Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra of nonhuman primates. Effect of saturated versus polyunsaturated dietary fat[1]

John S. Parks[2] and Lawrence L. Rudel

Department of Comparative Medicine, Bowman Gray School of Medicine, Wake Forest University
Winston-Salem, NC 27103

Abstract Monkeys fed polyunsaturated fat had significantly lower plasma cholesterol (186 ± 18 vs. 276 ± 31 mg/dl) and high density lipoprotein (HDL) mass concentrations (466 ± 28 vs. 518 ± 34 mg/dl) than did animals fed saturated fat. Plasma apoA-I concentrations also were significantly lower and apoA-II levels were generally, though not significantly, lower in the group fed polyunsaturated fat. In vivo reinjection studies, using thoracic duct lymph chylomicra labeled with ^{131}I and HDL labeled with ^{125}I, were done in order to study the mechanism of plasma HDL-lowering by polyunsaturated dietary fat. The peak specific activity (SA) of HDL apoA-I derived from ^{131}I-labeled chylomicra occurred at 3 hr after injection (172 ± 11% of 1 min S.A.) and then an exponential decay occurred indicative of a precursor-product relationship between chylomicron apoA-I and HDL apoA-I. In contrast, HDL apoA-II derived from ^{131}I-labeled chylomicra had no early S.A. increase and began to die away immediately after injection. Labeled apoA-I from chylomicron and HDL origin had similar plasma fractional catabolic rates (FCR = 0.34–0.38 vs. 0.32–0.38 d^{-1}, respectively); apoA-II from chylomicron or HDL origin also had similar FCR (0.46–0.51 vs. 0.42–0.51 d^{-1}, respectively), which were significantly shorter than those for HDL apoA-I. There was a consistent trend toward a higher FCR for HDL apoA-I or A-II of polyunsaturated fat-fed recipients. Chylomicron apoA-I/triglyceride and apoA-II/triglyceride mass ratios were lower in polyunsaturated fat-fed animals (A-I/TG = 1.56×10^{-3}; A-II/TG = 1.47×10^{-3}) vs. saturated fat-fed animals (A-I/TG = 2.58×10^{-3}; A-II/TG = 2.77×10^{-3}). It was concluded that: (*1*) dietary polyunsaturated fat significantly lowered plasma cholesterol, HDL, and apoA-I concentrations relative to saturated fat; (*2*) the HDL-lowering effect of the dietary polyunsaturated fat may be due to the combined effects of decreased apoprotein production by the intestine and increased HDL catabolism; and (*3*) in the blood, chylomicron apoA-I and A-II differ in their metabolic fates.—**Parks, J. S., and L. L. Rudel.** Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra of nonhuman primates. Effect of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* 1982. **23:** 410–421.

Supplementary key words apolipoprotein • lymph lipoproteins • nonhuman primates • saturated dietary fat • polyunsaturated dietary fat

Chylomicra are large lipid-protein complexes that transport dietary lipids from the intestine to the plasma compartment (1). Triglyceride is the predominant lipid class, constituting approximately 90% of the total mass of the particle (1) and, together with cholesteryl ester, forms the core of the particle. A monolayer of polar constituents (phospholipid, cholesterol, and protein) surrounds the core and stabilizes the particle in an aqueous milieu (1). The apoprotein component of the chylomicron particle is heterogeneous and represents less than 1% of the total mass of the particle (2, 3).

The intestinal chylomicra have been shown to be the source of significant amounts of apoA-I, the major apoprotein of plasma HDL (4–8). ApoA-II has also been reported to be synthesized by the intestine (9), but its contribution to the plasma apoA-II pool is not well known. Chylomicron apoA-I has been shown by several laboratories to be a precursor of HDL apoA-I (10, 11) but there is a paucity of data regarding the metabolic fate of chylomicron apoA-II in the plasma. Studying one human patient with recurrent chylous pleural effusion, Schaefer, Jenkins, and Brewer (11) reported a similar kinetic pattern of appearance of apoA-I and A-II of injected chylomicra into HDL. Furthermore, several investigators have reported similar plasma decay rates for radiolabeled apoA-I and A-II of human HDL; these studies have indicated a $T_{1/2}$ of apoA-I and apoA-II ranging from 4.0 to 5.8 days (12–14).

Polyunsaturated dietary fat has been found by several investigators to lower plasma HDL concentration (15–17). In view of the apparent precursor fate of chylomicron apoA-I and phospholipid (10, 18) for plasma HDL,

Abbreviations: DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); IEF, isoelectric focusing; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; $T_{½}$, half-life; FCR, fractional catabolic rate; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyl transferase; HDL, high density lipoprotein.

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it might be reasoned that diets rich in polyunsaturated fat could alter the production of chylomicron apoproteins destined for HDL. In fact, Shepherd et al. (15) have suggested that intestinal apoA-I production was lowered by dietary polyunsaturated fat. This led to a decrease in plasma HDL and apoA-I concentrations of the patients consuming the polyunsaturated fat diet because the fractional catabolic rate of apoA-I was unaltered. No data are available on the role of unsaturated fat on intestinal chylomicron apoA-II production and metabolism.

In the present study we have attempted to determine if thoracic duct lymph chylomicron apoA-I and A-II have kinetic fates similar to those of apoA-I and A-II of plasma HDL and to determine if dietary unsaturated fat influences the kinetics of chylomicron apoA-I and A-II in plasma relative to those of saturated fat. We selected African green monkeys for these studies because their plasma lipoprotein response to dietary cholesterol and unsaturated fat is more similar to that of North American human beings than has been found in most of the other species of nonhuman primates (19, 20). Furthermore, we had previously characterized the HDL apoproteins in this species (21).

MATERIALS AND METHODS

The animals used for the metabolic studies were male African green monkeys of the vervet subspecies. All animals were adapted to chair restraint for at least 15 days prior to any experimental manipulation. At the end of this period, urinary 17-hydroxy steroids returned to normal levels indicating that restraint-induced stress had decreased.[3] The diets fed in these experiments were the 75-8 butter test diet and the 75-8 safflower test diet, which have been described in a separate publication (17). They are semipurified diets containing 0.79 mg of cholesterol/Kcal with 40% of calories as either safflower oil or butterfat. The animals had been maintained on their respective diets for several years prior to these studies.

