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Human plasma vitamin E kinetics demonstrate rapid recycling of plasma *RRR*-α-tocopherol

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ABSTRACT **A kinetic model of vitamin E transport in humans is described using data from our studies with deuterium-labeled stereoisomers of α-tocopherol (*RRR*- and *SRR*-). In normal subjects, both α-tocopherols are present at similar concentrations in chylomicrons, but by 24 hr, *RRR*-α-tocopherol is at higher plasma concentrations because *RRR*-α-tocopherol is preferentially incorporated into very low density lipoproteins, which are then secreted into plasma. In three nondiscriminator patients with familial isolated vitamin E deficiency, the fractional disappearance rates (mean ± SD) of deuterium-labeled *RRR*- and *SRR*-α-tocopherols in plasma were 1.4 ± 0.6 and 1.3 ± 0.3 pools per day, respectively (difference, 0.1 ± 0.3). In these patients, plasma concentrations of both *RRR*- and *SRR*-α-tocopherols decreased similarly to *SRR*-α-tocopherol in controls. In six controls, fractional disappearance rates of deuterium-labeled *RRR*-α-tocopherol (0.4 ± 0.1 pool per day) were significantly ($P < 0.01$) slower than for *SRR*- (1.2 ± 0.6). The differences (0.8 ± 0.6 pool per day) between these two rates in controls estimate the rate at which *RRR*-α-tocopherol, which had left the plasma, was returned to the plasma. Although plasma labeled *RRR*-α-tocopherol concentrations in controls appear to change slowly, these data show that both *RRR*- and *SRR*-α-tocopherols leave the plasma rapidly, but only *RRR*-α-tocopherol is returned to the plasma, likely in nascent very low density lipoproteins. This recycling of *RRR*-α-tocopherol accounts for nearly 1 pool of α-tocopherol per day.**

We have investigated lipoprotein transport of vitamin E in normal humans, in patients with genetic abnormalities in lipoprotein metabolism, and in cynomolgus monkeys (1–3) using deuterium-labeled tocopherols, including two stereoisomers of α-tocopherol (naturally occurring *RRR*- and synthetic *SRR*-) and γ-tocopherol. All three labels, when given in equal concentrations, appear in the chylomicrons in equal concentrations (1, 3). During chylomicron catabolism (the first 6–9 hr following the dose) the plasma contains equal concentrations of the three tocopherols. However, by 24 hr very low density lipoproteins (VLDLs) are preferentially enriched with *RRR*-α-tocopherol. Nascent VLDLs isolated from perfused monkey livers demonstrated that these lipoproteins are secreted from the liver preferentially enriched in *RRR*-α-tocopherol (2). These data suggest that human plasma becomes enriched in *RRR*-α-tocopherol (compared with *SRR*-α-tocopherol or γ-tocopherol) as a result of the secretion of nascent VLDLs enriched in *RRR*-α-tocopherol. We have suggested that a tocopherol binding protein is responsible for incorporating *RRR*-α-tocopherol into VLDLs during its assembly and secretion into the plasma by the liver (4, 5). The subsequent catabolism of VLDLs allows transfer of *RRR*-α-tocopherol to low and high density lipoproteins (6–8).

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In support of this hypothesis are studies in patients with familial isolated vitamin E deficiency (FIVE deficiency). These patients are vitamin E deficient yet have no abnormalities in gastrointestinal function and lipoprotein metabolism or other known cause of vitamin E deficiency that could account for their observed low plasma vitamin E concentrations (<1 μg/ml; normal 5–15), as reviewed (4, 5). FIVE deficiency patients do have a defect in the transport of vitamin E with an impaired secretion of α-tocopherol in VLDL (9, 10). Therefore, we have hypothesized that they lack or have a defective form of the tocopherol binding protein (9) and subsequently showed that some of these patients could not distinguish between *RRR*- and *SRR*-α-tocopherols (10). A recent report by Ben Hamida *et al.* (11) of vitamin E deficiency in patients mimicking Friedreich ataxia in inbred families in Tunisia documented, using homozygosity mapping, that their abnormality was on chromosome 8. It remains to be established that this is the locus of the tocopherol binding protein. The rat hepatic tocopherol binding protein (12, 13) transfers α-tocopherol in preference to other tocopherols (12, 14) and is a likely candidate to insert *RRR*-α-tocopherol into nascent VLDLs during assembly in the liver. This protein has been isolated from both rat (12, 13) and human liver (15) and the amino acid sequence of the rat protein has been reported (16).

