Discrimination between forms of vitamin E by humans with and without genetic abnormalities of lipoprotein metabolism

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Abstract To study the mechanisms of discrimination between various forms of vitamin E, four normal subjects, one patient with lipoprotein lipase deficiency, and three patients with abnormal apolipoprotein B-100 production were given an oral dose containing three tocopherols labeled with differing amounts of deuterium (2*R*,4'*R*,8'*R*-α-(5,7-(C^2H_3)$_2$)tocopheryl acetate (d_6-*RRR*-α-tocopheryl acetate), 2*S*,4'*R*,8'*R*-α-5-(C^2H_3)tocopheryl acetate (d_3-*SRR*-α-tocopheryl acetate), and 2*R*,4'*R*,8'*R*-γ-(3,4-^{2}H)tocopherol (d_2-*RRR*-γ-tocopherol). The tocopherol contents of plasma, red cells, and lipoproteins were measured up to 76 h after the dose. In normal subjects all three tocopherols were absorbed and secreted in chylomicrons with equal efficiencies. Both d_2-γ- and d_3-*SRR*-α-tocopherols peaked at similar concentrations in the other lipoprotein fractions, then decreased similarly, but 2–4 times more rapidly than did d_6-*RRR*-α-tocopherol. A lipoprotein lipase-deficient patient and a patient with prolonged production of chylomicrons with absent apolipoprotein B-100 also demonstrated the lack of discrimination between tocopherols during absorption. Despite abnormal apolipoprotein B-100 production in two patients, the "VLDL" was preferentially enriched in d_6-*RRR*-α-tocopherol. Our results show that there is no discrimination between the three tocopherols during absorption and secretion in chylomicrons, but subsequently there is a preferential enrichment of very low density lipoprotein (VLDL) with *RRR*-α-tocopherol. Catabolism of this VLDL results in the maintenance of plasma *RRR*-α-tocopherol concentrations.—**Traber, M. G., G. W. Burton, L. Hughes, K. U. Ingold, H. Hidaka, M. Malloy, J. Kane, J. Hyams, and H. J. Kayden.** Discrimination between forms of vitamin E by humans with and without genetic abnormalities of lipoprotein metabolism. *J. Lipid Res.* 1992. **33:** 1171–1182.

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Studies using deuterium-labeled tocopherols have produced new insights into the absorption and transport of vitamin E (1–9). In humans, orally administered deuterated α-tocopherol is first secreted from the intestine in chylomicrons, then is secreted from the liver in very low density lipoproteins (VLDL) and appears in the plasma simultaneously in low and high density lipoproteins (LDL and HDL, respectively) (4).

Studies in rats (1), monkeys (7), and humans (6) using two stereoisomers of α-tocopherol, *RRR*- and *SRR*-, labeled with different amounts of deuterium, have demonstrated that there is a marked preference for the naturally occurring *RRR*-stereoisomer. However, after oral administration of equal amounts of these two stereoisomers there were no differences in their concentrations in human chylomicrons, but subsequently the VLDL were found enriched with *RRR*-α-tocopherol (6). The catabolism of VLDL particles enriched the other plasma lipoproteins with d_6-*RRR*-α-tocopherol. This preferential secretion of d_6-*RRR*-α-tocopherol in VLDL has been demonstrated directly in nascent lipoproteins isolated from perfused livers of monkeys fed a combination of deuterated tocopherols (7).

On the basis of discrimination between tocopherols, we have suggested (1, 6, 10) that a protein, such as the hepatic tocopherol-binding protein first identified in rats by Catignani and Bieri (11), is involved in the discrimination process. Support for this hypothesis was obtained from studies of patients with familial isolated vitamin E deficiency. These patients are unable to maintain normal plasma tocopherol levels without vitamin E supplements (12). When they were given an oral dose of deuterated α-tocopheryl acetate, the maximum increase in deuterated α-tocopherol in their plasma took place during chylomicron catabolism, while in the controls the maximum increase took place during VLDL catabolism (8). In addi-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; GC–MS, gas chromatography–mass spectrometry.

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tion, the decrease in deuterated tocopherol levels in plasma was faster in the patients compared with the controls. Taken together, these results suggest that the patients have an impaired incorporation of α-tocopherol into VLDL (8). It is thus plausible that the mechanism(s) for discrimination between stereoisomers of α-tocopherol and for incorporation of tocopherols into VLDL are identical.

The physiological relevance of a protein that discriminates between stereoisomers that occur only in synthetic *all racemic* α-tocopherol is not obvious. However, naturally occurring vitamin E is present in the diet in eight different forms: α-, β-, γ-, and δ-tocopherols (which differ in the number and position of methyl groups attached to the chroman "head" group) and α-, β-, γ-, and δ-tocotrienols (which differ from the corresponding tocopherols in having a phytyl "tail" with three double bonds). The tocopherol-binding protein may discriminate between these natural forms of vitamin E, as *1*) the bioactivities of the tocopherols decrease in the order $\alpha > \beta > \gamma > \delta$ (13), and *2*) in in vitro competitive binding assays with the protein only α-tocopherol and, to a lesser extent, α-tocotrienol and γ-tocopherol were effective (11). In vivo, preference for α-tocopherol compared with γ-tocopherol has been observed in humans less than 24 h after a large (1 g) oral dose of both tocopherols (10).

To explore further the discrimination between tocopherols (such as *SRR*-α-tocopherol and γ-tocopherol) the present study was carried out in normal subjects and in patients with defined genetic abnormalities of lipid metabolism. A patient with lipoprotein lipase deficiency was studied because this defect results in extremely high plasma levels of triglyceride-rich chylomicrons and VLDL, and thus the slowed catabolism of these lipoproteins permits a more detailed analysis of tocopherols secreted in these fractions. To evaluate the role of VLDL secretion in the discrimination between tocopherols, three patients with abnormalities in apolipoprotein B-100 production were studied, although only one was found to have an apparent inability to secrete VLDL.

