# *RRR*- and *SRR*-α-tocopherols are secreted without discrimination in human chylomicrons, but *RRR*-α-tocopherol is preferentially secreted in very low density lipoproteins

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**Abstract** Five subjects ingested in a single 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) with natural stereochemistry, and of 2*S*,4'*R*,8'*R*-α-(5-$C^2H_3$)tocopheryl acetate ($d_3$-*SRR*-α-tocopheryl acetate). These are two of eight stereoisomers in synthetic vitamin E. By day 1 the plasma and red blood cells were enriched fourfold with $d_6$-*RRR*-α-tocopherol ($P < 0.004$). The ratio of $d_6$-*RRR*-/$d_3$-*SRR*- further increased over the succeeding 4 days, because the $d_3$-*SRR*- decreased at a faster rate than did the $d_6$-*RRR*-stereoisomer. Plasma and lipoproteins were isolated at intervals during the first day, and daily for 3 days, from four additional subjects fed a mixture of equal amounts of the deuterated tocopherols. The plasma contained similar concentrations of the two forms until 11 h, when the $d_6$-*RRR*-α-tocopherol concentration became significantly greater ($P < 0.05$). The chylomicrons contained similar concentrations of the two deuterated tocopherols, but the VLDL (very low density lipoproteins) became preferentially enriched in $d_6$-*RRR*-α-tocopherol by 11 h. The pattern of the deuterated tocopherols shows that during chylomicron catabolism all of the plasma lipoproteins were labeled equally with both tocopherols, but that during the subsequent VLDL catabolism the low and high density lipoproteins became enriched in $d_6$-*RRR*-α-tocopherol. These results suggest the existence of a mechanism in the liver for assembling VLDL preferentially enriched in *RRR*- relative to *SRR*-α-tocopherol. — **Traber, M. G., G. W. Burton, K. U. Ingold, and H. J. Kayden.** *RRR*- and *SRR*-α-tocopherols are secreted without discrimination in human chylomicrons, but *RRR*-α-tocopherol is preferentially secreted in very low density lipoproteins. *J. Lipid Res.* 1990. **31:** 675–685.

**Supplementary key words** vitamin E • stable isotopes • biodiscrimination

We have recently studied the transport of deuterium-labeled *RRR*-α-tocopherol (with natural stereochemistry) in human plasma lipoproteins (1). The deuterated vitamin E was absorbed from the intestinal lumen and secreted in chylomicrons. During chylomicron catabolism and lipoprotein remodeling, the deuterated tocopherol appeared in the other lipoprotein fractions, demonstrating that there is a rapid exchange of tocopherol between the lipoproteins in vivo (1).

Studies carried out in intact rats (2), as well as in isolated rat hepatocytes (2, 3), have demonstrated that tocopherol is secreted from hepatocytes in nascent VLDL. Our studies using deuterated tocopherol in humans also showed that, after its appearance in chylomicrons, the VLDL became enriched in deuterated tocopherol (1). During the catabolism of VLDL, deuterated tocopherol was found to increase markedly in low and high density lipoproteins, which contain the largest amounts of tocopherol in human plasma (1).

The absorption and transport of α- and γ-tocopherols, two naturally occurring forms of vitamin E, have also been studied in humans (4). The data showed that both tocopherols are absorbed equally from the gastrointestinal tract and secreted in chylomicrons, and that subsequently α-tocopherol predominates in the plasma.

Studies in rats by Ingold et al. (5) have demonstrated that the preference for α-tocopherol may be even more specific than that found in humans for α- versus γ-tocopherols (which differ in the number of methyl groups on the chromanol ring). Rats were fed up to 154 days with diets containing an equimolar mixture of $d_6$-*RRR*-α- and $d_3$-*SRR*-α-tocopheryl acetates, two stereoisomers of α-tocopherol that differ only in the chirality of the carbon atom at position 2. All of the rat tissues were preferentially enriched in $d_6$-*RRR*-α-tocopherol, except the liver, which initially had twice as much $d_3$-*SRR*- as $d_6$-*RRR*-α-tocopherol. During the following 16 days the *RRR*-/*SRR*-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

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ratio gradually increased to a value slightly greater than one. By the end of the study, the brain had the largest enrichment of natural α-tocopherol by a factor of five.

The studies described herein were designed to test whether humans also exhibit a strong preference for *RRR*- over *SRR*-α-tocopherol. This question is of interest because synthetic vitamin E (α-tocopherol) preparations contain an approximately equimolar mixture of eight stereoisomers, one of which has the *RRR*-structure and another the *SRR*-structure. Our work provides the first demonstration of chiral discrimination between *RRR*- and *SRR*-α-tocopherols in humans. Furthermore, we demonstrate that *RRR*- and *SRR*-α-tocopherols are equally well absorbed, but that subsequently there is an increase in the *RRR*-α-tocopherol content of the plasma. We suggest that this increase occurs because the liver secretes nascent VLDL preferentially enriched in *RRR*-α-tocopherol, and that the catabolism of this VLDL results in the enrichment of LDL and HDL with *RRR*-α-tocopherol.

## METHODS

### Deuterated α-tocopherols

The syntheses and analysis of deuterated α-tocopherols have been described previously (5, 6). 2*R*,4'*R*,8'*R*-α-(5,7-($C^2H_3$)$_2$)tocopheryl acetate ($d_6$-*RRR*-α-tocopheryl acetate) was provided to the subjects as 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) was provided as a source of trideuterated α-tocopherol with unnatural stereochemistry. 2-*ambo*-α-(5,7,8-($C^2H_3$)$_3$)tocopherol ($d_9$-α-tocopherol), used as an internal standard, was added in known amounts to each plasma, red cell, or lipoprotein sample immediately prior to lipid extraction (1, 7). These lipid extracts were purified by passage through an analytical, high-performance, silica gel chromatography column. The amounts of $d_3$-, $d_6$-, $d_9$-, and nondeuterated ($d_0$) α-tocopherol in the collected tocopherol fraction were determined by gas-liquid chromatography–mass spectrometry after conversion to their trimethylsilyl ethers. The absolute concentrations of $d_0$-, $d_3$-, and $d_6$-α-tocopherols in the original plasma and lipoprotein samples were obtained by comparing the respective peak areas with the peak area of the added $d_9$-α-tocopherol.

### Experimental protocols

This study was carried out with the approval of the Institutional Review Board of New York University Medical Center. The subjects gave written, informed consent, and had no abnormalities of lipid or lipoprotein metabolism. Five male subjects (numbered 1–5) each consumed a capsule containing both deuterated tocopherols (50 mg of each) with the evening meal, then blood samples were drawn on days 1, 2, 4, and 5 into EDTA tubes (Becton Dickinson, Rutherford, NJ). The plasma was separated by centrifugation, frozen at −70°C, and the tocopherol content was analyzed within 1–2 days.

One female subject (#6) participated twice at a 6-month interval. In one study the subject consumed a capsule containing a mixture of 75 mg of each of the two deuterated tocopherols after the evening meal at 10 PM, then blood was collected in EDTA tubes at 12, 14, 16, 18, 20, 36, 60, and 84 h. In the other study this subject consumed the same amounts of the two deuterated tocopherols with breakfast at 6 AM, then blood was collected in EDTA tubes at 0, 3, 5, 7, 9, 11, 28, 53, and 76 h.

Three subjects (#7 female; #8 male; #9 male) consumed a capsule containing a mixture of equal amounts of the two deuterated tocopherols (the exact amounts of each tocopherol were: #7 (40 mg), #8 (50 mg), and #9 (75 mg)) after an overnight fast immediately prior to breakfast. Blood samples (15 ml/time point) were collected in EDTA tubes at 0, 3, 5, 7, 9, 11, 28, 53, and 76 h after taking the tocopherols. In these latter studies (subjects 6–9), the plasma was immediately separated by centrifugation. A 1.0-ml aliquot was frozen for subsequent tocopherol analysis while the remainder was used for isolation of the lipoprotein fractions.

All subjects were allowed to eat ad libitum.