Studies of the discrimination between *RRR*- and *SRR*-α-tocopherol in FIVE deficiency patients demonstrated that they have an abnormal transport of *RRR*-α-tocopherol; *SRR*-α-tocopherol was transported similarly in controls and in patients (10). This suggests that *SRR*-α-tocopherol is transported in a nonspecific manner and can be used as a tracer of the removal of α-tocopherol from the plasma.

In the present paper we have devised a mathematical model for the turnover of plasma vitamin E. We propose that α-tocopherol leaves the plasma quickly, that only *RRR*-α-tocopherol is incorporated into VLDLs, and that VLDL secretion from the liver can account for the nearly complete *daily* recycling of plasma *RRR*-α-tocopherol.

METHODS

Protocol. Complete descriptions of the studies using deuterated tocopherols in humans have been published (1, 3, 10). Briefly, subjects were given an oral dose containing equal concentrations of the deuterated tocopherols with breakfast; then blood samples were taken at 2–3 hourly intervals up to 12 hr, then daily up to 96 hr. Deuterated tocopherol contents were measured by gas chromatography/mass spectrometry.

Mathematical Model. For purposes of mathematical modeling, we have used data from (*i*) normal humans [six were given 20 mg each (10) and seven were given 50 mg each (1,

Abbreviations: VLDL, very low density lipoprotein; FIVE deficiency, familial isolated vitamin E deficiency.

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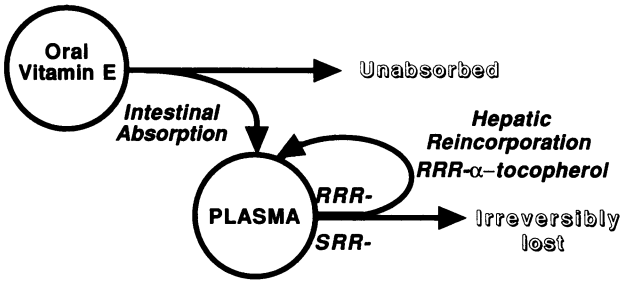

FIG. 1. Model of vitamin E kinetics. We have assumed that both *RRR*- and *SRR*-α-tocopherols labeled with deuterium are absorbed and secreted in chylomicrons entering the plasma similarly. Vitamin E then leaves the plasma. Based on studies of patients with FIVE deficiency who cannot discriminate between these stereoisomers and have a rapid loss of plasma vitamin E, we have also assumed that the rate of disappearance of plasma deuterated *SRR*-α-tocopherol is a measure of the rate of irreversible removal of α-tocopherol from the plasma. The difference in the rates of disappearance of labeled *RRR*- and *SRR*-α-tocopherols is a measure of the recycling or secretion by the liver of *RRR*-α-tocopherol, preferentially incorporated into VLDLs, which is secreted into the plasma.

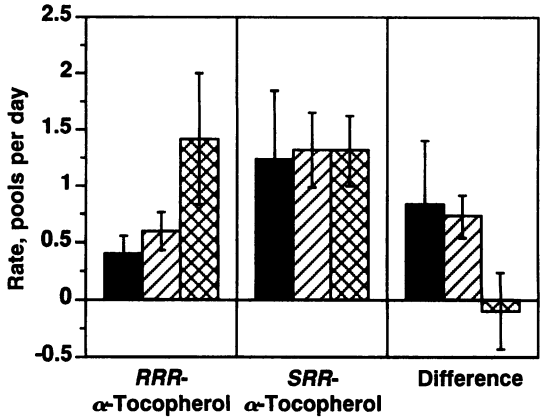

FIG. 2. Kinetic parameters: Rates of disappearance of labeled *RRR*- and *SRR*-α-tocopherols from the plasma. Shown are the means ± SD of the rates of disappearance of naturally occurring *RRR*-α-tocopherol and its synthetic stereoisomer *SRR*-α-tocopherol; both tocopherols were labeled with deuterium. Control subjects are indicated by solid bars, patients who could discriminate between stereoisomers of α-tocopherol are indicated by diagonal bars, and nondiscriminators are indicated by cross-hatched bars.

3) of *RRR*-α-tocopherol (labeled with six deuterium atoms) and *SRR*-α-tocopherol (labeled with three deuterium atoms)] and (*ii*) seven patients with FIVE deficiency given 20 mg of each labeled α-tocopherol (10). Descriptive data about the subjects has been published (1, 3, 10). Data from each individual were fitted by the model shown in Fig. 1.

