Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency

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Abstract We assessed whether patients with familial isolated vitamin E deficiency could discriminate between natural (*RRR*-) and synthetic (*SRR*-) stereoisomers of α-tocopherol labeled with six (d_6) or three (d_3) deuterium atoms, respectively. After oral administration of 20 mg of each of the stereoisomers, patients (seven) and controls (seven) had similar concentrations of both in chylomicrons, similar initial increases of both, and similar rates of decrease of d_3-*SRR*-α-tocopherol in plasma. Patients and controls differed in their abilities to maintain plasma d_6-*RRR*-α-tocopherol concentrations. Controls maintained plasma d_6-*RRR*-α-tocopherol concentrations by preferentially secreting it in very low density lipoprotein (VLDL). Three of seven patients did not discriminate between the two stereoisomers and their plasma and lipoprotein d_6-*RRR*-α-tocopherol concentrations declined rapidly. The remaining patients were intermediate between non-discriminators and controls in their ability to discriminate and maintain plasma d_6-*RRR*-α-tocopherol concentrations. The degree of discrimination between the two stereoisomers in the patients was correlated with the age of onset of the neurologic disability ($r^2 = 0.64$, $P < 0.03$). Estimates based on the rate of decrease of plasma d_6-*RRR*-α-tocopherol in non-discriminators suggest that the entire plasma α-tocopherol pool of normal subjects is replaced daily. We suggest *1)* that a hepatic α-tocopherol binding protein, which preferentially incorporates *RRR*-α-tocopherol into VLDL, is required to maintain plasma *RRR*-α-tocopherol concentrations; *2)* that non-discriminators are lacking this protein, or have a marked defect in the *RRR*-α-tocopherol binding region of the protein; and *3)* that patients who discriminate, but have difficulty maintaining plasma *RRR*-α-tocopherol concentrations, have a less severe defect, or perhaps a defect in the transfer function of the protein.—**Traber, M. G., R. J. Sokol, A. Kohlschütter, T. Yokota, D. P. R. Muller, R. Dufour, and H. J. Kayden.** Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency. *J. Lipid Res.* 1993. **34:** 201–210.

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Over the last 10 years, 11 patients with familial isolated vitamin E (FIVE) deficiency have been described worldwide (1–11). They do not have generalized lipid malabsorption syndromes, abnormalities in lipoprotein metabolism that could result in vitamin E deficiency, or any other known cause of vitamin E deficiency. When consuming normal dietary amounts of vitamin E, they have low to undetectable plasma α-tocopherol concentrations and develop neurologic abnormalities typical of vitamin E-deficient experimental animals. These abnormalities are similar to those in patients with severe vitamin E deficiency as a consequence of chronic lipid malabsorption, such as abetalipoproteinemia or cholestasis (12, 13). When FIVE deficiency patients consume daily vitamin E supplements (800–1200 IU/day), normal plasma α-tocopherol concentrations are maintained and the progression of their neurologic abnormalities is either halted or reversed.

Traber et al. (11) demonstrated in four patients with FIVE deficiency that, when they were given a 15-mg oral dose of deuterated *RRR*-α-tocopherol, they secreted the labeled vitamin E into chylomicrons in a similar manner to normal subjects. However, impaired secretion of labeled α-tocopherol by the patients into very low density lipoproteins (VLDL) resulted in a faster disappearance of

Abbreviations: FIVE, familial isolated vitamin E; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; PBS, phosphate-buffered saline; t_{moc}, time of maximum observed concentration.

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the label from their plasma (11). These results suggested that the hepatic α-tocopherol binding protein (14–18), which putatively transfers α-tocopherol into hepatic-derived lipoproteins (11), was defective or absent in the FIVE deficiency patients. These data further suggested that one function of this protein is to regulate plasma α-tocopherol levels.

Dietary vitamin E includes: α-, β-, γ-, δ-tocopherols, which have a phytyl tail and differ in the number and position of methyl groups on the chromanol ring, and α-, β-, γ-, δ-tocotrienols, which have unsaturated tails. Synthetic α-tocopherol, sold as vitamin E supplements, contains equal amounts of eight different stereoisomers arising from the three chiral centers of the phytyl tail. Naturally occurring *RRR*-α-tocopherol has the highest biologic activity, as shown in rats (19–24), monkeys (25), and humans (26–31). *RRR*- and *SRR*-α-tocopherols labeled with deuterium have been used to investigate discrimination between stereoisomers of α-tocopherol because most of the differences in activity of the stereoisomers results from differences in the chirality at the 2 position, where the phytyl tail meets the chromanol ring (21).

