# Differences in the Plasma Transport and Tissue Concentrations of Tocopherols and Tocotrienols: Observations in Humans and Hamsters (43546)

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*Abstract.* **Certain aspects of tocopherol and tocotrienol absorption, plasma transport, and tissue distribution were examined in humans and hamsters. Plasma transport differed in that tocopherols were found primarily in low density lipoprotein and high density lipoprotein in association with plasma surface components, whereas tocotrienols disappeared from plasma with chylomicron clearance. In keeping with transport by triglyceride-rich lipoproteins, tocotrienols were deposited in conjunction with triglycerides in the adipose tissue of hamsters. In hamsters, tocopherols were the only tocol readily detected in all tissues, except adipose during tocotrienol supplementation. In fasting humans, the plasma tocotrienol concentration was not significantly increased after tocotrienol supplementation, whereas the platelet concentration of δ-tocotrienol doubled. Furthermore, tocotrienol intake did not appear to modulate the plasma cholesterol concentration in normolipemic hamsters. Thus, the transport, tissue concentration, and relative biologic function of tocopherol and tocotrienol appear somewhat disparate and possibly unrelated.** [P.S.E.B.M. 1993, Vol 202]

Tocopherol has long been recognized as the most important lipid antioxidant in mammalian systems. Recent attention has turned to the possible biologic importance of tocotrienols and whether they might function in an antioxidant capacity to modulate the pathway for cholesterol biosynthesis (1) and actually lower plasma cholesterol in certain hypercholesterolemic individuals (2, 3). It has been proposed that tocotrienols may be involved in specialized microsomal systems (4, 5), as opposed to the general membrane antioxidant function of tocopherols (6). For example, in the original antifetal resorption assay in rats, α-tocotrienol revealed only 10–30% of the activity expressed by α-tocopherol, indicating its relative ineffectiveness in that system (7, 8). On the other hand, tocotrienols added to subcellular hepatic microsomal redox systems *in vitro* are claimed to have 40 × the antioxidant capacity of α-tocopherol (5). Whether this *in vitro* effect can be extrapolated to *in vivo* biologic functions is open to question.

For tocotrienols to exert an important biologic function, they should have a demonstrable transport system with deposition in appropriate tissues. In this report, we compare certain aspects of lipoprotein transport and tissue storage of tocopherols and tocotrienols for possible clues to the significance of the tocotrienols. For these experiments, we examined a variety of systems involving humans and hamsters.

## Materials and Methods

**Hamster Study.** Male Syrian hamsters (Lakeview strain; Charles River, Wilmington, MA) weighing 130 g were used to compare tocol plasma transport in detail and to assess tissue concentrations and relative distribution. Hamsters were fed from 4 to 11 weeks purified diets containing 20% dietary fat as either tocotrienol-stripped palm oil (diet with 3 ppm of tocotrienols), regular refined, bleached, and deodorized palm oil (diet containing 36 ppm of tocotrienols), or crude palm oil (diet with 223 ppm of tocotrienols) (Table I). Groups of three to five hamsters were maintained in 12:12-hr

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**Table I.** Composition of Purified Hamster Diets

| Ingredient | Diet[a] | | |
|---|---|---|---|
| | 182A (223 ppm)[b] (%) | 182B (36 ppm)[b] (%) | 182C (3 ppm)[b] (%) |
| Casein | 22 | 22 | 22 |
| Rice flour | 23.3 | 23.3 | 23.3 |
| Glucose | 13.3 | 13.3 | 13.3 |
| Cellulose | 10 | 10 | 10 |
| Wheat bran | 5 | 5 | 5 |
| Fat | | | |
| Crude palm oil | 18 | — | — |
| Palm oil RBD[c] | — | 18 | — |
| Palm oil (tocotrienol stripped)[d] | — | — | 18 |
| Soybean oil | 2 | 2 | 2 |
| Mineral mix[e] | 5 | 5 | 5 |
| Vitamin mix[f] | 1.1 | 1.1 | 1.1 |
| Choline chloride | 0.3 | 0.3 | 0.3 |

