Interrelationships of α-tocopherol with plasma lipoproteins in African green monkeys: effects of dietary fats

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Abstract The distributions of plasma lipoprotein α-tocopherol and lipids were studied in African green monkeys consuming diets enriched in saturated, monounsaturated, or polyunsaturated fatty acids. Plasma total α-tocopherol concentrations were not different among the animals fed the three diets, whereas plasma total cholesterol concentrations were significantly different among the diet groups. The α-tocopherol: total lipid molar ratio in plasma high density lipoproteins (HDL) was significantly higher compared to low density lipoproteins (LDL) and very low plus intermediate-sized low density lipoprotein (VLDL + ILDL) of each diet group, suggesting that HDL may exhibit a greater affinity for α-tocopherol. The presence of a positive correlation between HDL α-tocopherol and plasma apoA-I concentration and the absence of a correlation between HDL α-tocopherol and total lipid in HDL suggested that α-tocopherol associates with the protein moiety of HDL on the surface of the particle. A direct relationship between the plasma apoA-I: apoB molar ratio and the percentage of α-tocopherol found in the HDL fraction indicated that a greater proportion of α-tocopherol associates with HDL as the number of HDL particles in plasma increases relative to LDL particles. LDL from monkeys fed diets high in saturated fat contained 40% and 33% fewer α-tocopherol molecules per particle than LDL from monkeys fed polyunsaturated and monounsaturated fats, respectively. The phase transition temperature of LDL cholesteryl esters, indicative of the physical state of the lipids in the particle core, was well above body temperature in LDL from saturated fat-fed monkeys and was significantly higher compared to the other diet groups. This difference in the physical state of the cholesteryl esters in LDL from the group of monkeys fed saturated fat may contribute to the ability of α-tocopherol to interact with lipoproteins. Our data indicate that α-tocopherol does not distribute uniformly with plasma lipid among lipoprotein particles; rather, physical and dietary factors influence the interaction of α-tocopherol with lipoproteins.—**Carr, T. P., M. G. Traber, J. L. Haines, H. J. Kayden, J. S. Parks, and L. L. Rudel.** Interrelationships of α-tocopherol with plasma lipoproteins in African green monkeys: effects of dietary fats. *J. Lipid Res.* 1993. **34:** 1863–1871.

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Vitamin E, a lipid-soluble vitamin, is transported in the plasma solely by lipoproteins; there are no other specific plasma transport proteins. It is absorbed and secreted from the intestine in chylomicrons (1), which contain various forms of vitamin E (*RRR*-, *SRR*-, or *all rac*-α-tocopherols, and γ-tocopherol (2–5)) in proportion to their relative abundance in the diet. Tocopherols can be transferred to high density lipoproteins (HDL) during chylomicron clearance (1, 3, 5, 6), and HDL tocopherols can subsequently transfer to other circulating lipoproteins (7–11). After chylomicron remnant uptake by the liver, the hepatic tocopherol-binding protein (12, 13) is thought to preferentially transfer *RRR*-α-tocopherol to nascent very low density lipoprotein (VLDL) (3–5, 14). Other forms of vitamin E (such as γ-tocopherol or *SRR*-α-tocopherol) are not effectively incorporated into VLDL (3–5, 14). Thus, secretion of *RRR*-α-tocopherol in nascent VLDL by the liver and the exchange of tocopherols among lipoproteins act together to determine total plasma and individual lipoprotein α-tocopherol concentrations (7–9, 11).

Studies in humans have indicated that plasma α-tocopherol concentrations are highly correlated with plasma total lipid levels (15–17); this relationship appears to reflect a homogeneous distribution of α-tocopherol among the lipid components of the lipoproteins. When individual lipoprotein classes were considered, VLDL and LDL α-tocopherol levels were also highly correlated with the total lipid concentrations of each of these fractions,

Abbreviations: VLDL, very low density lipoproteins; ILDL, intermediate-sized low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; V + ILDL, VLDL + ILDL; SAT, saturated fatty acid; POLY, polyunsaturated fatty acid; MONO, monounsaturated fatty acid.

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but a similar relationship was less apparent in HDL (18). HDL α-tocopherol concentrations were most strongly correlated with HDL protein concentrations (19, 20). In populations where HDL concentrations are elevated, as in pre-menopausal women (18–22) and children (18, 23), the majority of plasma α-tocopherol was found within HDL, even though less than half of the total plasma lipid resided in HDL. Thus, it appears that α-tocopherol may interact differently with HDL than with VLDL and LDL. This observation may also help explain the disparities in the literature concerning the relative distribution of α-tocopherol among plasma lipoprotein fractions (10, 15, 24–31).