### Lipoprotein isolation

Chylomicrons and lipoproteins were isolated as described previously (1). Briefly, chylomicrons were isolated from duplicate samples of 1 ml plasma overlayered with 1 ml saline (0.15 M NaCl, 0.3 mM EDTA, pH 7.4) by centrifugation for 8 min at 40,000 rpm using a swinging bucket rotor (TLS 55) and a TL 100 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). Subsequently, the indicated lipoprotein fractions were isolated by centrifugation for 2 h at 100,000 rpm using a fixed angle rotor (TLA 100.2) with sequential density changes achieved by adding solid KBr. The density ranges used were: VLDL $d<1.006$, LDL $1.006<d<1.063$, and HDL $d>1.063$ g/ml. The HDL were not separated from the serum proteins because the $d>1.21$ fraction contains negligible amounts of tocopherol. Immediately upon isolation, each of the lipoprotein fractions was divided into two equal fractions (for quantitation of the deuterated tocopherols and of the triglycerides—see below). The lipoprotein samples were frozen and stored at −70°C. The lipoprotein fractions and the 1-ml plasma samples were taken by courier on dry ice to the National Research Council in Ottawa, Canada for analyses of the $d_0$-, $d_3$-, and $d_6$-α-tocopherols. The data from the analyses of the chylomicron fractions are presented only for the first 24 h after administration of the test dose. Although deuterium-labeled chylomicrons could be isolated at 24 h and later,

it is not likely that this is representative of newly absorbed tocopherols. The data are presented per ml of plasma with corrections for aliquots taken, but not for losses due to the ultracentrifugation procedure. These have been assumed to be negligible.

### Triglyceride analysis

Known aliquots of the chylomicron and VLDL fractions were extracted with chloroform–methanol 2:1 (8). The total fatty acids in the chloroform layer were estimated using the hydroxylamine assay (9). The triglyceride fatty acids were estimated by subtracting from the total fatty acids: *1*) the fatty acids present in cholesteryl esters, as estimated by gas–liquid chromatography of the free and total cholesterol in the sample (10), and *2*) the fatty acids present in phospholipids, as estimated from the determination of lipid phosphorus (11). The triglyceride ($\mu$mol/ml plasma) was calculated assuming that the molecular weights of the triglycerides were equal to that of triolein.

### Statistical analysis

The statistical significance of the results was determined using the statistical analysis program, Stat View 512+ (Brain Power Associates, Calabasus, CA) using two-factor analysis of variance (ANOVA) with repeated measures and comparisons at individual time points by a two-tailed Student's *t*-test. Results of the statistical tests were considered to be significant at the 95% confidence level ($P < 0.05$).

## RESULTS

**Fig. 1A** shows the mean plasma concentrations of $d_6$-*RRR*- and $d_3$-*SRR*-$\alpha$-tocopherols in the five subjects on 4 days in the week after they each consumed equal amounts (50 mg) of $d_6$-*RRR*-$\alpha$-tocopherol and $d_3$-*SRR*-$\alpha$-tocopherol in a single oral dose taken with their Sunday evening meal. (Data for each individual will be presented and discussed in detail elsewhere.) The plasma from each individual at all of the time points was preferentially enriched in $d_6$-*RRR*-$\alpha$-tocopherol. Furthermore, by the first day (approximately 15–18 h after ingestion of the tocopherols) the average concentration of $d_6$-*RRR*-$\alpha$-tocopherol (7.7 ± 2.9 nmol/ml) in the plasma of the five subjects was nearly four times greater than the $d_3$-*SRR*-$\alpha$-tocopherol (2.2 ± 1.0; $P < 0.004$). During the subse-

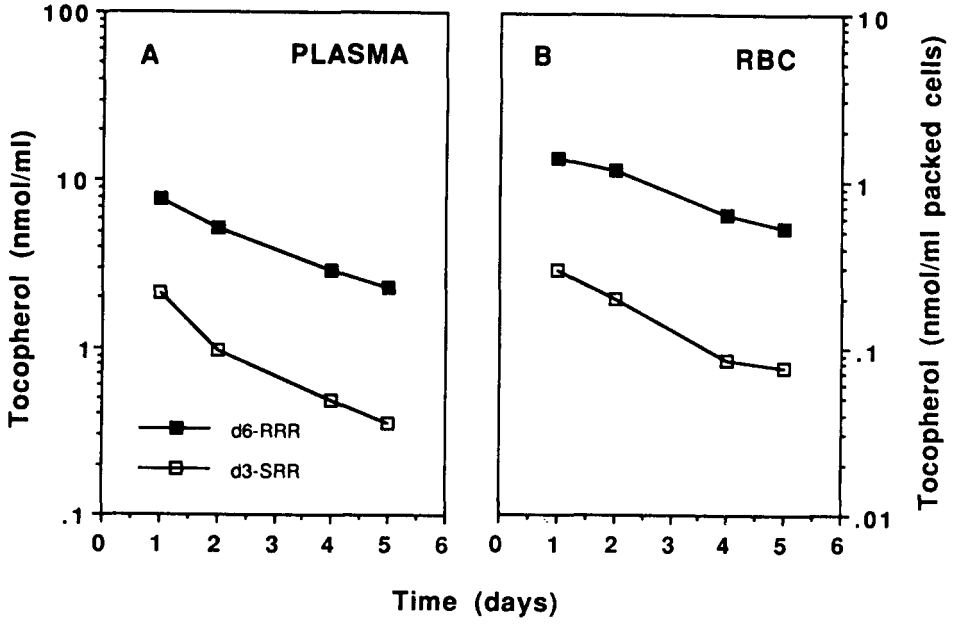


**Fig. 1.** Plasma and red blood cell deuterated tocopherol concentrations. Five normal humans (#1–5) ingested 50 mg each of 2*R*,4'*R*,8'*R*-$\alpha$-(5,7-($C^2H_3$)$_2$)tocopheryl acetate ($d_6$-*RRR*-$\alpha$-tocopheryl acetate) and 2*S*,4'*R*,8'*R*-$\alpha$-(5-$C^2H_3$)tocopheryl acetate ($d_3$-*SRR*-$\alpha$-tocopheryl acetate) with the evening meal, then blood samples were drawn on the indicated days. After the addition of 2-*ambo*-$\alpha$-(5,7,8-($C^2H_3$)$_5$)tocopherol ($d_9$-tocopherol), as an internal standard, to the plasma or washed red blood cells, the deuterated tocopherols were measured in an aliquot of a heptane extract of the plasma or red blood cells by gas–liquid chromatography–mass spectrometry and were quantitated by comparing the areas of the peaks of the unlabeled-, $d_6$-, and $d_3$-$\alpha$-tocopherols to that of the internal standard, as described in the Methods section. The means of the $d_6$-*RRR*- (closed symbol) and $d_3$-*SRR*- (open symbol) $\alpha$-tocopherol concentrations (nmol/ml) of the plasma (A) and the red blood cells (nmol/ml packed cells) (B) from the five subjects are shown. The statistical comparisons between $d_6$-*RRR*- and $d_3$-*SRR*- in the plasma were significant at all time points (day 1, $P < 0.004$; day 2, $P < 0.001$; day 4, $P < 0.003$; day 5, $P < 0.003$), as were the comparisons in the red blood cells (day 1, $P < 0.002$; day 2, $P < 0.003$; day 4, $P < 0.001$; day 5, $P < 0.001$).

quent days the $d_6$-*RRR*-α-tocopherol concentration remained significantly greater (day 2, $P < 0.001$; day 4, $P < 0.003$; day 5, $P < 0.003$). The disappearance of the tocopherols from the plasma followed approximately first order kinetics during this interval. In each of the individuals studied the $d_3$-*SRR*-α-tocopherol disappeared at a faster rate than the $d_6$-*RRR*- form, as estimated from the slopes of the semi-logarithmic plots of the tocopherol concentrations versus time past the peak in the deuterated tocopherol concentration (**Table 1**). The red cells isolated from these subjects also showed a statistically significant enrichment of $d_6$-*RRR*-α-tocopherol (Fig. 1B).

Since the discrimination between *RRR*- and *SRR*-α-tocopherols must occur within the first 15–18 h of ingestion of the dose, a study was carried out in a subject (#6) who took 75 mg of each of the $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols on two separate occasions, 6 months apart, once at 6 AM and once at 10 PM. The deuterated tocopherol concentrations of plasma from blood samples drawn following the doses taken at 6 AM and 10 PM are shown in **Figs. 2A and B,** respectively. When the dose of labeled tocopherols was taken at 10 PM (Fig. 2B) with the first blood sample drawn the following day, the plasma was already enriched in $d_6$-*RRR*-α-tocopherol, as was the case for the subjects shown in Fig. 1A.