[a] Diets were fed as flour gels, prepared by withholding 60 g of rice flour (per kg mix) from the formulation and premixing it with 800 ml of boiling water to form a gel to which the remaining ingredients were added.
[b] Tocotrienol content of the diet.
[c] RBD, refined, bleached, deodorized.
[d] Tocotrienol-stripped palm oil was obtained by extracting palm oil (eight times) with hexane.
[e] Ausman-Hayes mix contained (g/kg of mix): magnesium oxide, 32; calcium carbonate, 290; potassium phosphate dibasic, 314; calcium phosphate dibasic, 73; magnesium sulphate, 99; sodium chloride, 162; ferric citrate, 27; potassium iodide, 0.077; manganese sulfate, 1.22; zinc chloride, 0.92; cupric sulfate, 0.29; chromium acetate, 0.044; sodium fluoride, 0.023; and sodium selenite, 0.004.
[f] Hayes-Cathcart vitamin mix contained (g/kg of mix): DL-$\alpha$-tocopheryl acetate, 500 IU/g, 15; inositol, 5; niacin, 3; calcium pantothenate, 1.6; vitamin A palmitate, 500,000 IU/g, 1.5; cholecalciferol, 400,000 IU/g, 0.100; menadione, 0.200; biotin, 0.020; folic acid, 0.200; riboflavin, 0.700; thiamin, 0.600; pyridoxine HCl, 0.700; cyanocobalamin, 0.001; and dextrin, 972.

light:dark cycles, with the dark cycle beginning at 6 AM. The purified diets were fed *ad libitum* with free access to water.

At the end of the experimental period, all hamsters were fasted overnight (about 15 hr) in individual wire-bottom cages, and blood samples were obtained using an EDTA-wetted (0.268 *M*) syringe by cardiac puncture under light anesthesia with a gaseous mixture of 50%:50% $CO_2$ and $O_2$. After refeeding for 3 hr, hamsters were again anesthetized and mesenteric lymph was collected by aspiration (insulin syringe) under a dissecting microscope without contamination of blood or plasma. Animals were exsanguinated, and the small intestine was opened, all surface materials were rinsed away, and mucosal cells were collected by gently scraping with a scalpel blade. Several other tissues were sampled and frozen at −30°C until analyzed, whereas plasma, lymph, and urine samples were analyzed fresh. To study postprandial chylomicron transport, several hamsters (fed Diet 182A, Table I) were fed for 3 hr after an overnight fast and then injected under light anesthesia (as above) via the jugular vein with Triton WR 1339 at 240 mg/kg body wt. Two hours later, blood was collected by cardiac puncture, as described.

All protocols and procedures used in hamster experiments were approved by the Institutional Animal Use Committee.

**Human Study.** To assess transport and distribution of tocopherols and tocotrienols in plasma, a group of 14 healthy, normolipemic male and female volunteers, 20–50 years of age, were studied. All subjects were included by informed consent, and protocols were approved by the Institutional Human Use Committee.

To analyze the distribution of tocopherols among lipoproteins, blood samples were drawn from overnight-fasted subjects into EDTA-containing vacuum tubes (Vacutainer Systems, Rutherford, NJ), and plasma (combined from two subjects/tube) was separated by centrifugation at 3000 rpm at 4°C for 20 min.

To study transport and concentration of tocopherols and tocotrienols in plasma and platelets, semi-fasted (6 hr) or fed (2 hr postprandial) subjects were bled (into EDTA Vacutainers) before (baseline plasma) and after (supplemented plasma) 10 days of consuming two tocotrienol-rich Palm Vitee (PORIM, Malaysia) capsules, containing 80 mg of tocotrienols (16.9 mg $\delta$, 36.6 mg $\gamma$, and 26.5 mg $\alpha$) and 64 mg of tocopherols (all $\alpha$), per day with meals. Blood was collected 6 hr after a light, low-fat breakfast before and after the 10 days of Palm Vitee supplementation. Tocotrienol and tocopherol concentrations also were determined in platelets isolated from citrated blood samples (1 vol of 3.8% trisodium citrate/9 vol of blood).

**Preparation of Lipoproteins.** Lipoproteins were isolated from human plasma (EDTA) by sequential ultracentrifugation (9) in Quick Seal tubes (Beckman,

Palo Alto, CA) using a Ti 70.1 rotor at 12°C. Four lipoprotein fractions were isolated: very low density lipoproteins ([VLDL] $d < 1.006$ g/ml), intermediate density lipoproteins ([IDL] $1.006 < d < 1.019$ g/ml), low density lipoproteins ([LDL] $1.019 < d < 1.063$ g/ml), and high density lipoproteins ([HDL] $1.063 < d < 1.21$ g/ml). All lipoprotein fractions were dialyzed for 24 to 36 hr at 4°C, against 0.15 *M* NaCl and 1 m*M* EDTA (pH 7.4).

