVA ([a-d]$p < 0.05$).
For VLDL particle diameters[e,f,g] $0.05 < p < 0.10$.
Values are the mean ± SEM, $n = 4$.

TABLE 2. VERY LOW DENSITY LIPOPROTEIN AND CHYLOMICRON CONCENTRATIONS

Time	*0*	*2 h*	*4 h*	*6 h*	*8 h*
Triglyceride concentrations (mg/dl plasma)					
Total TG	35 ± 10[a,b]	91 ± 11[a,c]	87 ± 20[b,d]	52 ± 15[f]	29 ± 8[c,d,f]
VLDL/CM	8 ± 1[a–c]	55 ± 10[a,d,e]	40 ± 5[b,f]	31 ± 11[c,d]	14 ± 5[e,f]
Medium VLDL	14 ± 1	24 ± 6	30 ± 12	8 ± 4	8 ± 2
Small VLDL	13 ± 3	12 ± 5	17 ± 5	13 ± 6	7 ± 2
Chylomicrons	3.7 ± 1.2[a]	16.6 ± 4.0[a–d]	9.5 ± 0.4[b]	8.2 ± 1.8[c]	4.4 ± 0.8[d]
V6 (150 ± 70 nm)*	3.3 ± 1.3[a,b]	11.0 ± 2.1[a]	13.1 ± 2.9[b,c]	8.8 ± 1.9	5.2 ± 0.6[c]
V5 (70 ± 10 nm)	0.8 ± 0.3[a]	27.3 ± 5.1[a,c]	17.7 ± 2.1[b]	14.4 ± 3.1	4.2 ± 3.9[c]
V4 (50 ± 10 nm)	7.8 ± 4.1	20.4 ± 6.3	25.2 ± 10.0[a,b]	4.7 ± 2.9[a]	4.9 ± 1.8[b]
V3 (38 ± 3 nm)	6.5 ± 2.6	3.9 ± 2.2	5.2 ± 2.1	3.9 ± 2.0	2.9 ± 1.2
V2 (33 ± 2 nm)	10.8 ± 3.7	9.8 ± 3.6	14.2 ± 3 .9	10.8 ± 4.7	6.0 ± 1.9
V1 (29 ± 2 nm)	1.8 ± 0.8	2.2 ± 0.8	2.3 ± 1.0	1.9 ± 1.2	1.1 ± 0.6
Particle concentrations (nmol/L)					
Total	40 ± 16	48 ± 12	60 ± 18	37 ± 13	23 ± 7
VLDL/CM	0.2 ± 0[a–c]	4.2 ± 0.7[a,d]	2.8 ± 0.4[b,e]	2.3 ± 1.2[c]	0.8 ± 0.5[d,e]
Medium VLDL	12 ± 6	17 ± 4	21 ± 8	7 ± 3	6 ± 3
Small VLDL	28 ± 10	27 ± 9	36 ± 11	28 ± 13	16 ± 6
Chylomicrons	0[a,b]	0.2 ± 0.1[a,c,d]	0.1 ± 0[b]	0.08 ± 0.02[c]	0.03 ± 0.03[d]
V6	0.1 ± 0.1[a–c]	0.3 ± 0.1[a,d]	0.3 ± 0.1[b,e]	0.3 ± 0.1[c]	0.1 ± 0.03[d,e]
V5	0.1 ± 0.1[a,b]	3.7 ± 0.7[a,c]	2.4 ± 0.3[b]	2.0 ± 1.1	0.6 ± 0.5[c]
V4	4.9 ± 2.6	12.6 ± 3.9	15.6 ± 6.2[a,b]	2.9 ± 1.8[a]	3.0 ± 1.1[b]
V3	7.1 ± 2.9	4.3 ± 2.5	5.7 ± 2.3	4.2 ± 2.2	3.3 ± 1.3
V2	21 ± 8	19 ± 7	28 ± 8	21 ± 9	12 ± 4
V1	6.5 ± 3.0	7.7 ± 2.6	8.0 ± 3.4	6.6 ± 4.2	3.8 ± 1.9

*Diameter of particles for particular subfraction. Values are the mean ± SEM, $n = 4$.

