Nascent VLDL from liver perfusions of cynomolgus monkeys are preferentially enriched in *RRR*- compared with *SRR*-α-tocopherol: studies using deuterated tocopherols

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Abstract The transport and secretion of vitamin E in lipoproteins have been studied in cynomolgus monkeys fed tocopherols labeled with different amounts of deuterium. The animals were fed a single dose of vitamin E containing 60 μmol of each 2*R*,4'*R*,8'*R*-α-(5,7-(C^2H_3)$_2$)tocopheryl acetate (d_6-*RRR*-α-tocopheryl acetate; α-tocopherol with natural stereochemistry), 2*S*,4'*R*,8'*R*-α-5-(C^2H_3)tocopheryl acetate (d_3-*SRR*-α-tocopheryl acetate; α-tocopherol with unnatural stereochemistry), and 2*R*,4'*R*,8'*R*-γ-(3,4-^{2}H)tocopherol (d_2-*RRR*-γ-tocopherol; γ-tocopherol with natural stereochemistry). Chylomicrons, as well as the other plasma lipoproteins, contained equal concentrations of all three tocopherols at the earliest time points after feeding suggesting that all three tocopherols were absorbed equally. At later times plasma lipoproteins became preferentially enriched in d_6-*RRR*-α-tocopherol. This is likely to be due to hepatic secretion of VLDL (very low density lipoproteins) and other lipoproteins, which were enriched in d_6-*RRR*-α-tocopherol, as demonstrated in the lipoproteins isolated from perfused livers that had been obtained 24 h following the administration of the deuterated tocopherols. Taken together these data demonstrate that the liver, not the intestine, is the likely site of discrimination between tocopherol isomers and that the liver secretes nascent lipoproteins preferentially enriched in d_6-*RRR*-α-tocopherol. — **Traber, M. G., L. L. Rudel, G. W. Burton, L. Hughes, K. U. Ingold, and H. J. Kayden.** Nascent VLDL from liver **perfusions of cynomolgus monkeys are preferentially enriched in** *RRR*- compared to *SRR*-α-tocopherol: studies using deuterated tocopherols. *J. Lipid Res.* 1990. **31:** 687-694.

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We have been investigating the transport of vitamin E in human lipoproteins. Using vitamin E labeled with deuterium, we have demonstrated that α-tocopherol is first secreted in chylomicrons by the intestine, then appears in VLDL (very low density lipoproteins) secreted by the liver (1). The increase in the concentration of deuterated tocopherols in low and high density lipoproteins (LDL and HDL, respectively), lipoproteins that contain the bulk of vitamin E in the plasma (1-3), occurred after the secretion of the deuterated tocopherol in VLDL (1). Thus, the largest concentrations of deuterated tocopherol in LDL and HDL occurred during, or as a result of, the catabolism of VLDL.

Our studies have also shown that humans discriminate between naturally occurring forms of vitamin E, α-tocopherol versus γ-tocopherol (4), and between two of the eight stereoisomers of α-tocopherol present in synthetic vitamin E, the naturally occurring 2*R*,4'*R*,8'*R*,α-tocopherol (*RRR*-α-tocopherol) and 2*S*,4'*R*,8'*R*-α-tocopherol (*SRR*-α-tocopherol) (5). Importantly, the discrimination between these forms of vitamin E does not occur during absorption and secretion of the vitamins in chylomicrons, but occurs after their absorption. This suggested that the liver might be the source of the discrimination between tocopherols. Therefore, we sought a direct test of the hypothesis that the liver secretes nascent VLDL preferentially enriched in natural *RRR*-α-tocopherol.

A primate liver perfusion system for studying nascent lipoprotein secretion by the liver has been devised by Johnson, St. Clair, and Rudel (6). The lipoproteins isolated from the perfusate have been demonstrated to include VLDL, discoidal HDL, and apolipoprotein B-containing lipoproteins of d > 1.006 g/ml that are deficient in core lipids and have redundant surface com-

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

ponents (6–9). The ability to isolate lipoproteins reproducibly from the primate liver suggested that this experimental system might be suitable for studies of vitamin E transport, and in particular the discrimination between forms (α- versus γ-tocopherols) and between stereoisomers of α-tocopherol. Therefore, we have used this system to study the secretion of tocopherols in nascent lipoproteins, especially nascent VLDL, by perfused monkey livers.