Seven hours after administration of the tocopherols at 6 AM, both forms of deuterated tocopherols appeared in nearly equal concentrations in the plasma (Fig. 2A). (At the earlier time points the deuterated tocopherols were below detectable levels in the plasma of this subject.) The 9- and 11-h samples contained increases in the concentration of $d_6$-*RRR*-, while the $d_3$-*SRR*-α-tocopherol decreased. At subsequent time points the concentrations of both deuterated tocopherols declined. At equivalent postdose intervals in the two studies, the concentrations of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols, respectively, were virtually equal (compare Figs. 2A and B), thereby demonstrating the consistency of this method of measuring transport of vitamin E within a given subject. The red cells in this subject at time points after 9 h, during both studies, were preferentially enriched in $d_6$-*RRR*-α-tocopherol (data not shown).

Because the preferential incorporation of $d_6$-*RRR*-α-tocopherol into plasma occurred so soon after ingestion, additional studies were carried out in three normal subjects who were given the deuterated tocopherols with breakfast and from whom blood samples were obtained every 2 h from 3–11 h, then daily for 3 days. The size of

TABLE 1. Slopes of the semi-logarithmic plots of the plasma *RRR*- and *SRR*-α-tocopherol concentrations from the five subjects shown in Fig. 1

| | $d_6$-*RRR*-α-tocopherol | | $d_3$-*SRR*-α-tocopherol | |
|---|---|---|---|---|
| Subject | Slope[a] | $r^2$ | Slope[a] | $r^2$ |
| 1 | −0.1341 | 0.979 | −0.2829 | 0.969 |
| 2 | −0.1501 | 0.994 | −0.2331 | 0.997 |
| 3 | −0.1664 | 0.986 | −0.2254 | 0.991 |
| 4 | −0.0866 | 0.985 | −0.1075 | 0.790 |
| 5 | −0.1205 | 0.986 | −0.1505 | 0.999 |

[a] Log(nmol/ml) · $day^{-1}$.

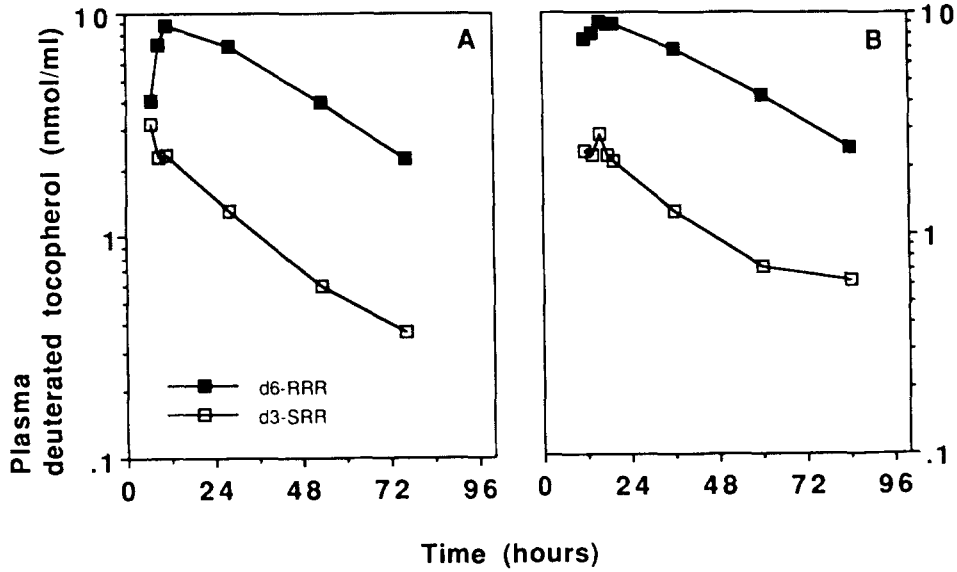


**Fig. 2.** Plasma deuterated tocopherol concentrations after ingestion of the dose at 10 PM and at 6 AM. Subject #6 took 75 mg each of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopheryl acetates on two separate occasions 6 months apart, once at 10 PM and once at 6 AM. The mean of the plasma $d_6$-*RRR*- (closed symbol) and $d_3$-*SRR*- (open symbol) α-tocopherol concentrations (nmol/ml plasma) of blood samples drawn following the dose taken at 10 PM and 6 AM were analyzed as described in Fig. 1 and are shown in A and B, respectively.

the dose of each tocopherol was varied from 75 to 50 to 40 mg in an attempt to correlate the dose with plasma concentrations of the deuterated tocopherols. However, subjects 6 and 9, who both ingested 75 mg of each deuterated tocopherol, had the highest and lowest plasma deuterated tocopherol levels, respectively. Therefore, the data from all four subjects were combined for statistical analysis. **Fig. 3** shows the means of the plasma tocopherol concentrations; the data for the individual subjects are given in **Table 2.** There were no statistically significant differences in the concentrations of the two deuterated tocopherols in the plasma at any time point up to 11 h; thereafter the $d_6$-*RRR*-α-tocopherol concentrations were statistically greater than the $d_3$-*SRR*-α-tocopherol concentrations (11 h, $P < 0.05$; 28 h, $P < 0.02$; 53 h, $P < 0.006$; 76 h, $P < 0.001$).

To assess the sequential appearance of the tocopherols in the lipoproteins, the fraction of each deuterated tocopherol relative to the total tocopherol was calculated for chylomicrons, VLDL, LDL, and HDL (**Fig. 4**). The concentrations of the tocopherols in the lipoprotein fractions isolated from subjects 6–9 are shown in Table 2. The chylomicrons contained similar amounts of both tocopherols with a similar pattern of increase and decrease in the fractions of $d_6$-*RRR*-/total and $d_3$-*SRR*-/total α-tocopherols (Fig. 4A). The VLDL (Fig. 4B) contained similar increases of both tocopherols during the first 9 h, thereafter the $d_6$-*RRR*- fraction was statistically significantly greater than the $d_3$-*SRR*-fraction (see legend to Fig. 4). The LDL and HDL also contained similar increases of the two tocopherols during the initial portion of the study, then the $d_6$-*RRR*- fraction became statistically greater than the $d_3$-*SRR*- fraction during the 9–76 h period (see the legend to Fig. 4). The $d_3$-*SRR*-α-tocopherol decreased faster than did the $d_6$-*RRR*- in the plasma, as well as in the VLDL, LDL, and HDL, in all subjects, as determined from the slopes of the curves shown in Figs. 3 and 4 and **Table 3.**

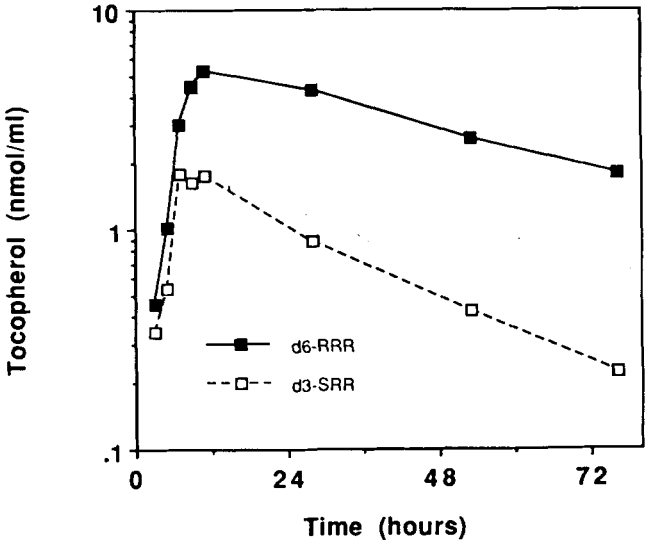


**Fig. 3.** Plasma deuterated tocopherol concentrations in four subjects following ingestion of the tocopherols with breakfast. Four subjects consumed a single dose of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopheryl acetates with breakfast. Blood samples were collected at 0, 3, 5, 7, 9, 11, 28, 53, and 76 h after taking the tocopherols, the plasma was immediately separated by centrifugation, a 1.0-ml aliquot was taken for tocopherol analysis, as described in Fig. 1, and the remainder was used for isolation of the lipoprotein fractions shown in Fig. 4. The means of the plasma $d_6$-*RRR*- (closed symbol) and $d_3$-*SRR*- (open symbol) α-tocopherol concentrations (nmol/ml plasma) are shown. There were no statistically significant differences in the concentrations of the two tocopherols in the plasma at any time point up to 11 h; thereafter the $d_6$-*RRR*-α-tocopherol concentrations were statistically greater than the $d_3$-*SRR*-α-tocopherol concentrations (11 h, $P < 0.05$; 28 h, $P < 0.02$; 53 h, $P < 0.006$; 76 h, $P < 0.001$). (Data for individual subjects are given in Table 2).