**Preparation of Platelets.** Citrated blood was centrifuged in plastic tubes at room temperature at 200*g* for 10 min. The top 1–2 ml of platelet-rich plasma were transferred to another tube, and platelet concentration in platelet-rich plasma was determined using a hemocytomer (Improved Neubauer; American Optical Co., Buffalo, NY). Platelets were pelleted by centrifugation at room temperature at 1000*g* for 10 min. The supernatant was discarded and sedimented platelets were washed two times with 3 ml of phosphate-buffered saline containing EDTA (1 m*M*). "Washed" platelets were homogenized with 0.4 ml of water and 0.1 ml of 1% ascorbate and frozen on dry ice for storage at −30°C. Samples were thawed by sonication for analysis of tocopherol and tocotrienol concentrations.

**Lipid Analyses.** Plasma and lipoprotein fractions, total cholesterol, triglycerides, and phospholipids were measured by enzymatic assays, total cholesterol and triglyceride with Sigma kits (No. 362 and No. 336, respectively; Sigma Diagnostics Co., St. Louis, MO), and phospholipid by Wako kit (No. 996-5400; Wako Pure Chemical Ind. LTD, Osaka, Japan).

α- And γ-tocopherol concentrations in lipoprotein fractions were determined by the high-performance liquid chromatography method of Bieri *et al.* (10). Standards for *all-trans*-retinol, *all-trans*-retinyl acetate, D-α-tocopherol, and D-α-tocopheryl acetate were obtained from Hoffman-La Roche, Inc. (Nutley, NJ). In brief, 200 μl of plasma were mixed with 200 μl of ethanol solution (containing an internal standard of tocopheryl acetate) and extracted with 600 μl of hexane. A portion of the hexane extract was evaporated under nitrogen and redissolved in 100 μl of methanol. About 90 μl of methanol solution were injected into a $C_{18}$ reverse-phase, 150- × 4.6-mm, high-performance liquid chromatography column (Supelcosil LC-18, 5 μm of particle size; Supelco, Inc., Bellefonte, PA) equipped with a Rhodyne 7010 injection valve and 100-μl loop (Rainin Instrument Co., Inc., Emeryville, CA) and developed with methanol:water (95:5) using 2 ml/min flow rate (Beckman model 110A pump; Beckman Instruments Inc., Berkeley, CA). Retinol and α- and γ-tocopherol were detected at 290 nm in a Beckman UV detector (Beckman Instruments Inc., Palo Alto, CA) eluting in about 8 min. Retinol and α-tocopherol were quantified in relation to peak areas of corresponding internal standards.

Individual tocopherol and tocotrienol isomers in plasma, platelets, lymph, urine, bile, and various tissues were determined using an high-performance liquid chromatography procedure based on the method of Tan and Brzustkiewicz (11). In brief, 200–500 μl of plasma, bile, urine, lymph, or platelet suspension were mixed with the same amount of ethanol solution and extracted with 600–1500 μl of hexane. For tissue analysis, 0.3–1.0 g of sample was ground with anhydrous sodium sulphate to obtain a fine, dry powder followed by two to three extractions of lipids with hexane. A portion of the hexane extract was evaporated under $N_2$ and dissolved in 200–800 μl of methanol, and 100 μl were injected into a Zorbax PRO-10 ODS, 25 cm- × 4.6-mm, reverse-phase column (DuPont Co., Wilmington, DE) equipped with a Rhodyne 7010 injection valve and 100-μl loop (Rainin). The individual classes of tocopherols and tocotrienols were eluted from the column by an isocratic mobile phase of acetonitrile/methanol (95:5 v/v) using 1.6 ml/min flow rate (rabbit HP solvent delivery system; Rainin) and detected using fluorescence at 295 nm for excitation and 330 nm for emission (FD-300 Dual monochromator fluorescence detector; Spectro-Vision, Chelmsford, MA). Chromatography peaks were identified and sample concentration was determined by comparing with appropriate tocopherol and tocotrienol standards. All four tocopherol standards (α, β, γ, and δ) and α-tocotrienol were gifts from Hoffman-La Roche.