METHODS

Deuterated α-tocopherols

The syntheses and analysis of deuterated α-tocopherols have been described previously (10, 11). A deuterated γ-tocopherol was specially synthesized for this study (12). 2*R*,4'*R*,8'*R*-α-(5,7-(C^2H_3)$_2$)tocopheryl acetate (d_6-*RRR*-α-tocopheryl acetate), hexadeuterated α-tocopherol with natural stereochemistry; 2*S*,4'*R*,8'*R*-α-5-(C^2H_3)tocopheryl acetate (d_3-*SRR*-α-tocopheryl acetate), trideuterated α-tocopherol with unnatural stereochemistry; and 2*R*,4'*R*,8'*R*-γ-(3,4-^{2}H)tocopherol (d_2-*RRR*-γ-tocopherol), a dideuterated γ-tocopherol with natural stereochemistry; were fed to each monkey. 2-*ambo*-α-(5,7,8-(C^2H_3)$_3$)Tocopherol (d_9-tocopherol), used as an internal standard, was added in known amount to each plasma, or lipoprotein sample immediately prior to lipid extraction (1, 13). These lipid extracts were purified and absolute concentrations of d_0-, d_3-, and d_6-α-tocopherols and d_0- and d_2-γ-tocopherols in the original plasma and lipoprotein samples were determined by gas chromatography–mass spectrometry as described previously (1, 5, 13, 14).

Experimental protocols

The cynomolgus monkeys used in these studies were adult males between 7 and 10 years of age. They were randomly selected from larger groups of animals that were part of a study on the effects of the type of dietary fat on atherosclerosis. Experimental diets contained 0.2 mg of cholesterol per kcal and 40% of calories as fat, in either a saturated fat diet (41%, 47%, and 12% as saturated, monounsaturated, or polyunsaturated fatty acids, respectively) or a polyunsaturated fat diet (22%, 30%, and 48%). The animals studied had been fed their experimental diets for 5 years, and in the case of the liver perfusion animals were being terminated for evaluation of the extent of atherosclerosis. **Table 1** shows a list of parameters describing the six animals used in these studies.

Each animal consumed a single dose of vitamin E containing all three deuterated tocopherols (60 μmol of each dissolved in vegetable oil) disguised in a favorite food (apples or cookies) followed by the regular morning meal. Two monkeys, #1182 and #918, were used for studies of lipoprotein transport in vivo. These animals had been surgically fitted with indwelling femoral artery cannulae, and were maintained in restraining jackets with tethers so that repeated blood samples could be obtained without disturbing the animals. The procedures associated with this technique have been described in detail (15). Blood samples (5 ml) were obtained at 3, 6, 9, 12, 24, 36, 48, and 72 h after the dose had been fed. Blood was collected over EDTA (1 mg/ml) and immediately refrigerated and centrifuged to remove red blood cells. Plasma was then isolated, refrigerated until all samples were collected, then promptly sent on wet ice via Federal Express to New York University for lipoprotein isolation.

Four monkeys fed the dose of deuterated tocopherols were used for liver perfusion studies. These animals were anesthetized 24 h after receiving the vitamin E, blood samples were taken, and the liver was isolated and removed for perfusion (7). The perfusion medium consisted of Krebs-Henseleit original Ringer bicarbonate buffer, pH 7.4, containing D-glucose, amino acids, 3% albumin, insulin, hydrocortisone, streptomycin, penicillin, and washed human erythrocytes at 22% hematocrit. Oleic acid was added to the perfusate at a rate of 0.1 μmol/min per g liver and the recirculating perfusion was carried out, as described previously (16).

Lipoprotein isolation

Chylomicrons and lipoproteins were isolated from monkey plasma as described previously for human

TABLE 1. Monkey characteristics

Animal #	Diet Group	Body Weight	Age	Plasma Triglycerides	Plasma Cholesterol			
					VLDL + IDL	LDL	HDL	Total
		kg	*months*	*mg/dl*		*mg/dl*		
1171	Sat	6.4	101	12	37	196	64	297
918	Poly	5.1	117	10	31	167	42	240
1115	Poly	6.0	106	10	22	98	62	182
1121	Poly	5.4	105	13	27	210	73	310
1182	Poly	4.1	101	5	50	345	26	421
1433	Poly	5.0	90	55	20	56	44	120

plasma (1). Lipoproteins were isolated from the perfusate according to the procedures described previously (8). Tocopherol analyses were carried out on all of the lipoprotein fractions. Triglyceride, cholesterol, and apolipoprotein B distributions in the perfusate lipoprotein fractions were measured as described (16). **Table 2** shows some lipid and lipoprotein secretion parameters in the perfusions from these studies.

The lipoprotein fractions were frozen and stored at −70°C (less than 60 days) until they, along with the plasma samples, were taken by courier on dry ice to the National Research Council in Ottawa, Canada for analyses of the tocopherols.