The time at which the fraction of $d_6$-*RRR*-/total tocopherol reached a peak in VLDL occurred prior to that in LDL or HDL. The highest ratio of $d_6$-*RRR*-/total in any lipoprotein fraction occurred in the chylomicrons at 9 h (0.35 ± 0.04 nmol deuterated/nmol total α-tocopherol). At 9 h, this fraction was only 0.27 ± 0.10 in the VLDL and this was the maximum value reached. This implies that the tocopherol is transported first in the chylomicrons, then in the VLDL. The peak in the $d_6$-*RRR*-/total occurred at 11 h in the LDL and HDL with values of 0.22 ± 0.08 and 0.22 ± 0.04, respectively, indicating that the catabolism of VLDL results in the concurrent enrichment of LDL and HDL with *RRR*-α-tocopherol.

The triglyceride content of the chylomicrons and VLDL from subjects 6–9 were also estimated. The amounts of triglycerides calculated in each fraction, as well as the time of the maximum triglyceride secretion, were quite variable between subjects (Table 2). A representative plot of the triglyceride and tocopherol concentrations is shown in **Fig. 5.** In this subject (#7), up to the time of maximum secretion of triglycerides into the chylomicrons (9 h), the $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherol concentrations increased equally. By contrast, at the time of the maximum triglyceride concentration in the VLDL (26 h), the *RRR*-α-tocopherol concentration was nearly four times greater than the concentration of *SRR*-α-tocopherol. These data emphasize the differences in the deuterated tocopherol concentrations of the chylomicrons and VLDL at the times of the maxima in triglyceride secretion.

## DISCUSSION

We have demonstrated that humans discriminate strongly between *RRR*- and *SRR*-α-tocopherols, stereo-

TABLE 2. Concentrations (nmol/ml) of unlabeled ($d_0$), deuterated ($d_3$-*SRR* and $d_6$-*RRR*) and total α-tocopherol VLDL at various times after subjects ingested a mixture of

| | | Plasma | | | | Chylomicrons | | | | | VLDL | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Time | $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total | $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total | TG | $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total | TG |
| | *h* | | | | | | | | | | | | | | |
| 6 | 0 | 22.0 | 0.00 | 0.00 | 22.0 | 0.09 | 0.00 | 0.00 | 0.09 | 0.11 | 1.26 | 0.00 | 0.00 | 1.3 | 0.13 |
| 6 | 3 | 20.0 | 0.00 | 0.00 | 20.0 | 0.21 | 0.00 | | | 0.05 | 2.51 | 0.04 | 0.14 | 2.7 | 0.58 |
| 6 | 5 | 20.6 | 0.00 | 0.00 | 20.6 | 2.49 | 0.20 | 0.15 | 2.84 | 0.19 | 1.40 | 0.08 | 0.22 | 1.7 | 0.02 |
| 6 | 7 | 19.4 | 3.23 | 4.05 | 26.7 | 2.49 | 0.97 | 1.05 | 4.51 | 0.20 | 2.08 | 1.35 | 1.48 | 4.9 | 0.71 |
| 6 | 9 | 18.6 | 2.30 | 7.30 | 28.2 | 0.35 | 0.22 | 0.25 | 0.82 | 0.06 | 1.67 | 0.49 | 1.02 | 3.2 | 0.34 |
| 6 | 11 | 18.2 | 2.33 | 8.83 | 29.4 | 0.09 | 0.06 | 0.05 | 0.20 | 0.08 | 1.60 | 0.40 | 0.94 | 2.9 | 0.37 |
| 6 | 28 | 20.8 | 1.31 | 7.13 | 29.2 | 0.17 | 0.05 | 0.04 | 0.26 | 0.17 | 2.28 | 0.28 | 0.91 | 3.5 | 0.14 |
| 6 | 53 | 20.2 | 0.60 | 3.99 | 24.8 | 0.07 | 0.00 | | | | 3.32 | 0.19 | 0.79 | 4.3 | 0.27 |
| 6 | 76 | 17.6 | 0.37 | 2.25 | 20.2 | 0.15 | 0.00 | | | | 1.74 | 0.08 | 0.31 | 2.1 | 0.18 |
| 7 | 0 | 20.5 | 0.00 | 0.00 | 20.5 | 0.37 | 0.00 | 0.00 | 0.37 | 0.10 | 1.24 | 0.00 | 0.00 | 1.2 | 0.30 |
| 7 | 3 | 20.2 | 1.12 | 1.64 | 22.9 | | | | 0.00 | 0.12 | 2.51 | 0.48 | 0.52 | 3.5 | 0.18 |
| 7 | 5 | 19.7 | 1.20 | 2.63 | 23.5 | 0.16 | 0.12 | 0.16 | 0.44 | 0.16 | 1.66 | 0.38 | 0.48 | 2.5 | 0.07 |
| 7 | 7 | 18.9 | 2.14 | 4.95 | 25.9 | 0.22 | 0.10 | 0.17 | 0.49 | 0.46 | 2.30 | 0.96 | 1.25 | 4.5 | 0.37 |
| 7 | 9 | 19.0 | 1.79 | 5.86 | 26.7 | 0.25 | 0.39 | 0.48 | 1.11 | 0.68 | 2.63 | 0.63 | 1.40 | 4.7 | 0.46 |
| 7 | 11 | 18.4 | 1.37 | 5.61 | 25.4 | 0.57 | 0.06 | 0.28 | 0.91 | 0.20 | 1.69 | 0.31 | 0.79 | 2.8 | 0.29 |
| 7 | 28 | 19.4 | 0.77 | 4.84 | 25.0 | 0.18 | | | 0.18 | 0.17 | 2.51 | 0.26 | 1.02 | 3.8 | 0.76 |
| 7 | 53 | 17.6 | 0.28 | 2.77 | 20.7 | 0.26 | | | 0.26 | 0.21 | 2.60 | 0.00 | 0.59 | 3.2 | 0.29 |
| 7 | 76 | 19.9 | 0.05 | 2.22 | 22.2 | 0.06 | | | 0.06 | 0.17 | 1.83 | 0.00 | 0.41 | 2.2 | 0.14 |
| 8 | 0 | 32.5 | 0.00 | 0.00 | 32.5 | 0.11 | 0.00 | | 0.11 | 0.05 | 3.39 | 0.00 | 0.00 | 3.4 | 0.18 |
| 8 | 3 | 32.9 | 0.11 | 0.00 | 33.0 | 0.18 | 0.01 | 0.01 | 0.20 | 0.10 | 4.01 | 0.10 | 0.00 | 4.1 | 0.26 |
| 8 | 5 | 23.9 | 0.73 | 1.17 | 25.8 | 0.31 | 0.02 | 0.02 | 0.34 | 0.03 | 5.76 | 0.39 | 0.53 | 6.7 | 0.76 |
| 8 | 7 | 24.0 | 1.40 | 2.38 | 27.8 | 0.20 | 0.02 | 0.02 | 0.24 | 0.03 | 9.13 | 0.86 | 1.26 | 11.2 | 0.94 |
| 8 | 9 | 23.0 | 0.97 | 2.84 | 26.8 | 0.40 | 0.07 | 0.08 | 0.54 | 0.13 | 8.06 | 0.52 | 1.08 | 9.7 | 0.56 |
| 8 | 11 | 25.4 | 2.08 | 4.52 | 32.0 | 0.84 | 0.24 | 0.27 | 1.34 | 0.31 | 9.89 | 0.95 | 2.08 | 12.9 | 1.04 |
| 8 | 28 | 23.0 | 0.69 | 3.27 | 26.9 | 0.32 | 0.02 | 0.04 | 0.37 | 0.06 | 3.62 | 0.20 | 0.67 | 4.5 | 0.27 |
| 8 | 53 | 21.0 | 0.28 | 1.95 | 23.3 | 0.14 | 0.01 | | 0.15 | 0.04 | 2.95 | 0.20 | 0.30 | 3.4 | 0.03 |
| 8 | 76 | 21.7 | 0.21 | 1.61 | 23.5 | 0.19 | 0.01 | 0.00 | 0.20 | | 2.86 | 0.00 | 0.23 | 3.1 | 0.15 |
| 9 | 0 | 12.6 | 0.00 | 0.00 | 12.6 | 0.11 | | | 0.11 | 0.09 | 1.12 | | 0.00 | 1.1 | 0.16 |
| 9 | 3 | 9.1 | 0.12 | 0.17 | 9.4 | 0.21 | 0.04 | 0.15 | 0.41 | 0.04 | 0.50 | 0.06 | | 0.6 | 0.54 |
| 9 | 5 | 9.1 | 0.21 | 0.28 | 9.6 | 0.29 | 0.03 | 0.07 | 0.39 | 0.09 | 0.35 | 0.05 | 0.11 | 0.5 | 0.45 |
| 9 | 7 | 10.1 | 0.34 | 0.53 | 11.0 | 0.13 | 0.07 | 0.09 | 0.28 | 0.14 | 0.24 | | 0.14 | 0.4 | 0.46 |
| 9 | 9 | 10.0 | 1.43 | 1.98 | 13.4 | 0.15 | 0.15 | 0.31 | 0.61 | 0.15 | 0.51 | 0.29 | 0.41 | 1.2 | 0.29 |
| 9 | 11 | 9.4 | 1.20 | 1.99 | 12.6 | 0.11 | 0.12 | 0.09 | 0.32 | 0.44 | 1.01 | 0.18 | 0.29 | 1.5 | 0.47 |
| 9 | 28 | 10.7 | 0.73 | 2.07 | 13.5 | 0.06 | | 0.00 | 0.06 | 0.08 | 1.33 | 0.23 | 0.32 | 1.9 | 0.22 |
| 9 | 53 | 11.3 | 0.53 | 1.66 | 13.5 | 0.04 | | 0.03 | 0.07 | 0.10 | 1.14 | 0.08 | 0.21 | 1.4 | 0.09 |
| 9 | 76 | 10.6 | 0.27 | 1.10 | 12.0 | 0.07 | | 0.00 | 0.07 | 0.10 | 1.14 | 0.06 | 0.24 | 1.4 | 0.63 |