Vitamin E is a chain-breaking antioxidant, sequestering free-radicals and preventing the auto-oxidation of susceptible compounds, such as polyunsaturated fatty acids, proteins, and DNA (32). The oxidative modification hypothesis of atherosclerosis proposes that oxidation of LDL may be one of the initiating factors in arterial plaque formation (33). LDL from rabbits fed a diet rich in linoleic acid are more susceptible to oxidation than LDL from rabbits fed a diet rich in oleic acid (34). Paradoxically, we have found that while African green monkeys fed a diet rich in linoleic acid have linoleic acid-enriched LDL, these animals exhibit less atherosclerosis than monkeys with oleic acid-enriched LDL (35). Vitamin E, whether added in vitro (36) or administered orally in vivo (37–39), can make LDL more resistant to oxidation. Because plasma antioxidants, especially α-tocopherol, may play a critical role in protecting against LDL oxidation, this apparent discrepancy in African green monkeys might be attributed to differences in LDL α-tocopherol. It is not known how the degree of saturation of fatty acids in the diet and, hence, in plasma lipoproteins affects the metabolism of lipoprotein-associated α-tocopherol. Therefore, the present study was conducted to examine the distribution and interaction of α-tocopherol with plasma lipoproteins in African green monkeys consuming diets rich in saturated, monounsaturated, and polyunsaturated fat.

MATERIALS AND METHODS

Animals and diets

Adult male African green monkeys of the vervet (*Cercopithecus aethiops pygerythrus*) and grivet (*C. aethiops aethiops*) subspecies were obtained from Primate Imports, Port Washington, NY, and from the breeding colony at Hahnemann Medical School. Animals were individually caged in rooms containing approximately 24 animals. All animal procedures were approved by the Animal Care and Use Committee of the Bowman Gray School of Medicine. Three diet groups were established containing 35% of calories as fat (40). Palm oil, linoleic-rich safflower oil, and oleic-rich safflower oil were used to represent fats high in saturated, polyunsaturated, and monounsaturated fatty acids, respectively (41). The diets also contained 0.8 mg cholesterol/kcal. The three experimental groups were established after all animals were challenged with the saturated fat diet for 8 weeks; each group had similar means and standard deviations for plasma total and HDL cholesterol. Animals were fed 90 kcal (30 g) per kg body weight per day for approximately 3 years. The vitamin E content of the diets was measured and found to be 30, 50, and 40 mg α-tocopherol/kg diet in the saturated, polyunsaturated, and monounsaturated fat diets, respectively. Dietary vitamin E was naturally present in the oils used in diet preparation; some vitamin E was also added to the diets in the vitamin mixture.

Plasma lipid and lipoprotein characterization

Plasma lipoproteins were characterized from 30-ml blood samples taken after an overnight fast. Animals were restrained with ketamine (10 mg/kg) and blood was drawn from the femoral vein into tubes containing 1 mg/ml EDTA as an anticoagulant. Red blood cells were removed by centrifugation for 30 min at 1000 *g* in a refrigerated centrifuge. The plasma was adjusted to contain 0.1% EDTA, 0.1% NaN_3, 80 μg/ml phenylmethylsulfonyl fluoride (PMSF), and 1 μg/ml aprotinin. Total cholesterol concentrations were determined enzymatically using the method of Allain et al. (42). Plasma apolipoprotein concentrations were measured by enzyme-linked immunosorbent assay (43, 44). The density of the plasma (9-ml aliquots) was adjusted to 1.225 g/ml and the lipoproteins were isolated by ultracentrifugation at 50,000 rpm for 24 h (15°C) in a Beckman Ti 70.1 rotor. The lipoproteins were then separated into fractions containing very low density lipoproteins plus intermediate-sized low density lipoproteins (V + ILDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) by gel filtration chromatography using 4% agarose, and the LDL molecular weights were determined as described previously (45). For the purpose of calculating cholesterol distributions among the lipoproteins, total cholesterol concentrations in the lipoprotein fractions were measured enzymatically (42).

The chemical compositions of the lipoproteins were determined in the fractions isolated by the agarose column. Phospholipids were estimated by measuring inorganic phosphorus (46) and total protein was determined according to the method of Lowry et al. (47) using bovine serum albumin (fraction V) as the reference standard. Lipoprotein total lipids were extracted into chloroform–methanol 2:1 according to the method of Folch, Lees, and Sloane Stanley (48) and triglycerides, free cholesterol, and cholesteryl esters were isolated from the extract by thin-layer chromatography using a solvent system of hexane–diethyl ether–acetic acid 70:30:1. The lipid

classes were scraped from the TLC plate and extracted into chloroform–methanol 2:1. Triglycerides were measured using the chemical assay of Sardesai and Manning (49) and free and esterified cholesterol were quantified by the *o*-phthalaldehyde procedure (50). Plasma concentration of total lipoprotein lipid was determined by the summation of the molar concentration of the individual lipid classes. The phase transition temperatures of the LDL were determined by differential scanning calorimetry (51).