The hepatic α-tocopherol binding protein may be involved in discrimination between tocopherols. Discrimination does not occur during absorption of vitamin E by the intestine, because chylomicrons contain a similar distribution of tocopherols to that fed (30–32). Discrimination between α- and γ-tocopherols did occur when the intestine was bypassed by giving humans intravenous lipid emulsions, which are metabolized similarly to chylomicrons (33), suggesting the liver was involved in discrimination. Studies using perfused monkey livers provided direct evidence of the liver's role in the preferential secretion of α-tocopherol in lipoproteins (25). Monkeys consumed equimolar amounts of *RRR*- and *SRR*-α-tocopheryl acetates and *RRR*-γ-tocopherol labeled with different amounts of deuterium 24 h before killing and liver perfusion. Labeled *RRR*-α-tocopherol accounted for more than 75% of the deuterated tocopherols secreted by the liver into perfusate lipoproteins. Sato et al. (18) demonstrated that the isolated tocopherol binding protein(s) from rat liver transfer(s) α-tocopherol, in preference to β, δ, or γ-tocopherols, between liposomes and microsomes in vitro. Recently, Yoshida et al. (34) demonstrated that immunoreactivity to the rat hepatic tocopherol binding protein was detected only in rat liver cytosol and lysate of hepatocytes, but not in a variety of other tissues or in plasma. 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). Taken together, the available data suggest that the hepatic tocopherol binding protein is required to discriminate between forms of dietary vitamin E for secretion in VLDL and thus regulate plasma α-tocopherol levels.

FIVE deficiency patients have an impaired ability to maintain normal plasma levels of α-tocopherol, and may therefore lack, or have a defective form of, the tocopherol binding protein (11). We, therefore, hypothesized that if FIVE deficiency patients have a defective or absent hepatic tocopherol binding protein, then they may have an impaired ability to discriminate between different tocopherols. This study, therefore, was designed to test the ability of FIVE deficiency patients to discriminate between *RRR*- and *SRR*-α-tocopherols.

METHODS

Deuterated tocopherols

The syntheses and analysis of the deuterated tocopherols have been described previously (24, 35, 36).

Each subject consumed 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, and 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 inverted stereochemistry at position 2. An internal standard, 2-*ambo*-α-(5,7,8-(C^2H_3)$_3$)tocopherol (d_9-α-tocopherol) was added in known amounts to each sample immediately before extraction of the lipids into heptane (37, 38). These heptane extracts were purified by passage through an analytical, high-performance, silica gel chromatography column. The amounts of d_3-, d_6-, d_9-α-tocopherols and unlabeled (d_0-) α-tocopherol in the isolated tocopherol fraction were determined by gas chromatography–mass spectrometry after conversion to their trimethylsilyl ethers. The absolute concentrations of d_0-, d_3-, and d_6-α-tocopherols in the plasma and lipoprotein samples were obtained by comparing their respective peak areas with the peak area of the internal standard d_9-α-tocopherol (11, 37, 38).

Experimental protocols

This study was carried out with the approval of the Institutional Review Boards of the New York University Medical Center and the University of Colorado Health Sciences Center. All subjects gave written, informed consent. The control subjects had no abnormalities of lipid or lipoprotein metabolism. **Table 1** shows the characteristics of the patients and references to previous publications in which each patient has been reported. For easier comparison between the present and our previous study (11), we have used the same patient numbers. Our previous patient #3 chose not to participate in this study. The same numbers as used previously (11) were retained for control subjects 5, 7–10.

The neurologic status of the patients shown in Table 1 was assessed using the neurologic scoring method previously described by Sokol et al. (39). Twelve neurologic

TABLE 1. Patient characteristics

Patient #	Reference	Study Age	Age Neurologic Abnormality Detected	Sex	Present Neurologic Score[a]	Discriminator or Non-Discriminator
		yr	*yr*			
1	7, 9, 11	24	6	F	8	Discriminator
2	7, 9, 11	28	27	M	4	Discriminator
4	5, 7, 9, 11	33	2	F	20	Non-discriminator
5	3	29	13	F	16	Discriminator
6	1, 8	21	3	M	15	Non-discriminator
7	6	65	52	M	13	Discriminator
8	2	18	5	M	14	Non-discriminator

[a]Refer to the text for the description of the methods for determining the neurologic score.

signs were rated as absent (0), mildly abnormal (+), moderately abnormal (++), or severely abnormal (+++). The neurologic signs were hyporeflexia or areflexia, truncal ataxia, limb ataxia, ophthalmoplegia, decreased proprioception, decreased vibratory sensation, proximal-muscle weakness, decreased light-touch sensation, decreased pain sensation, dysarthria, pes cavus, and scoliosis. A total neurologic score was calculated by summing the scores (0 to +++) for the 12 signs, for a maximum total score of 36.

Each subject consumed a capsule containing 20 mg of d_6-*RRR*- and 20 mg of d_3-*SRR*-α-tocopheryl acetates with breakfast after an overnight fast. Blood samples were drawn into EDTA tubes (Becton Dickinson, Rutherford, NJ) at intervals between 0 and 72 h after consumption of the tocopherols. Subjects were allowed to eat ad libitum.

Plasma was separated from blood cells by centrifugation at 2,000 *g* for 5 min, and an aliquot (1.0 ml) was kept frozen at −70°C until analyzed for tocopherol. The remainder of the plasma was kept at 4°C until sample collection was completed. These conditions inhibit the exchange of tocopherol between lipoproteins (11). Previously, we have found that lipoproteins isolated from fresh plasma, or from the same plasma samples stored at 4°C for 2 weeks, contained the same distribution and concentrations of labeled tocopherols (unpublished observations, Traber, M. G.). The plasma samples were then shipped on wet ice by overnight courier to NYU Medical Center for lipoprotein isolation, as described previously (30). In all cases chylomicrons and lipoproteins were isolated within 10 days of tocopherol administration, and kept frozen at −70°C until analyzed.