Statistical analysis

The statistical significance of the results was determined by the statistical analysis program, Stat View 512+ (Brain Power Associates, Calabasus, CA) using analysis of variance (ANOVA). Results of the statistical tests were considered to be significant at the 95% confidence level ($P < 0.05$). The slopes of the lines fitted to the linear portion of the plot of the log of the tocopherol concentration in nmol/ml ([tocopherol]) versus time past the peak in tocopherol concentrations were calculated using Cricket graph (Cricket Software, Malvern, PA).

RESULTS

Transport of deuterated tocopherol in plasma lipoproteins

The plasma of two monkeys given the three deuterated tocopherols was preferentially enriched in *RRR*-α-tocopherol by 24 h. All three tocopherols, d_6-*RRR*-α-, d_3-*SRR*-α-, and d_2-γ-, increased up to 12 h in the plasma of monkey #1182, with d_6-*RRR*-α- continuing to increase until 24 h (**Fig. 1**). In contrast, the plasma of monkey #918 contained maximum levels of d_3-*SRR*-α- and d_2-γ-tocopherols at 3 h; d_6-*RRR*-α-tocopherol reached a steady, maximum level during the interval 6–24 h (see appendix: **Supplementary Data Table 1**). In both animals, once the peak levels of the deuterated tocopherols had been achieved, d_3-*SRR*-α- and d_2-γ-tocopherols decreased in the plasma at faster rates than did d_6-*RRR*-α-tocopherol. This can also be seen from the slopes of the linear portions of the plots of the log [tocopherol] in the plasma versus time after the peak in the tocopherol concentrations (i.e., $\log[\text{nmol} \cdot \text{ml}^{-1}] \cdot t^{-1}$, **Table 3**).

The distribution of deuterated tocopherols in the lipoprotein fractions was similar to that in humans (4, 5). As shown in **Fig. 2,** monkey #1182 showed increases of all three deuterated tocopherols in the chylomicron fraction during the first 9 h. (The tocopherol concentrations of the chylomicron fractions are not shown beyond 24 h as this fraction would no longer contain newly absorbed tocopherols). The VLDL fraction contained similar increases of the three deuterated tocopherols during the first 6 h, but by 9 h the VLDL fraction was preferentially enriched in d_6-*RRR*-α-tocopherol. Similarly, both the LDL and the HDL fractions from monkey #1182 contained all three deuterated tocopherols during the first 6 h, after which they were preferentially enriched in d_6-*RRR*-α-tocopherol. In monkey #918 the lipoproteins, other than the chylomicrons, also became preferentially enriched in *RRR*-α-tocopherol (see Supplementary Data Table 1).

Table 3 lists the slopes of the disappearance portions of the tocopherol concentration curves ($\log[\text{nmol} \cdot \text{ml}^{-1}] \cdot t^{-1}$) for VLDL, LDL, and HDL from monkeys #918 and #1182. (The chylomicron fractions have been excluded from this table because these plots were not linear.) The values of the slopes for each fraction were similar for the two animals. Furthermore, in all three lipoprotein fractions the slopes were similar for d_3-*SRR*-α- and d_2-γ-tocopherols, and were greater than those for d_6-*RRR*-α-tocopherol.

TABLE 2. Liver perfusate measurements

	Animal Number		
	1171	1115	1433
Diet (saturated or polyunsaturated fat)	Saturated	Poly	Poly
Triglyceride accumulation rate (μg/100 g liver/min)	227	158	90
Cholesterol accumulation rate (μg/100 g liver/min)	148	105	98
Apolipoprotein B accumulation rate (μg/100 g liver/min)	38	14	16
Cholesterol (% in VLDL)	71%	66%	57%
Apolipoprotein B (% in VLDL)	77%	73%	46%