isomers of vitamin E that differ only in the chirality of the carbon atom at position 2. This discrimination occurs, not during absorption and chylomicron secretion, but subsequently during VLDL secretion by the liver. Evidence from this study that discrimination between tocopherols does not occur during absorption includes: *1*) the chylomicron fractions were labeled approximately equally with both deuterated tocopherols (Fig. 4A), and *2*) during chylomicron secretion and catabolism (the first 6–9 h) the concentrations of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols were nearly equal in the plasma and all lipoprotein fractions (Figs. 3, 4, and Table 2). This latter observation also implies that the two tocopherols are absorbed and are transferred without discrimination to other lipoproteins during chylomicron catabolism.

Our previous studies using α- and γ-tocopherols demonstrated that these two compounds are also equally well absorbed by humans from the intestinal lumen and are secreted in chylomicrons (4). Furthermore, our studies in rats support this hypothesis. Supplementation of the infusate into the duodenum of thoracic duct cannulated rats with a 50-fold excess of α-tocopherol compared to γ-tocopherol did not change the concentration of γ-tocopherol in the secreted chylomicrons, suggesting that α-tocopherol did not compete with γ-tocopherol for absorption (12). Moreover, in rats fed an equal mixture of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols, the ratio of the two tocopherols in the lymph was approximately 1 (H. A. Zahalka, G. W. Burton, and K. U. Ingold, unpublished observations). Thus, all of the studies that have been carried out

in plasma, lipoproteins, and red cells along with concentrations of triglycerides (TG; μmol/ml) in chylomicrons and equal amounts of the $d_3$-*SRR* and $d_6$-*RRR*-α-tocopheryl acetates

| LDL | | | | HDL | | | | RBC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total | $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total | $d_0$ | $d_3$-*SRR* | $d_6$-*RRR* | Total |
| 7.0 | 0.00 | 0.00 | 7.0 | 11.4 | 0.00 | 0.00 | 11.4 | 4.1 | 0.00 | 0.00 | 4.1 |
| 9.6 | 0.00 | 0.00 | 9.6 | 12.9 | 0.00 | 0.02 | 12.9 | 3.0 | 0.00 | 0.00 | 3.0 |
| 12.9 | 0.22 | 0.47 | 13.6 | 12.4 | 0.19 | 0.13 | 12.7 | 3.8 | 0.00 | 0.06 | 3.9 |
| 9.4 | 0.78 | 1.21 | 11.4 | 11.9 | 1.11 | 1.54 | 14.6 | 4.2 | 0.00 | 0.05 | 4.3 |
| 7.4 | 1.03 | 3.10 | 11.6 | 12.6 | 1.35 | 5.25 | 19.2 | 4.9 | 0.29 | 0.56 | 5.8 |
| 9.3 | 1.12 | 4.65 | 15.1 | 12.7 | 1.42 | 6.27 | 20.4 | 4.8 | 0.38 | 1.09 | 6.3 |
| 11.3 | 0.86 | 4.05 | 16.2 | 14.4 | 0.99 | 5.14 | 20.5 | 4.2 | 0.21 | 1.43 | 5.8 |
| 9.0 | 0.31 | 1.96 | 11.2 | 16.8 | 0.58 | 3.82 | 21.2 | | | | |
| 10.0 | 0.00 | 1.25 | 11.3 | 13.5 | 0.25 | 1.78 | 15.5 | 5.5 | 0.00 | 0.78 | 6.3 |
| 5.7 | 0.00 | 0.00 | 5.7 | 10.0 | 0.00 | 0.00 | 10.0 | 4.4 | 0.00 | 0.00 | 4.4 |
| 5.3 | 0.00 | 0.58 | 5.9 | 9.2 | 0.27 | 0.55 | 10.0 | 4.1 | 0.03 | 0.22 | 4.4 |
| 3.4 | 0.00 | 0.67 | 4.1 | 12.1 | 0.45 | 1.46 | 14.0 | 4.2 | 0.07 | 0.36 | 4.6 |
| 3.7 | 0.39 | 0.92 | 5.0 | 12.1 | 0.92 | 2.54 | 15.5 | 4.0 | 0.12 | 0.49 | 4.6 |
| 4.0 | 0.27 | 1.36 | 5.7 | 11.6 | 0.78 | 3.47 | 15.9 | 4.5 | 0.15 | 0.85 | 5.5 |
| 4.4 | 0.39 | 1.50 | 6.3 | 11.1 | 0.83 | 3.65 | 15.6 | | | | |
| 4.7 | 0.23 | 1.09 | 6.0 | 8.5 | 0.44 | 2.32 | 11.2 | | | | |
| 5.1 | 0.07 | 0.92 | 6.1 | 11.4 | 0.25 | 1.95 | 13.6 | 4.7 | 0.10 | 1.05 | 5.9 |
| 6.8 | 0.20 | 0.37 | 7.3 | 12.4 | 0.15 | 1.43 | 14.0 | 4.0 | 0.11 | 0.67 | 4.8 |
| 10.3 | 0.00 | 0.00 | 10.3 | 11.9 | 0.00 | 0.00 | 11.9 | 3.4 | 0.00 | 0.00 | 3.4 |
| 8.2 | 0.09 | 0.06 | 8.3 | 10.5 | 0.04 | 0.43 | 11.0 | 4.5 | 0.00 | 0.01 | 4.5 |
| 11.4 | 0.46 | 0.54 | 12.4 | 8.5 | 0.34 | 0.49 | 9.3 | 3.6 | 0.02 | 0.03 | 3.7 |
| 8.6 | 0.56 | 0.79 | 10.0 | 8.2 | 0.34 | 0.94 | 9.5 | 3.3 | 0.30 | 0.17 | 3.8 |
| 8.2 | 0.40 | 1.14 | 9.8 | 9.6 | 0.31 | 1.25 | 11.2 | 2.8 | 0.07 | 0.15 | 3.0 |
| 10.7 | 0.59 | 1.72 | 13.0 | 8.1 | 0.44 | 1.62 | 10.1 | 2.8 | 0.18 | 0.34 | 3.4 |
| 11.8 | 0.30 | 1.43 | 13.5 | 8.2 | 0.16 | 1.35 | 9.7 | 3.0 | 0.08 | 0.43 | 3.5 |
| 9.9 | 0.18 | 1.08 | 11.2 | 8.3 | 0.05 | 1.08 | 9.4 | 3.1 | 0.04 | 0.32 | 3.5 |
| 9.0 | 0.07 | 0.56 | 9.7 | 7.4 | 0.03 | 0.73 | 8.1 | 2.2 | 0.08 | 0.20 | 2.5 |
| 3.8 | | 0.00 | 3.8 | 6.4 | 0.00 | | 6.4 | | | | |
| 3.6 | 0.07 | | 3.6 | 4.8 | 0.03 | | 4.8 | 2.9 | | 0.00 | 2.9 |
| 2.8 | 0.09 | 0.15 | 3.1 | 4.7 | 0.05 | 0.16 | 4.9 | 2.0 | 0.32 | 0.00 | 2.3 |
| 3.5 | 0.14 | 0.22 | 3.9 | 4.6 | 0.09 | 0.24 | 4.9 | 2.6 | 0.05 | 0.13 | 2.8 |
| 3.6 | 0.54 | 0.64 | 4.8 | 4.7 | 0.46 | 0.86 | 6.0 | 2.0 | 0.07 | 0.13 | 2.2 |
| 3.9 | 0.46 | 0.96 | 5.3 | 4.8 | 0.54 | 1.04 | 6.4 | 2.8 | 0.30 | 0.38 | 3.4 |
| 5.1 | 0.39 | 1.11 | 6.6 | 5.8 | 0.69 | 1.21 | 7.7 | 2.5 | 0.18 | 0.55 | 3.3 |
| 4.6 | 0.23 | 0.73 | 5.6 | 5.8 | 0.15 | 0.91 | 6.9 | 2.0 | 0.12 | 0.49 | 2.6 |
| 4.8 | 0.16 | 0.52 | 5.5 | 9.7 | 0.10 | 0.55 | 10.3 | 2.2 | 0.20 | 0.53 | 3.0 |