Red cells were isolated from the blood samples of one control subject and two patients by centrifugation at 2000 *g* for 5 min, and washed 3 times by repeated resuspension in cold isotonic phosphate-buffered saline (PBS) and centrifugation at 2000 *g* for 5 min. After resuspension in PBS the hematocrit was measured (approximately 50%), and 1-ml aliquots were stored frozen at −70°C.

The frozen plasma, red cells, and lipoprotein fractions were shipped on dry ice to Drs. Keith U. Ingold and Graham W. Burton at the National Research Council, Ottawa, Ontario Canada, where they were kept frozen at −70°C until analysis of the various tocopherols.

Mathematical and statistical analysis

Calculations were carried out using a Macintosh II computer (Apple Computers, Cupertino, CA). The times of maximum observed concentration (t_{moc}) were chosen manually. The linear regression function (linest) of a spreadsheet program (Microsoft Excel, Microsoft Corp., Redmond, WA) was used to calculate the slope of the curve of the logarithms of the deuterated tocopherol concentrations versus time using the data past the t_{moc} in plasma, VLDL, LDL, and HDL. The area under the curve (AUC) was calculated for the plasma deuterated tocopherols by summing the trapezoids estimated from the X-Y data points using KaleidaGraph (Synergy Software (PCS Inc.) Reading, PA). The statistical significance of the differences was determined using the statistical analysis program, Super Anova (Abacus Concepts, Berkeley, CA), using analysis of variance (ANOVA) and least square means comparisons. Results of the statistical tests were considered to be significant at the 95% confidence level ($P < 0.05$). Correlations were calculated using StatView II (Brain Power, Calabasas, CA).

RESULTS

The means of the plasma deuterated tocopherol concentrations from seven control subjects after the oral administration of d_3-*SRR*- and d_6-*RRR*-α-tocopherols are shown in **Fig. 1A**. (Individual values are available from the corresponding author upon request.) Concentrations

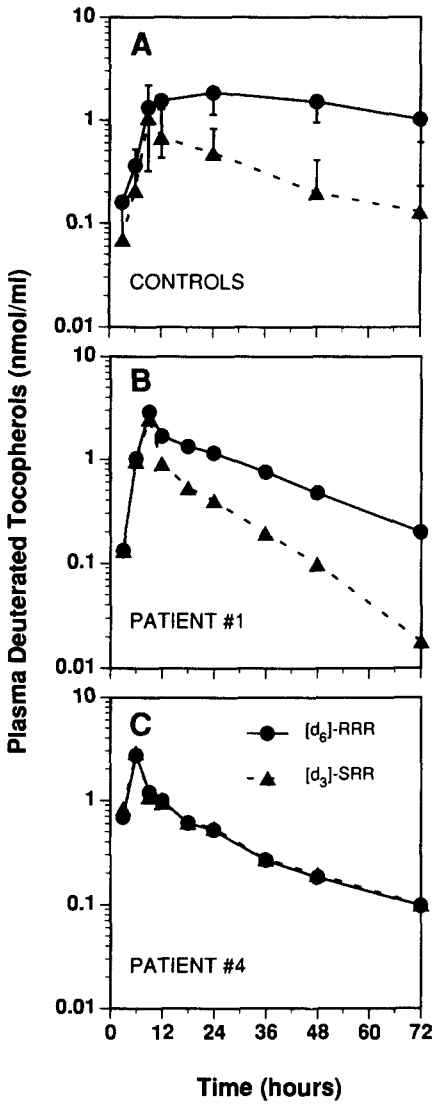

Fig. 1. Plasma concentrations of d_6-*RRR*- and d_3-*SRR*-α-tocopherols in controls (A, mean of seven subjects, error bars show standard deviations), patient # 1 (B), and patient #4 (C). Each subject consumed a mixture containing 20 mg d_6-*RRR*-α-tocopheryl acetate and 20 mg d_3-*SRR*-α-tocopheryl acetate.

of both tocopherols initially increased, after 9 h d_3-*SRR*-α-tocopherol began to decrease, while the concentration of d_6-*RRR*-α-tocopherol did not decrease until about 24 h. The patients displayed two different patterns. Four of the seven patients studied had the pattern, as illustrated by patient #1 (Fig. 1B) and, like the controls, discriminated between the two stereoisomers of α-tocopherol. In marked contrast, the other group of three patients did not discriminate between the stereoisomers. A representative patient (#4) of this latter group is shown in Fig. 1C. These two patient groups will be termed discriminators and non-discriminators, respectively.