Apolipoprotein B-100 is a major apolipoprotein in VLDL secreted by the liver, while apolipoprotein B-48 (48% of the amino terminal portion of B-100) is secreted in chylomicrons by the intestine (14). If discrimination between tocopherols occurred solely during the synthesis and secretion of VLDL, then patients who are able to secrete chylomicrons, but have an impaired production of apolipoprotein B-100, should not be able to discriminate between tocopherols. Therefore, a single oral dose containing γ-tocopherol, and *RRR*- and *SRR*-α-tocopheryl acetates, labeled with different amounts of deuterium, was administered to patients and controls, so that comparisons of the lipoprotein patterns of deuterated tocopherols would indicate which specific steps of lipoprotein secretion and catabolism result in discrimination between tocopherols.

METHODS

Deuterated tocopherols

The syntheses of all but one of the deuterated tocopherols have been described previously (1, 15, 16). For this work an additional deuterated γ-tocopherol was prepared for use as an internal standard. *RRR*-γ-tocotrienol (0.25 g) dissolved in $CH_3CH_2O^2H$ (7 ml) was allowed to stand overnight under deuterium gas at one atmosphere in the presence of Adams catalyst (0.03 g). The crude deuterated γ-tocopherol was purified by chromatography on silica gel using 5% ethyl acetate in hexane as an eluent. Analysis by gas chromatography–mass spectrometry (GC–MS) showed the γ-tocopherol to be a mixture containing a continuous distribution of 0 to 33 atoms of deuterium. d_{17}-γ-Tocopherol was the most abundant peak in this mixture (10%) and was used to determine the amounts of d_0- and d_2-γ-tocopherol in each sample. As the amounts of d_0- and d_2-γ-tocopherol present in the internal standard mixture were less than 1% and 2% of the d_{17}-γ-tocopherol, respectively, their contributions to the d_0- and d_2-γ-tocopherol peak areas in each sample were ignored. Also, the more highly deuterated forms in the γ-tocopherol mixture did not interfere with the unlabeled and deuterated forms of α-tocopherol because the two forms of tocopherol were well resolved under the GC–MS conditions.

A mixture containing 2*R*,4'*R*,8'*R*-α-(5,7-(C^2H_3)$_2$)tocopheryl acetate (d_6-*RRR*-α-tocopheryl acetate), a source of hexadeuterated α-tocopherol with natural stereochemistry, 2*S*,4'*R*,8'*R*-α-5-(C^2H_3)tocopheryl acetate (d_3-*SRR*-α-tocopheryl acetate), a source of trideuterated α-tocopherol with reversed stereochemistry at position 2, and 2*R*,4'*R*,8'*R*-γ-(3,4-^{2}H)tocopherol (d_2-*RRR*-γ-tocopherol), a source of dideuterated γ-tocopherol with natural stereochemistry, were administered orally to each subject. Internal standards, 2-*ambo*-α-(5,7,8-(C^2H_3)$_3$)tocopherol (d_9-α-tocopherol) and "d_{17}"-γ-tocopherol were added in known amounts to each sample immediately prior to extraction of the lipids into heptane (4, 5). These heptane extracts were purified by passage through an analytical, high-performance, silica gel chromatography column. The amounts of d_2-γ-tocopherol, d_3-, d_6-, d_9-α-tocopherols and unlabeled (d_0-) α- and γ-tocopherols in the collected tocopherol fraction were determined by GC-MS after conversion to their trimethylsilyl ethers. The absolute concentrations of d_0-, d_3-, and d_6-α-tocopherols and d_0- and d_2-γ-tocopherols in the plasma and lipoprotein samples were obtained by comparing their respective peak areas with the peak areas of the corresponding added d_9-α- and d_{17}-γ-tocopherols (4, 5, 8).

Experimental protocols

This study was carried out with the approval of the Institutional Review Board of New York University Medical

Center. All of the subjects gave written, informed consent. The control subjects had no abnormalities of lipid or lipoprotein metabolism; numbers 1–3 were male, aged 24–26 years and subject 4 was female, aged 13 years. The lipoprotein lipase-deficient patient, DP (15-year-old female), has normal apolipoprotein C-II levels and nondetectable lipoprotein lipase activity (H. J. Kayden, unpublished observations); her ability to transport tocopherols has been studied previously (subject 9, ref. 10). The three patients with abnormal apolipoprotein B production are as follows: *1*) AF (female, aged 22 years), the first patient reported to have normal apolipoprotein B-48 but to lack apolipoprotein B-100, was described by Malloy et al. (17). She is now known to synthesize apolipoprotein B-48 and a protein corresponding to the N-terminal 50% of the sequence of apolipoprotein B-100 (18). She is homozygous for a C to T substitution in codon 2252 of the apolipoprotein B-100 gene producing an in-frame stop codon, which results in a truncated protein. No lipid composition of her lipoproteins is available, but electron photomicrographs demonstrate the presence of both VLDL and LDL particles (18). She was reported to have neurologic abnormalities (mainly ataxia) resulting from vitamin E deficiency, which were alleviated by administration of supplemental vitamin E (17). *2*) DL (male, age 8 years), was described by Herbert et al. (19) to have a genetic defect that abolishes production of apolipoprotein B-100 and a prolonged, but defective, production of chylomicrons containing apolipoprotein B-48. The VLDL/chylomicron fraction contained 62% triglyceride, 7% total cholesterol, 25% phospholipids, and 5% protein on a dry weight basis; the LDL fraction contained no measurable protein or lipid (19). This child has been given large oral supplements of vitamin E from infancy and has no symptoms of vitamin E deficiency. *3*) KK (male, age 48 years), a member of a large kindred was described by Harano et al. (case 4, ref. 20) to have an abnormal apolipoprotein B-100 production. It was suggested that this patient has homozygous hypobetalipoproteinemia with spared chylomicron formation (20), but because of the variations in the symptomatology of the kindred, he may be a compound heterozygote with two different abnormalities in apolipoprotein B-100 production. His VLDL contained cholesterol (11 mg/dl plasma), triglycerides (10 mg/dl), and apolipoprotein B (1 mg/dl); his LDL contained cholesterol (1 mg/dl), triglycerides (4 mg/dl), and apolipoprotein B (1 mg/dl) (20). No neurologic abnormalities were observed in this patient; of the family members only the father (now deceased) had neurologic abnormalities (ataxia) that could have been the result of vitamin E deficiency.