## Results

**Tocopherols.** Absorption and transport of tocopherols versus tocotrienols were compared on the chance that the tocols might have different lipid affinities in plasma or distributions among hamster tissues. In the case of tocopherol, hamster gut revealed proportional absorption of all the dietary isomers of tocopherol from intestinal lumen into the mucosal cells. However, secretion by intestinal mucosa cells into lymph was decidedly (10:1) in favor of α-tocopherol over γ-tocopherol. δ-Tocopherol was poorly secreted into lymph (Table II). This preferential transport and uptake of α-tocopherol over other tocopherol isomers also was noted in most tissues (except adipose), i.e., α-tocopherol represented 81–100% of all isomers. Adipose tissue was the only tissue that accumulated a substantial amount (33% of total tocopherols) as β + γ + δ-tocopherols (Table III). Plasma of fasted humans contained only about 10% (of total tocotrienols) as β + γ + δ-tocopherols, and plasma of fasted hamsters only about 3% as the non-α-tocopherol isomers (Table II).

In terms of the mass distribution of tocopherol among lipoproteins in our population of adult humans, VLDL transported 19%, IDL 3%, LDL 42%, and HDL 36%. α-Tocopherol represented 87–89% of the total tocopherol pool in each lipoprotein, with the balance

**Table II.** Absorption and Transport of Tocotrienols and Tocopherols as Evidenced by Concentration in Intestinal Mucosa, Lymph, and Plasma of Hamsters Fed a Diet Enriched with Tocotrienols[a] and in Plasma of Humans Supplemented with Tocotrienols[b]

| | *n* | Tocotrienol | | | | Tocopherol | | | | Tocotrienol to tocopherol |
|---|---|---|---|---|---|---|---|---|---|---|
| | | α | γ | δ | Total | α | β + γ | δ | Total | |
| Hamster (μg/g or μg/dl) | | | | | | | | | | |
| Diet | 4 | 80 ± 2 | 113 ± 4 | 30 ± 1 | 223 ± 6 | 12 ± 1 | 13 ± 0 | 2 ± 0 | 80 ± 1[c] | 3:1 |
| Intestinal mucosa (postprd; 3 hr) | 2 | 11 ± 1 | 19 ± 2 | 7 ± 1 | 37 ± 3 | 6 ± 0 | 6 ± 1 | 4 ± 1 | 16 ± 1 | 2:1 |
| Lymph (postprd; 3 hr) | 2 | 250 ± 60 | 400 ± 7 | 360 ± 20 | 1010 ± 15 | 3160 ± 530 | 220 ± 80 | 70 ± 20 | 3450 ± 620 | 1:3 |
| Plasma (over-night fast)[d] | 4 | <1 | <1 | 0 | <2 | 2520 ± 470 | 60 ± 10 | 30 ± 10 | 2610 ± 480 | 1:1300 |
| Plasma (postprd; 3 hr) | 3 | 30 ± 40 | 10 ± 1 | <1 | 40 ± 10 | 870 ± 110 | 70 ± 20 | 40 ± 10 | 980 ± 100 | 1:25 |
| Plasma (postprd; 3 hr + Triton)[e] | 3 | 220 ± 40 | 110 ± 20 | 20 ± 2 | 350 ± 40 | 1440 ± 150 | 180 ± 60 | 70 ± 20 | 1690 ± 130 | 1:5 |
| Human plasma (μg/dl) | | | | | | | | | | |
| Baseline (fasted 6 hr) | 5 | 0 | 0 | 11 ± 2 | 11 ± 2 | 1070 ± 190 | 120 ± 40 | 4 ± 4 | 1190 ± 240 | 1:110 |
| Supplemented (fasted 6 hr) | 5 | <1 | <1 | 13 ± 2 | 13 ± 2 | 1594 ± 330 | 50 ± 20 | 11 ± 1 | 1640 ± 320 | 1:130 |
| Supplemented (2 hr postprd) | 5 | 16 ± 10 | 13 ± 5 | 12 ± 1 | 41 ± 16 | 1940 ± 210 | 54 ± 8 | 18 ± 4 | 2010 ± 200 | 1:49 |