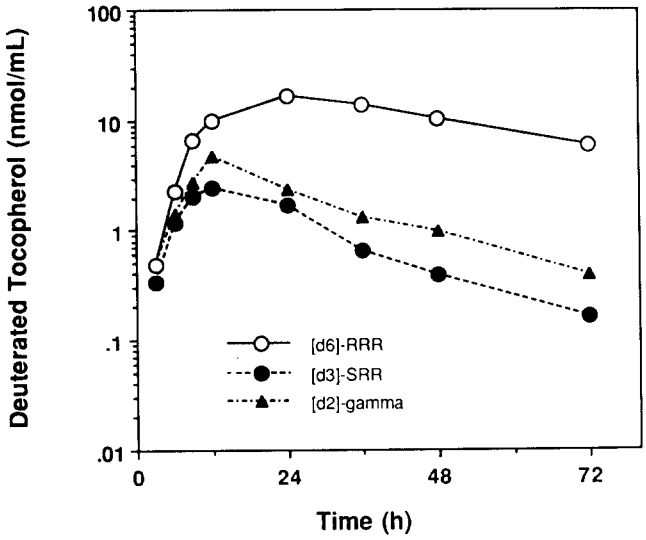

Fig. 1. Monkeys were fed a single dose of vitamin E containing 60 μmol of each [d_6]-*RRR*-α-tocopheryl acetate, [d_3]-*SRR*-α-tocopheryl acetate, and [d_2]-*RRR*-γ-tocopherol, then blood samples were obtained at the indicated intervals following the dose. The nmol/ml plasma of [d_6]-*RRR*-α-tocopherol (○), [d_3]-*SRR*-α-tocopherol (●), and [d_2]-γ-tocopherol (▲) from monkey #1182 are shown.

Tocopherol contents of lipoproteins isolated from liver perfusions

Although four monkeys were given the dose of deuterated tocopherols, one of the monkeys (#1121) ate only part of the dose and the amounts of tocopherol in the lipoproteins from that liver perfusion were below the limits of detection. The data from the other three monkeys are shown in **Fig. 3** and are given in **Supplementary Table 2** (appendix). Plasma, obtained at the time of killing, approximately 24 h after the dose had been consumed, was preferentially enriched in d_6-*RRR*-α-tocopherol. The VLDL fraction isolated from the perfusate was also enriched in d_6-*RRR*-α-tocopherol, as were the lipoproteins isolated from the d > 1.006 g/ml fraction. Because the amounts of deuterated tocopherols in the plasma and the liver perfusion fractions were different in each of the animals, the percentage of each of the labeled tocopherols relative to the total deuterated tocopherol was also calculated from the data shown in Supplementary Table 2. The plasma at the time of killing contained 77% ± 3% (mean ± SD, n = 3) d_6-*RRR*-α-tocopherol, significantly greater ($P < 0.001$) than the 10% ± 6% found for d_3-*SRR*-α- and 14% ± 2% found for d_2-γ-tocopherols; these latter two percentages are not significantly different from each other. Both the VLDL and the d>1.006 g/ml fractions isolated from the 4-h perfusate were both significantly ($P < 0.001$) enriched in d_6-*RRR*-α-tocopherol. The VLDL contained 71% ± 7% d_6-*RRR*-α-tocopherol, but only 16% ± 10% d_3-*SRR*-α- and 14% ± 7% d_2-γ-tocopherols. The d>1.006 g/ml fraction contained 70% ± 6% d_6-*RRR*-α-tocopherol, but only 15% ± 6% d_3-*SRR*-α- and 15% ± <1% d_2-γ-tocopherols.

DISCUSSION

This study is the first to demonstrate that nascent lipoproteins, both the d<1.006 and the d>1.006 g/ml fractions, secreted by the liver are preferentially enriched in *RRR*-α-tocopherol. This study provides further support for our hypothesis that discrimination between different forms of tocopherol occurs in the liver. The d>1.006 g/ml fraction isolated from the perfusate, which contains apolipoprotein B-containing particles that are triglyceride-poor (6, 7, 9) and nascent HDL (8, 17), contained a distribution of deuterated tocopherols similar to that in the VLDL (d<1.006 g/ml) fraction. It is likely that the apolipoprotein B-containing lipoproteins are the major transporters of tocopherol from the liver because the distribution of the deuterated tocopherols appears to be related to the density distribution of apolipoprotein B. As shown in Table 2, monkeys #1115 and #1171 had 73% and 77%, respectively, of the perfusate apolipoprotein B in the

TABLE 3. Slopes of the linear portions of the curves past the peak in the tocopherol concentrations

		[d_6]-*RRR*-α-Tocopherol		[d_3]-*SRR*-α-Tocopherol		[d_2]-*RRR*-γ-Tocopherol	
Fraction	Animal #	Slope[a]	r^2	Slope[a]	r^2	Slope[a]	r^2
Plasma	1182	−0.0096	0.995	−0.0205	0.964	−0.0158	0.991
	918	−0.0087	0.931	−0.0234	0.961	−0.0212	0.990
VLDL	1182	−0.0178	0.915	−0.0212	0.791	−0.0219	0.894
	918	−0.0150	0.933	−0.0253	0.842	−0.0201	0.927
LDL	1182	−0.0115	0.992	−0.0215	0.971	−0.0226	0.992
	918	−0.0082	0.928	−0.0239	0.978	−0.0205	0.994
HDL	1182	−0.0081	0.996	−0.0169	0.923	−0.0198	0.999
	918	−0.0073	0.853	−0.0220	0.974	−0.0188	0.982