With one exception, each subject consumed a capsule containing 50 mg each of d_6-*RRR*-α-tocopheryl acetate, d_3-*SRR*-α-tocopheryl acetate, and d_2-*RRR*-γ-tocopherol with breakfast after an overnight fast. The exception was control subject #1 who consumed 75 mg of each tocopherol and whose results for *RRR*- and *SRR*- have been reported previously (as subject #9, ref. 6). The 50-mg dose was chosen because we have found in our previous studies (6) that adequate amounts of the labeled tocopherols are present in plasma lipoproteins for measurement of deuterated tocopherols after oral administration of this amount of labeled vitamin E. Smaller doses (20 mg of each *RRR*- and *SRR*-α-tocopheryl acetates labeled with 6 and 3 atoms of deuterium, respectively) in normal subjects have yielded qualitatively similar results (21). Although α-tocopheryl acetates and γ-tocopherol were used in this study, the acetate forms of α-tocopherol have previously been shown to be equivalent to feeding the free tocopherol forms (5).

Blood samples were drawn into EDTA tubes (Becton Dickinson, Rutherford, NJ) at approximately 0, 3, 6, 9, 12, 27, 51, and 75 h after consumption of the tocopherols (the precise times at which blood was drawn are shown in the figures). Subjects were allowed to eat ad libitum.

The plasma was immediately separated from blood cells by centrifugation. An aliquot (1.0 ml) was frozen for subsequent tocopherol analysis and the remainder was used for isolation of the lipoprotein fractions, as described previously (6). For patients not studied at NYU Medical Center, the remaining plasma was refrigerated until all of the samples had been obtained. These plasma samples were then shipped on wet ice by overnight courier to NYU Medical Center for lipoprotein isolation. In all cases chylomicrons and lipoproteins were isolated within 10 days of tocopherol administration and were frozen at −70 to −80°C. We have determined previously that refrigeration at 4°C retards the exchange of tocopherol between lipoproteins (8) and we have found no change in the distribution of tocopherols when the lipoproteins were isolated immediately after drawing blood compared with plasma that had been stored in the refrigerator for 2 weeks prior to lipoprotein isolation (M. G. Traber and G. W. Burton, unpublished observations).

Red cells were isolated by centrifugation at 1700 rpm for 5 min, washed 3 times by repeated resuspension in cold isotonic phosphate-buffered saline (PBS), followed by centrifugation at 1700 rpm for 5 min. They were resuspended in PBS to approximately a 50% hematocrit, the actual hematocrit of the samples was measured, then 1-ml aliquots were frozen at −80°C until shipped.

The frozen plasma, red cells, and lipoprotein fractions were shipped on dry ice to the National Research Council of Canada where they were kept frozen at −80°C until analyzed.

Mathematical and statistical analysis

The linear regression function (linest) of a spreadsheet

program (Microsoft Excel) was used to calculate the slope of the curve of the logarithms of the deuterated tocopherol concentrations versus time using the data past the maximum concentration for the plasma, VLDL, LDL, and HDL for each individual normal subject. The statistical significance of the slopes of the plasma and the lipoprotein fractions for all three tocopherols was determined simultaneously using a Macintosh II computer (Apple Computers, Cupertino, CA) and the statistical analysis program, Super Anova, (Abacus Concepts, Berkeley, CA) using two factor analysis of variance (ANOVA) and least square means comparisons. Results of the statistical tests were considered to be significant at the 95% confidence level ($P < 0.05$).

RESULTS

Studies of tocopherol discrimination in control subjects

The mean ± standard error of the concentrations of each of the deuterated tocopherols over 3 days in the plasma, red blood cells, and lipoproteins of four controls are shown as semi-log plots in **Fig. 1**. (A complete set of the individual data is available from the authors upon request.) By 28 h the plasma in all four control subjects showed a marked discrimination between deuterated tocopherols with a preferential retention of d_6-*RRR*- as compared with d_3-*SRR*-α- and d_2-γ-tocopherols. Plasma d_3-*SRR*- (−0.013 ± 0.002 log[nmol/ml]/h) and d_2-γ-tocoph-