to assess the ability of the intestine to discriminate between α- and γ-tocopherols, or between stereoisomers of α-tocopherols, by measuring the tocopherols present in thoracic duct lymph (rats), in lymph chylomicrons (rats), or in plasma chylomicrons (humans) have shown that there is no appreciable discrimination by the intestine between the tocopherols during absorption and subsequent secretion in chylomicrons.

In contrast to the chylomicrons, the VLDL appear to contain approximately equal concentrations of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols only during the first few hours after ingestion, after which time these particles become enriched in $d_6$-*RRR*-α-tocopherol. Discrimination between the two tocopherols was greatest during the period of maximal VLDL secretion, as assessed by measuring the triglyceride levels (Fig. 5B; Table 2). Previously, the secretion of tocopherol in nascent VLDL has been demonstrated in rat hepatocytes (2, 3). The present report is the first direct demonstration that the VLDL are preferentially enriched with *RRR*-α-tocopherol as compared with *SRR*-α-tocopherol after oral administration of the two forms of α-tocopherol. We believe the apparent equal increases in the concentrations of $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols in the VLDL fractions from the early time points are due to one or more of the following. *1*) Contamination of the VLDL fraction by chylomicron remnants, i.e., this is an artifact of the lipoprotein isolation process and is not a result of secretion of VLDL containing both tocopherols; *2*) transfer of tocopherol to circulating VLDL from HDL (13) that had become labeled with

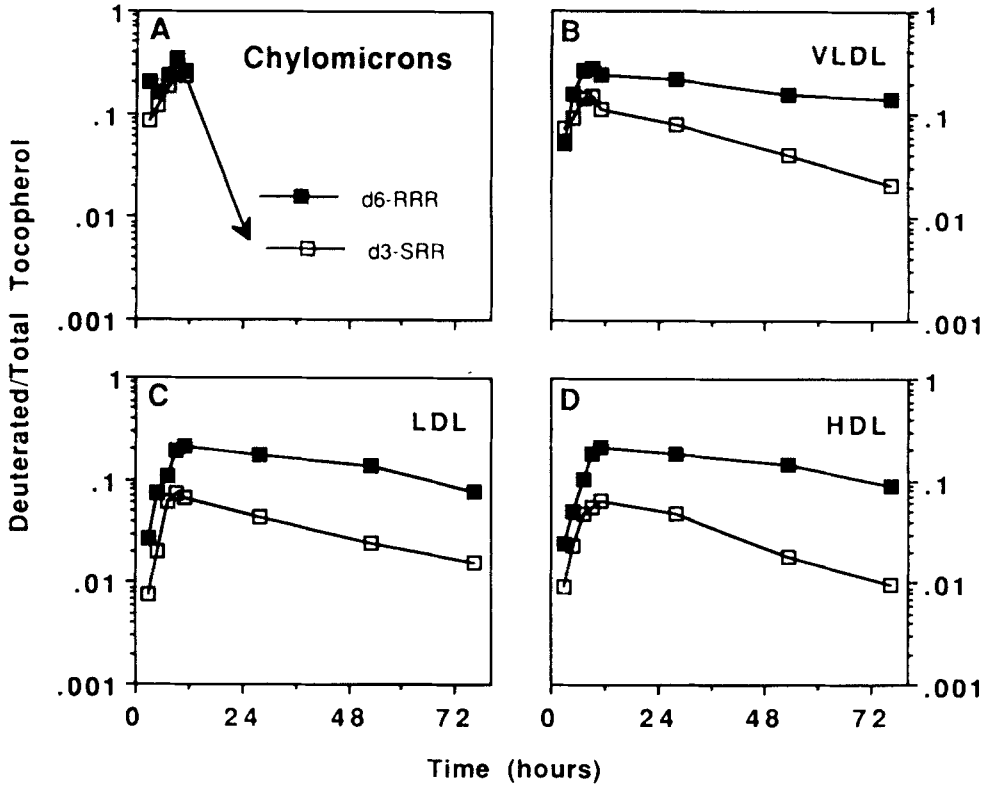


**Fig. 4.** Deuterated/total α-tocopherol ratios in lipoproteins. The lipoproteins were isolated from the plasma of the subjects shown in Fig. 3. Chylomicrons were isolated from duplicate samples of 1 ml plasma overlayered with 1 ml saline (0.15 M NaCl, 0.3 mM EDTA, pH 7.4) by centrifugation for 8 min at 40,000 rpm using a swinging bucket rotor (TLS 55) and a TL 100 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). Subsequently, the indicated lipoprotein fractions were isolated by centrifugation for 2 h at 100,000 rpm using a fixed angle rotor (TLA 100.2) at the following density intervals: VLDL $d<1.006$, LDL $1.006<d<1.063$ and HDL $d>1.063$ g/ml, as described in the Methods section. The tocopherol contents were analyzed as described for Fig. 1. Shown are the ratios of deuterated/total tocopherol in each of the lipoprotein fractions. The chylomicrons contained similar ratios of each deuterated/total α-tocopherol (A). VLDL (B) contained a similar fraction of both $d_6$-*RRR*-/total and $d_3$-*SRR*-/total α-tocopherols during the first 9 h, thereafter the $d_6$-*RRR*- fraction was significantly greater (11 h, $P < 0.02$; 28 h, $P < 0.01$; 53 h, $P < 0.01$; 76 h, $P < 0.004$). The LDL (C) and HDL (D) also contained similar fractions of both $d_6$-*RRR*-/total and $d_3$-*SRR*-/total α- tocopherols during the initial portion of the study, thereafter the $d_6$-*RRR*- fraction became statistically greater than the $d_3$-*SRR*-fraction during the 9–76 h period. The results of the statistical comparison for LDL were: 9 h, $P < 0.05$; 11 h, $P < 0.01$; 28 h, $P < 0.005$; 53 h, $P < 0.001$; 76 h, $P < 0.01$, and for HDL were: 9 h, $P < 0.01$; 11 h, $P < 0.005$; 28 h, $P < 0.003$; 53 h, $P < 0.0001$; 76 h, $P < 0.001$.

the two tocopherols during chylomicron catabolism; and *3*) secretion of some VLDL by the intestine.