The mean ± SD of the plasma d_6-*RRR*-α-tocopherol concentration at 72 h in the controls was 1.0 ± 0.4 nmol/ml, but was 0.39 ± 0.25 in the discriminators and only 0.05 ± 0.04 in the non-discriminators. These low concentrations in the patients are the result of major differences in tocopherol kinetics between the patients and the controls. Differences occurred in the times at which the d_6-*RRR*-α-tocopherol concentration peaked in the plasma and the rapidity with which it decreased. The time of maximum observed concentration (t_{moc}) of d_6-*RRR*-α-tocopherol in the control plasma was 19 ± 10 h, which was significantly later ($P < 0.05$) than in the discriminators (11 ± 2 h) and non-discriminators (6 h) (**Table 2**). Although the maximum concentrations of the labeled α-tocopherols were not significantly different, the length of time that the concentrations remained at, or near, the maximum were different for the three groups. This is seen in the differences in the mean areas under the curve (AUC) (**Table 3**). The control d_6-*RRR*-α-tocopherol AUC (87 ± 35 nmol/ml · h) was more than triple that of the non-discriminators (27 ± 7), and slightly, but not significantly, greater than the discriminators (69 ± 31). By contrast, the mean d_3-*SRR*-α-tocopherol AUC was about 25 nmol/ml · h in all three groups. The mean ± SD ratios of the plasma AUC d_6-*RRR*-/AUC d_3-*SRR*-α-tocopherols were 4.2 ± 1.7 in controls, 2.5 ± 0.4 in discriminators, and 1.0 ± 0.1 in non-discriminators (Table 3). The non-discriminator ratio was significantly different ($P < 0.05$) from that of the controls.

In part, the differences in plasma AUCs of the labeled tocopherols in the controls and the two patient groups were due to differences in the rates of decrease of the tocopherols after they had reached their maximum concentrations (**Table 4**). In the control subjects, plasma d_6-

TABLE 2. Time (h) of maximum observed concentration (t_{moc}) of d_6-*RRR*-α-tocopherol in controls and in patients, who do and do not discriminate between stereoisomers of α-tocopherol

	Controls (n = 7)	Discriminators (n = 4)	Non-Discriminators (n = 3)
		t_{moc}, h	
Chylomicrons	11 ± 6	8 ± 3	6
VLDL	22 ± 11	8 ± 2	6
LDL	20 ± 8	11 ± 2	6
HDL	21 ± 10	11 ± 2	6
Plasma	19 ± 10	11 ± 2	6

Means ± standard deviations of the t_{moc} (h) of d_6-*RRR*-α-tocopherol, except for the non-discriminators in whom t_{moc} = 6 h in all fractions for all three patients. Chylomicron t_{mocs} were not statistically different among the three groups. For controls, chylomicron t_{mocs} occurred significantly earlier ($P < 0.05$) than any of the other lipoproteins. Control t_{mocs} in plasma, VLDL, LDL, and HDL were significantly later ($P < 0.05$) than those of either patient group. The t_{mocs} of the discriminators were not significantly different from those of the non-discriminators in any of the fractions.

TABLE 3. Area under the curve (AUC) of the plasma deuterated tocopherols in control subjects and in patients who do and do not discriminate between stereoisomers of α-tocopherol

Group	No.	d_3-*SRR*-α	d_6-*RRR*-α	d_6/d_3
Control	5	34	89	2.6
	7	9	60	6.9
	8	19	76	3.9
	9	42	145	3.4
	10	13	35	2.8
	11	29	100	3.4
	12	17	106	6.2
Mean ± SD		23 ± 12	87 ± 35	4.2 ± 1.7
Discriminators	1	26	58	2.2
	2	13	30	2.4
	5	38	94	2.5
	7	31	94	3.0
Mean ± SD		27 ± 11	69 ± 31	2.5 ± 0.4
Non-discriminators	4	36	34	1.0
	6	17	20	1.2
	8	26	26	1.0
Mean ± SD		26 ± 9	27 ± 7	1.0 ± 0.1

The AUC (nmol/ml · h) of d_3-*SRR*- and d_6-*RRR*-α-tocopherols are shown. The d_3-*SRR*-α-tocopherol AUC was not significantly different among the three groups. The d_6-*RRR*-α-tocopherol AUC was significantly greater ($P < 0.05$) than the d_3-*SRR*-α-tocopherol AUC for both controls and discriminators, but not non-discriminators. It was significantly smaller ($P < 0.005$) in the non-discriminators compared with the controls, or the discriminators.

RRR-α-tocopherol concentrations decreased about one-third as fast as d_3-*SRR*- (−0.007 ± 0.002 vs. −0.019 ± 0.007 log[nmol/ml]/h); in the discriminators, plasma d_6-*RRR*-α-tocopherol decreased one-half as fast as d_3-*SRR*-(−0.011 ± 0.003 vs. −0.021 ± 0.007); and in the non-discriminators, plasma d_6-*RRR*-α-tocopherol decreased at the same rate as d_3-*SRR*- (−0.024 ± 0.011 vs. −0.022 ± 0.007). It should be noted that plasma d_3-*SRR*-α-tocopherol decreased at similar rates in all three groups.

The appearance and disappearance patterns of the deuterated tocopherols in the different lipoprotein fractions provide valuable information to elucidate the mechanisms for these observations. The means of the deuterated tocopherol concentrations in control lipoproteins are shown in **Fig. 2.** The chylomicrons contained similar concentrations of both tocopherols, indicating similar absorption and incorporation of the two stereoisomers into chylomicrons. The t_{moc} of the VLDL, LDL, and HDL occurred subsequent to that of the chylomicrons (Table 2) demonstrating that as the VLDL became enriched in d_6-*RRR*-α-tocopherol, so did the low and high density lipoproteins (LDL and HDL). After reaching its maximum concentration d_6-*RRR*-α-tocopherol decreased more slowly than did d_3-*SRR*- in the VLDL, LDL, and HDL of the controls (Table 4). These data are consistent with our previous observations on the discrimination between tocopherols in normal subjects (30, 31). That is, there is virtually no discrimination between *RRR*- and *SRR*-α-tocopherols during absorption and secretion of tocopherols into chylomicrons. Subsequently, the liver secretes nascent VLDL enriched in *RRR*-α-tocopherol. It is the catabolism of this VLDL that leads to the preferential enrichment of LDL and HDL with *RRR*-α-tocopherol.