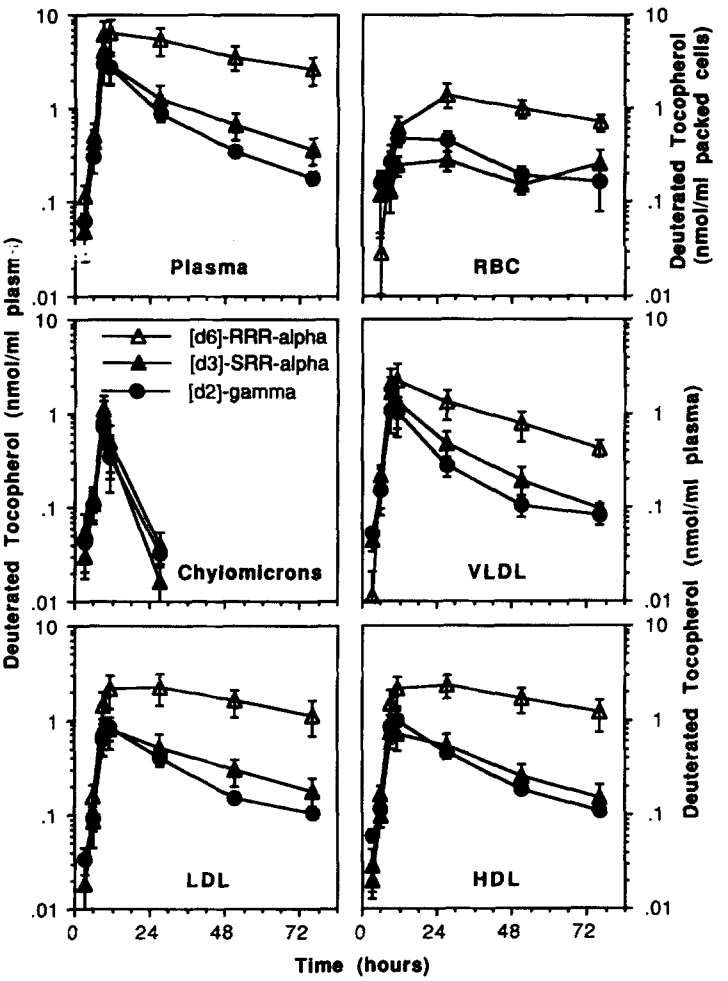

Fig. 1. Four control subjects were given an oral dose containing 50 mg each of 2*R*,4'*R*,8'*R*-α-(5,7-(C^2H_3)$_2$)tocopheryl acetate (d_6-*RRR*-α-tocopheryl acetate), 2*S*,4'*R*,8'*R*-α-5-(C^2H_3)tocopheryl acetate (d_3-*SRR*-α-tocopheryl acetate), and 2*R*,4'*R*,8'*R*-γ-(3,4-^{2}H)tocopherol (d_2-*RRR*-γ-tocopherol), then blood samples were obtained at the indicated intervals. Lipoproteins were isolated as described in the Methods section. The mean ± standard error (SE) of the deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, red cells, chylomicrons, VLDL (d<1.006), LDL (1.006<d<1.063), and HDL (d>1.063 g/ml).

erol (−0.017 ± 0.005) decreased at rates roughly three times faster than did d_6-*RRR*-α-tocopherol (−0.005 ± 0.003) (**Table 1**). By 28 h the red cells also showed a preferential enrichment with d_6-*RRR*-α-tocopherol as compared with the other two labeled tocopherols (Fig. 1).

The chylomicron fraction contained approximately equal concentrations of the three labeled tocopherols in all four control subjects, while the other lipoprotein fractions became preferentially enriched in d_6-*RRR*-α-tocopherol by 28 h (Fig. 1). The rates at which d_3-*SRR*-α-tocopherol and d_2-γ-tocopherol decreased in plasma and each of the nonchylomicron lipoprotein fractions were significantly ($P < 0.05$) faster than that of d_6-*RRR*-α-tocopherol (Table 1). There were no significant differences in the rates of decrease between d_3-*SRR*-α- and d_2-γ-tocopherols with the exception of those in LDL, where d_2-γ-tocopherol decreased faster (Table 1).

To determine whether the lack of discrimination between tocopherols in VLDL during the first 12 h was due to an incomplete separation of chylomicron remnants from VLDL, triglyceride-rich lipoproteins containing apolipoprotein B-100 were isolated using apolipoprotein B-100 affinity columns provided by Dr. Ross Milne (Hospital St. Justine, Montreal, Quebec, Canada). The ratio of all three deuterated tocopherols to each other in the apolipoprotein B-100 particles during the first several hours of the study remained close to unity, but by 28 h there was a marked enrichment in d_6-*RRR*-α-tocopherol (data not shown), similar to the results from the isolation of VLDL by ultracentrifugation. It appears that during chylomicron catabolism all of the plasma lipoproteins, including VLDL, rapidly acquire the tocopherols present in the oral dose. Only after the intestinally derived particles are removed from the plasma and the VLDL, newly secreted from the liver, are the dominant particles in this density range, does the plasma become preferentially enriched in α-tocopherol, as was observed previously (6, 7, 10).

Studies in patients with defined genetic abnormalities of lipoprotein metabolism

Lipoprotein lipase deficiency. After an oral dose of the three labeled tocopherols, the plasma from a patient with lipoprotein lipase deficiency, DP, contained higher concentrations of tocopherols than did the controls, but it took longer for the plasma of this patient to become preferentially enriched with d_6-*RRR*-α-tocopherol (~48 h) (compare **Fig. 2** with Fig. 1). The peak concentrations of the deuterated tocopherols in the chylomicrons were almost 10-fold higher than in the controls. Furthermore, the deuterated tocopherols in this fraction did not decrease precipitously, but remained elevated for 72 h. It is, therefore, likely that the chylomicron fraction also contains large, lipid-rich, liver-derived VLDL (22). Preferential enrichment of both the chylomicrons and the VLDL with d_6-*RRR*-α-tocopherol occurred by 48 h. The concentrations in LDL and HDL, which are minor carriers of plasma vitamin E in this patient, were within the normal range, and both lipoproteins were enriched with d_6-*RRR*-α-tocopherol by 24 h. The labeled tocopherol concentrations in the red cells were comparable to those in the controls, although the degree of discrimination was less pronounced.

Abnormalities in apolipoprotein B production. To evaluate the role of VLDL secreted by the liver in the discrimination between tocopherols, three patients with abnormalities in apolipoprotein B-100 production were studied. Although these patients were not expected to have apolipoprotein B-100-containing lipoproteins (VLDL and LDL), nonetheless lipoproteins were isolated by density gradient ultracentrifugation at $d < 1.006$ and at $1.006 < d < 1.063$ g/ml. The actual protein and lipid composition of the particles from these patients have not been analyzed; therefore, we have noted that the particles isolated in these density ranges are abnormal by designating them "VLDL" and "LDL," respectively.