In vivo reinjection studies were performed using twelve recipient animals. Two chylomicron doses from butterfat-fed donors (butter-derived chylomicra) were injected into two or four recipient animals in two separate experiments and the same was true for two doses of chylomicra obtained from safflower oil-fed donors (safflower-derived chylomicra). Recipient animals for each chylomicron dose were chosen from both dietary fat groups. Autologous HDL doses were used in most of the in vivo reinjection studies; however, three of twelve recipient animals were reinjected with HDL from another animal within the same dietary group with no apparent effect on the outcome of the experiment.

Lymph chylomicra were collected from donor animals after cannulation of the thoracic duct. During surgery the animal was immobilized with ketamine (10 mg/kg body weight) and 5 mg of curare (tubocurarine chloride). The thoracic duct was exposed and cannulated, first with the direction of flow using a 15-inch (0.02 I.D. × 0.037 inch O.D.) piece of silastic tubing (Dow Corning, Midland, MI) that had been previously heparinized with TDMAC-Heparin (Polyscience, Inc., Warrington, PA). The tubing was passed through the thoracic wall musculature underneath the skin and then looped back through the thoracic wall. The thoracic duct was subsequently cannulated against the direction of flow. The tubing was secured and the incision was closed with the silastic tubing positioned just under the skin.

A duodenal tube (42 inch, size 8 French Feeding Tube, Cutter Lab, Berkeley, CA), was passed into the stomach through a midline abdominal incision and positioned 2–4 inches into the duodenum. The stomach was sutured to the abdominal wall to anchor the tubing, and the abdominal incision was closed. The animal was given prostigmine (Neostigmine®) as needed at the end of surgery to reverse paralysis. All animals were allowed to recover from surgery at least 10 days prior to the start of an experiment.

Lymph collection was begun by exposing the cannula through a small incision in the skin. After the cannula was cut, two pieces of heparinized silastic tubing (0.025 I.D. × 0.047 inch O.D.) were attached as extensions. Lactated Ringer's solution was dripped into the inflow end of the thoracic duct, while lymph was collected at room temperature from the outflow end into a tube containing 0.1% EDTA, 0.02% azide, and 0.04% DTNB, pH 7.4 (final concentration). The flow rate of lymph was 15–20 ml/hr and was regulated by the infusion rate of the lactated Ringer's solution. During collection of thoracic duct lymph, the appropriate liquid diet (similar in composition to the safflower oil or butterfat solid diet) was infused at a rate of 12 ml/hr. This rate of diet administration was calculated to match the basic caloric requirements of the animal over a 24-hr period.

Lymph samples were stored at 15°C until chylomicron isolation. Chylomicra were isolated within 24 hr after collection of the lymph. Lymph chylomicra ($S_f > 400$) were obtained by centrifugation in the SW 27 rotor at 7.5×10^6 g-min, i.e., 30 ml of lymph were overlayered with 8 ml of d 1.006 mg/ml saline solution and centrifuged at 27,000 rpm for 78 min at 15°C. The chylomicra were then isolated by tube slicing, collected, and then resuspended in d 1.006 g/ml saline. A small amount of sucrose was added to raise the density to facilitate overlayering. Ten ml of the solution of resuspended chy-

[3] Klein, R., and L. L. Rudel. Unpublished observations.

lomicra was overlayered with d 1.006 g/ml saline and centrifuged in the SW 40 rotor at 15,000 rpm for 15 min (1.42×10^6 g-min) at 15°C to remove the $S_f > 2000$ chylomicra; this fraction was then discarded. Five ml of the solution containing $S_f < 2000$ chylomicra was overlayered with d 1.006 g/ml saline and was refloated in the SW 40 rotor at 27,000 rpm for 108 min (7.5×10^6 g-min) at 15°C to isolate the $400 < S_f < 2000$ chylomicra that were used in the metabolic studies. All centrifugations were carried out in a Beckman L5-50 ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA). HDL was isolated from individual recipient animals by the combined ultracentrifugation and agarose column separation method of Rudel et al. (22).

Concentrated samples of chylomicra and HDL (2–4 mg/ml protein) were iodinated according to the method of McFarlane (23) as modified by Bilheimer, Eisenberg, and Levy (24). Chylomicra were iodinated with ^{131}I and HDL was iodinated with ^{125}I using carrier-free ^{131}I and ^{125}I, respectively, obtained from New England Nuclear (Boston, MA). Enough ICl was added so that 0.5 mole of ^{131}I was added for every 20,000 g of chylomicra protein or 0.5 mole of ^{125}I for every 28,000 g of HDL protein. After iodination, specific activities of the HDL doses ranged from 1.6 to 4.1×10^5 (mean = 2.19×10^5) cpm/μg protein while chylomicron protein specific activities were 1.4–3.1 $\times 10^5$ (mean = 2.42×10^5) cpm/μg. For HDL doses in all four experiments, the mean ± S.E.M. labeling efficiency was 50 ± 4% with 1.2% of the label in lipid and 0.6 ± 0.1% of the label in TCA-soluble. Labeling patterns of the chylomicra doses were comparable except that 18% (range = 13–23%) of the label was lipid-bound.

The ^{131}I-labeled chylomicra (1.3–1.5 mg of protein) and autologus ^{125}I-labeled HDL (0.8–3.6 mg of protein) doses were mixed at 4°C 15–30 min prior to injection into the saphenous vein of chair-restrained recipient animals. The animals were fasted 16 hr prior to dose injection and were not fed again until 12 hr after injection. A solution (0.5 ml) containing 0.5% I_2 and 1.0% KI (Lugol's solution) was administered through a gastric tube to each recipient animal 1 day prior to dose injection. During the study, recipient animals were given ad libitum a solution containing 0.45% NaCl and 0.05% NaI. Blood samples (2.5 ml) were withdrawn from the femoral artery through heparinized silastic tubing (0.04 I.D. × 0.085 inch O.D.) which had been surgically implanted at least 24 hr prior to the experiment. The samples were collected in 0.1% EDTA, 0.02% azide, 0.04% DTNB, pH 7.4 (final concentration) and placed on ice immediately. Sampling times were 1, 5, 10, 20, 40 min; 1, 3, 5, 8, 12 hr; and 1, 2, 3, 4, and 5 days after dose injection. Between blood collections, the cannulae were maintained patent by pumping a solution of 0.9% NaCl, 0.1% EDTA, pH 7.4 at a rate of 0.5 ml/hr. Complete daily urine samples were collected throughout the 5-day study for six of twelve animals to determine the fractional catabolic rate of HDL protein as described by Berson and Yalow (25). Plasma volume was calculated from the plasma dilution of the ^{125}I-labeled HDL dose 10 min after injection.