[a–e]Values sharing a common superscript in any given row were significantly different from each other ($p < 0.05$), except for the particle concentrations in the V4 subfraction ($p < 0.07$). The total particle concentration is equal to either (a) the sum of the particles in the Large, Medium and Small CM/VLDL subfractions, or (b) the sum of the particles in the CM, V6, V5, V4, V3, V2, and V1 subfractions.

TABLE 3. LOW DENSITY LIPOPROTEIN AND HIGH DENSITY LIPOPROTEIN CONCENTRATIONS

Time	*0*	*2 h*	*4 h*	*6 h*	*8 h*
LDL Cholesterol Concentrations (mg/dl plasma)					
Total LDL cholesterol	87 ± 3	81 ± 4	84 ± 6	89 ± 5	98 ± 9
IDL (25 ± 2 nm)*	4.5 ± 2.1	2.0 ± 2.0	0.5 ± 0.5	0.5 ± 0.5	3.5 ± 2.8
Large LDL (22 ± 0.7 nm)	50 ± 9	54 ± 6	50 ± 7	64 ± 6	58 ± 6
Small LDL	32 ± 10	25 ± 8	34 ± 11	25 ± 6	37 ± 11
LDL Particle Concentrations (nmol/L)					
Total LDL particles	943 ± 108	811 ± 109	940 ± 130	873 ± 76	1,069 ± 154
IDL	36 ± 18	17 ± 17	6 ± 5	3 ± 1	28 ± 22
Large LDL	375 ± 69	410 ± 44	375 ± 50	480 ± 52	431 ± 90
Small LDL	534 ± 168	384 ± 136	559 ± 173	390 ± 108	609 ± 168
HDL Cholesterol Concentrations (mg/dl plasma)					
Total	51 ± 3	52 ± 3	54 ± 3	56 ± 3	57 ± 3
Large HDL	27 ± 5	29 ± 6	30 ± 2	31 ± 6	31 ± 6
Medium HDL	3.6 ± 1.5	3.5 ± 0.33	5.7 ± 1.3	8.1 ± 1.8	9.0 ± 3.3
Small HDL	20 ± 3	20 ± 3	18 ± 4	16 ± 3	17 ± 4
HDL Particle Concentrations (μmol/L)					
Total	31 ± 2	31 ± 2	31 ± 2	31 ± 3	33 ± 3
Large HDL	8.1 ± 1.2	8.7 ± 1.3	8.9 ± 1.7	9.0 ± 1.7	9.1 ± 1.6
Medium HDL	2.8 ± 1.2	2.7 ± 0.25	4.4 ± 1.0	6.2 ± 1.5	7.0 ± 2.6
Small HDL	20.3 ± 3.7	19.7 ± 3.0	18.2 ± 4.1	16.2 ± 2.9	16.9 ± 2.1

*Diameter of particles for particular subfraction.

The total LDL particle concentration is equal to the sum of the particles in the IDL, Large LDL, and Small LDL subfractions. The total HDL particle concentration is equal to the sum of the particles in the Large, Medium and Small HDL subfractions. There was no significant difference for any of the measured parameters.

Values are the mean ± SEM, $n = 4$.

TABLE 4. PLASMA VITAMIN E CONCENTRATIONS (MICROMOL/L PLASMA)

Time	*0*	*2 h*	*4 h*	*6 h*	*8 h*
α-TCT	0[a–c]	2.07 ± 0.56[a]	2.94 ± 0.68[b,d,e]	1.38 ± 0.21[c,d]	0.89 ± 0.17[e]
γ-TCT	0[a,b]	2.63 ± 0.80[a,c]	3.16 ± 0.96[b,d,e]	1.03 ± 0.16[d]	0.57 ± 0.12[c,e]
δ-TCT	0.19 ± 0.03[a,b]	1.63 ± 0.51[a,c,d]	1.41 ± 0.39[b,e,f]	0.42 ± 0.06[c,e]	0.25 ± 0.06[d,f]
α-TCP	7.30 ± 0.13	7.37 ± 0.33	7.51 ± 0.28	7.10 ± 0.22	7.01 ± 0.26
γ-TCP	1.96 ± 0.38[a]	2.57 ± 0.52	3.83 ± 0.58[a]	3.73 ± 0.24[b]	3.84 ± 0.49[c]

In a given row, values sharing a common superscript were significantly different from each other by ANOVA ($p < 0.05$).