AF synthesizes and secretes apolipoprotein B-48 and a protein corresponding to the N-terminal 50% of apolipoprotein B-100 (18). As shown in **Fig. 3**, 12 h after oral administration of the three labeled tocopherols, her plasma and red cells were preferentially enriched in d_6-*RRR*-α-tocopherol. Nearly equal concentrations of the three tocopherols were present in the "chylomicron fraction," while the "VLDL" fraction was preferentially enriched in d_6-*RRR*-α-tocopherol by 12 h. Furthermore, the amounts

TABLE 1. Slopes (log[nmol/ml]/h) of the disappearance portion of the logarithms of the deuterated tocopherol concentrations versus time curves

Fraction	[d_2]-γ-Tocopherol	[d_3]-*SRR*-α-Tocopherol	[d_6]-*RRR*-α-Tocopherol
VLDL	−0.015 ± 0.004[a]	−0.015 ± 0.005[b]	−0.009 ± 0.006[a,b]
LDL	−0.015 ± 0.004[a,c]	−0.010 ± 0.001[b,c]	−0.004 ± 0.001[a,b]
HDL	−0.015 ± 0.002[a]	−0.011 ± 0.002[b]	−0.004 ± 0.001[a,b]
Plasma	−0.017 ± 0.005[a]	−0.013 ± 0.002[b]	−0.005 ± 0.003[a,b]

For each lipoprotein fraction within each row, slopes for the different labeled tocopherols sharing the same superscript letter are significantly different, $P < 0.05$.

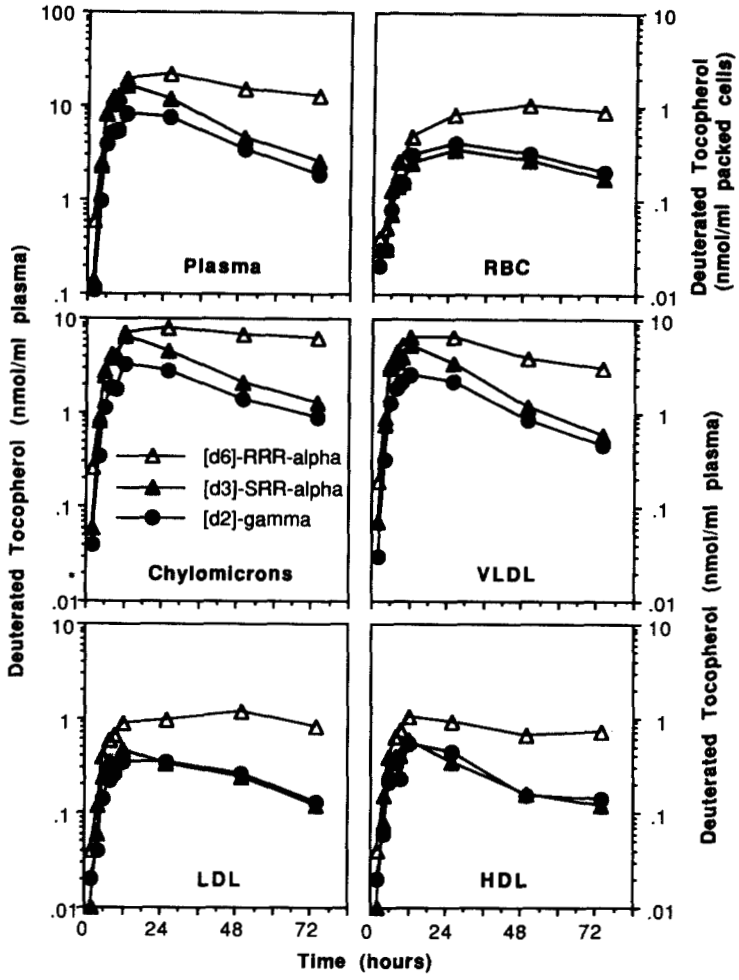

Fig. 2. DP, a lipoprotein lipase-deficient patient, was studied under the conditions described in Fig. 1. The deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, red cells, chylomicrons, VLDL ($d < 1.006$), LDL ($1.006 < d < 1.063$), and HDL ($d > 1.063$ g/ml). Note that the y-axis for the labeled tocopherol concentrations in plasma is 10-fold higher than in the other figures.

of d_6-*RRR*-α-tocopherol in the "VLDL" fraction continued to increase from 12 to 72 h, suggesting continued production of nascent "VLDL" particles by the liver. These data show that AF discriminates between tocopherols and secretes an apolipoprotein B-50, VLDL-density particle, preferentially enriched in d_6-*RRR*-α-tocopherol.

DL has a genetic defect that abolishes production of apolipoprotein B-100, and has a prolonged, but defective, production of chylomicrons containing apolipoprotein B-48 (19). As shown in **Fig. 4**, after oral administration, the plasma contained similar, but increasing, concentrations of the three deuterated tocopherols over the 48 h that this patient was studied. Furthermore, the deuterated tocopherols in all of the lipoproteins increased throughout the study, but the concentrations were lower than those observed in the controls (compare Fig. 4 with Fig. 1). In addition, the red cells contained much lower levels of tocopherols than those of the controls or the other patients.

KK has a genetic defect in apolipoprotein B-100 production (20). As shown in **Fig. 5**, 12 h after oral administration of the three deuterated tocopherols, his plasma and red cells were preferentially enriched with d_6-*RRR*-α-tocopherol. Compared with the controls, the patient had a more prolonged secretion of deuterated tocopherols in the chylomicron fraction with a second peak occurring at 36 h (compare Fig. 5 with Fig. 1). Although the concentrations of the deuterated tocopherols in the "VLDL" and the "LDL" fractions were markedly lower than those of the controls, these lipoproteins were preferentially enriched with d_6-*RRR*-α-tocopherol. KK's "LDL" contained more d_6-*RRR*-α-tocopherol than did those of AF (Fig. 3). KK's HDL not only were preferentially enriched in d_6-*RRR*-α-tocopherol, but the concentrations were within the control HDL range.