*Note:* Values represent mean ± SD (1 μmol of α-tocotrienol or α-tocopherol is equivalent to 425 μg and 431 μg, respectively).
[a] Hamsters were fed for 4 weeks a purified diet (Diet 182A, Table I) containing 223 ppm of tocotrienols and 70 ppm of tocopherols, of which 53 ppm were added with vitamin mix in the form of DL-α-tocopheryl acetate.
[b] Humans were supplemented with two Palm Vitee capsules (80 mg of tocotrienols and 64 mg of tocopherols) per day for 10 days.
[c] Value includes tocopheryl acetate added to diet in vitamin mix at 53 ppm.
[d] Plasma of hamsters fed for 11 weeks a purified diet described in Footnote *a*. Mean plasma cholesterol, 144 ± 62 mg/dl; triglyceride, 192 ± 78 mg/dl.
[e] Plasma obtained 2 hr after Triton WR 1339 injected into 3-hr-postprandial hamsters.

as γ-tocopherol. The concentration of α-tocopherol (expressed per total lipids for each lipoprotein) was significantly higher in HDL than in LDL or VLDL (Table IV). Plasma α-tocopherol was well correlated ($r = 0.74$) with phospholipids and cholesterol ($r = 0.73$), but not with core lipids, i.e., triglycerides. By contrast, plasma γ-tocopherol was correlated with triglycerides but not with phospholipids or cholesterol (Table V).

**Tocotrienols.** When tocotrienol absorption and transport were assessed in hamsters, it was observed that the tocotrienols behaved somewhat differently than the tocopherols. Minimal preference was observed for absorption of tocotrienol isomers from the lumen into the intestinal mucosa. However, the lymph contained 4-fold more δ-tocotrienol than would be expected from its relative concentration to α- and γ-tocotrienols in the diet or intestinal mucosa (Table II). Only in adipose tissue of supplemented hamsters did the tocotrienols maintain their dietary concentration advantage over the tocopherols (Table III). This is corroborated by the fact that the tocotrienol to tocopherol ratio was greater than 1.0 in adipose, which was the *only* tissue to accumulate more tocotrienols than tocopherols and only in *supplemented* hamsters. Tissue concentrations of tocotrienols were typically much lower than tocopherol (1:180 on average) in tocotrienol-supplemented hamsters and were undetectable in tissues from normal hamsters (with the exception of γ-tocotrienol in adipose tissue) (Table III). In contrast to tocopherols, the fasting hamster plasma contained no detectable tocotrienols, even after several weeks of supplementation. This was also true for the normal, fasting human, for whom values ranged between 5 and 15 μg/dl of γ-tocotrienol compared with 1190 μg/dl of tocopherol. Even after supplementation with 80 mg of tocotrienols/day for 10 days, the fasting plasma level in humans averaged only 13 μg/dl of tocotrienol (all δ-tocotrienol), compared with 1640 μg/dl for tocopherols (Table II).

Since tocotrienols were not detected in fasting hamster plasma, postprandial plasma samples were collected 3 hr after feeding and again 2 hr later after Triton blockade. These samples indicate that α-, γ-, and δ-tocotrienols were transported by postprandial lipoproteins, with α-tocotrienol now being twice as concentrated as γ-tocotrienol and 10 times greater than δ-tocotrienol (Table II). In contrast to plasma, the platelet concentration of tocotrienols doubled after supplementation with Palm Vitee in humans, and the increase in tocotrienols (entirely as δ-tocotrienol) was greater than the percentage of increase observed for tocopherols (Table VI).

**Tocotrienols and Plasma Cholesterol.** The poten-

**Table III.** Content of Tocotrienols and Tocopherols in Different Tissues of Hamsters Fed a Diet Enriched with Tocotrienols[a]