Each subject received eight 50 mg capsules containing a total of 77 mg α-TCT, 96 mg δ-TCT, 3 mg γ-TCT, 62 mg α-TCP, 96 mg γ-TOC. α-TCT represented 33% of total tocotrienols and α-TCP represented 95% of total tocopherols. α-TCT and α-TCP represented 26% and 21% of total Vitamin E, respectively.

Values are the mean ± SEM, $n = 4$.

TCT accounted for 0%, 13%, 16%, 10%, and 7% of total vitamin E in the 0, 2, 4, 6, and 8 h plasma samples, respectively.

Table 5 lists the vitamin E concentrations in TGRL, LDL, and HDL fractions isolated by ultracentrifugation. For TGRL, α-TCP accounted for 87% of total vitamin E in the fasting sample. Two hours postprandially this value had declined to 57%, primarily because of a 13-fold increase in α-TCT and a 20-fold increase in γ-TCT. This pattern was maintained at 4 h. The subsequent time-points were characterized by declining values of the tocotrienols. For LDL, α-TCP accounted for 88% of total vitamin E in the fasting sample. No α-TCT was detected in the fasting LDL sample. However, 2 h postprandially, α-TCT concentrations in LDL were in excess of 1400 nmol/L and this represented 26% of total vitamin E. For HDL, no tocotrienols were detected in the fasting sample. α-TCP accounted for 88% of the total vitamin E. Tocotrienols were detected in the 2 h to 8 h postprandial samples of HDL and accounted for up to 25% of total vitamin E in the 4 h HDL sample.

Based on the data from Tables 4 and 5, Fig. 1 depicts the time-course of the changes in α-TCT concentrations in plasma, TGRL, LDL, and HDL. Oral supplementation with the tocotrienol-enriched vitamin E capsules resulted in peak α-TCT levels in plasma and all lipoproteins at 4 h after supplementation, followed by a return to baseline (0 h). All tocotrienol and tocopherol isomers were primarily transported in the TGRL fraction (Fig. 1B). This is illustrated in Fig. 1C, which depicts the changes in the two α isomers found in TGRL. The kinetics of transport for the two α isomers was comparable in TGRL (Fig. 1C).

To obtain additional information on the physiology of α-TCT transport. we correlated α-TCP and α-TCT concentrations measured in TGRL, LDL, and HDL isolated by ultracentrifugation, with the TG concentration of the lipoprotein subfractions measured by NMR. Plasma TG concentrations ($r = 0.76$), VLDL TG ($r = 0.805$), VLDL CM TG ($r = 0.79$), medium-sized VLDL TG ($r = 0.60$) as well as the TG content of V6, V5, and V4 subfractions (measured by NMR), showed significant correlations with the α-TCT content of TGRL iso-

TABLE 5. VITAMIN E ISOMER CONCENTRATIONS IN LIPOPROTEIN FRACTIONS (NMOL/L)