In KK's plasma and lipoproteins, unlike those of the

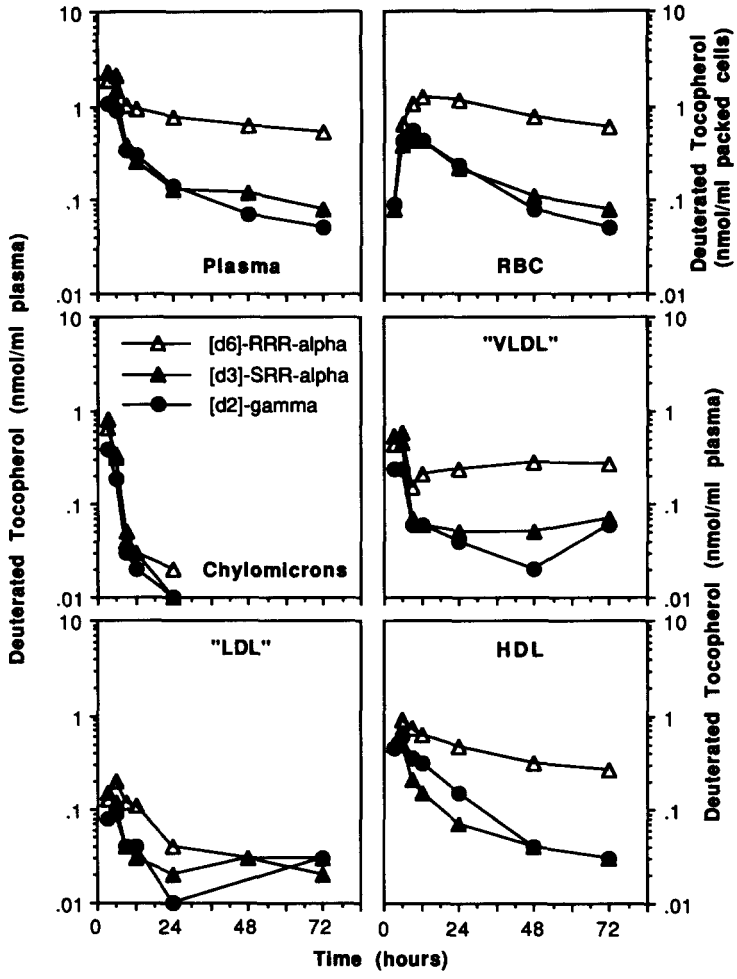

Fig. 3. AF, a patient with normotriglyceridemic abetalipoproteinemia, was studied under the conditions described in Fig. 1. The deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, red cells, chylomicrons, "VLDL" (d<1.006), "LDL" (1.006<d<1.063), and HDL (d>1.063 g/ml).

other subjects, the d_3-*SRR*-α- and d_2-γ-tocopherols did not decrease similarly; d_2-γ-tocopherol appears to decrease faster. Throughout the 72-h period of the study, the mean (± standard deviation) concentrations of unlabeled γ-tocopherol in KK's plasma (0.21 ± 0.14 nmol/ml) were about half of those observed in either AF or DL (0.46 ± 0.17 and 0.40 ± 0.09, respectively) and less than one tenth of the control subjects (3.05 ± 1.19). The low levels of γ-tocopherol may be the result of supplementation with vitamin E, which has been shown to reduce serum γ-tocopherol levels (23, 24). Or perhaps it results from substantially different dietary intakes of γ-tocopherol of this subject who lives in Japan, while the others live in the United States.

DISCUSSION

The preferential enrichment of the plasma with *RRR*-α-tocopherol is associated with VLDL secretion and catabolism as demonstrated in normal subjects, the patient with lipoprotein lipase deficiency, and the patients with abnormalities in apolipoprotein B-100 production. In normal subjects the concentrations and the rates of disappearance of d_3-*SRR*-α- and d_2-*RRR*-γ-tocopherols were similar, and both of these tocopherols disappeared faster than did d_6-*RRR*-α-tocopherol (Fig. 1 and Table 1).

Patients with defined genetic abnormalities of lipoprotein metabolism

Lipoprotein lipase deficiency. The patient with lipoprotein lipase deficiency, DP, had elevated plasma deuterated tocopherols and a slowed discrimination in favor of d_6-*RRR*-α-tocopherol (compare Fig. 2 with Fig. 1) consistent with impaired hydrolysis of triglyceride-rich lipoproteins. The preference for d_6-*RRR*-α-tocopherol became apparent in the plasma by 48 h, which is the length of time required for chylomicron removal, measured using retinyl palmitate in type I hyperlipidemic patients (25). Thus, the plasma was not preferentially enriched with d_6-*RRR*-

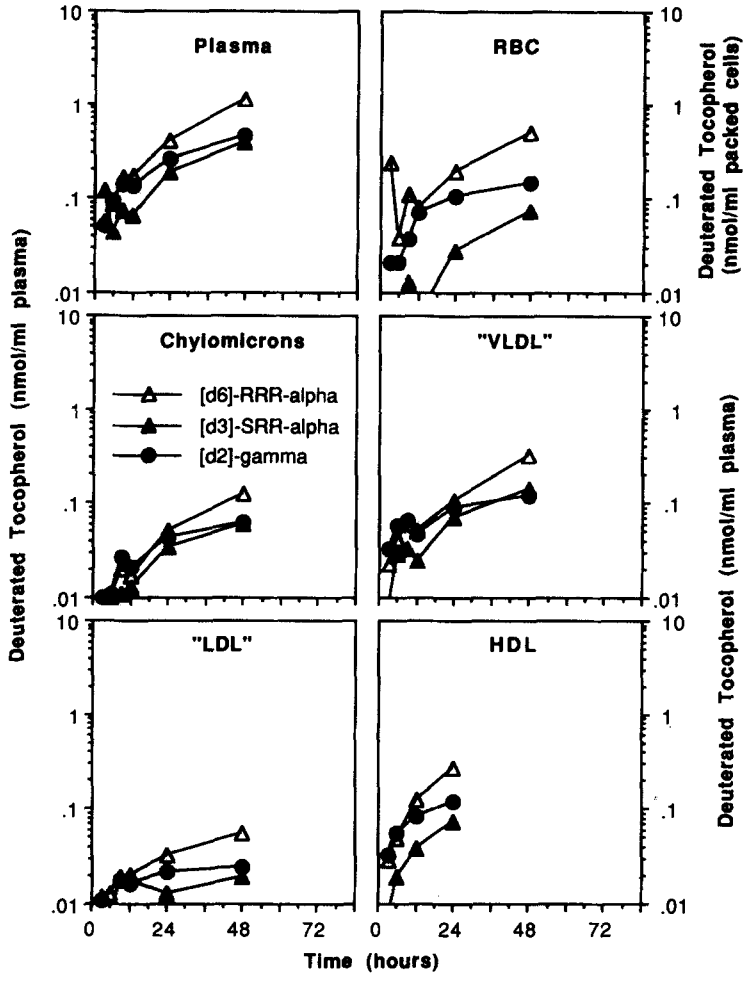

Fig. 4. DL, a patient with absent apolipoprotein B-100 and impaired chylomicron production, was studied under the conditions described in Fig. 1. The deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, red cells, chylomicrons, "VLDL" ($d < 1.006$), "LDL" ($1.006 < d < 1.063$), and HDL ($d > 1.063$ g/ml).