The mathematical model was designed with the following assumptions. It was assumed that the absorption and secretion of the two deuterated tocopherols (*RRR*- and *SRR*-α-tocopherols) into chylomicrons were similar. Initial inputs into the plasma were assumed to occur simultaneously for the two labels. The plasma concentrations were fitted to the following functions:

$$\text{Plasma } [SRR] = \frac{u}{\rho - \alpha}(e^{-\alpha t} - e^{-\rho t}),$$

$$\text{Plasma } [RRR] = \frac{v}{\rho - \beta}(e^{-\beta t} - e^{-\rho t}),$$

where u and v are constants, ρ is the rate constant of absorption, α is the rate constant of disappearance of *SRR*, and β is the rate constant of disappearance of *RRR*-α-tocopherol. All of the model parameters, including the rate constants and an absorption delay, were estimated from the individual data by fitting a three-pool model (a plasma pool each for *RRR*- and *SRR*-α-tocopherols and a common absorption pool) to the plasma concentrations of *RRR*- and *SRR*-α-tocopherols simultaneously with the help of a modeling program developed by us (17). Fitting was by nonlinear least squares, assuming measurement errors to have a constant coefficient of variation. *RRR*- and *SRR*-α-tocopherol rate constants were compared in controls by a paired *t* test. Half-lives were calculated as $t_{1/2}$ = ln2/disappearance rate constant.

To calculate the rate of preferential input of *RRR*-α-tocopherol into the plasma, it was assumed that *RRR*- and *SRR*-α-tocopherols leave the plasma at similar rates. There-

Table 1. Kinetic parameters from normal subjects and patients (discriminators and nondiscriminators) with FIVE deficiency given 20 mg each of *RRR*- and *SRR*-α-tocopherols labeled with differing amounts of deuterium

Subject	k_{SRR}, pools per day	k_{RRR}, pools per day	Difference ($k_{RRR} - k_{SRR}$)	*SRR* $t_{1/2}$, hr	*RRR* $t_{1/2}$, hr
Control					
5	0.78	0.48	0.30	16.2	51.0
7	2.41	0.56	1.85	12.8	87.7
8	1.09	0.54	0.56	21.4	34.8
10	1.02	0.33	0.70	6.9	29.6
11	0.75	0.33	0.42	15.2	31.1
12	1.30	0.19	1.11	22.1	51.0
Mean ± SD	1.23 ± 0.62*	0.40 ± 0.15*	0.82 ± 0.58	15.8 ± 5.7	47.5 ± 21.9
Discriminator					
P1	1.63	0.80	0.83	10.2	20.7
P2	1.13	0.53	0.60	14.7	31.7
P5	1.57	0.64	0.93	17.5	40.5
P7	0.95	0.41	0.54	10.6	26.2
Mean ± SD	1.32 ± 0.33	0.59 ± 0.17	0.72 ± 0.18	13.3 ± 3.5	29.8 ± 8.5
Nondiscriminator					
P4	1.00	1.05	−0.05	16.6	15.9
P6	1.31	1.10	0.20	12.7	15.1
P8	1.63	2.08	−0.45	10.2	8.0
Mean ± SD	1.31 ± 0.31	1.41 ± 0.58	−0.10 ± 0.33	13.2 ± 3.2	13.0 ± 4.4

*Values are significantly different ($P < 0.01$).

Table 2. Kinetic parameters of normal subjects given 50 mg each of *RRR*- and *SRR*-α-tocopherols labeled with differing amounts of deuterium

Control	k_{SRR}, pools per day	k_{RRR}, pools per day	Difference ($k_{RRR} - k_{SRR}$)	*SRR* $t_{1/2}$, hr	*RRR* $t_{1/2}$, hr
RB	1.43	0.36	1.07	11.6	46.2
MD	0.95	0.30	0.66	17.5	56.4
FT	0.82	0.21	0.61	20.3	78.8
JL	1.35	0.57	0.78	12.3	29.4
MT	0.79	0.44	0.35	21.1	38.1
MT*	0.58	0.44	0.14	28.6	37.7
VL	1.81	0.76	1.05	9.2	21.9
Mean ± SD	1.10 ± 0.44†	0.44 ± 0.18†	0.67 ± 0.34	17.2 ± 6.8	44.1 ± 18.9

*Dose given at 6 p.m. instead of 6 a.m.
†Values are significantly different ($P < 0.001$).

fore, the difference between the two rates should yield the rate of reincorporation of *RRR*-α-tocopherol into the plasma.