Plasma obtained by low speed centrifugation of the blood samples was subjected to heparin-manganese precipitation according to the method of Warnick and Albers (26), using a final plasma manganese concentration of 92 mM. The supernatant solution was isolated after low speed centrifugation and its density was adjusted to 1.225 g/ml with solid KBr. After centrifugation at 40,000 rpm for 40 hr in the SW 40 rotor at 15°C, HDL was collected from the top of the tube after isolating this layer by tube slicing. No qualitative difference, as judged by IEF gels, could be seen in the apoprotein pattern of HDL isolated by the heparin-manganese precipitation or by the agarose column method (22). HDL was dialyzed, lyophilized, and delipidated as described previously (21). Subsequently, SDS-PAGE gel electrophoresis of apoHDL was performed (21) after which the apoA-I band was sliced out and the ^{131}I and ^{125}I radioactivity was measured; duplicate determinations on each sample were made in a Beckman Gamma 4000 (Beckman Instruments, Fullerton, CA) which was preprogrammed for double isotope counting. Isoelectric focusing of apoHDL also was performed (21), and then the apoA-II band was sliced and counted for ^{131}I and ^{125}I radioactivity. The amount of apoA-I and apoA-II in each sample was determined by radial immunodiffusion as described below. Recovery of radioactivity was 97 ± 2% for SDS-PAGE and 100 ± 2% for IEF gels, as determined using samples with a known amount of radioactivity.

Radial immunodiffusion was done by the method of Mancini, Carbonara, and Heremans (27) as modified by Albers et al. (28). Briefly, solvent-delipidated HDL or tetramethylurea-delipidated plasma samples and purified apoA-I and A-II standards were dissolved and diluted in 0.01 M Tris-HCl buffer (pH 8.0) containing 8 M urea. The immunoassay was carried out in 1% agarose gels containing the appropriate antiserum. The production of antiserum has been detailed elsewhere (29). The apoA-I assay was linear from 150 to 500 ng/sample/well with an intraassay and interassay coefficient of variation of 1.9% and 2.6%, respectively (n = 6). The assay of apoA-II was linear from 150 ng to 650 ng/sample/well with an intraassay and interassay coefficient of variation of 1.5 and 3.3%, respectively (n = 6). In some experiments, the results obtained from radial immunodiffusion were corroborated by rocket immunoelectrophoresis by a method similar to that of Weeke (30).

TABLE 1. Effects of the type of dietary fat on plasma concentrations of cholesterol, HDL, and HDL apoproteins

Diet Group	Cholesterol[a]	HDL-Chol[a]	HDL[b]	apoA-I[c]	apoA-II[c]
	plasma concentration (mg/dl)				
Safflower	186 ± 18	64 ± 6	466 ± 28	153 ± 15	41 ± 5
Butter	276 ± 31	75 ± 6	518 ± 34	187 ± 15	48 ± 4
	$P < 0.02$	$P < 0.003$	$P < 0.005$	$P < 0.05$	N.S.

[a] For each group, values represent mean ± SEM for at least 50 monthly observations (seven to nine animals sampled per month). HDL-chol was determined by the heparin-manganese method (26).

[b] Values represent mean ± SEM for duplicate determinations on each of twelve animals. Concentrations were determined by complete chemical analysis of HDL as described in the Materials and Methods section.

[c] Values represent mean ± SEM for four observations on seven animals (S) and eight animals (B).

In vitro incubations of chylomicra and HDL were carried out in cellulose nitrate tubes for the SW 41 rotor (Beckman Instruments, Inc., Fullerton, CA). To each tube was added a constant amount (150–200 μg) of chylomicron protein with increasing amounts of HDL protein (150–12,000 μg). The control had only chylomicron protein. The total volume was adjusted to 1 ml with d 1.006 g/ml saline. The mixtures were incubated at 37°C for 1 hr in an oscillating water bath. Incubation was stopped by placing the tubes on ice. One ml of d 1.006 g/ml saline was adjusted to 1.225 g/ml with solid KBr and then added to each tube; the solutions were mixed and were overlayered with d 1.006 g/ml saline. Centrifugation was carried out in the SW 41 rotor at 27,000 rpm for 126 min at 15°C (6.5×10^6 g-min). The chylomicra were isolated from the top of the centrifuge tube after tube slicing and were then dialyzed, lyophylized, and delipidated with chloroform–methanol 2:1. Chylomicron apoproteins were analyzed by radial immunodiffusion, SDS-PAGE, and IEF as described previously for HDL. Results were expressed as mass of apoprotein relative to control incubations. Chylomicron protein recovery for each incubation was corrected for losses based on triglyceride recovery, which averaged 72 ± 2% of that of the unincubated control.

Protein determinations were performed by the method of Lowry et al. (31) using bovine serum albumin, fraction V (Sigma Chemical Co., St. Louis, MO) as the standard. Cholesterol determinations were done according to the procedure of Rudel and Morris (32). Phospholipids were quantitated by the method of Fiske and SubbaRow (33), while triglyceride assays were done according to the method of Sadesai and Manning (34). Free and esterified cholesterol were quantitated after thin-layer chromatographic separation of HDL and chylomicron lipids as detailed previously (35).

Statistical analyses of the data were done by the paired *t*-test, the Student's *t*-test, or by comparison of regression lines (36). Standard error of the mean is given with all averages.

RESULTS

Dietary fat effect on lipoprotein concentrations and compositions

Vervet monkeys consuming a diet rich in polyunsaturated fat (safflower oil) had significantly lower plasma cholesterol concentrations than did animals fed the saturated (butter) fat diet (**Table 1**). HDL cholesterol concentrations were also significantly lower in the safflower oil-fed animals as were total HDL concentrations. The whole plasma concentrations of apoA-I and A-II in safflower oil-fed animals were lower than in the butter fat-fed animals. The diet-related difference in plasma concentration for apoA-I was statistically significant while the difference in apoA-II was smaller and did not reach statistical significance.