Separate blood samples were drawn for the determination of α-tocopherol concentrations in plasma and individual lipoprotein fractions so that special precautions could be taken to minimize the oxidation of α-tocopherol. Plasma was isolated from 20 ml blood by low speed centrifugation and adjusted to contain 0.1% EDTA, 0.1% NaN_3, 80 μg/ml PMSF, 1 μg/ml aprotinin, and 1% ascorbic acid (pH 6.6). Aliquots of plasma were then applied to the 4% agarose column and lipoproteins were eluted with saline (containing 0.01% EDTA and 0.01% NaN_3) that was continuously sparged with nitrogen. As the ascorbic acid in the plasma sample was separated from the lipoproteins by gel filtration, elution fractions were collected into tubes that contained an ascorbic acid solution to make the final concentration 1%. Fractions corresponding to V + ILDL, LDL, and HDL were immediately frozen in liquid nitrogen and shipped to New York University Medical Center for analysis. α-Tocopherol in the plasma and column fractions was extracted in hexane from samples saponified with alcoholic KOH containing 1% ascorbic acid and quantified by high performance liquid chromatography using fluorescence detection as described (52). The coefficient of variation of the α-tocopherol assay was approximately 7%. Total cholesterol and α-tocopherol concentrations were measured in each lipoprotein fraction recovered from the agarose column. Corrections were made for nonspecific losses from the agarose column by multiplying the percent distribution of cholesterol and α-tocopherol among the lipoprotein fractions with the concentration of total cholesterol and α-tocopherol in whole plasma. Recovery of total cholesterol and α-tocopherol was 87.7 ± 1.4% and 88.3 ± 1.7%, respectively (mean ± SEM; n = 36). The number of α-tocopherol molecules per LDL particle was calculated using the plasma molar concentrations of α-tocopherol and apoB in the LDL fraction and assuming one apoB molecule per LDL particle.

Statistical analyses

Data in the tables are presented as means ± SEM. Statistical comparison among diet groups was determined by one-way analysis of variance with Fisher's post-hoc least significant difference test using StatView™ II (Abacus Concepts, Inc., Berkeley, CA). Correlation and multiple regression analyses were also performed using StatView™ II.

RESULTS

α-Tocopherol and cholesterol concentrations and distributions within plasma lipoproteins of African green monkeys are presented in **Table 1.** Plasma α-tocopherol concentrations were not different among animals fed saturated (SAT), polyunsaturated (POLY), or monounsaturated (MONO) fat-containing diets. Likewise, V + ILDL and LDL α-tocopherol concentrations were not significantly different among the groups; however, HDL α-tocopherol concentrations were significantly lower in the POLY group compared to the SAT group ($P < 0.05$ using one-way analysis of variance). In all diet groups, the highest percentage of plasma α-tocopherol was found in the HDL fraction (~50%) whereas a smaller proportion (~42%) was associated with LDL and less than 10% was found with the V + ILDL fraction.

TABLE 1. α-Tocopherol and cholesterol concentrations and distributions among plasma lipoproteins

	α-Tocopherol				Cholesterol			
Group	Plasma	V + ILDL	LDL	HDL	Plasma	V + ILDL	LDL	HDL
	μg/ml				*mg/dl*			
SAT	12.3 ± 1.2	0.70 ± 0.09 *(5.6 ± 0.5)*	5.08 ± 0.74 *(40.9 ± 4.0)*	6.49 ± 0.75[a] *(53.4 ± 4.3)*	379 ± 32[a]	52 ± 7[a] *(13.1 ± 0.9)*	255 ± 25[a] *(65.7 ± 2.1)*	73 ± 6[a] *(21.2 ± 2.7)*
POLY	10.7 ± 1.2	0.80 ± 0.17 *(8.1 ± 1.6)*	5.20 ± 0.96 *(44.4 ± 4.4)*	4.68 ± 0.45[b] *(47.5 ± 4.3)*	219 ± 26[b]	24 ± 4[b] *(10.3 ± 0.7)*	145 ± 23[b] *(62.5 ± 3.0)*	52 ± 4[b] *(27.2 ± 3.3)*
MONO	11.7 ± 1.6	1.44 ± 0.71 *(9.4 ± 2.6)*	5.07 ± 1.21 *(40.1 ± 5.3)*	5.20 ± 0.54[a,b] *(50.4 ± 5.3)*	292 ± 29[b]	40 ± 6[a,b] *(12.9 ± 1.2)*	171 ± 27[b] *(55.3 ± 3.5)*	82 ± 5[a] *(31.9 ± 3.9)*

Values represent means ± SEM; n = 12 for each diet group. SAT, saturated; POLY, polyunsaturated; MONO, monounsaturated. Values in parentheses indicate percent of plasma.

[a,b] Means within the same column bearing unlike superscripts are significantly different ($P < 0.05$) as determined by one-way analysis of variance and Fisher's post-hoc least significant difference test.