RESULTS

Previously, we reported that patients with FIVE deficiency could be categorized based on their abilities to discriminate between *RRR*- and *SRR*-α-tocopherols (10). Three patients were unable to discriminate; they had similar concentrations of the two labels at all time points. Using our model, the fractional disappearance rates (mean ± SD) of *RRR*- and *SRR*-α-tocopherols were 1.41 ± 0.58 pools per day for *RRR*-α-tocopherol and 1.31 ± 0.31 for *SRR*- (Fig. 2; individual data are shown Table 1). This yields a half-life of ≈13 hr for both *RRR*- and *SRR*-α-tocopherols in these patients and documents their rapid plasma disappearance of vitamin E.

In contrast to nondiscriminators, the control subjects demonstrated a significant difference in the fractional disappearance rate between *RRR*- and *SRR*-α-tocopherols (0.40 ± 0.15 and 1.23 ± 0.62 pools per day, difference = 0.82 ± 0.58, $P < 0.01$, Table 1). The apparent half-life of *RRR*-α-tocopherol in control subjects is ≈48 hr, consistent with the "slow" disappearance of α-tocopherol from the plasma. These data were from control subjects consuming 20 mg of each labeled tocopherol; data from studies in which control subjects consumed 50 mg (one subject, MT, who was studied twice, consumed 50 mg with breakfast in one study and also consumed 75 mg of each with dinner in a separate study) of each of the stereoisomers of α-tocopherol were also fitted using this model. Despite differences in dose size, the fractional disappearance rates of *RRR*- and *SRR*-α-tocopherol (0.44 ± 0.18 and 1.10 ± 0.44 pools per day, respectively) did not change (compare Table 1 with Table 2). A representative control subject is shown in Fig. 3.

Comparing controls and patients with FIVE deficiency, the fractional disappearance rate of *RRR*-α-tocopherol was faster in nondiscriminator patients (1.41 ± 0.58 compared with 0.40 ± 0.15 in controls), while discriminator patients had intermediate rate constants (0.59 ± 0.17). As expected from the similar plasma concentrations of *SRR*-α-tocopherol in controls, discriminators, and nondiscriminators (10), the fractional plasma disappearance rates calculated for these three groups were very close (Fig. 2, Table 1).

The similarity in the fractional disappearance rates for the two stereoisomers of α-tocopherol in the nondiscriminator patients, along with the similarity in *SRR* fractional catabolic rate between patients and controls, support the idea that *SRR*-α-tocopherol can be used in normal subjects to trace the irreversible loss of vitamin E from the plasma. Based on this assumption, the fractional disappearance rates of *RRR* and *SRR* were used to obtain the rate of resecretion of *RRR*-α-tocopherol into the plasma (Fig. 1). The difference between the two rates in six normal subjects given 20 mg was 0.82 ± 0.58 pool per day (or 0.034 ± 0.024 pool per hr) and in six normal subjects given 50 mg was 0.67 ± 0.34 pool per day (or 0.028 ± 0.014 pool per hr). To calculate the amounts of *RRR*-α-tocopherol resecreted into the plasma, we have assumed a plasma α-tocopherol concentration of 25 μM and a plasma volume of 4 liters, equaling a plasma pool size of 100 μmol of α-tocopherol; this then represents a recycling rate of about 74 μmol/day or 3 μmol/hr.

DISCUSSION

These studies demonstrate that although labeled *RRR*-α-tocopherol concentrations apparently leave the plasma slowly, the opposite is true. *RRR*-α-Tocopherol rapidly leaves the plasma but is incorporated into VLDLs and resecreted back into the plasma. The result of this process is the apparent slow disappearance of *RRR*-α-tocopherol from the plasma. Our conclusions are based on studies of the transport of stereoisomers of α-tocopherol labeled with deuterium in nondiscriminator patients with FIVE deficiency. In these patients the disappearance rates of *RRR* and *SRR* are similar; therefore we have made the assumption that *SRR* can