[a]nmol tocopherol · ml^{-1} · t^{-1}

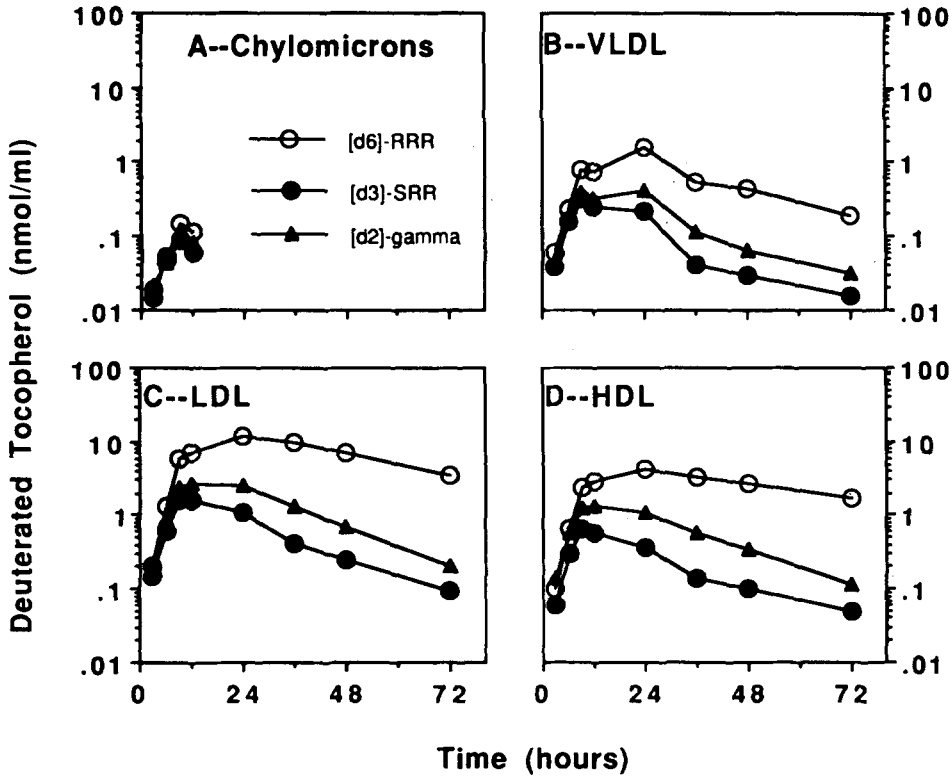

Fig. 2. The tocopherol contents of chylomicrons (A), VLDL (B), LDL (C), and HDL (D) isolated from plasma of monkey #1182 at the indicated intervals following oral administration of the single dose of deuterated tocopherols described in Fig. 1 are shown as the nmol/ml plasma of [d_6]-*RRR*-α-tocopherol (○), [d_3]-*SRR*-α-tocopherol (●), and [d_2]-γ-tocopherol (▲).

VLDL fraction, which also contained 79% and 81%, respectively, of the total deuterated tocopherols in the perfusate. By contrast, animal #1433 which had only 46% of the apolipoprotein B in its VLDL fraction also had only 38% of the deuterated tocopherols in this fraction. The reason some animals have a greater proportion of lipids and apolipoproteins in non-VLDL fractions is yet to be determined. Nevertheless, despite differences in the densities of the particles secreted, there is a marked preference for secretion of d_6-*RRR*-α-tocopherol in nascent hepatic lipoproteins.

Nonhuman primates absorb d_6-*RRR*- and d_3-SRR-α-tocopherols, and d_2-γ-tocopherol with roughly equal efficiencies (just as do humans (4, 5) and rats (10, 18)) as is clearly demonstrated by monkey #1182. The chylomicron fraction from this animal contained equal amounts of all three tocopherols during the first 9 h of the study (Fig. 2). Furthermore, the plasma, as well as the other lipoprotein fractions isolated at earlier times (3–6 h) contained equal amounts of all three tocopherols (Figs. 1 and 2). We have observed this uniform labeling of lipoproteins during chylomicron catabolism previously and have postulated that tocopherol is transferred along with excess surface components from chylomicron remnants to HDL during lipolysis by lipoprotein lipase (1, 4, 5, 14). The immediate transfer of HDL tocopherol to other lipoproteins results in the simultaneous labeling of all the lipoprotein fractions with virtually equal specific activities (labeled/total tocopherol) in LDL and HDL (1, 4, 5, 14). Since the plasma lipoproteins were equally labeled with all three tocopherols at the early time points, yet nascent hepatic lipoproteins isolated from the liver obtained 24 h after dosing were enriched with d_6-*RRR*-α-tocopherol, it appears that hepatic secretion of α-tocopherol is a lengthy process.