The pattern of deuterated tocopherols in the lipoprotein fractions from the discriminators is illustrated by patient #1, as shown in **Fig. 3.** As was observed in the controls, the chylomicrons from the discriminators contained similar concentrations of d_3-*SRR*- and d_6-*RRR*-α-tocopherol. During the first 9 h (the time of chylomicron catabolism), the other lipoprotein fractions also contained similar concentrations of both labels. Preferential secretion of d_6-*RRR*-α-tocopherol in VLDL occurred by 18 h, but by 24 h the VLDL from this patient contained only one-fourth the concentration of d_6-*RRR*-α-tocopherol found in the controls (0.1 compared with 0.4 ± 0.2 nmol/ml). Other discriminators (#2, 5, and 7) also preferentially secreted d_6-*RRR*-α-tocopherol into VLDL by 18 to 24 h (data not shown). As illustrated by patient #1, the LDL and HDL contained only one peak of deuterated tocopherols, which was coincident with the chylomicron peak (Fig. 3). The t_{mocs} for d_6-*RRR*-α-tocopherol in VLDL, LDL, and HDL in the discriminators were not significantly different from those of the chylomicrons (Table 2). Furthermore, the rates of decrease of d_6-*RRR*-

TABLE 4. Rates of decrease (mean ± SD) of deuterated tocopherols (log[nmol]/ml · h) in controls and in patients that do and do not discriminate between stereoisomers of α-tocopherol

	Controls (n = 7)		Discriminators (n = 4)		Non-Discriminators (n = 3)	
	d_3-*SRR*-α	d_6-*RRR*-α	d_3-*SRR*-α	d_6-*RRR*-α	d_3-*SRR*-α	d_6-*RRR*-α
Plasma	-0.019 ± 0.007^{a}	$-0.007 \pm 0.002^{a,c}$	-0.021 ± 0.007^{b}	$-0.011 \pm 0.003^{b,d}$	-0.022 ± 0.007	$-0.024 \pm 0.011^{c,d}$
VLDL	-0.020 ± 0.006^{a}	$-0.013 \pm 0.010^{a,c}$	-0.013 ± 0.005^{e}	-0.010 ± 0.004^{d}	-0.023 ± 0.008^{e}	$-0.029 \pm 0.014^{c,d}$
LDL	-0.017 ± 0.007^{a}	$-0.006 \pm 0.001^{a,c}$	-0.018 ± 0.005	-0.011 ± 0.003^{d}	-0.022 ± 0.004	$-0.024 \pm 0.006^{c,d}$
HDL	$-0.014 \pm 0.008^{a,f}$	$-0.006 \pm 0.002^{a,c}$	-0.019 ± 0.008	-0.012 ± 0.003^{d}	-0.027 ± 0.010^{f}	$-0.025 \pm 0.008^{c,d}$

Slopes of the disappearance portion of the deuterated tocopherol curves for control subjects, discriminator, and non-discriminator patients are shown, Slopes in the same row sharing the same letter are significantly different ($P < 0.05$).

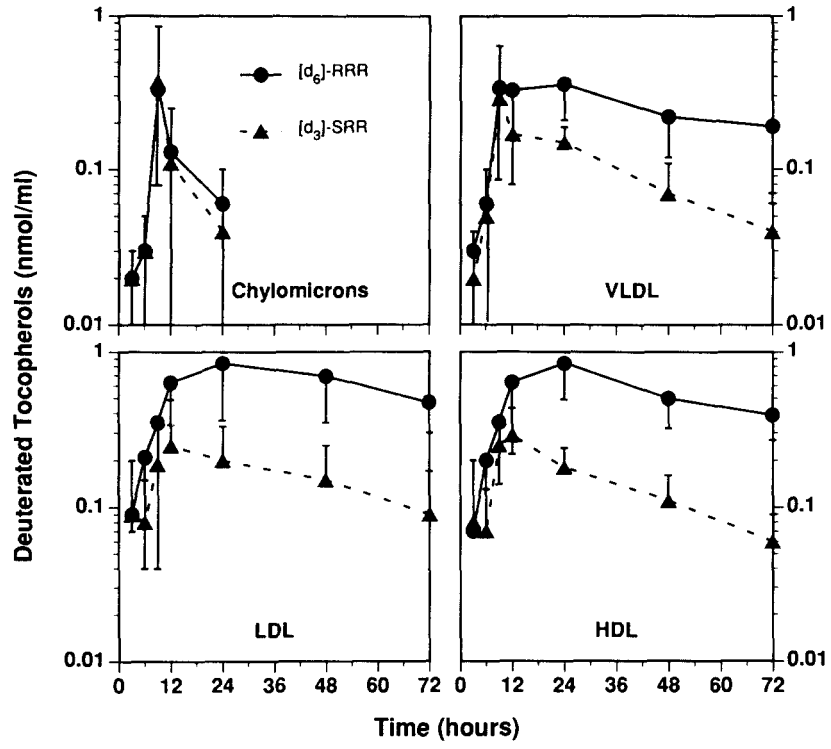

Fig. 2. The mean concentrations (nmol/ml plasma) of d_6-*RRR*- and d_3-*SRR*-α-tocopherols in lipoproteins isolated from the plasma obtained from the control subjects (n = 7, error bars show standard deviations) shown in Fig. 1A.