α-tocopherol until the chylomicrons had been catabolized. Previously, we demonstrated in vitro that lipoprotein lipase functions as a tocopherol transfer protein during triglyceride hydrolysis (26). However, lack of the protein does not abrogate delivery of tocopherol to tissues, as adipose tissue tocopherol levels of this patient were at the low end of the normal range (26). Thus, lipoprotein lipase deficiency leads to an elevation of plasma tocopherol with a majority of the plasma tocopherol in the triglyceride-rich lipoproteins, which results in a somewhat impaired delivery of tocopherol to tissues.

Abnormalities in apolipoprotein B production. Three patients who lack the ability to secrete apolipoprotein B-100 were also studied. AF discriminates between tocopherols. She was first described by Malloy et al. (17) as having "normotriglyceridemic abetalipoproteinemia" and lacking apolipoprotein B-100 but not B-48. Recently, she was shown to secrete a protein corresponding to the N-terminal 49.6% of the sequence of apolipoprotein B-100 (18). She has a normal VLDL catabolic rate, a normal apolipoprotein B production rate, but only a small amount of ^{125}I-labeled VLDL was converted to LDL, probably due to the rapid removal of abnormal VLDL particles (18). This patient's plasma was preferentially enriched in d_6-*RRR*-α-tocopherol within 12 h of consuming the dose of deuterated tocopherols (Fig. 3). Furthermore, the amounts of the d_6-*RRR*-α-tocopherol in the "VLDL" fraction continued to increase slightly from 12 to 72 h, demonstrating continued production of preferentially enriched "VLDL" particles by the liver. Because few "LDL" are produced from "VLDL" (18), it is reasonable that little deuterated tocopherol was found in this fraction. Only the HDL became enriched in d_6-*RRR*-α-tocopherol during "VLDL" catabolism. Thus, HDL and the abnormal VLDL transport the bulk of the plasma tocopherols in this patient. Lipoprotein lipase may transfer tocopherol from chylomicrons and "VLDL" to tissues in this patient. Alternatively, because HDL tocopherol readily exchanges between lipoproteins or red cells (27–29), HDL may also deliver tocopherol to tissues. However, neither of these

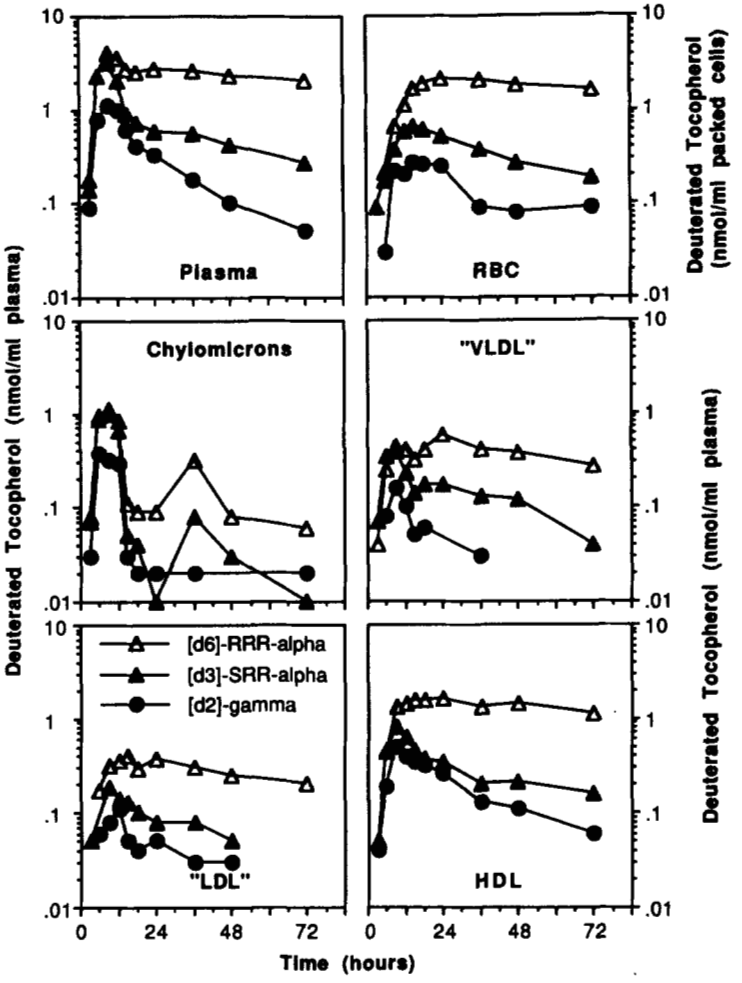

Fig. 5. KK, a patient with absent apolipoprotein B-100 and spared chylomicron production, was studied under the conditions described in Fig. 1. The deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, red cells, chylomicrons, "VLDL" ($d<1.006$), "LDL" ($1.006<d<1.063$), and HDL ($d>1.063$ g/ml).

mechanisms was sufficient to prevent vitamin E deficiency. When this child (age 8) first came to the attention of the Malloy group she had neurologic abnormalities due to vitamin E deficiency, but supplemental vitamin E did reverse the neurologic damage (17).