The preferential secretion of $d_6$-*RRR*-α-tocopherol in VLDL, which becomes very evident after a few hours, suggests that there is a specific mechanism in the liver that recognizes *RRR*-α-tocopherol and incorporates it into VLDL for secretion into the plasma. We propose that the incorporation of tocopherol into VLDL by the liver requires a specific "tocopherol-transfer protein." This protein may well be the tocopherol-binding protein that had been isolated from rat liver cytosol (14–16).

An alternative explanation for the relative increase of *RRR*-α-tocopherol in the VLDL might be that the liver preferentially catabolizes or excretes other forms of vitamin E (i.e., *SRR*-α- and γ-tocopherols). However, measurements of the $d_6$-*RRR*-/$d_3$-*SRR*- ratio in livers of rats fed equal concentrations of both tocopherols for prolonged periods indicate that this is not the case since for the first 16 days of the experiment the livers preferentially contained *SRR*-α-tocopherol (5). This result implies that *RRR*-α-tocopherol is preferentially exported in VLDL while the *SRR*-form remains in the liver.

Further evidence for the likelihood that a hepatic tocopherol-binding protein is involved in the maintenance of plasma levels of tocopherol is the existence of nine patients world-wide who have Familial Isolated Vitamin E Deficiency (17–24). These patients do not have any abnormalities of lipid or lipoprotein metabolism or gastroin-

TABLE 3. Slopes of the semi-logarithmic plots of the plasma and lipoprotein *RRR*-α-tocopherol/total tocopherol or *SRR*-α-tocopherol/total tocopherol ratios of the subjects shown in Fig. 4

| | *RRR*-α/Total | | *SRR*-α/Total | |
|---|---|---|---|---|
| Subject | Slope[a] | $r^2$ | Slope[a] | $r^2$ |
| Plasma | | | | |
| 6[b] | −0.0067 | 0.997 | −0.0099 | 0.966 |
| 7 | −0.0055 | 0.990 | −0.0214 | 0.947 |
| 8 | −0.0050 | 0.987 | −0.0129 | 0.923 |
| 9 | −0.0037 | 0.942 | −0.0092 | 0.975 |
| VLDL | | | | |
| 6[b] | −0.0053 | 0.996 | −0.0086 | 0.935 |
| 7 | −0.0033 | 0.877 | −0.0130 | 0.999 |
| 8 | −0.0056 | 0.928 | −0.0124 | 0.999 |
| 9 | −0.0012 | 0.468 | −0.0076 | 0.921 |
| LDL | | | | |
| 6[b] | −0.0068 | 0.991 | −0.0101 | 0.995 |
| 7 | −0.0046 | 0.961 | −0.0172 | 0.981 |
| 8 | −0.0051 | 0.912 | −0.0116 | 0.960 |
| 9 | −0.0044 | 0.959 | −0.0072 | 0.991 |
| HDL | | | | |
| 6[b] | −0.0065 | 0.988 | −0.0099 | 0.999 |
| 7 | −0.0057 | 0.987 | −0.0110 | 0.990 |
| 8 | −0.0038 | 0.994 | −0.0179 | 0.985 |
| 9 | −0.0071 | 0.785 | −0.0158 | 0.928 |

[a]$Day^{-1}$.
[b]Data from 6 AM experiment.

testinal function, but when consuming a normal diet, they have exquisitely low plasma vitamin E levels and develop neurologic abnormalities characteristic of vitamin E deficiency. We have recently investigated the absorption and transport of vitamin E using deuterated *RRR*-α-tocopherol in four patients with this disorder and in six control subjects (25) and concluded that the tocopherol content of the plasma lipoproteins in the patients increased only during chylomicron catabolism, while the tocopherol content increased in the control subjects during both chylomicron and VLDL catabolism. These results suggested that the patients might lack or have a defective hepatic α-tocopherol binding protein.

In the present study, during the catabolism of VLDL, the $d_6$-*RRR*-α-tocopherol increased preferentially in both the LDL and the HDL fractions. Enrichment of these latter two lipoproteins with *RRR*-α-tocopherol could occur during the remodeling and catabolism of VLDL. As LDL is a direct catabolic product of VLDL metabolism it is not surprising that the tocopherol present in VLDL would ultimately be found in the LDL fraction. In addition, the increase in the tocopherol content of HDL could occur either by the direct transfer of excess surface material and perhaps lipid core of VLDL during triglyceride hydrolysis by lipoprotein lipase, or by the transfer of tocopherol from tocopherol-rich LDL to HDL. Both of these mechanisms would serve to maintain the *RRR*-α-tocopherol content of the plasma by transferring tocopherol to lipoproteins with relatively long turnover times compared to the triglyceride-rich lipoproteins.

As shown in Tables 1 and 3, *SRR*-α-tocopherol was found to decrease in the plasma at a faster rate than *RRR*-

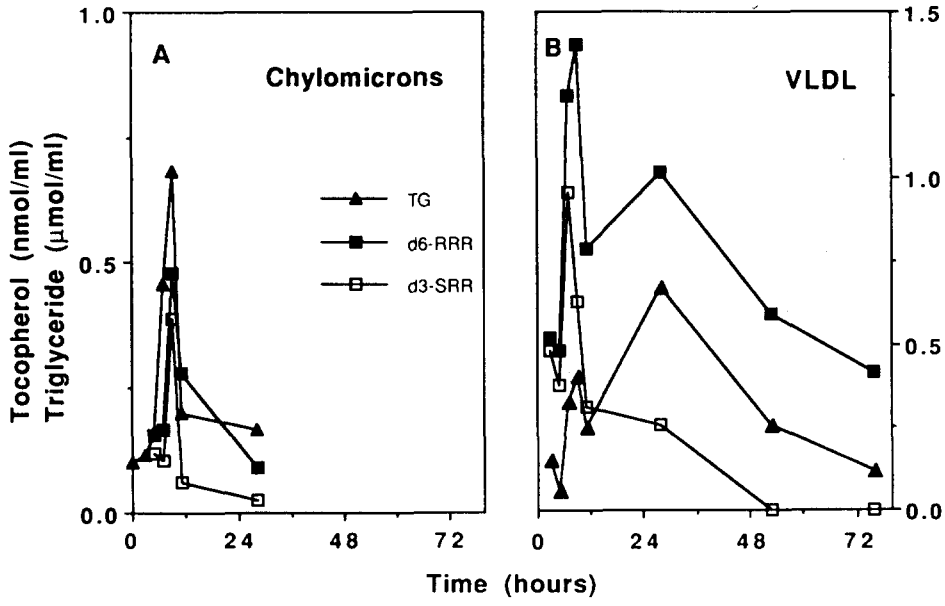


**Fig. 5.** The triglyceride and deuterated tocopherol contents of chylomicrons and VLDL from a representative subject. A representative plot of the data from one of the four subjects shown in Fig. 4 is shown. The deuterated tocopherol contents were analyzed as described in Fig. 1 and the triglyceride contents were estimated from a chloroform–methanol extract of chylomicrons and VLDL from subject 7, as described in the Methods section. A shows the chylomicron and B the VLDL concentrations of $d_3$-*SRR*-, $d_6$-*RRR*-α-tocopherol and the triglyceride concentrations of these two lipoprotein fractions expressed as nmol tocopherol or μmol triglyceride in the lipoprotein fraction/ml of plasma.

α-tocopherol. If the mechanism for the secretion of *RRR*-α-tocopherol is a transfer protein that recognizes the *SRR*-form poorly, then each time plasma lipoproteins containing the tocopherols are taken up by the liver there will be a discrimination against the *SRR*-form with the *RRR*-form being preferentially secreted into the plasma, resulting in a faster depletion of *SRR* from the plasma. Thus, the faster decrease in *SRR*-α-tocopherol is also consistent with the hypothesis that a transfer protein may be involved in the discrimination between tocopherols.

The red cells were also found to be enriched in *RRR*-α-tocopherol (Fig. 1), and from Table 2 it is apparent that these are preferentially enriched at time points subsequent to the enrichment in the plasma. It is likely that the red cells obtain tocopherol by exchange with the lipoproteins, most likely HDL. Studies of the transfer of the two stereoisomers of α-tocopherol to rat red cells from whole plasma have demonstrated that both forms exchange at roughly equal rates. However, the *SRR*-form leaves the red cell more readily than does the *RRR*-stereoisomer, thereby enriching the cells with *RRR*-α-tocopherol (26).