Time		*0 h*	*2 h*	*4 h*	*6 h*	*8 h*
α-TCT	TGRL	64 ± 27[a,b]	862 ± 220[a,c,d]	922 ± 295[b,e,f]	280 ± 90[c,e]	133 ± 46[d,f]
	LDL	0 ± 0[a–c]	1,410 ± 330[a,d]	1,757 ± 407[b,e,f]	827± 128[c,e]	531 ± 102[d,f]
	HDL	0 ± 0[a,b]	300 ± 114	549± 131[a,c]	397 ± 170[b]	206 ± 43[c]
γ-TCT	TGRL	31 ± 13[a,b]	588 ± 162[a,c,d]	567 ± 201[b,e,f]	135 ± 45[c,e]	55 ± 20[d,f]
	LDL	10 ± 9[a]	333 ± 93	451 ± 153[a]	327 ± 190	100 ± 22
	HDL	0 ± 0[a,b]	388 ± 143[a]	500 ± 111[b,c,d]	192 ±37[c]	140 ± 30[d]
δ-TCT	TGRL	0 ± 0[a,b]	156 ± 42[a,c,d]	135 ± 47[f]	18 ± 11[c,e]	0 ± 0[d,f]
	LDL	21 ± 12	115 ± 30	157 ± 50	242 ± 218	12± 6
	HDL	0 ± 0[a,b]	197 ± 70[a,c,d]	184 ± 33[b,e,f]	60 ± 14[c,e]	43± 8[d,f]
α-TCP	TGRL	2,006 ± 301[a,b]	2,877 ± 141[a,c]	2,982 ± 299[b,d]	2,131 ± 286	1,614 ± 339[c,d]
	LDL	3,179 ± 226	3,207 ± 339	3,017 ± 145	2,488 ± 534	3,011 ± 130
	HDL	2,964 ± 180	2,941 ± 137	3,029 ± 185	2,451 ± 532	3,251 ± 140
γ-TCP	TGRL	216 ± 23[a]	591 ± 81[b]	1,090 ± 259[a–c]	685 ± 73	511 ± 145[c]
	LDL	409 ± 72[a]	384 ± 81[b,c]	510 ± 72	646 ± 107[b]	697± 60[a,c]
	HDL	420 ± 96[a]	433 ± 107[b]	680 ± 168	725 ± 112	1,014 ± 165[a,b]

In a given row, values sharing a common superscript were significantly different from each other by ANOVA ($p < 0.05$).

Values are expressed as mean ± SEM, $n = 4$.