After 24 h the plasma samples obtained from all five monkeys were preferentially enriched in d_6-*RRR*-α-tocopherol (Figs. 1 and 3, and Supplementary Tables 1 and 2). The plasma levels seem to be dependent upon the secretion of VLDL enriched in d_6-*RRR*-α-tocopherol, with catabolism of VLDL resulting in the transfer of d_6-*RRR*-α-tocopherol to all lipoprotein fractions. The slopes of the disappearance portion of the tocopherol concentration curves (Table 3) demonstrate that the net loss of d_6-*RRR*-α-tocopherol in VLDL is faster than in plasma, LDL, or HDL, indicating that this tocopherol leaves the VLDL fraction more rapidly than it leaves the LDL or HDL. It is also of interest that the d_3-*SRR*-α-tocopherol

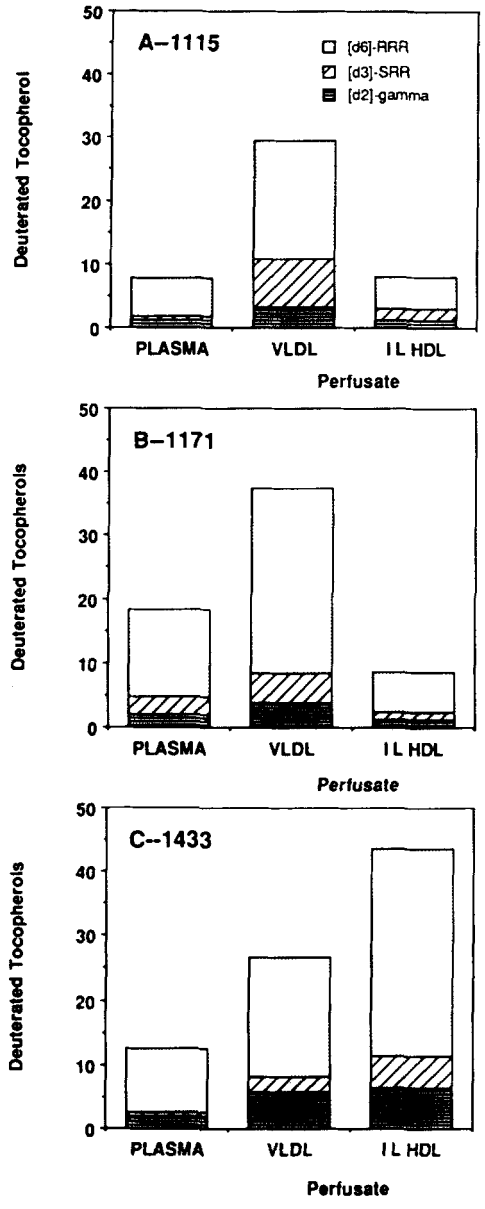

Fig. 3. Monkeys #1171 (A), #1115 (B), and #1433 (C) were fed a single dose of vitamin E containing 60 μmol of each [d_6]-*RRR*-α-tocopheryl acetate, [d_3]-*SRR*-α-tocopheryl acetate, and [d_2]-*RRR*-γ-tocopherol, then a plasma sample was obtained 24 h later at the time of killing. The liver was removed, washed, and perfused for 90 min. The perfusate was replaced with fresh perfusate, which was used to reperfuse the liver for an additional 4 h, then lipoproteins were isolated. The VLDL was isolated by ultracentrifugation at d < 1.006 g/ml, then the density was adjusted to 1.21 g/ml and the remaining lipoproteins (ILHDL) were isolated. The concentration of deuterated tocopherols in the plasma (nmol/ml), and the total amount of deuterated tocopherols isolated in each perfusion lipoprotein fraction (nmol) from the 4-h perfusate are shown.

and the d_2-γ-tocopherol were lost from the plasma and the lipoprotein fractions at essentially the same rates. This suggests that there is no specific mechanism to maintain the plasma concentrations of either of these two tocopherols. Indeed, we suggest that non-*RRR*-α-tocopherols are distributed into the plasma lipoproteins principally (or even entirely) during chylomicron catabolism and that following chylomicron remnant uptake by the liver only *RRR*-α-tocopherol is secreted in the nascent lipoproteins (see Fig. 3).