DL is unlike AF in that he has both a defective apolipoprotein B-100 production and a prolonged dietary fat absorption (19). Despite "limited formation" of chylomicrons containing apolipoprotein B-48, large, lipid-rich particles were found in his plasma in the fasting state, indicating that fat absorption was continuous, but occurred at a lower rate than in normal subjects (19). No discrimination between tocopherols was observed in this patient, and the delivery of adequate amounts of α-tocopherol into the plasma and red cells was impaired (Fig. 4). This child has taken large supplemental doses of vitamin E since infancy and does not have symptoms of vitamin E deficiency.

KK, who has abnormal apolipoprotein B-100 production (20), discriminated between the labeled tocopherols. Based on the deuterated tocopherol concentrations, he has a somewhat prolonged period of chylomicron secretion with a second peak occurring at 36 h. This latter peak might be the result of enterohepatic circulation of the deuterated tocopherols. Preferential enrichment of VLDL with d_6-*RRR*-α-tocopherol was observed by 24 h (Fig. 5). Since his "LDL" d_6-*RRR*-α-tocopherol was triple that of the other two patients with abnormalities in apolipoprotein B-100 production, it is likely that he can effectively transport α-tocopherol in apolipoprotein B-containing lipoproteins from the liver to tissues. Previously, we demonstrated that LDL delivers tocopherol to cells via the LDL receptor mechanism (30). In addition, his HDL d_6-*RRR*-α-tocopherol was in the normal range. Thus, it is not surprising that although he has not received supplemental vitamin E, he does not have symptoms of vitamin E deficiency.

Taken together, the data from the patients with genetic abnormalities of lipoprotein metabolism demonstrate that

when the chylomicrons are in the circulation for a prolonged period of time, irrespective of whether this is due to their impaired catabolism or prolonged secretion, then the plasma contains all three of the administered tocopherols in virtually equal concentrations. However, if VLDL particles are secreted, albeit abnormal VLDL particles, then the plasma becomes enriched in *RRR*-α-tocopherol. Supplemental vitamin E can alleviate problems of impaired transport because tocopherols are transferred to all of the plasma lipoproteins during chylomicron catabolism.

Red cell tocopherol concentrations

The four control subjects and three (of four) patients show a preferential enrichment of their red cells with d_6-*RRR*-α-tocopherol. Although plasma d_6-*RRR*-α-tocopherol concentrations differ substantially (from ~1 to ~20 nmol/ml) between controls and patients, the red cell concentrations are surprisingly similar (~1 nmol/ml packed cells). The only subject with a reduced red cell tocopherol content was DL, whose red cells required 3 days for d_6-*RRR*-α-tocopherol to reach ~1 nmol/ml (Fig. 4). We evaluated his vitamin E status by measuring his adipose tissue α-tocopherol concentration (31, 32), which was 370 ng α-tocopherol/mg triglyceride (normal range of 222 ± 111 (32)). Thus, the supplemental vitamin E (100 mg/kg per day) taken by the patient is sufficient to maintain tissue α-tocopherol concentrations in spite of the sluggish transport system.

Proposed mechanism for tocopherol discrimination

The hepatic tocopherol-binding protein (11) could be the mechanism responsible for both the discrimination between tocopherols and for incorporation of α-tocopherol into VLDL, and thus regulation of plasma tocopherol levels. Recently, this protein isolated from rat liver has been purified to homogeneity (33, 34). The purified protein preferentially transfers α-tocopherol between liposomes and mitochondria (33). Recently, Yoshida et al. (34) demonstrated that immunoreactivity to the rat hepatic tocopherol-binding protein was detected in rat liver cytosol and lysate of hepatocytes, but not in the cytosol of rat kidney, spleen, adrenals, testes, lung, stomach, intestines, heart, skeletal muscle, and brain, nor in the lysate of Ito cells, endothelial cells, and Kupffer cells isolated from rat liver, nor in rat plasma and lipoproteins. They found no evidence that the tocopherol-binding protein is involved in the biotransformation of tocopherol. The authors concluded that the tocopherol-binding protein is unlikely to be involved in storage of tocopherol and that a general intracellular transfer function seems unlikely. They suggested that the tocopherol-binding protein may be critical in the hepatic handling of α-tocopherol by specifically binding and retaining α-tocopherol, as well as possibly transferring α-tocopherol from an endocytic to a secretory compartment (34). The available data (1, 6–8, 10, 11, 33, 35–37) suggest that the tocopherol-binding/transfer protein has a pocket that recognizes the number of methyl groups on the chroman "ring" and the chirality of the phytyl "tail" of *RRR*-α-tocopherol. Perhaps, the protein transfers α-tocopherol from the lysosomes after chylomicron remnant uptake and inserts it into nascent VLDL during its assembly in the endoplasmic reticulum/Golgi in the liver (38, 39).

Regulation of plasma α-tocopherol by the tocopherol-binding protein has implications for supplementation of normal subjects with vitamin E. If α-tocopherol requires the tocopherol-binding protein for insertion into VLDL, then supplements beyond the carrying capacity of the protein would not be secreted into the plasma. There is some evidence for this limitation. When 400, 800, or 1200 IU of *all rac* α-tocopheryl acetate were administered daily for 28 days to control subjects, plasma α-tocopherol levels plateaued by 5 days at double the initial plasma α-tocopherol concentration irrespective of dose size (40). When 300 mg of each α- and γ-tocopherols were orally administered to a patient with an in-dwelling t-tube after gall bladder surgery, we observed that not only was there a preferential enrichment of the plasma with α-tocopherol, but coincident with the peak in the plasma there was an increased excretion of both α-tocopherol and γ-tocopherol in the bile (10). Possibly the large dose exceeded the capacity of the liver to secrete α-tocopherol and, therefore, it was excreted. This mechanism could be the basis of the relative safety of vitamin E (41) as compared with vitamins A or D, which are stored in the liver.

In conclusion, the present studies in patients with genetic abnormalities in apolipoprotein and lipoprotein metabolism document that discrimination for the preservation of levels of *RRR*-α-tocopherol in plasma appears to be solely a function of the liver; the intestinal tract has no detectable role in discrimination.

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