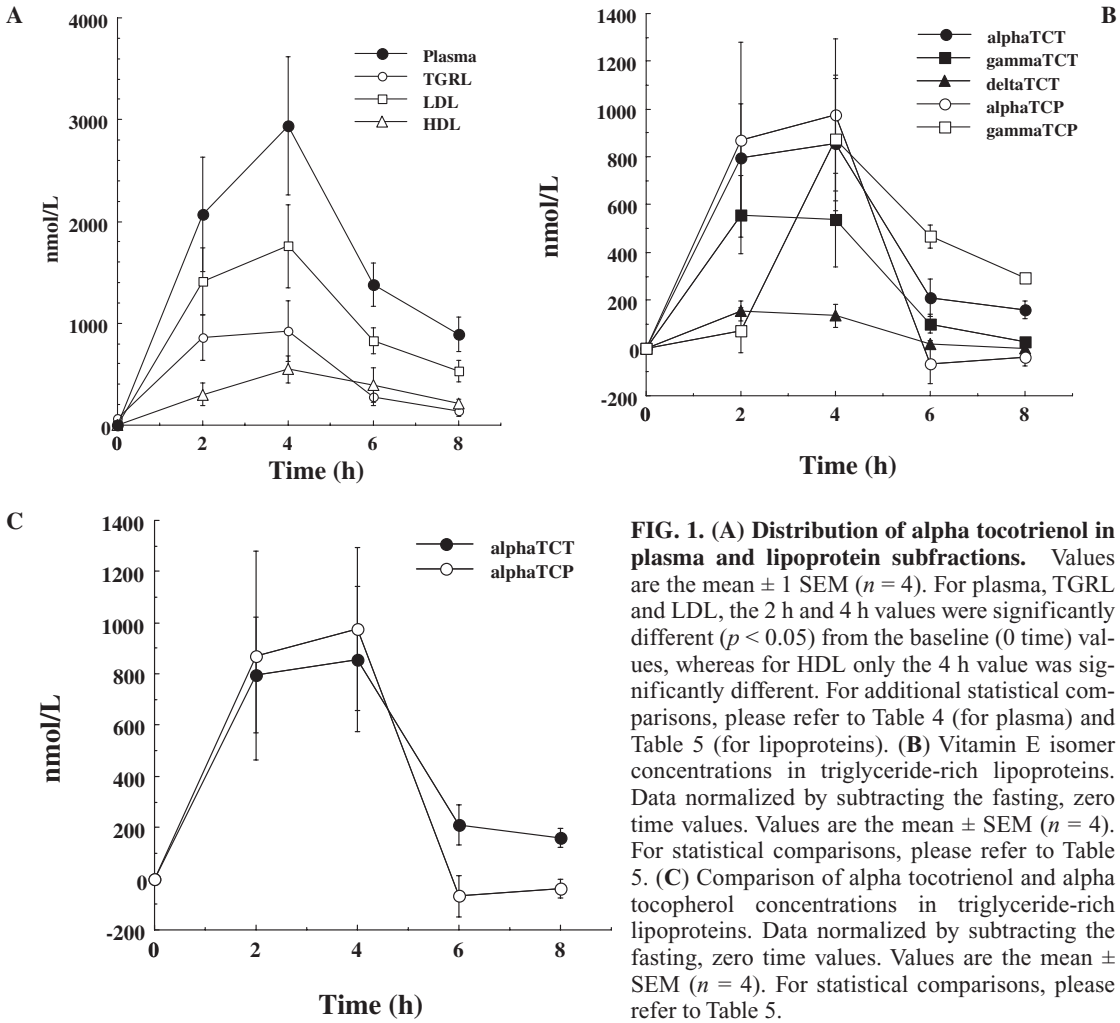

FIG. 1. (A) Distribution of alpha tocotrienol in plasma and lipoprotein subfractions. Values are the mean ± 1 SEM (n = 4). For plasma, TGRL and LDL, the 2 h and 4 h values were significantly different ($p < 0.05$) from the baseline (0 time) values, whereas for HDL only the 4 h value was significantly different. For additional statistical comparisons, please refer to Table 4 (for plasma) and Table 5 (for lipoproteins). (**B**) Vitamin E isomer concentrations in triglyceride-rich lipoproteins. Data normalized by subtracting the fasting, zero time values. Values are the mean ± SEM (n = 4). For statistical comparisons, please refer to Table 5. (**C**) Comparison of alpha tocotrienol and alpha tocopherol concentrations in triglyceride-rich lipoproteins. Data normalized by subtracting the fasting, zero time values. Values are the mean ± SEM (n = 4). For statistical comparisons, please refer to Table 5.

lated by ultracentrifugation. Similar relationships were also noted with the α-TCT content measured in LDL isolated by ultracentrifugation and the plasma TG concentrations, VLDL TG, VLDL-CM TG, medium-sized VLDL TG, as well as the TG content of the V6, V5, and V4 subfractions. In contrast, there were no correlations noted between α-TCP measured in isolated LDL or the TG content of any of the subfractions measured by NMR (Table 6).

DISCUSSION

The efficiency of α-TCP as chain-breaking antioxidants, combined with its prevalence in the human body, led biologists to almost completely discount the "minor" vitamin E molecules as topics for basic and clinical research. Recent discoveries have led to a serious reconsideration of this conventional wisdom (13). All eight tocols in the vitamin E family share close structural similarity and hence possess comparable antioxidant efficacy. Yet, current studies of the biological functions of vitamin E indicate that members in the vitamin E family possess unique biological functions often not shared by other family members (4, 5, 35, 51).