The plasma concentrations of individual HDL constituents and the percentage composition of HDL of animals from both dietary groups are shown in **Table 2.** The recipient animals used in the metabolic studies were a subset of these animals. Animals fed the butter diet had HDL with less protein and more phospholipid. A statistically significant difference could be seen in the higher phospholipid/protein ratio for the groups fed butter fat vs. safflower oil ($P < 0.05$; *t*-test). HDL from animals consuming the butter diet had a lower percentage of cholesterol ester and a higher percentage of free cholesterol. The ester to total cholesterol ratio was significantly higher in HDL of the unsaturated fat fed group ($P < 0.05$, *t*-test). The molar ratio of free cholesterol to phospholipid was slightly higher in butter-fed animals, but this difference was not statistically significant. The ratio of surface constituents (FC, PL, protein) to core

TABLE 2. Effect of the type of dietary fat on chemical composition of plasma HDL

	Butter Diet		Safflower Diet	
	Plasma Conc.	%	Plasma Conc.	%
	(mg/dl)		*(mg/dl)*	
Protein	227 ± 13[a]	44.3 ± 0.8	213 ± 12	45.8 ± 0.4
Cholesterol ester	96 ± 7	18.5 ± 0.4	91 ± 6	19.5 ± 0.5
Free cholesterol	18 ± 1	3.4 ± 0.1	13 ± 1	2.9 ± 0.2
Phospholipid	166 ± 14	31.4 ± 0.9	137 ± 9	29.3 ± 0.6
Triglyceride	11 ± 1	2.3 ± 0.4	12 ± 2	2.5 ± 0.4
Mass ratios				Significance[b]
PL/Pro[c]	0.715 ± 0.030		0.641 ± 0.018	$P < 0.05$
EC/TC	0.744 ± 0.016		0.787 ± 0.005	$P < 0.05$
Molar ratio				
FC/PL	0.221 ± 0.007		0.200 ± 0.015	NS

[a] All values, mean ± SEM for duplicate determinations on each of 12 animals.
[b] Significant differences were determined by the student's t-test. NS = not significant at p = 0.05.
[c] Abbreviations: PL, phospholipid; Pro, protein; EC, esterified cholesterol; TC, total cholesterol; FC, free cholesterol.

constituents (CE, TG) was higher for the HDL of butter-fed (3.84) vs. safflower oil-fed animals (3.52), indicating that the average size of the particles in the saturated fat-fed animals may have been smaller.

The chemical compositions of the chylomicron doses used in all four studies are shown in **Table 3.** The percentage compositions of the butterfat- and safflower oil-derived doses were very similar, but the chylomicra from butter-fed donors had slightly less triglyceride and more phospholipid than did those from the safflower oil-fed donors. The phospholipid/triglyceride ratio showed this trend; the two butterfat-derived chylomicron doses had ratios of 0.12 and 0.10 while those of the safflower oil-derived doses were 0.079 and 0.081. The higher percentage protein values for animals T6 and T223 appeared to result from albumin retained after isolation and washing. If it is assumed that all of the free cholesterol, phospholipid, and protein is located in the coat of the chylomicron particle, then the butter-derived chylomicra had a greater percentage of total mass in the coat (13.1 and 12.4%) than did the safflower oil-derived chylomicra (9.9 and 9.2%). Assuming both particles had an equivalent coat thickness, the chylomicra from safflower oil-fed animals would be predicted to have an average diameter 300–400 Å larger than the butter-derived particles, based on the relationship described by Sata, Havel and Jones (37). The larger diameter safflower oil-derived chylomicron particles therefore would be expected to have had less protein per mass of triglyceride transported than the butter-derived particles. This was the case as the safflower oil-derived chylomicron particles had smaller apoA-I/triglyceride and apoA-II/triglyceride ratios than did the butter-derived chylomicra (Table 3). The weight ratios of apoA-I to apoA-II in the chylomicron doses used in the metabolic studies averaged 1.1 ± 0.2, and were similar for both diet groups.

Injection studies of chylomicra from butterfat-fed donors

The pattern of radioactive apoA-I and apoA-II disappearance in vivo from plasma HDL after injection of ^{125}I-labeled HDL and ^{131}I-labeled chylomicra from but-

TABLE 3. Percentage composition of vervet lymph chylomicra (S_f 400–2000)

	% Composition[a]					Ratios					
	Lipid					Weight/Weight					
Donor[b]	Pro	EC	FC	PL	TG	PL/TG	PL/Pro	TC/PL	EC/TC	A-I/TG ×10^3	A-II/TG ×10^3
#137 (B)	1.9	0.8	0.8	10.4	86.9	0.120	5.3	0.15	0.51	2.31	2.77
#T6 (B)	2.5	1.4	0.8	8.7	86.6	0.100	3.5	0.25	0.65	2.85	2.77
#T223 (S)	2.4	0.8	0.4	7.0	89.4	0.079	2.9	0.17	0.64	1.69	0.77
#63 (S)	1.3	0.9	0.6	7.3	89.9	0.081	5.6	0.21	0.61	1.44	2.17

[a] Abbreviations: TG, triglyceride; A-I, apoA-I; A-II, apoA-II; others as given in Table 2.
[b] Animal number of chylomicron donor and dietary grouping. B, butterfat-fed; S, safflower oil-fed.

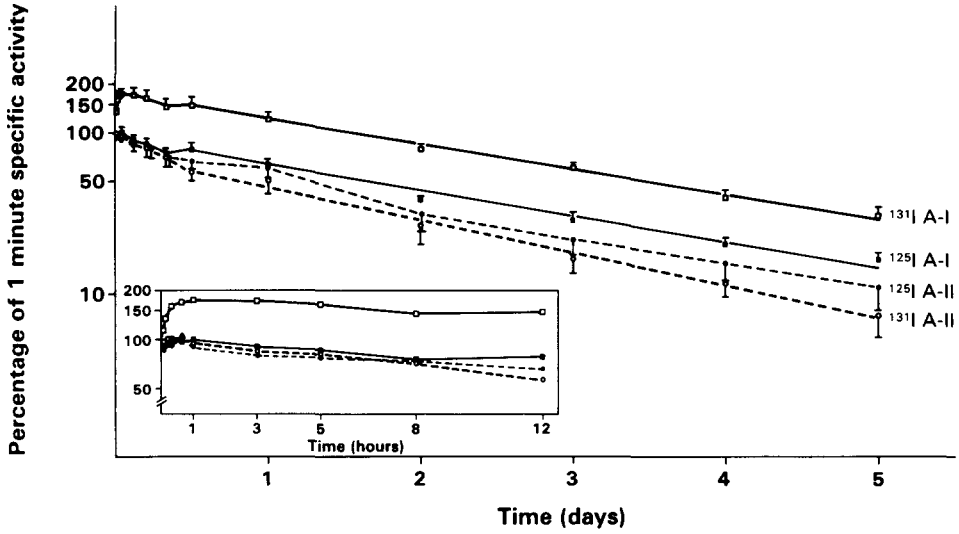

Fig. 1. Tracer amounts of butter-derived ^{131}I-labeled chylomicra (S_f 400–2,000) and autologous ^{125}I-labeled HDL were simultaneously injected and the patterns of change in specific activity vs. time are shown for apoA-I and apoA-II in plasma HDL isolated from recipient animals. HDL was isolated from plasma collected at each time point and the specific activity of the HDL apoproteins was determined after SDS-PAGE and IEF as described in the Methods section. Each point represents the mean ± standard error of results from six experiments, including three butter- and three safflower oil-fed recipient animals, using two different chylomicra doses in separate experiments. Early time points are shown with inset.