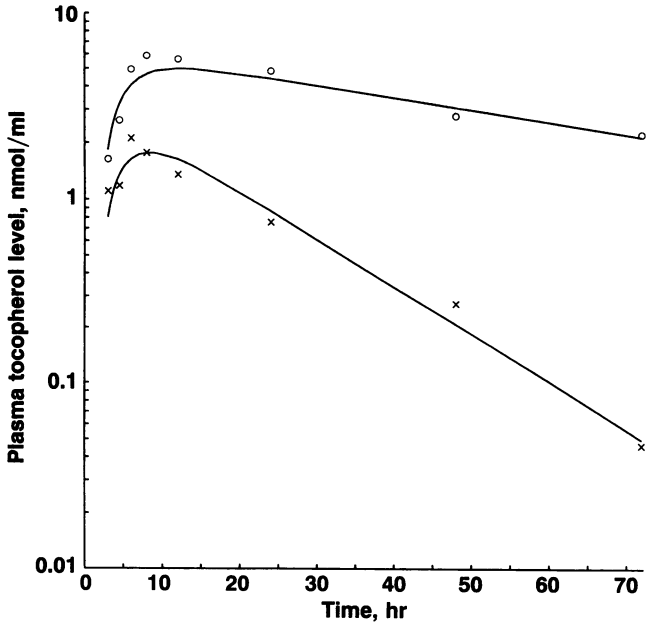

FIG. 3. Representative control subject. This control subject (RB) was given an oral dose containing 50 mg each of *RRR*-α-tocopherol (labeled with six deuterium atoms) and *SRR*-α-tocopherol (labeled with three deuterium atoms) with breakfast; then blood samples were taken at indicated intervals. Deuterated tocopherol contents of plasma samples were measured by gas chromatography/mass spectrometry (1). Shown are the individual data points (○, *RRR*-α-tocopherol; ×, *SRR*-α-tocopherol) and the line shows the computer-generated fit.

be used to trace α-tocopherol as it is lost from the plasma in normal subjects.

An estimate of the amount of *RRR*-α-tocopherol that had to have been recycled in order to obtain the observed disappearance rate of *RRR*-α-tocopherol was calculated using the disappearance rate of *SRR*-α-tocopherol in a total of 12 control subjects. From this we have estimated that *RRR*-α-tocopherol is reincorporated into the plasma at a rate of about 74 μmol/day or 3 μmol/hr.

To independently verify whether the liver is capable of secreting α-tocopherol at this rate we have made some estimates of the ability of the tocopherol binding protein to transfer tocopherol. The amount of the tocopherol binding protein in rat liver has been estimated by Sato *et al.* (12) as ≈70 μg/g of liver. The rate of α-tocopherol transfer by the purified protein was estimated to be 64 pmol/hr per μg of binding protein (table III from ref. 12). Thus, rat tocopherol binding protein could theoretically transfer 4480 pmol of α-tocopherol per hr per g of liver. Assuming that the human liver contains an equally active tocopherol binding protein and that the liver is ≈1800 g, it could transfer 8 μmol/hr or 190 μmol/day—a value in excess of what we have estimated (about 74 μmol/day or 3 μmol/hr).

Alternatively, the amount of tocopherol secreted can be estimated from the production rate of VLDL triglyceride (VLDL-TG) and the concentration of α-tocopherol in VLDL. The production rate of VLDL-TG has been estimated at 872 ± 71 mg/hr in normal humans and 1731 ± 166 mg/hr in hypertriglyceridemic patients (18) or 1091 ± 304 mg/hr in nonobese and 2028 ± 405 mg/hr in obese humans (19)—a range of VLDL-TG secretion of 1–2.3 mmol/hr. The concentration of *RRR*-α-tocopherol in VLDL in four of our subjects was 2 ± 1 μmol/mmol of TG (1). Combining these two estimates yields an estimated α-tocopherol secretion of 2–5 μmol/hr or 40–100 μmol/day, again consistent with our kinetic estimates of α-tocopherol secretion.

We have also attempted to estimate the amount of α-tocopherol that would obviate the necessity for the tocopherol binding protein. Given that the rate of loss of *RRR*-α-tocopherol in the nondiscriminator patients was 1.4 ± 0.58 pools per day and an estimated pool size of 100 μmol, the absolute catabolic rate is ≈140 μmol/day or 60 mg of α-tocopherol. The fractional absorption of vitamin E in humans has been estimated to be about 70%, based on the fecal recovery of an oral dose of radioactive α-tocopherol (20, 21). Likely, this is an overestimate; therefore, assuming slightly more than a 50% rate of absorption suggests that nearly 100 mg is needed. This value of 100 mg is a level of vitamin E found to be protective in epidemiologic studies (22, 23).

In conclusion, these studies demonstrate that plasma α-tocopherol is not static and slowly turning over, but rather is in a state of rapid flux into and out of the plasma. Estimates of VLDL production rates suggest that the predicted influx of α-tocopherol into the plasma can be accounted for by the secretion of nascent VLDLs into the plasma.

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