Delivery of orally taken vitamin E to vital organs is a key determinant of the overall efficacy of vitamin E in those tissues. Thus, mechanisms responsible for the transfer of absorbed vitamin E to the tissues have been the subject of active investigation (45). Current efforts to understand how dietary vitamin E is transported to the tissues have focused on α-TCP transport (3, 17, 44). α-TTP mediate α-TCP secretion into the plasma while other TCP-binding proteins seem to play a less important role (17). α-TCP selectively binds to TTP. The affinity of α-TCT to bind TTP has been estimated to be almost an order of magnitude less compared to the affinity for α-TCP (14, 28). The lack of relative specific affinity of TTP for TCT led to the notion that availability of dietary TCT to vital organs is negligible. Two recent lines of development provide a compelling rationale to revisit the transport of oral TCT. First, that trace nanomolar concentrations of TCT exhibits potent neuroprotective functions not shared by TCP (19, 20, 34–36). Second, that orally taken α-TCT may be successfully delivered to several vital organs by α-TTP independent mechanisms (18).

TABLE 6. CORRELATION OF α-TOCOTRIENOL AND α-TOCOPHEROL IN SPECIFIC LIPOPROTEIN FRACTIONS ISOLATED BY ULTRACENTRIFUGATION WITH TRIGLYCERIDE-RICH LIPOPROTEIN TRIGLYCERIDE CONCENTRATIONS AND TRIGLYCERIDE-RICH LIPOPROTEIN PARTICLE CONCENTRATIONS MEASURED BY NMR

	α-*TCT TGRL*	α-*TCP TGRL*	α-*TCT LDL*	α-*TCT HDL*	γ-*TCT TGRL*	γ-*TCP TGRL*	γ-*TCT LDL*	γ-*TCP LDL*	γ-*TCT HDL*	γ-*TCP HDL*
Plasma TG	0.763	0.775	0.695		0.769		0.520	0.672	0.640	0.543
VLDLTG (tot)	0.805	0.792	0.741		0.814	0.46	0.555	0.644	0.689	0.516
VLDLCM-TG	0.789	0.623	0.76		0.797	0.489	0.456		0.719	
Med VLDL-TG	0.601	0.694	0.526		0.623		0.550	0.503	0.520	
Small VLDL-TG		0.459						0.758		0.714
CYM-TG	0.732	0.519	0.669		0.761				0.681	
V6-TG	0.867	0.615	0.872	0.674	0.849	0.681	0.694	0.867		
V5-TG	0.684	0.593	0.66		0.690			0.466	0.587	
V4-TG	0.697	0.698	0.618	0.454	0.719		0.586	0.481	0.624	
V3-TG										
V2-TG		0.451						0.743		0.708
V1-TG		0.452						0.775		0.701
VLDL(tot)		0.63						0.766		0.684
Large V/CM	0.715	0.606	0.693		0.721			0.452	0.627	
Medium VLDL	0.528	0.673	0.455		0.549		0.514	0.501		
Small VLDL		0.461						0.765		0.718
CY nmol/L	0.687	0.527	0.633		0.71				0.609	
V6 nmol/L	0.813	0.548	0.833	0.632	0.799			0.656		0.807
V5 nmol/L	0.683	0.594	0.659		0.689			0.468	0.587	
V4 nmol/L	0.697	0.698	0.617	0.454	0.718		0.586	0.481	0.624	
V3 nmol/L										
V2 nmol/L		0.453						0.743		0.708
V1 nmol/L		0.456						0.774		0.700
VLDL (nm)			0.441							

Table shows *r* values for the respective correlations. All *r* values were significant ($p < 0.05$). Blank entries indicate no significant correlations ($p > 0.05$). No significant correlations were noted between the indicated parameters and α-TCP in TGRL or α-TCP in HDL.

Studies in humans have found that tocotrienols are either not detected in fasting plasma or are present in minute concentrations (21, 22, 42, 49). Absorption of tocotrienols was increased when supplements were administered with food (49). We were therefore led to investigate a postprandial model, providing a high-fat challenge to facilitate tocotrienol absorption. Gender-based differences in the transport of dietary vitamins are known to exist (10). In a recent study of rodents, we noted more efficient tissue delivery of oral TCT in females compared to males (18). In this study, we chose to investigate the outcomes of vitamin E supplementation in women. Previous studies have shown that in humans subjected to oral supplementation, plasma α-TCT rises to 1 μ*M* concentration (26). In this study, we observed that the maximal α-TCT concentrations in the blood plasma of supplemented individuals averaged almost 3 μ*M* in plasma, 1.7 μ*M* in LDL, 0.9 μ*M* in TGRL, and 0.5 μ*M* in HDL. Given that 100–250 n*M* α-TCT is sufficient to completely prevent stroke-related neurodegeneration (19, 20, 36), it may be concluded that oral supplementation of TCT-enriched vitamin E results in plasma concentration of α-TCT that is 12- to 30-fold higher than the concentration required for its reported neuroprotective function.