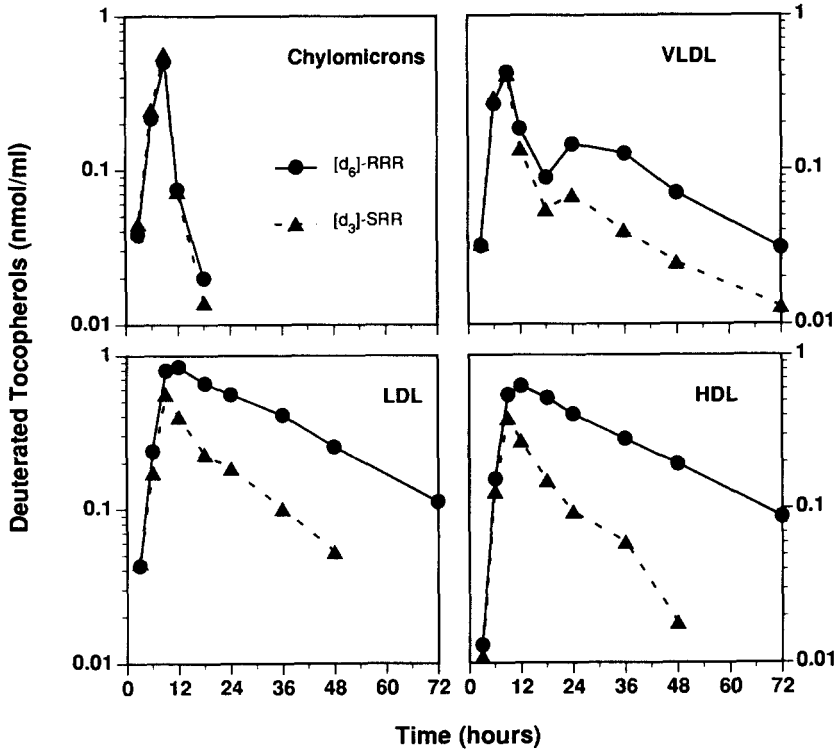

Fig. 3. The concentrations (nmol/ml plasma) of d_6-*RRR*- and d_3-*SRR*-α-tocopherols in lipoproteins isolated from the plasma obtained from patient #1 shown in Fig. 1B.

and d_3-*SRR*-α-tocopherol in VLDL, LDL, and HDL were not significantly different (Table 4). Taken together with the observation that the disappearance rates of d_3-*SRR*-α-tocopherol in control and discriminator lipoproteins were similar, it is apparent that the secretion of d_6-*RRR*-α-tocopherol in VLDL by the discriminators was not as vigorous as in the controls.

The pattern of deuterated tocopherols in the lipoprotein fractions from the non-discriminators is illustrated by patient #4, as shown in **Fig. 4.** Both labeled tocopherols peaked simultaneously at 6 h in all fractions of all of the non-discriminators (Table 2). Both labels decreased similarly in VLDL, LDL, and HDL and at rates similar to those of control d_3-*SRR*- (Table 4). Although in all three non-discriminators there was a slight increase in the VLDL tocopherol concentrations at 24 h, discrimination between the two tocopherols did not take place (data not shown).

The tocopherol content of red blood cells (RBC) from two non-discriminators and one control were analyzed. Control RBCs contained 6.9 ± 0.6 nmol unlabeled α-tocopherol/ml packed RBC, while those of the patients contained 0.18 ± 0.04. Up to 9 h, both the patients' and control cells contained similar concentrations of deuterated tocopherols (**Fig. 5**). However, d_6-*RRR*-α-tocopherol in the patients' RBC peaked at lower concentrations (patient #6, 0.04 nmol/ml and patient #8, 0.16) than did control cells (0.48). RBC d_3-*SRR*-α-tocopherols decreased similarly in the control (−0.015 log[nmol/ml]/h) and in patient #8 (−0.016), but not as fast as in patient #6 (−0.042). RBC d_6-*RRR*-α-tocopherol decreased at rates similar to d_3-*SRR*- in each non-discriminator (−0.016 log[nmol/ml]/h in patient #8, and −0.039 in patient #6), while it decreased more slowly in the control (−0.006). Although only one control subject was studied, the values obtained were similar to those we have obtained previously in three control subjects who consumed 50 mg, and one who consumed 75 mg, of each of d_6-*RRR*- and d_3-*SRR*-α-tocopherols (31). Previously, the mean ± standard deviation of the unlabeled α-tocopherol in control RBCs was 4.4 ± 1.8 nmol/ml packed cells; the maximum d_6-*RRR*- and d_3-*SRR*-α-tocopherol concentrations were 1.4 ± 0.8 nmol/ml and 0.27 ± 0.12, respectively; and the rates of decrease for d_6-*RRR*- and d_3-*SRR*-α-tocopherols were −0.004 ± 0.004 and −0.011 ± 0.004, respectively. These in vivo results in the non-discriminator RBC contrast with in vitro studies in which deuterated *RRR*- and *SRR*-α-tocopherols entered RBC at similar rates, but *SRR*- left more rapidly (40). These preliminary data suggest that discrimination between tocopherols by cell membranes in vivo is dependent upon the differences in plasma lipoprotein concentrations of the stereoisomers.