A tocopherol binding protein has been described in rat liver cytosol (but not intestine) (19), which promotes the transfer of tocopherol between microsomes and liposomes (20, 21). We have also described tocopherol transport in a group of patients with Familial Isolated Vitamin E Deficiency and our results are consistent with the hypothesis that the patients may lack, or have a defective, tocopherol transfer protein (14). We suggest that the tocopherol binding protein is an *RRR*-α-tocopherol binding protein and that it recognizes and transfers *RRR*-α-tocopherol from the site of chylomicron remnant catabolism in the liver to the site of VLDL assembly. Thus, *RRR*-α-tocopherol is inserted into nascent VLDL, and it is during the catabolism of this lipoprotein that *RRR*-α-tocopherol is distributed to the other lipoproteins, along with excess surface components. In its simplest form this scheme predicts that every time a lipoprotein is catabolized by the liver all the non-*RRR*-α-tocopherols are excreted, while the *RRR*-α-tocopherol is returned to the plasma in nascent VLDL.

An alternative, but much less probable, explanation for the preferential enrichment of the plasma with *RRR*-α-tocopherol is that the tissues take up non-*RRR*-α-tocopherols more readily than *RRR*-α-tocopherol. This is unlikely because *RRR*-α-tocopherol is the form of vitamin E with the highest biological activity (22) and, compared with the naturally occurring forms of tocopherol (α-, β-, γ-, δ-), has the highest antioxidant activity (23). In addition, human adipose tissue tocopherol contains mostly α-, not γ-tocopherol (M. G. Traber, H. J. Kayden, unpublished data), although human diets contain more γ- than α-tocopherol (24). Furthermore, tissues from rats fed equal amounts of *RRR*- and *SRR*-α-tocopherols up to 154 days were (with the exception of the liver for just the first 16 days) preferentially enriched in *RRR*-α-tocopherol (10). Moreover, *RRR*- and *SRR*-α-tocopherols have been shown to transfer from plasma to red blood cell membranes at equal rates, but *SRR*-α-tocopherol transfers from these membranes to plasma more rapidly than *RRR*-α-tocopherol (25).

The more rapid disappearance from the plasma of *SRR*-α- and γ-tocopherols compared with *RRR*-α-tocopherol may be related to the greater ease with which these forms of vitamin E leave membranes. It is known that triglyceride-rich lipoproteins, which are rapidly catabolized by the liver, readily take up tocopherol (26), but do not readily transfer tocopherol to other lipoproteins (27). If the tocopherols transfer from membranes onto HDL and then onto triglyceride-rich lipoproteins, the catabolism of

these latter lipoproteins by the liver would provide a mechanism for the removal of tocopherols from plasma and tissues. If only *RRR*-α-tocopherol is subsequently secreted back into the plasma, *RRR*-α-tocopherol levels would be maintained in the plasma and tissues, while non-*RRR*-α-tocopherols would decrease rapidly. Another possibility is that the non-*RRR*-α-tocopherol forms of vitamin E are more rapidly catabolized, and that only *RRR*-α-tocopherol remains in the liver. This possibility would require a mechanism for the prevention of the catabolism of *RRR*-α-tocopherol, perhaps a binding protein that protects the *RRR*-α-tocopherol form from degradation. Since the metabolism of tocopherols is unknown, this possibility cannot be ruled out. But it seems unlikely because, in the rat studies discussed above (10), the liver was found to be enriched in *SRR*-α-tocopherol during the first 16 days of the study, arguing against the possibility of preferential catabolism of non-*RRR*-α-tocopherols.

In conclusion, this study has demonstrated that non-human primates absorb *RRR*-, *SRR*-α-tocopherols and *RRR*-γ-tocopherol with roughly equal efficiencies, and that subsequently there is marked discrimination between the tocopherols with preferential enrichment of the plasma with *RRR*-α-tocopherol. We sought to establish the origin of this enrichment by measuring the tocopherol content of nascent lipoproteins secreted by perfused livers and demonstrated that these nascent lipoproteins are preferentially enriched in *RRR*-α-tocopherol.

APPENDIX

SUPPLEMENTARY TABLE 1. Plasma and lipoprotein deuterated tocopherols (nmol/ml plasma)