ter-fed donors into recipient animals (three butter- and three safflower oil-fed) is shown in **Fig. 1.** No consistent difference among recipients from the two dietary groups was observed in the short term (1 min–12 hr) patterns of apoprotein die-away, and the data for the animals from both diet groups were averaged. The pattern of appearance of chylomicron ^{131}I-labeled apoA-I in plasma HDL was distinctly different from that of chylomicron ^{131}I-labeled apoA-II. The specific activity of ^{131}I-labeled apoA-I increased to a peak between 1–3 hr (172 ± 11% of 1 min specific activity), while the ^{131}I-labeled apoA-II specific activity began to decrease immediately after injection. The long term (0.5–5 d) die-away of the two ^{131}I-labeled proteins was also different with the ^{131}I-labeled apoA-II curve having the more rapid decay (see below).

It can also be seen in Fig. 1 that the pattern of appearance in plasma HDL of ^{131}I-labeled apoA-I (from chylomicra) was different from that of ^{125}I-labeled apoA-I (from injected HDL). The specific activity of HDL ^{125}I-labeled apoA-I was highest at 10 min after injection and decreased in a log-linear manner thereafter. This was different from the time pattern for the specific activity of HDL ^{131}I-labeled apoA-I, which increased to a peak value during the first three hours after injection and was succeeded by a log-linear decrease. The slopes of the lines describing the decay from plasma of HDL ^{131}I-labeled apoA-I were similar.

In contrast to the data for apoA-I, there was no discernable difference in the die-away pattern of ^{131}I-labeled apoA-II vs. ^{125}I-labeled apoA-II in the reinjection studies using butter-derived chylomicra (Fig. 1). Both had maximal specific activities at 1 min after injection and the subsequent kinetic behavior of both proteins was similar over the course of the entire study.

Injection studies of chylomicra from safflower oil-fed donors

Results similar to those of the butter-derived chylomicron reinjection experiments were obtained when two different doses of chylomicra from safflower oil-fed donors were studied. In vivo die-away curves of apoA-I and apoA-II after injection of safflower-derived ^{131}I-labeled chylomicra and autologous ^{125}I-labeled HDL into six recipient animals (three butter- and three safflower oil-fed) are shown in **Fig. 2.** No consistent difference in short term (1 min–12 hr) kinetic pattern for either apoprotein due to the dietary group of the recipient was seen, and data from both groups were averaged. The specific activity of HDL ^{131}I-labeled apoA-I increased to 148 ± 39% at 1 hr and remained elevated for 5 hr. The specific activity of HDL ^{131}I-labeled apoA-II was highest 10 min after injection, then began to decay. As in the studies using butter-derived chylomicra, ^{131}I-labeled apoA-II had a more rapid decay than did ^{131}I-labeled apoA-I (see below). After injection of safflower oil-derived chylomicra, the HDL ^{131}I-labeled apoA-I and ^{125}I-labeled apoA-I showed analogous kinetic behavior to that seen when butter-derived chylomicra were injected. Log-linear decay curves for HDL ^{125}I-labeled

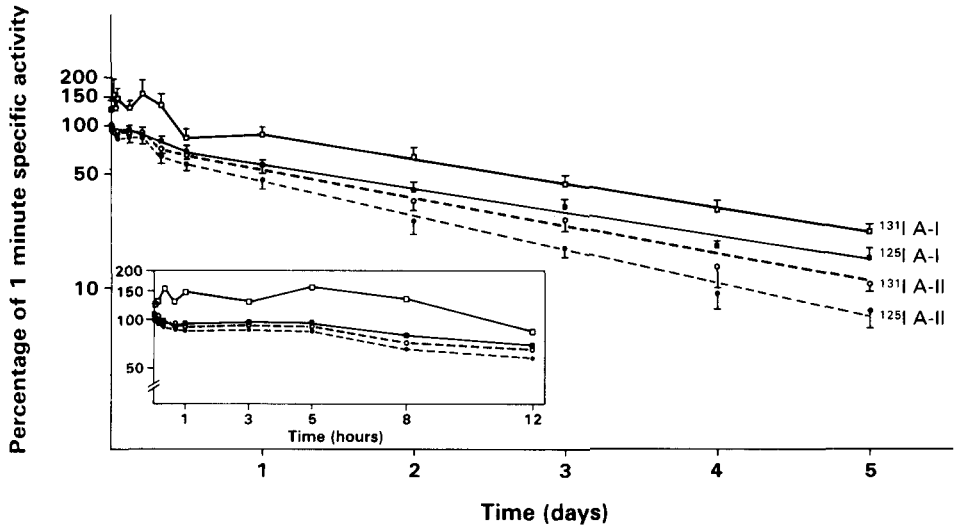

Fig. 2. Trace amounts of safflower-derived ^{131}I-labeled chylomicra (S_f 400–2,000) and autologous ^{125}I-labeled HDL were simultaneously injected and the patterns of change in specific activity vs. time are shown for apoA-I and apoA-II in plasma HDL isolated from recipient animals. Each point represents the mean ± standard error of results from six experiments, including three butter- and three safflower oil-fed recipient animals, using two different chylomicra doses in separate experiments. Experimental details are the same as for Fig. 1. Early time points are shown with inset.

apoA-I and ^{125}I-labeled apoA-II were found from 12 hr to 5 days after injection (Fig. 2). The kinetic behavior of plasma HDL apoA-II of safflower-derived chylomicron (^{131}I) or HDL (^{125}I) origin (Fig. 2) was similar at all times.