| Diet | n | Tocotrienol (μg/g or μg/dl) | | | | Tocopherol (μg/g or μg/dl) | | | | Tocotrienol to tocopherol |
|---|---|---|---|---|---|---|---|---|---|---|
| | | α | γ | δ | Total | α | β + γ | δ | Total | |
| Adipose[b] | 7 | 3.8 ± 0.4 | 5.3 ± 0.7 | 2.1 ± 0.3 | 11.2 ± 1.3 | 4.9 ± 0.7 | 1.4 ± 0.3 | 1.0 ± 0.4 | 7.3 ± 0.5 | 2:1 |
| Heart | 7 | 2.0 ± 0.4 | 0.5 ± 0.1 | <0.01 | 2.5 ± 0.4 | 28.2 ± 2.5 | 1.5 ± 0.3 | 2.8 ± 0.6 | 32.6 ± 3.0 | 1:13 |
| Testes | 7 | 0.8 ± 0.1 | 0.6 ± 0.1 | <0.01 | 1.4 ± 0.1 | 14.4 ± 2.9 | 1.2 ± 0.2 | 1.3 ± 0.1 | 17.6 ± 3.0 | 1:13 |
| Kidney | 4 | 0.5 ± 0.1 | <0.01 | <0.01 | 0.5 ± 0.1 | 16.8 ± 2.9 | 0.9 ± 0.3 | 1.5 ± 0.4 | 19.8 ± 3.5 | 1:40 |
| Adrenal glands | 6 | 2.6 ± 0.8 | 2.7 ± 0.8 | 0 | 5.3 ± 1.6 | 42.9 ± 2.3 | 3.7 ± 1.7 | 6.2 ± 0.6 | 52.8 ± 2.9 | 1:10 |
| Liver | 4 | 0.6 ± 0.0 | <0.01 | <0.01 | 0.6 ± 0.0 | 23.7 ± 3.2 | 1.2 ± 0.5 | 2.1 ± 0.6 | 27.4 ± 4.1 | 1:46 |
| Cerebellum | 5 | <0.01 | 0 | 0 | <0.01 | 18.9 ± 2.1 | 0.9 ± 0.1 | 0.4 ± 0.2 | 20.2 ± 1.9 | 1:>2000 |
| Cerebrum | 4 | <0.01 | 0 | 0 | <0.01 | 25.5 ± 4.4 | 0.3 ± 0.1 | 0.8 ± 0.3 | 26.6 ± 4.3 | 1:>2000 |
| Spleen | 3 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0 | 0.3 ± 0.1 | 35.8 ± 7.0 | 0.5 ± 0.2 | 0.4 ± 0.1 | 36.7 ± 6.7 | 1:120 |
| Pancreas | 3 | 1.6 ± 0.7 | 1.2 ± 0.1 | 0.2 ± 0.1 | 3.0 ± 0.7 | 78.4 ± 2.8 | 1.3 ± 0.5 | 0.9 ± 0.3 | 80.6 ± 3.2 | 1:27 |
| Retina | 4 | 0 | 0 | 0 | 0 | 79.0 ± 20.6 | 1.1 ± 0.6 | 1.0 ± 0.4 | 81.1 ± 21.2 | — |
| Seminal vesicle | 3 | 0.2 ± 0.1 | 0.2 ± 0.0 | <0.1 | 0.5 ± 0.4 | 9.1 ± 1.4 | 0.3 ± 0.2 | 0.2 ± 0.0 | 9.6 ± 1.2 | 1:19 |
| Muscle (gastrocnemius) | 3 | 0.9 ± 0.3 | 0.3 ± 0.2 | 0.3 ± 0.1 | 1.4 ± 0.5 | 35.2 ± 7.9 | 1.4 ± 0.4 | 0.7 ± 0.1 | 37.3 ± 7.5 | 1:27 |
| Bile | 4 | 0 | 0 | 0 | 0 | 64 ± 17 | 0 | 0 | 64 ± 17 | — |
| Urine | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — |

*Note:* Values represent mean ± SD.
[a] Hamsters were fed for 11 to 15 weeks a purified diet containing 223 ppm of tocotrienols and 70 ppm of tocopherols.
[b] Only adipose tissue of control animals (3 ppm of dietary tocotrienol) revealed a detectable level of tocotrienols as γ-tocotrienol only (0.8 ± 0.1 μg/g).

**Table IV.** Distribution of Tocopherols in Human Lipoproteins

| Lipoprotein fraction | n | α-Tocopherol to total lipids[a] (μg/mg) | α-Tocopherol (μg/dl) | γ-Tocopherol (μg/dl) | α + γ-Tocopherol (μg/dl) | α + γ-Tocopherol (% distribution) |
|---|---|---|---|---|---|---|
| VLDL | 7 | 1.58 ± 0.17[b] | 167 ± 63 (88 ± 4)[d] | 22 ± 12 | 189 ± 70 | 19 ± 7 |
| LDL | 7 | 1.50 ± 0.16[c] | 369 ± 47 (87 ± 1) | 57 ± 11 | 426 ± 56 | 42 ± 5 |
| HDL | 7 | 1.91 ± 0.18[b,c] | 323 ± 62 (87 ± 4) | 46 ± 18 | 369 ± 68 | 36 ± 5 |
| All fractions (VLDL + IDL + LDL + HDL) | 7 | 1.74 ± 0.15 | 891 ± 88 (87 ± 3) | 127 ± 34 | 1004 ± 89 | 100 |

*Note:* Values are expressed as mean ± SD.
[a] Total lipids = total cholesterol + triglyceride + phospholipid in each lipoprotein fraction.
[b,c] Means with common superscript are significantly different by one factorial analysis of variance and Fisher's protected least significant difference analysis ($P < 0.05$).
[d] α-Tocopherol as percentage of total (α + γ-tocopherol) in each lipoprotein.