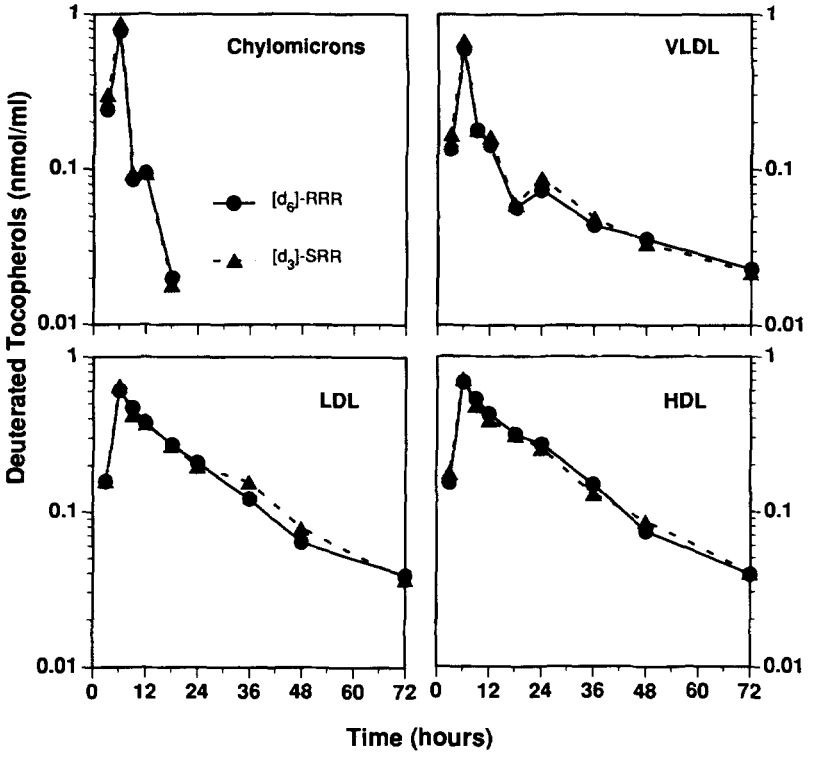

Fig. 4. The concentrations (nmol/ml plasma) of d_6-*RRR*- and d_3-*SRR*-α-tocopherols in lipoproteins isolated from the plasma obtained from patient #4 shown in Fig. 1C.

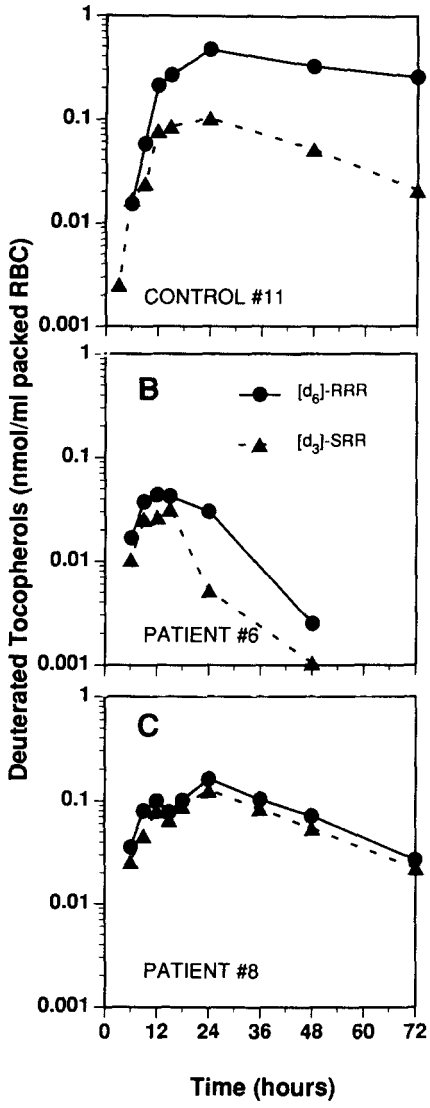

Fig. 5. The concentrations (nmol/ml packed cells) of d_6-*RRR*- and d_3-*SRR*-α-tocopherols in erythrocytes (RBC) from control subject #11 (A), patient #6 (B), and patient #8 (C).

DISCUSSION

Patients with FIVE deficiency have an impaired incorporation of α-tocopherol into VLDL, probably resulting from a defective, or absent, hepatic tocopherol binding protein (11). Because the putative role of this protein is to preferentially incorporate *RRR*-α-tocopherol into VLDL, we tested whether patients with FIVE deficiency could discriminate between two orally administered stereoisomers of α-tocopherol. The seven patients that we studied segregated into two groups, as to whether they could or could not discriminate between *RRR*- and *SRR*-α-tocopherols (discriminators (n = 4) and non-discriminators (n = 3)). All of the subjects (both patients and controls) transported d_3-*SRR*-α-tocopherol in a similar manner. Thus, the differences in plasma concentrations between the two stereoisomers depended upon the subject's ability to enhance the transport of d_6-*RRR*-α-tocopherol.