TABLE 2. Chemical compositions of plasma lipoproteins

	PL	FC	TG	CE	Pr	PL/Pr	FC/Pr	TG/Pr	CE/Pr
	% mass					*mass ratio*			
V + ILDL									
SAT	21.6 ± 0.6[a]	11.1 ± 0.3	11.9 ± 2.3[a]	40.4 ± 2.7[a]	14.9 ± 0.9	1.49 ± 0.09	0.77 ± 0.04	0.85 ± 0.21[a]	2.83 ± 0.27
POLY	22.3 ± 0.4[a]	11.2 ± 0.5	8.5 ± 1.6[a,b]	40.7 ± 1.8[a]	17.3 ± 1.3	1.36 ± 0.09	0.69 ± 0.05	0.53 ± 0.11[a,b]	2.53 ± 0.24
MONO	19.8 ± 0.7[b]	10.7 ± 0.4	6.4 ± 1.2[b]	47.5 ± 1.0[b]	15.7 ± 0.7	1.29 ± 0.08	0.69 ± 0.04	0.43 ± 0.09[b]	3.09 ± 0.14
LDL									
SAT	21.9 ± 0.3[a]	9.5 ± 0.3	1.5 ± 0.4	46.1 ± 0.9[a,b]	21.0 ± 0.5[a]	1.05 ± 0.02[a]	0.45 ± 0.02[a]	0.067 ± 0.015	2.22 ± 0.09[a]
POLY	20.1 ± 0.5[b]	9.3 ± 0.4	1.0 ± 0.3	45.1 ± 1.0[a]	24.5 ± 0.5[b]	0.82 ± 0.02[b]	0.38 ± 0.02[b]	0.041 ± 0.011	1.86 ± 0.08[b]
MONO	20.8 ± 0.3[b]	9.0 ± 0.2	1.4 ± 0.2	48.8 ± 0.9[b]	19.9 ± 0.5[a]	1.05 ± 0.03[a]	0.46 ± 0.01[a]	0.069 ± 0.012	2.48 ± 0.10[c]
HDL									
SAT	24.9 ± 1.0	2.5 ± 0.1	1.2 ± 0.2	21.8 ± 0.6	49.6 ± 1.2[a,b]	0.51 ± 0.03	0.053 ± 0.004	0.025 ± 0.005	0.44 ± 0.02
POLY	22.4 ± 1.1	2.4 ± 0.1	0.7 ± 0.1	22.2 ± 0.55	52.3 ± 1.0[a]	0.43 ± 0.03	0.046 ± 0.003	0.014 ± 0.003	0.43 ± 0.01
MONO	24.6 ± 0.9	2.5 ± 0.1	1.0 ± 0.1	22.8 ± 0.7	49.1 ± 0.7[b]	0.51 ± 0.02	0.051 ± 0.003	0.020 ± 0.003	0.47 ± 0.02

Values represent means ± SEM; n = 12 for each diet group and lipoprotein class. PL, phospholipid; FC, free cholesterol; TG, triglyceride; CE, cholesteryl ester; Pr, protein. SAT, saturated; POLY, polyunsaturated; MONO, monounsaturated.

[a,b,c] Means within the same column and lipoprotein class bearing unlike superscripts are significantly different ($P < 0.05$) as determined by one-way analysis of variance and Fisher's post-hoc least significant difference test.

Plasma total cholesterol concentrations were significantly elevated in monkeys fed the SAT diet compared with those fed the POLY and MONO diets (Table 1). The cholesterol concentrations of the V + ILDL, LDL, and HDL fractions in the SAT group also were significantly higher than in the POLY group. LDL cholesterol concentrations in the MONO group were lower ($P < 0.05$) than those of the SAT group and were similar to those of the POLY group. Conversely, HDL cholesterol concentrations in the MONO group were similar to those of the SAT group and were significantly higher than those of the POLY group. In all groups, the majority of the plasma cholesterol was associated with LDL (~61%), a smaller proportion was recovered with HDL (~27%), and ~12% was associated with the V + ILDL fraction.

The chemical compositions of the plasma lipoproteins are shown in **Table 2.** Cholesteryl esters were the major component (40–49%) of the LDL and V + ILDL fractions, while protein comprised about half of the mass of HDL. Triglycerides accounted for approximately 10% of the mass in the V + ILDL fraction and less than 2% of the mass in the LDL and HDL fractions. Dietary fat appeared to significantly alter the percentage composition of the lipoproteins as noted in Table 2. In the V + ILDL fraction, a greater percentage of cholesteryl ester and a lower percentage of phospholipid were found in the MONO group compared to the other diet groups. In the LDL fraction, the percentage of phospholipid was greatest in the SAT group, whereas the percentage of protein was greater in the POLY group. In the HDL fraction, the percentage of protein was greatest in the POLY group compared to the MONO group.