**Table V.** Correlation Coefficients between Concentration of Fasting Human Plasma Lipid Components and α- and γ-Tocopherol[a]

| | r | P |
|---|---|---|
| Plasma components | | |
| Cholesterol vs α-tocopherol | 0.73 | <0.005 |
| Cholesterol vs γ-tocopherol | 0.36 | NS |
| Triglyceride vs α-tocopherol | 0.34 | NS |
| Triglyceride vs γ-tocopherol | 0.58 | <0.05 |
| Phospholipids vs α-tocopherol | 0.74 | <0.005 |
| Phospholipids vs γ-tocopherol | 0.51 | NS |

[a] Because the fasting plasma concentration of tocotrienols was so low, their correlation with lipid fractions was not attempted.

tial for tocotrienols to lower the plasma cholesterol was explored in normolipemic hamsters. The results (Table VII) indicate that neither intermediate (36 ppm) nor high level (223 ppm) supplementation of tocotrienols in diets fed to hamsters for 4 weeks altered total plasma cholesterol, HDL-cholesterol, or triglycerides.

## Discussion

It is well appreciated that the four isomers of tocopherol (α, β, γ, and δ) are not equally absorbed by the intestine or transported by lipoproteins, nor are they equally sequestered by certain tissues (12–14). By virtue of a specific α-tocopherol binding protein in hepatocytes (and possibly in other cell types as well), the α-isomer is preferentially retained by the liver and subsequently secreted in VLDL (15, 16). The α-tocoph-

**Table VI.** Effect of Tocol Supplementation[a] on Tocotrienol and Tocopherol Concentration in Human Platelets

| | *n* | Platelet | | | | | | | | Tocotrienol to tocopherol |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Tocotrienol ($\mu g/10^{11}$) | | | | Tocopherol ($\mu g/10^{11}$) | | | | |
| | | $\alpha$ | $\gamma$ | $\delta$ | Total | $\alpha$ | $\beta + \gamma$ | $\delta$ | Total | |
| Baseline | 3 | 0 | 0 | 2.5 ± 0.4 | 2.5 ± 0.4 | 59 ± 3.6 | 7.7 ± 0.8 | 0.1 ± 0.1 | 67 ± 3.5 | 1:27 |
| Supplemented | 3 | 0 | 0 | 4.9 ± 0.9[b] | 4.9 ± 0.9[b] | 86 ± 20 | 4.4 ± 2.0 | 0.4 ± 0.2 | 91 ± 23 | 1:19 |

*Note:* Values are mean ± SD.
[a] Humans were supplemented with two Palm Vitee capsules (80 mg of tocotrienols and 64 mg of tocopherol) per day for 10 days and bled in a semifasted state (6 hr after a light breakfast).
[b] Significantly different from baseline value by paired *t* test ($P < 0.05$).

**Table VII.** Effect of Tocotrienol Supplementation on Hamster Growth and Plasma Lipids[a]

| | Dietary tocotrienols | | |
|---|---|---|---|
| | 3 ppm ($n = 7$) | 36 ppm ($n = 8$) | 223 ppm ($n = 8$) |
| Body wt (g) | | | |
| Initial | 58 ± 4 | 59 ± 2 | 60 ± 3 |
| Final | 118 ± 9 | 121 ± 13 | 116 ± 10 |
| Liver wt (g) | 3.9 ± 0.5 | 4.0 ± 0.5 | 3.7 ± 0.3 |
| Plasma cholesterol (mg/dl) | | | |
| Total | 120 ± 28 | 125 ± 18 | 124 ± 33 |
| HDL | 63 ± 13 | 65 ± 15 | 66 ± 17 |
| Plasma triglyceride (mg/dl) | 168 ± 97 | 106 ± 46 | 125 ± 68 |

[a] Syrian hamsters were fed for 4 weeks semipurified diets (see Table I) containing either 3 ppm, 36 ppm, or 223 ppm of tocotrienols. Values are mean ± SD.