Kinetic analysis of die-away curves

Attempts were made to fit the die-away data to the classical two-pool compartmentalization model without success. Instead, a monoexponential function gave a better description of the die-away process, i.e., the log-linear plots from days 0.5–5 did not deviate significantly from a straight line as determined by regression analysis (36). Thus, analysis of the die-away data of the HDL apoproteins was made assuming the monoexponential model and a metabolic steady state. Since there was no significant difference in the long term kinetic behavior of HDL apoproteins with respect to the dietary origin of the chylomicron dose, the data were grouped with respect to the diet of the recipient animals as shown in **Table 4.**

The pool size of apoA-I for the saturated fat-fed recipients was significantly larger than that of the unsaturated fat-fed animals; the pool size of apoA-II was not significantly different between diet groups. Statistically significant diet-related differences in the FCR or synthetic rate of apoA-I and apoA-II were not found except for the FCR of ^{125}I-labeled apoA-I that just reached statistical significance. However, a higher average FCR was consistently found for the group of unsaturated fat-fed recipients. This was true for ^{131}I- vs. ^{125}I-labeled apoA-I and apoA-II regardless of the lipoprotein origin of the radiolabeled apoprotein (chylomicron vs. HDL). When apoA-I versus apoA-II catabolism was compared, we found that apoA-II had a significantly higher FCR than apoA-I regardless of the dietary fat of the recipient animal or the lipoprotein origin of the radiolabeled apoprotein. On the other hand, there was no significant difference in the FCR of plasma HDL apoproteins derived from chylomicra versus endogenous HDL as seen by comparing the FCR of ^{131}I- vs. ^{125}I-labeled apoA-I and apoA-II.

Fractional catabolic rates were also calculated from the urine to plasma ratio of ^{125}I radioactivity. The mean FCR of HDL protein for six animals (three butter- and three safflower oil-fed) was 0.44 ± 0.07 day^{-1}. This value represents an average of the FCR of all HDL apoproteins. Since apoA-I is the major apoprotein of HDL (60–70% of total protein; ref. 21), a comparison was made of FCR derived via the urine/plasma ratio vs. plasma ^{125}I-labeled apoA-I die-away. The two independent measurements of FCR were not significantly different as judged by a paired *t*-test.

Three of the recipient animals used to study the butter-derived chylomicron dose were also used to study the safflower-derived chylomicron dose. These recipients served as their own controls from one experiment to the next, and the possibility of detecting differences related to the type of fat from which the chylomicra originated was maximized. However, no statistically significant FCR difference was found for the apoproteins of the butter- vs. safflower oil-derived chylomicra.

In vitro studies

In light of the divergent metabolic fates of vervet HDL apoA-I and A-II of chylomicron origin that we observed in the reinjection studies, in vitro incubations of chylomicra and HDL were undertaken. The conditions of the incubations were set to achieve maximal exchange of apoproteins (38). The results are shown in **Fig. 3.** It can be seen that when the amounts of HDL protein increased relative to chylomicra protein, the mass of apoA-II in chylomicra decreased while that of apoA-I increased. Chylomicron apoA-II content decreased in spite of the fact that HDL apoA-II concentrations increased in the incubation medium. No apparent difference was noted using chylomicra and HDL from either dietary group. It should be noted that the HDL to chylomicron protein ratios used in these in vitro incubations are at least 1–2 orders of magnitude lower than one would expect to find in vivo.

DISCUSSION

The plasma cholesterol lowering effect of polyunsaturated fat in vervet monkeys has been reported previously (17) and is similar to the effect reported by Shepherd et al. (15) in man. The percentage decrease in HDL cholesterol concentration was also similar. The present studies were designed to investigate the mechanism of the HDL-lowering effect. In the study of human subjects (15), it was proposed that apoA-I synthetic rate must have been altered since the apoA-I fractional turnover rate was unaffected by the saturation of dietary fat. Since apoA-I has been demonstrated to be synthesized by the intestine and transported on chylomicra (4–8) and since the chylomicron protein-phospholipid coat has been shown to transfer into HDL (10, 11, 18), we investigated the possibility that the apoA-I derived from chylomicra may behave in a distinct fashion relative to the degree of dietary fat saturation. In addition, we examined the fate of apoA-II since it is the other major apoprotein of HDL and was found to be a major chylomicron apoprotein with the molar ratio of apoA-II/apoA-I of lymph chylomicra being greater than 1.

The amount of apoA-I transported on safflower oil-derived chylomicra, expressed as the apoA-I/TG mass ratio, was decreased relative to that of butter-derived chylomicra. Since the chylomicron particles were isolated from lymph during continuous infusion of isocaloric amounts of either saturated (butter) or unsaturated (safflower oil) fat, differences in the chylomicron apoA-I/TG ratio probably reflect differences in the relative propensity of the intestine to transport apoA-I on chylomicra. Since plasma apoA-I concentrations are lower,

TABLE 4. Whole body kinetic parameters of HDL apoA-I and A-II die-away[a]

Diet of Recipients	N	HDL A-I					HDL A-II				
		^{131}I-apoA-I[b]			^{125}I-apoA-I			^{131}I-apoA-II		^{125}I-apoA-II	
		Pool Size	FCR	Synthetic Rate	FCR	Synthetic Rate	Pool Size	FCR	Synthetic Rate	FCR	Synthetic Rate
		(mg/kg)	*(d^{-1})*	*(mg/kg/d)*	*(d^{-1})*	*(mg/kg/d)*	*(mg/kg)*	*(d^{-1})*	*(mg/kg/d)*	*(d^{-1})*	*(mg/kg/d)*
Butter	6	77[c] ± 2	0.343 ± 0.019	26.6 ± 2.0	0.320 ± 0.017	24.9 ± 1.8	19 ± 1	0.460 ± 0.035	8.7 ± 1.0	0.422 ± 0.040	7.8 ± 0.6
Safflower	6	64 ± 4	0.377 ± 0.026	24.1 ± 1.9	0.382 ± 0.023	24.4 ± 1.7	17 ± 1	0.510 ± 0.042	8.4 ± 0.9	0.508 ± 0.022	8.3 ± 0.3
Significance[d] Butter vs. safflower		$P < 0.01$	NS	NS	$P = 0.05$	NS	NS	NS	NS	NS	NS
Significance[e] A-I vs. A-II			$P < 0.01$		$P < 0.01$						
Significance[f] ^{131}I vs. ^{125}I			NS					NS			

[a] Values were calculated assuming a monoexponential specific activity die-away of HDL apoproteins and a metabolic steady state. Fractional catabolic rate (FCR) is the slope of the log-linear plots (Figs. 1 and 2) from days 0.5–5. Pool size was calculated as the plasma volume × plasma concentration of apoprotein. Plasma volume was estimated as 4% of body weight or by isotopic dilution of ^{125}I-HDL label at 10 min. Synthetic rate = fractional catabolic rate × pool size.