The lipid:protein ratio for each lipid class was highest in the V + ILDL fraction and lowest in the HDL fraction (Table 2). Dietary fat appeared to significantly alter the lipid:protein ratios in the fractions containing V + ILDL and LDL, but not in HDL. In the V + ILDL fraction, a higher triglyceride:protein ratio was found in the SAT group compared to the MONO group. In the LDL fraction, lower ratios of phospholipid and free cholesterol to protein were found in the POLY group compared to the other groups. The ratio of cholesteryl ester:protein was significantly different in each diet group, with MONO having the highest and POLY having the lowest.

The distributions of α-tocopherol and cholesterol among plasma lipoproteins were dissimilar (Table 1), indicating that the extent with which α-tocopherol and total cholesterol associated with the various lipoproteins were not the same. Previous studies have suggested that α-tocopherol concentrations are correlated with plasma total lipid (15–17). To determine whether any single lipid class was a better predictor of α-tocopherol concentrations in the plasma, regression analyses were performed between the molar concentrations of plasma α-tocopherol and the following lipids: phospholipids, esterified and unesterified cholesterol, triglycerides, and total lipid. The strongest correlation was between α-tocopherol and total lipid concentration ($r = 0.57$, $P = 0.0003$).

The relationship between α-tocopherol and total lipid was examined within each of the lipoprotein fractions (**Table 3**). The α-tocopherol:total lipid ratio in the V + ILDL fraction was lowest in the SAT group, and was similar in the POLY and MONO groups. In the LDL fraction, the ratio was similar in the SAT and MONO groups, whereas the SAT group was significantly lower compared to the POLY group. In the HDL fraction, the ratio was lowest in the MONO group and similar in the SAT and POLY groups. However, for all diet groups the HDL α-tocopherol:total lipid ratios were significantly higher than

TABLE 3. Ratios of plasma lipoprotein α-tocopherol to total lipid

Group	α-Tocopherol:Total Lipid Ratio		
	V + ILDL	LDL	HDL[a]
		mmol:mol	
SAT	0.91 ± 0.11[b]	1.33 ± 0.11[b]	4.85 ± 0.56[b,c]
POLY	1.92 ± 0.27[c]	2.65 ± 0.41[c]	5.46 ± 0.63[b]
MONO	1.50 ± 0.20[c]	1.91 ± 0.23[b,c]	3.54 ± 0.39[c]

Values represent means ± SEM; n = 12 for each diet group. SAT, saturated; POLY, polyunsaturated; MONO, monounsaturated.

[a] HDL values within each diet group are significantly greater ($P < 0.05$) than LDL and V + ILDL values as determined by one-way analysis of variance and Fisher's post-hoc least significant difference test. No significant differences were detected between V + ILDL and LDL values.

[b,c] Means within the same column bearing unlike superscripts are significantly different ($P < 0.05$) as determined by one-way analysis of variance and Fisher's post-hoc least significant difference test.

those in the LDL and V + ILDL fractions. The α-tocopherol:total lipid ratios in the LDL compared to the V + ILDL fractions were not significantly different in any diet group as determined by one-way analysis of variance. The concentrations in plasma of α-tocopherol and total lipid in the V + ILDL and LDL fractions from each diet group were highly correlated (**Fig. 1**). V + ILDL and LDL values are shown together because data from both lipoprotein fractions fit the same regression line. A similar, statistically significant relationship was not detected in the HDL fraction from any of the diet groups.

To test the hypothesis that HDL α-tocopherol was associated with protein, the correlations of plasma concentrations of apoA-I or apoA-II with HDL α-tocopherol were calculated (**Fig. 2**). (More than 98% of the total plasma apoA-I is contained within the HDL fraction in each of the diet groups.) The results from all diet groups show that HDL α-tocopherol concentrations were correlated with plasma apoA-I concentrations ($r = 0.60$, $P = 0.0001$), but no relationships were observed between HDL α-tocopherol and plasma apoA-II concentrations.

The percent distribution of α-tocopherol in the plasma HDL fraction was plotted against the plasma apoA-I:apoB molar ratio (**Fig. 3**). The apoA-I:apoB molar ratio estimates the relative number of HDL and LDL particles in plasma, irrespective of the lipid content of the lipoprotein fractions. A positive correlation ($r = 0.64$, $P = 0.0001$) was observed, indicating that a greater proportion of plasma α-tocopherol associated with HDL when the number of HDL particles was increased relative to LDL particles.