erol binding protein would appear to be present in hamster intestine too, since even though all isomers of tocopherol were absorbed from the gut lumen into the intestinal mucosal cell, $\alpha$-tocopherol was preferentially secreted in chylomicrons, as detected in lymph and postprandial lipoproteins. Once returned to the liver in chylomicron remnants, $\alpha$-tocopherol is selectively bound to the cellular binding protein (17) for resecretion in nascent hepatic lipoproteins, whereas $\beta$-, $\delta$-, and $\gamma$-tocopherol disappear rapidly, except for a minor component of $\gamma$-tocopherol representing about 10–15% of the $\alpha$-tocopherol mass (15). The rapid disappearance of $\beta$- and $\delta$-tocopherol may reflect the methylation of these two dimethyl isomers to the trimethyl $\alpha$-tocopherol by tissues, or they may be secreted in bile (15), although only $\alpha$-tocopherol could be detected in hamster bile. High $\alpha$-tocopheryl acetate supplementation results in further displacement of plasma $\gamma$-tocopherol (18).

Although it is generally agreed that tocopherol is transported by lipoproteins, reports vary in their assertion that most tocopherol is carried either by LDL (19) or HDL (20). In our population of adults, VLDL transported 19%, IDL 3%, LDL 42%, and HDL 36%. In infants, who usually have a higher percentage of HDL than adults, the HDL transports more tocopherol than LDL (21). Because HDL is relatively phospholipid-rich, the high concentration of $\alpha$-tocopherol in HDL and the correlation between $\alpha$-tocopherol and plasma phospholipids suggest that $\alpha$-tocopherol is associated with phospholipids on the surface of lipoproteins, presumably with the polyunsaturated fatty acids in the phospholipid molecule (22). By contrast, $\alpha$-tocopherol was not associated with the triglyceride content (i.e., core lipid) of the lipoproteins, whereas $\gamma$-tocopherol did appear associated with plasma triglyceride. Thus, the phospholipid-rich, triglyceride-poor HDL has a higher concentration of $\alpha$-tocopherol/mg of lipid than LDL or VLDL. Because adults, in comparison with infants (21) have a relatively expanded mass of LDL (e.g., the LDL-C in these adults was 124 ± 7 mg/dl compared with 65 ± 19 mg/dl in the infants described previously [18]), the phospholipids in the LDL fraction usually transport the largest mass of tocopherol in adults. This distinction between tocopherol concentration (depending on phospholipid concentration) and bulk transport (depending on lipoprotein pool size) would appear to explain much of the discrepancy concerning which lipoprotein fraction carries the most tocopherol.

In contrast to $\alpha$-tocopherol, our data suggest that tocotrienols are transported nonspecifically like any lipid-soluble compound (similar to the bulk of $\gamma$-tocopherol), most likely being incorporated with triglyceride in the core of the triglyceride-rich chylomicron.

Once transported to the adipose tissue, a modest level of tocotrienol (principally γ-tocotrienol) appears to be deposited and stored with the triglyceride, presumably to be released during lipolysis. Unlike α-tocopherol, none of the tocotrienols appeared to be sequestered by a cytosolic binding protein in the liver to enhance their conservation and resecretion into lipoproteins. The essential lack of tocotrienols in LDL or HDL, in contrast with α-tocopherol, points to the striking difference in their transport and underscores the likelihood that the two classes of tocols associate differently with lipoprotein lipid moieties during transport in plasma and with cellular lipids in tissues. It is unexplained why δ-tocotrienol was the only isomer detectable in fasting human plasma and represented the only form in platelets, although its concentration was only 3–5% that of the α-tocopherol in platelets.

Aside from the demonstrated *in vitro* antioxidant potential of tocotrienols (4, 5), it has been reported (2, 3) that tocotrienol supplementation of hypercholesterolemic humans can lower the plasma cholesterol 5–36%, especially the LDL fraction. A reduction in LDL cholesterol would be an obvious benefit to reducing risk to atherosclerosis. However, neither the normolipemic hamsters in the present study nor a double-blind cross-over study in hyperlipemic humans (23) has confirmed a change in plasma cholesterol during tocotrienol supplementation. Our inability to demonstrate reasonable transport or tissue accumulation of tocotrienols seriously questions whether a generalized biologic role for this tocol can be justified.

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