[b] ^{131}I count originated from ^{131}I-labeled chylomicra dose while ^{125}I count was from ^{125}I-labeled HDL dose.

[c] Values reported are mean ± S.E.M.

[d] Significant differences determined by Students' *t*-test. NS, not significant at $P = 0.05$.

[e] Significant differences determined by paired *t*-test of ^{131}I-apoA-I vs. ^{131}I-apoA-II or ^{125}I-apoA-I vs. ^{125}I-apoA-II.

[f] Significant differences determined by paired *t*-test of ^{131}I vs. ^{125}I-apoA-I or ^{131}I vs. ^{125}I-apoA-II. NS, not significant at $P = 0.05$.

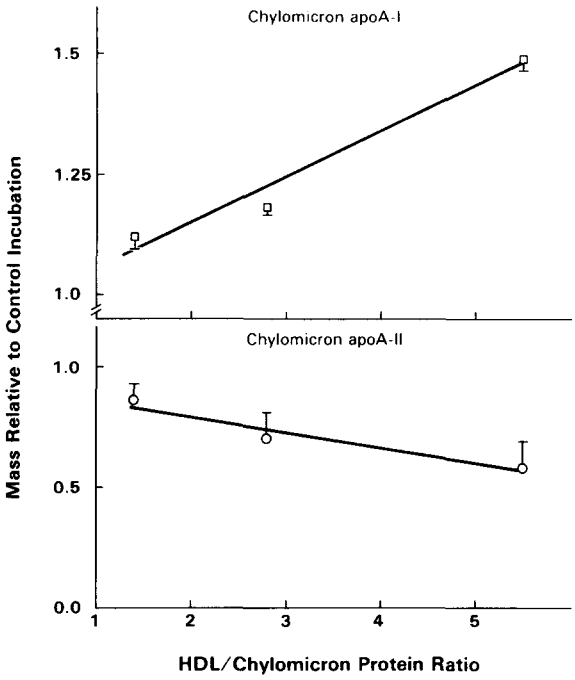

Fig. 3. Chylomicra apoA-I (top panel) and A-II (bottom panel) mass after in vitro incubation of chylomicra (0.2 mg protein) with increasing amounts of HDL (0.2–1.2 mg protein). After incubation at 37°C for 1 hr, chylomicra were separated from HDL by raising the density of the incubation medium (0.9% NaCl, 0.01% EDTA, 0.01% azide, pH 7.4) with 1 ml of d 1.225 g/ml solution and centrifuging for 120 min at 27,000 rpm in a SW 40 rotor at 15°C. Amounts of apoA-I and A-II were determined by radial immunoassay as described in the Materials and Methods section. All values expressed are relative to the control incubation in which no HDL protein was added. Four experiments were done using chylomicra and HDL from both dietary groups.

exchange of apoA-I from HDL onto chylomicra in lymph would also be expected to be lower and it seems that the decreased amount of apoA-I on chylomicra in safflower oil-fed animals accurately reflects decreased intestinal synthesis. To substantiate this conclusion, measurement of apoA-I transport rates in other lymph lipoproteins and estimation of the secretion rate into blood, as proposed by Windmueller, Herbert, and Levy (39), will need to be carried out.

We have also analyzed the die-away curves assuming a metabolic steady state and a monoexponential die-away rate. This analysis showed a trend towards a greater FCR for HDL apoproteins of polyunsaturated fat-fed recipients although only the difference in the FCR for ^{125}I-labeled apoA-I reached statistical significance. Calculated synthetic rates of HDL apoproteins were similar among recipients even though a decreased apoA-I/TG and apoA-II/TG ratio was found for the polyunsaturated vs. saturated chylomicron doses. This contradiction could occur if the kinetic model for whole body apoprotein metabolism is not sensitive enough to detect a selective effect on the rates of synthesis or catabolism in one tissue if other tissues are not similarly affected. ApoA-I is known to be synthesized in several tissues including liver, intestine, and kidney.[4] Since the apoA-I/triglyceride ratio in chylomicra was decreased in polyunsaturated fat-fed animals, perhaps an effect of the type of fat on intestinal production of apoA-I was present that did not occur in other tissues.

Taken together, our data suggest that the plasma HDL-lowering effect of polyunsaturated dietary fat may be mediated both by a decreased intestinal production and by an increased catabolism of HDL apoproteins. Additional studies are needed to determine if there are differences among individuals or diets that will further clarify the effects on synthesis or catabolism. For example, the saturated vs. unsaturated fat diets of the recipients in this study contained a relatively high cholesterol content (0.8 mg/kcal); dietary cholesterol and saturated fat have been shown to have contrasting effects on HDL concentrations depending on the individual African green monkey (17).

A second aspect of the study was the comparison of apoA-II turnover with that of apoA-I. No statistically significant difference due to dietary fat saturation was found in the plasma concentration of apoA-II, although the mean value for plasma apoA-II concentration in the safflower oil-fed group was lower by the same proportional amount, as was the case for the concentrations of HDL cholesterol, HDL mass, and apoA-I (Table 1). The lower apoA-II/triglyceride ratio in chylomicra of safflower oil-fed animals suggested that the apparent polyunsaturated fat-induced lowering of apoA-II concentration was real, but more data are needed to establish this point. The data are consistent in showing a different time course of chylomicron ^{131}I-labeled apoA-II movement into plasma HDL relative to that of chylomicron ^{131}I-labeled apoA-I (Figs. 1 and 2). The apoA-II appeared to be rapidly (almost instantaneously) transferred from injected chylomicron particles into HDL, from whence the ^{131}I-labeled apoA-II began to decay at a rate identical to that of the ^{125}I-labeled apoA-II of the simultaneously injected autologous HDL. Since autologous ^{125}I-labeled HDL was injected together with the chylomicron dose (^{131}I), the difference in the pattern of ^{131}I label in HDL relative to that of ^{125}I must represent net apoprotein transfer and not mere exchange. In contrast to the data for apoA-II, the ^{131}I-labeled apoA-I specific activity in HDL gradually rose during the first 1–3 hr after chylomicron injection, after which the ^{131}I-labeled apoA-I decayed from HDL at a rate identical to that of the ^{125}I-labeled apoA-I of the autologous HDL.