LDL from animals of each diet group were further characterized and the results are presented in **Table 4.** LDL apoB concentrations were highest in monkeys fed the SAT diet and were lowest in monkeys fed the MONO diet. Assuming that plasma LDL contain one apoB molecule in the protein moiety of each particle, the SAT-fed animals exhibited a higher plasma concentration of LDL particles than the MONO group; the POLY group contained an intermediate number of LDL particles. LDL molecular weights, determined using agarose column chromatography, tended to be highest in the SAT group and lowest in the POLY group, and this result is likely to be biologically significant (40). The average numbers of α-tocopherol molecules per LDL particle were cal-

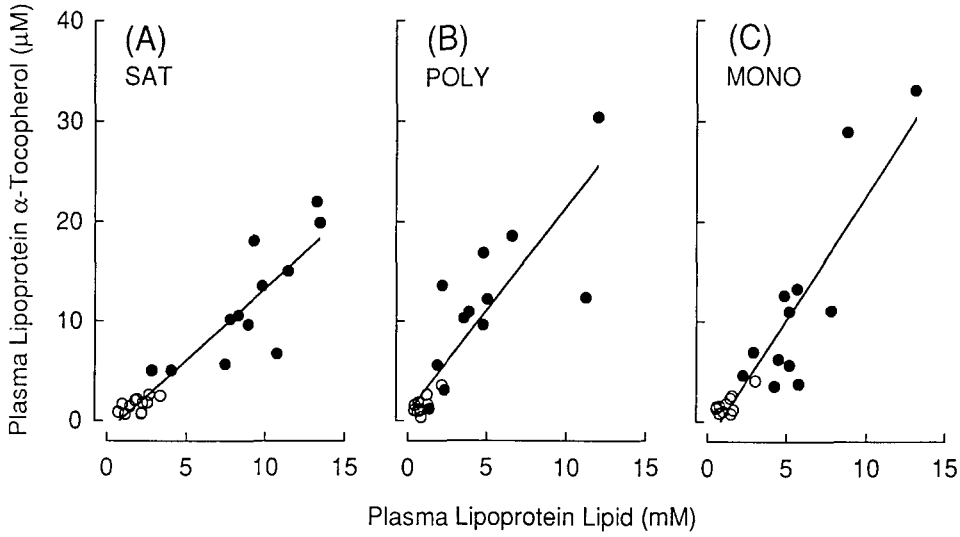

Fig. 1. Relationship between concentrations of α-tocopherol and total lipid in plasma LDL (filled circles) and V + ILDL (open circles) fractions. Lipoproteins were isolated from animals consuming saturated (A), polyunsaturated (B), and monounsaturated (C) fat-enriched diets. The linear regression equations and correlation coefficients were: y = −1.15 + 1.45x, $r = 0.93$ (A); y = 0.86 + 2.06x, $r = 0.87$ (B); and y = −2.02 + 2.46x, $r = 0.91$ (C).

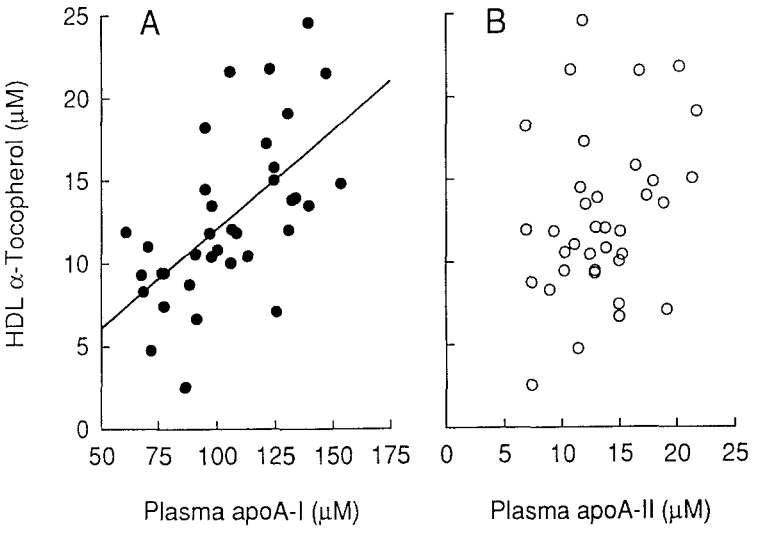

Fig. 2. Relationship between HDL α-tocopherol concentrations and plasma apoA-I (A) and apoA-II (B) concentrations. Data have been combined from all diet groups. A significant correlation (r = 0.60, P = 0.0001) was observed between HDL α-tocopherol and plasma apoA-I concentrations. No correlation was observed between HDL α-tocopherol and plasma apoA-II concentrations.

culated for each diet group and are also presented in Table 4. LDL from the SAT group contained an average of 4.2 ± 0.4 α-tocopherol molecules per particle, significantly fewer than LDL from the MONO or the POLY groups (6.3 ± 0.8 and 6.7 ± 0.9, respectively).