		Plasma					Chylomicrons				
Monkey #	Time	d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α	d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α
	h										
1182	3	17.25	0.50	13.97	0.34	0.49	0.28	0.02	0.20	0.02	0.02
1182	6	18.47	1.42	23.90	1.17	2.28	0.15	0.04	0.10	0.06	0.05
1182	9	16.01	2.72	18.77	2.07	6.59	0.30	0.11	0.22	0.09	0.15
1182	12	20.36	4.72	17.03	2.48	10.08	0.19	0.09	0.13	0.06	0.12
1182	24	15.65	2.34	19.60	1.72	16.66	0.28	0.10	0.21	0.07	0.25
1182	36	15.68	1.32	19.65	0.66	13.77	0.17	0.02	0.11	0.01	0.07
1182	48	16.90	1.00	20.56	0.40	10.12	0.44	0.03	0.36	0.01	0.17
1182	72	17.69	0.39	22.70	0.16	5.86	0.25	0.01	0.19	0.01	0.05
918	3	24.13	2.56	32.93	4.45	5.38	0.46	0.15	0.37	0.23	0.25
918	6	20.12	2.84	26.48	2.63	8.58	0.37	0.08	0.29	0.09	0.15
918	9	21.08	2.15	28.00	1.43	9.13	0.51	0.02	0.43	0.06	0.15
918	12	22.90	1.90	30.93	1.15	9.40	0.22	0.00	0.16	0.02	0.06
918	24	22.42	1.15	30.15	0.63	9.03	0.12	0.01	0.08	0.01	0.03
918	36	18.90	0.48	24.57	0.23	5.64	0.31	0.01	0.24	0.01	0.05
918	48	21.71	0.30	29.00	0.18	4.64	0.17	0.01	0.12	0.00	0.02
918	72	20.51	0.11	27.10	0.06	2.48	0.04	0.01	0.02	0.00	0.01

supplementary table 1 continued

VLDL					LDL					HDL				
d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α	d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α	d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α
1.65	0.05	1.51	0.04	0.06	10.16	0.22	13.40	0.15	0.21	5.99	0.14	7.12	0.06	0.10
1.79	0.18	1.68	0.16	0.24	11.12	0.92	14.94	0.62	1.32	6.45	0.58	7.77	0.30	0.65
2.00	0.39	1.87	0.32	0.79	12.44	2.32	16.72	1.67	5.76	6.11	1.23	7.13	0.67	2.39
1.28	0.32	1.12	0.25	0.74	9.80	2.66	12.83	1.59	7.22	4.62	1.36	5.21	0.59	2.82
1.89	0.42	1.79	0.22	1.65	10.39	2.60	13.76	1.10	12.04	4.31	1.07	4.80	0.38	4.18
0.95	0.11	0.78	0.04	0.56	10.13	1.33	13.35	0.42	9.72	4.12	0.59	4.55	0.14	3.29
1.05	0.06	0.88	0.03	0.43	10.43	0.69	13.83	0.24	6.91	4.80	0.34	5.46	0.10	2.77
0.88	0.03	0.72	0.02	0.20	9.97	0.21	13.10	0.09	3.47	5.50	0.12	6.42	0.05	1.70
3.37	0.42	3.57	0.87	0.94	10.40	0.99	13.78	1.55	2.05	7.38	0.70	9.14	1.00	1.31
2.24	0.31	2.19	0.33	0.85	10.41	1.30	13.79	1.34	4.66	6.43	0.80	7.74	0.72	2.59
3.46	0.32	3.69	0.25	1.31	9.92	1.00	13.03	0.64	4.44	6.96	0.64	8.52	0.41	2.91
2.30	0.18	2.26	0.14	0.74	11.48	0.96	15.51	0.56	4.83	8.18	0.06	10.33	0.40	3.27
2.02	0.11	1.94	0.05	0.62						8.30	0.45	10.51	0.21	3.24
1.43	0.04	1.28	0.02	0.30	10.42	0.25	13.82	0.19	3.74	7.34	0.18	9.08	0.09	2.17
2.32	0.04	1.99	0.04	0.32	10.63	0.16	12.33	0.09	2.12	8.50	0.11	9.43	0.06	1.60
1.04	0.02	0.88	0.01	0.09	10.73	0.06	14.31	0.03	1.37	8.24	0.05	10.42	0.02	0.98

SUPPLEMENTARY TABLE 2. Liver perfusion study

Monkey #	Fraction	d_0-γ	d_2-γ	d_0-α	d_3-α	d_6-α
1115	Plasma	1.01	1.14	15.79	0.73	5.87
1115	VLDL	3.19	3.14	25.35	7.62	18.74
1115	ILHDL	1.27	1.23	13.84	1.75	5.02
1171	Plasma	1.01	1.92	31.13	2.87	13.51
1171	VLDL	2.83	3.80	18.80	4.61	29.03
1171	ILHDL	0.93	1.26	15.57	1.06	6.26
1433	Plasma	0.88	1.93	19.89	0.64	10.06
1433	VLDL	4.55	5.81	11.30	2.29	18.65
1433	ILHDL	3.86	6.38	37.14	4.88	32.29

Shown are the nmol/ml plasma, and the total nmol in the VLDL ($d < 1.006$ g/ml) and ILHDL ($1.006 < d > 1.21$ g/ml) isolated from the perfusate.

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