The peak in ^{131}I-labeled apoA-I specific activity in the HDL fraction from butter chylomicra was greater and

[4] Williams, D. L. Personal communication.

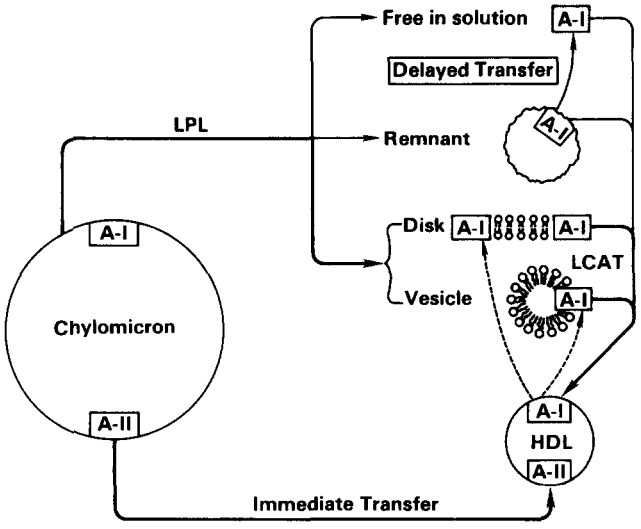

Fig. 4. Schematic diagram representing hypothetical metabolic fates of chylomicron apoA-I that distinguish it from the metabolic fate of chylomicron apoA-II in the plasma. Once chylomicra reach the plasma from lymph, apoA-II immediately (<1 min) transfers to plasma HDL. One consequence of apoA-II transfer into HDL is the displacement of some apoA-I (44) that is then transferred to the surface remnants to assist in LCAT mediated metabolism of these particles. The chylomicron particle is acted on by lipoprotein lipase (LPL) resulting in an immediate appearance in HDL of some chylomicron apoA-I via disk or vesicle formation from redundant surface (40). However, some chylomicron apoA-I also remains associated with chylomicra, remnant particles, and lipoproteins other than HDL and some exists free in the plasma for a finite period of time. This results in a delayed (1–3 hr) appearance in HDL of a fraction of chylomicron apoA-I. Darker lines depict rapid events (5 min), lighter lines indicate slower events (1–3 hr) and the dashed lines represent displacement of apoA-I from HDL by chylomicron apoA-II.

was sustained longer than that for safflower chylomicra (Figs. 1 and 2). The explanation for this difference is not known, but may be related to intrinsic chemical or physical differences between the two types of chylomicron particles. It also may be that this difference in the metabolism of saturated fat- vs. unsaturated fat-derived chylomicra is related to the relatively greater amount of surface material converted into HDL.

The integration of our findings with those of others can be summarized in the hypothetical pathways for apoA-I and apoA-II metabolism shown in **Fig. 4.** The in vivo behavior of ^{131}I-labeled apoA-II of chylomicra suggested that an immediate transfer into the HDL fraction may occur when chylomicra enter the circulation. On the other hand, the ^{131}I-labeled apoA-I of chylomicra did not appear to transfer immediately into HDL. Some of the chylomicron apoA-I may have remained associated with remnant particles formed during chylomicron clearance, thereby delaying appearance in the plasma HDL. Chylomicrons may have two domains of apoA-I, one that becomes associated with the surface remnants or vesicles (40) and one that remains associated with the core remnant, for example. In such a case, appearance of apoA-I originating in chylomicra would depend upon completion of metabolism of both remnant fractions. In support of such a possibility, El-Maghrabi et al. (41) have found apoA-I-enriched remnant particles that are isolated from in vitro incubation mixtures containing monkey chylomicrons and purified bovine lipoprotein lipase. Shepherd et al. (42) have presented evidence for two domains of apoA-I in plasma HDL. In addition, Redgrave and Small (18) have shown that 30% of the chylomicron non-apoB radioactivity remained associated with remnant fractions 30 min after injection into a hepatectomized rat. Thus, the possibility that some of the apoA-I remains associated with a type of chylomicron remnant particle is not unprecedented.

It is also possible that ^{131}I-labeled apoA-I may have been released from the chylomicron to exist free in plasma before association with HDL took place. Tall and co-workers (43) have shown that there is little difference in the thermodynamic stability of apoA-I in lipid-free form versus that associated with HDL. In contrast, apoA-I associated with phospholipid bilayers is four times more stable than either free apoA-I or apoA-I associated with HDL. These findings imply that it is possible for apoA-I that is not immediately transferred to HDL via disk or vesicle formation to exist free in solution for a finite period of time.

The finding that apoA-II had a significantly faster turnover rate in plasma HDL than did apoA-I suggests that the physiologic role of these two HDL apoproteins is different. Lagocki and Scanu (44) have shown that human apoA-II will displace apoA-I from canine HDL. Since we have found higher molar amounts of apoA-II relative to apoA-I on vervet chylomicra, and since the fate of chylomicron apoA-II once in the circulation was to move rapidly into HDL, it seems likely that chylomicron apoA-II may have displaced apoA-I from HDL, with the displaced apoA-I moving to its more thermodynamically stable location, namely chylomicron surface vesicles or disks formed during lipolysis of chylomicra (40). The fact that chylomicra can actually increase apoA-I content during in vitro incubation with HDL (Fig. 3), while losing apoA-II to HDL, attests to the feasibility of this hypothesis. The decay rate of apoA-II may then reflect the decay rate of HDL due to its apparent high affinity for association with HDL particles (44). In contrast, apoA-I would appear to have a more cyclic pathway in circulation. It would move onto phospholipid disks or vesicles where it could serve as a cofactor for LCAT during the metabolism and conversion of these particles into HDL. ApoA-II (from chylomicra) could then displace apoA-I, which would return to a disk or vesicle, and the process would be repeated. Were such a hypothesis to be correct, apoA-I turnover might be expected to be slower than apoA-II turnover, as was

observed (Table 4). It may be that in species such as the rat where little apoA-II is present in chylomicra, the behavior of apoA-I would be different because the apoA-II displacement of apoA-I from HDL would not occur.

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