In the lipoproteins of the non-discriminators, concentrations of *RRR*- and *SRR*-α-tocopherols were similar at all times. In these three patients, the deuterated tocopherol concentrations peaked at 6 h in all of the lipoprotein fractions including the chylomicrons (Table 2). (One of the patients (#8) had multiple peaks, but the first of these was at 6 h.) Preferential transport of d_6-*RRR*-α-tocopherol in VLDL (or other lipoproteins) did not occur. We suggest that the non-discriminators are either *1)* lacking the tocopherol binding protein, or *2)* have a defect in the portion of the protein that recognizes α-tocopherol and thus does not bind α-tocopherol. Either defect would prevent effective incorporation of *RRR*-α-tocopherol into nascent VLDL for secretion by the liver.

Four patients discriminated between *RRR*- and *SRR*-α-tocopherols with plasma d_6-*RRR* decreasing more slowly than d_3-*SRR*-α-tocopherol (Table 4). However, the secretion of d_6-*RRR*-α-tocopherol into VLDL was not as effective as in the controls; the differences in the rates of decrease of the two stereoisomers in the lipoproteins of the discriminators did not reach statistical significance, while those in control lipoproteins did (Table 4). Unlike the controls, in the discriminators the t_{moc} of the chylomicron d_6-*RRR*-α-tocopherol was not statistically different from the t_{moc} of the other lipoproteins (Table 2). Thus, lipoproteins in the discriminators acquired most of the d_6-*RRR*-α-tocopherol during chylomicron catabolism with minimal input of d_6-*RRR*-α-tocopherol during VLDL secretion and catabolism. It is possible that in these patients the tocopherol binding protein can bind *RRR*-α-tocopherol, but insertion into nascent VLDL is defective.

One of the discriminators, patient #5, is heterozygous for familial hypercholesterolemia, and has elevated levels of VLDL and LDL (3). Previously, Harding et al. (3) suggested that she had impaired absorption of vitamin E; however, the present studies demonstrate normal absorption and transport of deuterated tocopherols in chylomicrons. Furthermore, plasma concentrations of d_6-*RRR*-α-tocopherol at 24 h were similar to those of the control subjects (2.0 nmol/ml compared with 1.8 ± 0.7), d_6-*RRR*-AUC was within the normal range (94 nmol/ml · h compared with 87 ± 35), and the rate of decrease of d_6-*RRR*- was similar to that of the controls (−0.009 log[nmol/ml]/h compared with −0.007 ± 0.002). It is likely that as a result of the hypercholesterolemia she has decreased LDL receptor activity and an over-production of VLDL (41). This increased VLDL secretion would compensate for the impaired incorporation of d_6-*RRR*-α-tocopherol in VLDL, and thus plasma d_6-*RRR*- could be maintained at control concentrations over the 3 days of the study. Nevertheless, on presentation at the age of 23, and when consuming a

normal diet, this patient had undetectable plasma concentrations of α-tocopherol and had the characteristic neurologic abnormalities of vitamin E deficiency. The progression of these abnormalities was halted by administration of supplemental vitamin E (3).

In FIVE patients the degree of neurologic dysfunction due to vitamin E deficiency is dependent upon *1)* the severity of the defect in the transport and delivery of vitamin E to affected tissues, *2)* the age at which supplementation is initiated, *3)* the degree of impairment prior to supplementation, and *4)* the amount of supplementation. It is, therefore, not surprising that the degree of neurologic impairment (Table 1) observed in the patients was not obviously different between discriminators and non-discriminators. However, we assumed that a patient with a more severe impairment in neurologic function would present at a younger age. It is interesting to note that presentation with neurologic dysfunction was at an earlier age in the non-discriminators than discriminators (Table 1). The patient's age at presentation of dysfunction was significantly correlated ($r^2 = 0.640$, $P < 0.03$) with the ability to discriminate between *RRR*- and *SRR*-α-tocopherols, as assessed by the ratio of the AUCs of the two stereoisomers. Thus, the severity of the impairment in the putative function of the tocopherol binding protein correlated with the ability to transport α-tocopherol to target neurologic tissues and hence, with the age of onset of neurologic injury.

In conclusion, patients with FIVE deficiency differ in their abilities to discriminate between stereoisomers of α-tocopherol. The similar abilities of controls and patients to transport the unnatural stereoisomer, *SRR*-α-tocopherol, suggests that it is transported nonspecifically. The patients lack or have a defect in the normally enhanced transport of d_6-*RRR*-α-tocopherol via VLDL secretion by the liver. Characterization of the precise genetic defect in these patients awaits isolation of the human tocopherol binding protein and cloning of the gene. However, from these studies it would appear that there is heterogeneity in the genetic defect; non-discriminators may lack the protein or it may have a severely defective tocopherol binding region, while discriminators may have a protein with residual tocopherol binding or with a defective transfer function.

Note added following submission of the manuscript: We have had the opportunity of studying one additional patient described by Trabert et al. (10), using the same protocol described in this study in collaboration with Dr. U. Mielke and Dr. K. Schimrigk at the Universitäts Nervenklinik, Homburg/Saar. This patient was a non-discriminator—he did not discriminate between stereoisomers of α-tocopherol, and the observed maximum in the tocopherol concentrations was coincident with that in the chylomicrons.

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