One other aspect of the present study that deserves comment is the variation in the absolute concentrations of deuterated-α-tocopherol in relation to dose size. Of the five subjects who were given 50 mg of each $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols (Fig. 1), four had $d_6$-*RRR*-α-tocopherol levels >8 nmol/ml on day 1, while one subject (#5) had a concentration <3 nmol/ml. The two subjects (#6 and #9) who were given 75 mg of each of the $d_6$-*RRR*- and $d_3$-*SRR*-α-tocopherols had a nearly fourfold difference in the peak in the plasma $d_6$-*RRR*-α-tocopherol concentration. We have shown that there is outstanding reproducibility in our quantitative analyses of deuterated tocopherols (25). Moreover, the data shown for the subject who was investigated twice, 6 months apart, demonstrates that in a given subject the measurements of the deuterated α-tocopherol concentrations are consistent. Therefore, we conclude that there is a wide variation in the responses of different individuals to the tocopherol dose. Since our subjects were not fasted before blood taking and were encouraged to eat a meal when they took the dose of deuterated tocopherols, the levels of plasma tocopherols may reflect variations in the ability of the subjects to secrete and catabolize triglyceride-rich lipoproteins, a phenomenon that has also been noted by Cohn et al. (27). Although the tocopherol intake was not controlled in these experiments, it does not seem likely that it caused these variations because the usual daily dietary intake is less than 15 mg—an amount far less than the amount of deuterated tocopherols administered, which ranged from 40 to 75 mg of each of the two stereoisomers.

In conclusion, this study provides the first evidence that humans strongly discriminate between the naturally occurring *RRR*-α-tocopherol form of vitamin E and *SRR*-α-tocopherol, one of the eight stereoisomers present in synthetic vitamin E. Discrimination between these two forms of the vitamin appears not to occur during absorption, but rather as a postabsorptive phenomenon in the liver. Our data suggest that there is a mechanism in the liver for preferentially increasing the amount of *RRR*- relative to *SRR*-α-tocopherol in nascent VLDL. This finding is of potential importance because VLDL are catabolized to form LDL and the amount of α-tocopherol, a potent antioxidant, present in LDL may well have a significant bearing on the atherogenicity of the LDL (28).

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## REFERENCES

1. Traber, M. G., K. U. Ingold, G. W. Burton, and H. J. Kayden. 1988. Absorption and transport of deuterium-substituted 2R,4'R,8'R-α-tocopherol in human lipoproteins. *Lipids.* **23:** 791–797.
2. Cohn, W., F. Loechleiter, and F. Weber. 1988. α-Tocopherol is secreted from rat liver in very low density lipoproteins. *J. Lipid Res.* **29:** 1359–1366.
3. Bjørneboe, A., G-E. A. Bjørneboe, B. F. Hagen, J. O. Nossen, and C. A. Drevon. 1987. Secretion of α-tocopherol from cultured rat hepatocytes. *Biochim. Biophys. Acta.* **922:** 199–205.
4. Traber, M. G., and H. J. Kayden. 1989. Preferential incorporation of α-tocopherol vs γ-tocopherol in human lipoproteins. *Am. J. Clin. Nutr.* **49:** 517–526
5. Ingold, K. U., G. W. Burton, D. O. Foster, L. Hughes, D. A. Lindsay, and A. Webb. 1987. Biokinetics of and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat. *Lipids.* **22:** 163–172.
6. Ingold, K. U., L. Hughes, M. Slaby, and G. W. Burton. 1987. Synthesis of 2R,4'R,8'R-α-tocopherols selectively labelled with deuterium. *J. Labelled Comp. Radiopharm.* **24:** 817–831.
7. Burton, G. W., K. U. Ingold, D. O. Foster, S. C. Cheng, A. Webb, L. Hughes, and E. Lusztyk. 1988. Comparison of free α-tocopherol and α-tocopheryl acetate as sources of vitamin E in rats and humans. *Lipids.* **23:** 834–840.

8. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497–509.
9. Stern, E., and B. Shapiro. 1953. A rapid and simple method for the determination of esterified fatty acids and for total fatty acids in blood. *J. Clin. Pathol.* **6:** 158–160.
10. Kayden, H. J., L. J. Hatam, and M. G. Traber. 1983. The measurement of nanograms of tocopherol from needle aspiration biopsies of adipose tissue: normal and abetalipoproteinemic subjects. *J. Lipid Res.* **24:** 652–656.
11. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28:** 1756–1759
12. Traber, M. G., H. J. Kayden, J. B. Green, and M. H. Green. 1986. Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in rats. *Am. J. Clin. Nutr.* **44:** 914–923.
13. Massey, J. B. 1984. Kinetics of transfer of α-tocopherol between model and native plasma lipoproteins. *Biochim. Biophys. Acta.* **793:** 387–392.
14. Catignani, G. L., and J. G. Bieri. 1977. Rat liver α-tocopherol binding protein. *Biochim. Biophys. Acta.* **497:** 349–357.
15. Murphy, D. J., and R. D. Mavis. 1981. Membrane transfer of α-tocopherol. *J. Biol. Chem.* **256:** 10464–10468.
16. Behrens, W. A., and R. Madere. 1982. Transfer of α-tocopherol to microsomes mediated by a partially purified liver α-tocopherol binding protein. *Nutr. Res.* **2:** 611–618.
17. Burck, U., H. H. Goebel, H. D. Kuhlendahl, C. Meier, and K. M. Goebel. 1981. Neuromyopathy and vitamin E deficiency in man. *Neuropediatrics.* **12:** 267–278.
18. Kohlschutter, A., C. Hubner, W. Jansen, and S. G. Lindner. 1988. A treatable familial neuromyopathy with vitamin E deficiency, normal absorption, and evidence of increased consumption of vitamin E. *J. Inherited Metab. Dis.* **11:** 149–152.
19. Laplante, P., M. Vanasse, J. Michaud, G. Geoffroy, and P. Brochu. 1984. A progressive neurological syndrome associated with an isolated vitamin E deficiency. *Can. J. Neurol. Sci.* **11:** 561–564.
20. Harding, A. E., S. Matthews, S. Jones, C. J. K. Ellis, I. W. Booth, and D. P. R. Muller. 1985. Spinocerebellar degeneration associated with a selective defect of vitamin E absorption. *N. Engl. J. Med.* **313:** 32–35.
21. Krendel, D. A., J. M. Gilchrest, A. O. Johnson, and E. H. Bossen. 1987. Isolated deficiency of vitamin E with progressive neurologic deterioration. *Neurology.* **37:** 538–540
22. Yokota, T., Y. Wada, T. Furukawa, H. Tsukagoshi, T. Uchihara, and S. Watabiki. 1987. Adult-onset spinocerebellar syndrome with idiopathic vitamin E deficiency. *Ann. Neurol.* **22:** 84–87.
23. Stumpf, D. A., R. Sokol, D. Bettis, H. Neville, S. Ringel, C. Angelini, and R. Bell. 1987. Freidreich's disease. V. Variant form with vitamin E deficiency and normal fat absorption. *Neurology.* **37:** 68–74.
24. Sokol, R. J., H. J. Kayden, D. B. Bettis, M. G. Traber, H. Neville, S. Ringel, W. B. Wilson, and D. A. Stumpf. 1988. Isolated vitamin E deficiency in the absence of fat malabsorption—familial and sporadic cases: characterization and investigation of causes. *J. Lab. Clin. Med.* **111:** 548–559
25. Traber, M. G., R. J. Sokol, G. W. Burton, K. U. Ingold, A. M. Papas, J. E. Huffaker, and H. J. Kayden. 1990. Impaired ability of patients with familial isolated vitamin E deficiency to incorporate α-tocopherol into lipoproteins secreted by the liver. *J. Clin. Invest.* **85:** 397–407.
26. Cheng, S. C., G. W. Burton, K. U. Ingold, and D. O. Foster. 1987. Chiral discrimination in the exchange of α-tocopherol stereoisomers between plasma and red blood cells. *Lipids.* **22:** 469–473.
27. Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. 1988. Postprandial plasma lipoprotein changes in human subjects of different ages. *J. Lipid Res.* **29:** 469–479
28. Steinberg. D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320:** 915–924.