Vitamin E enters the circulation from the intestine in chylomicrons. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed vitamin E to all of the circulating lipoproteins and ultimately to tissues. This enrichment of lipoproteins with vitamin E is a key mechanism by which vitamin E is delivered to tissues (45). In the liver, newly absorbed dietary lipids are incorporated into nascent very low density lipoproteins. Results of this study demonstrate that TCT is efficiently delivered to all lipoprotein fractions despite the fact that TTP has a significantly lower affinity for α-TCT than α-TCP. This observation lends further credence to the hypothesis proposing a TTP-independent transport of TCT (18).

Lipid alterations during the postprandial phase result mainly from changes in TGRL (chylomicrons and chylomicron remnants). Our data are consistent with this notion because the major changes in triacylglycerol following the oral fat load were observed in the large VLDL/CM particles (greater than 50 nm diameter) measured by NMR. NMR is not able to distinguish between intestinally and hepatically-derived lipoproteins. However, it is expected that the majority of these particles are of intestinal origin, especially during the first 4 h after supplementation, when plasma and lipoprotein triacylglycerol concentrations increased. It may thus be hypothesized that the supplement-derived TCT entered the circulation associated with these larger particles. Following delivery of chylomicron remnants to the liver, newly secreted VLDL is known to contain α-TCP. Once in the circulation,

lipase action, the lipoprotein delipidation cascade, as well as cholesterol ester transfer protein-mediated exchanges between HDL and apoB containing lipoproteins, result in a distribution of the α-TCP between all lipoproteins. In the case of TCT, it is apparent from our data that transport to the liver is rapid (within 2 h) and largely confined to the particles of >50 nm diameter. Once in the liver, TCT are presumably repackaged into nascent VLDL for secretion. Subsequent appearance in LDL may be due to preferential packaging of TCT in the core of VLDL, which would be retained as the particle is hydrolyzed to LDL. Alternatively, TCT may be secreted in VLDL as part of phospholipids species in the surface of the lipoprotein. The latter could subsequently be exchanged to HDL.

Smaller and denser lipoprotein particles are being increasingly recognized as an important risk factor for cardiovascular disease. Specific dietary modifications and supplements can beneficially alter cardiovascular disease risk by influencing the size phenotype of lipoproteins beyond their known effects on plasma LDL cholesterol concentrations (9). A novel method for detailed analyses of lipoprotein subclass sizes and particle concentrations that uses nuclear magnetic resonance of whole sera has recently become available (11). We utilized this technique in the current study to derive information on postprandial effects on different lipoprotein subfractions. Our overall results are in agreement with the literature (40) showing that postprandial changes in triacylglycerol are mainly confined to the TGRL particles of >50 nm.

In sum, this work presents first evidence demonstrating the postabsorptive fate of tocotrienol isomers and their association with lipoprotein subfractions in humans. In supplemented women, maximal α-TCT levels averaged almost 3 μ*M* in blood plasma, 1.7 μ*M* in LDL, 0.9 μ*M* in TRL, and 0.5 μ*M* in HDL. Dietary tocotrienol was rapidly delivered to the lipoprotein subfractions of human plasma at concentrations sufficient to support its reported neuroprotective functions.

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ABBREVIATIONS

BMI, Body Mass Index; CM, chylomicrons; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; NMR, nuclear magnetic resonance; TC, total cholesterol; TCP, tocopherol; TCT, tocotrienol; TGRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

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