To test the possibility that interactions of α-tocopherol with LDL might be dependent upon the physical properties of LDL, the phase transition temperatures of LDL were determined (Table 4). LDL from the SAT group exhibited a mean transition temperature well above body temperature, and was significantly greater than that of the two other groups. The average transition temperature of LDL from the POLY group was well below body temperature, while the MONO group exhibited a mean transition temperature slightly below body temperature.

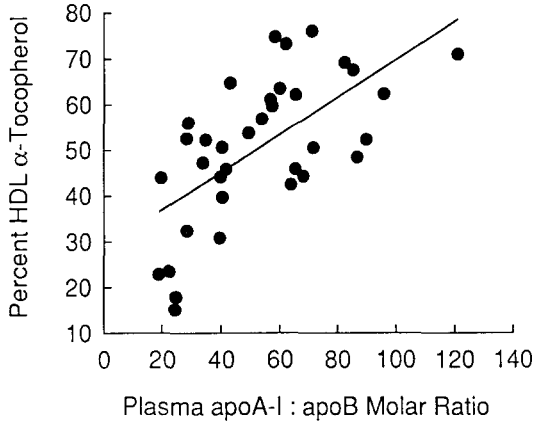

Fig. 3. Relationship between plasma apoA-I: apoB molar ratio and the percent of α-tocopherol in the plasma HDL fraction. Data have been combined from all diet groups. The apoA-I: apoB molar ratio represents the relative number of HDL and LDL particles in plasma. The correlation coefficient was r = 0.64 (P = 0.0001).

DISCUSSION

The distributions of plasma lipoprotein α-tocopherol and lipids were studied in African green monkeys consuming diets enriched in saturated, monounsaturated, or polyunsaturated fat. Despite similar plasma α-tocopherol concentrations in each diet group, dietary fat saturation clearly affected the α-tocopherol distribution among and enrichment within the lipoproteins. LDL from monkeys fed diets high in saturated fat contained significantly fewer α-tocopherol molecules per particle as LDL from polyunsaturated or monounsaturated fat-fed animals (Table 4). Within each diet group, strong correlations (r = 0.9) between plasma concentrations of α-tocopherol and total lipid in the V + ILDL and LDL fractions suggested that α-tocopherol in the apoB-containing lipoproteins was virtually all lipid-associated. However, HDL was disproportionately enriched in α-tocopherol relative to total lipid in each of the diet groups. The absence of a correlation between α-tocopherol and total lipid in HDL and the presence of a positive correlation between HDL α-tocopherol and plasma apoA-I concentration suggests that α-tocopherol associates with the protein moiety of HDL on the surface of the particle.

Several lines of evidence suggest a high affinity component of HDL for plasma α-tocopherol. Behrens and coworkers (19, 20) reported that HDL α-tocopherol concentrations were strongly correlated with HDL protein concentrations, but that a similar relationship was much less apparent in VLDL and LDL. Studies that examined the α-tocopherol distribution among plasma lipoproteins have reported that when HDL concentrations are elevated, the majority of α-tocopherol is associated with HDL (18–23). In the present study, we found a direct relationship between the plasma apoA-I:apoB molar ratio and the percentage of α-tocopherol found in the HDL fraction

TABLE 4. Plasma LDL measurements

Group	n	ApoB	n	Molecular Weight	n	α-Tocopherol	n	Transition Temperature
		mg/dl		*g/μmol*		*molecules/particle*		*°C*
SAT	14	131 ± 15[a]	14	3.84 ± 0.10	12	4.2 ± 0.4[a]	11	40.2 ± 0.8[a]
POLY	13	101 ± 16[a,b]	13	3.54 ± 0.13	12	6.7 ± 0.9[b]	12	32.8 ± 0.7[b]
MONO	14	89 ± 11[b]	14	3.76 ± 0.11	12	6.3 ± 0.8[b]	14	36.4 ± 0.6[c]

Values represent means ± SEM. SAT, saturated; POLY, polyunsaturated; MONO, monounsaturated.
[a,b,c] Means within the same column bearing unlike superscripts are significantly different ($P < 0.05$) as determined by one-way analysis of variance and Fisher's post-hoc least significant difference test.

(Fig. 3), suggesting that a greater proportion of α-tocopherol associated with HDL as the number of HDL particles in plasma increased relative to LDL particles.

Mechanisms that regulate the partitioning of α-tocopherol among plasma lipoprotein classes are not well understood. Granot, Tamir, and Deckelbaum (9) recently reported that neutral lipid transfer protein does not mediate the transfer of α-tocopherol among lipoproteins, suggesting that factors other than lipid mass influenced partitioning of α-tocopherol among lipoproteins. Our results (Fig. 2) implicate apoA-I as a protein responsible for increasing the affinity of HDL for α-tocopherol. A significant positive correlation was observed between the concentrations of HDL α-tocopherol and plasma apoA-I ($r = 0.60$, $P = 0.0001$), but no relationship was observed between HDL α-tocopherol and plasma apoA-II.

We have used our data to test two models of the interactions of HDL and α-tocopherol. Using the linear regression equations in Fig. 1 and assuming that all of the α-tocopherol in the apoB-containing lipoproteins is lipid-associated, the amount of lipid-associated HDL α-tocopherol can be estimated for each animal (model 1). We found that less than 60% of HDL α-tocopherol in any diet group was apparently associated with lipid, leaving approximately 40% associated with protein. We also used multiple linear regression analysis to investigate the effects of HDL surface lipids (i.e., phospholipid and free cholesterol), core lipids (i.e., cholesteryl ester and triglyceride), and proteins on HDL α-tocopherol concentrations (model 2). This model was highly significant, and of the variance in HDL α-tocopherol levels described by the model, approximately 67% was due to the surface lipid component and 33% was due to the protein moiety, while virtually none of the variance could be ascribed to the core lipid component of HDL. While this partitioning of the variance is not necessarily indicative of the distribution of α-tocopherol among the compositional constituents of HDL, it is interesting to note the similarity between the two models in the fraction of protein-associated HDL α-tocopherol.

The mode of interaction between HDL apoA-I and α-tocopherol is not known, but could involve hydrophobic interactions given the amphipathic natures of α-tocopherol and apoA-I. Differences between HDL and the apoB-containing lipoproteins may result from differences in the mechanisms by which tocopherols are incorporated into the lipoproteins. From our studies in vivo using deuterated tocopherols, we have suggested that the tocopherol-binding protein in the liver is responsible for the preferential incorporation of *RRR*-α-tocopherol into nascent VLDL during assembly (3–5, 14), and thus, α-tocopherol could be inserted into the forming lipid core. In this case, α-tocopherol in V + ILDL and LDL would appear to be associated primarily with lipid, as observed in Fig. 1. By contrast, nascent HDL is secreted by the liver (and intestine) in a relatively lipid-poor form, and in the circulation acquires its cholesteryl ester core as a result of the action of lecithin:cholesterol acyltransferase (LCAT) (53). α-Tocopherol is thought to be transferred to HDL either during the delipidation cascade of triglyceride-rich lipoproteins with the transfer of surface components (1, 3, 5, 6) or as the result of exchange of tocopherol between lipoproteins (7–11). In these cases, HDL α-tocopherol would seem likely to be located on the surface of the particle perhaps in association with apoA-I, as the protein moiety is believed to cover a significant portion of the HDL particle surface.

While the previous observations apply to all monkeys in the study, comparison among the different dietary fat groups was also informative. Previous studies have shown that LDL cholesteryl esters exhibit thermotropic transitions indicative of liquid crystalline to liquid transitions in the particle core (54, 55). LDL from monkeys fed saturated fat, unlike those fed polyunsaturated fat, exhibited cholesteryl ester transition temperatures well above body temperature, indicating that the lipids in the particle core of these animals were in a liquid crystalline state (51, 55, 56). The increase in LDL transition temperature was attributed to an enrichment of saturated and monounsaturated cholesteryl esters in the LDL of the monkeys fed saturated fat. This change in the physical state of the core lipids has been proposed to be a potentially atherogenic feature of plasma LDL. The results of the present study confirm the observation that a diet rich in saturated fat

elevates the phase transition temperature of LDL above body temperature (Table 4). Furthermore, monkeys fed polyunsaturated fat exhibited mean transition temperatures well below body temperature, indicating that the core lipids were in a liquid state. LDL cholesteryl esters in individual monounsaturated fat-fed animals may have existed in both liquid crystalline and liquid states, although the mean value was slightly below body temperature, suggesting that most of the cholesteryl esters were in a liquid state. As the dietary effect on the α-tocopherol molecule per particle content of LDL appears similar to the effect on LDL core lipid physical state, this property of LDL particles may contribute to the ability of α-tocopherol to interact with lipoproteins.

It has been suggested that another atherogenic feature of plasma LDL is its susceptibility to oxidation (33). Recent clinical studies have demonstrated that dietary supplementation with α-tocopherol produces an α-tocopherol enrichment in LDL that is directly correlated with the resistance of LDL to oxidation in vitro (37, 38). In the current study, plasma LDL from polyunsaturated fat-fed monkeys were significantly enriched in α-tocopherol relative to saturated fat-fed monkeys (Table 4). We have previously shown that African green monkeys fed polyunsaturated fat exhibit less atherosclerosis than animals fed saturated fat (35). It is possible that α-tocopherol enrichment of LDL in monkeys fed polyunsaturated fat may contribute to the decreased susceptibility to atherosclerosis by protecting against LDL oxidation. While this is a potential explanation for the dietary fat effects on the development of atherosclerosis in African green monkeys, data relating these observations with LDL oxidation and with the actual development of atherosclerosis have not been shown, and further study is required to elucidate any such relationships.

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