Metabolism of larger high density lipoproteins accumulating in some families of baboons fed a high cholesterol and high saturated fat diet

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Abstract Progeny of certain baboon sires accumulate lipoproteins in high density lipoprotein-1 (HDL_1) when challenged with a high cholesterol, high saturated fat diet. These studies were conducted to determine the apoprotein composition and metabolic fate of HDL_1 in the plasma. HDL_1 particles containing apoA-I with and without apoE were detected. The majority of particles, however, contained apoA-I without any detectable apoE. To determine the metabolic fate of HDL_1 in plasma, HDL_1 labeled with iodinated apoA-I from animals with high levels of HDL_1 and iodinated apoA-I-labeled autologous HDL were coinjected into both high and low HDL_1 animals. The data for the decay of radioactivity in HDL_1 and HDL were analyzed by multicompartment modelling. The radioactivity from HDL_1 was cleared from the plasma either via direct removal (9.1 ± 4.7% in low and 21.7 ± 8.3% in high HDL_1 animals) or via its conversion to HDL. A large proportion of radioactivity from HDL_1 was rapidly transferred to HDL directly or metabolized via an intermediate compartment. Most of the radioactivity from apoE-poor HDL_1, however, was transferred to HDL. Both high and low HDL_1 animals catabolized HDL_1 and HDL similarly. Low HDL_1 animals transferred HDL_1 radioactivity to HDL much faster. No detectable radioactivity from HDL was transferred to HDL_1. Thus, HDL_1 that accumulates in high HDL_1 animals is mainly a precursor for HDL. Our hypothesis is that this accumulation of HDL_1 is due to the slower cholesteryl ester transfer from HDL to lower density lipoproteins, thus affecting reverse cholesterol transport in high HDL_1 baboons.—**Kushwaha, R. S., D. M. Foster, V. N. Murthy, K. D. Carey, and H. C. McGill, Jr.** Metabolism of larger high density lipoproteins accumulating in some families of baboons fed a high cholesterol and high saturated fat diet. *J. Lipid Res.* 1989. **30:** 1147–1159.

Supplementary key words cholesterol • high fat diet • lipoprotein metabolism

Selective breeding of baboons by positive assortative mating of high and low responders to an atherogenic diet has produced families of baboons with distinctive lipoprotein phenotypes (1,2). The response of the serum cholesterol concentration to a fat- and cholesterol-enriched diet of these baboons is heritable (3,4), and genetic analyses have shown major gene effects on LDL cholesterol and HDL cholesterol concentrations (5). Most of these dyslipoproteinemic patterns are modulated by diet as well as by pedigree. One dyslipoproteinemia is characterized by the presence of larger high density lipoproteins (HDL), intermediate to low density lipoproteins (LDL), and HDL. These unusual lipoproteins, called HDL_1 due to their density and particle size (originally termed $F_{1.20}$ 9-28 by Nichols et al. [6]), contain apolipoprotein (apo) A-I, apoE, and apoC, but not apoB (7). HDL_1 appears when the dyslipoproteinemic baboons (baboons with high HDL_1) are challenged with dietary saturated fat and cholesterol, and disappear when they are fed polyunsaturated fat with or without cholesterol, or chow, which is low in fat and cholesterol (8). The objective of these studies was to characterize the heterogeneity of HDL_1 and to determine its metabolic fate in the plasma of dyslipoproteinemic (high HDL_1) and control (low HDL_1) baboons.

METHODS

Animals and diets

Heterogeneity studies. Six adult (7- to 15-year-old) male baboons (*Papio* sp.) were used for characterizing heterogeneity of lipoproteins. Three of these were feral animals (X-832, X-1946, and X-2887). The other three animals were progeny of sire X-102 (X-2033, X-2036, and X-

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HPLC, high performance liquid chromatography.

3475). Two of the progeny of X-102 were also used for turnover studies. These animals were maintained on a cholesterol- and fat-enriched diet as described below.

Turnover studies. Eight adult (5- to 7-year-old) baboons were used for the turnover studies. Three baboons with high HDL_1 were progeny of sire X-102, a sire with high plasma levels of HDL_1. This sire had transmitted the trait to many of his progeny. We selected five animals with low levels of plasma HDL_1 from the progeny of other sires and dams. The lipoprotein profiles of representative animals with high and low HDL_1 are shown in **Fig. 1**. Their plasma cholesterol values and other characteristics are given in **Table 1**. The high HDL_1 animals selected for these studies were negative for LP[a].

In vitro studies. One baboon with high HDL_1 (X-3475) and another with low HDL_1 (X-3566), used for turnover studies, were also used for in vitro studies.

Diet. Since HDL_1 was induced on a high cholesterol and high saturated fat diet (1), all animals were fed this diet for at least 10 weeks prior to the turnover studies and maintained on the same diet throughout these studies. The composition of the HCHF diet is given in **Table 2**. Saturated fat was provided from lard and USP cholesterol was added to provide 1.7 mg cholesterol/kcal.

The protocol for this experiment was approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research.

Heparin-Sepharose chromatography of HDL_1

To determine compositional heterogeneity of HDL_1, the plasma from high HDL_1 animals was fractionated by density gradient ultracentrifugation using an SW-41 Ti rotor and a Beckman ultracentrifuge model L5-70 (Beckman Co., Palo Alto, CA). The density gradient fractionation was a modification of the method described by Redgrave, Roberts, and West (9) and described by us earlier (8). Five fractions (0.4 ml each) between d 1.045 and 1.090 g/ml were dialyzed against 0.15 M NaCl and further

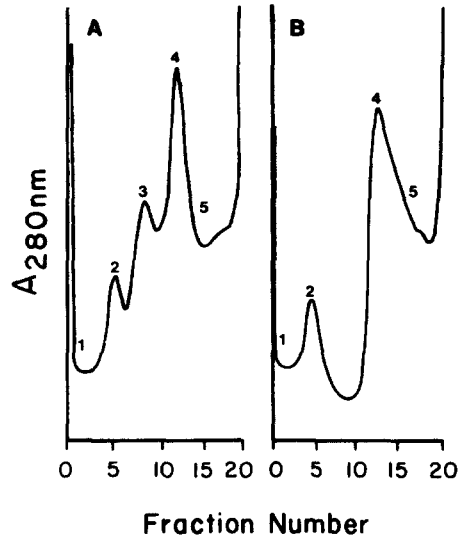

Fig. 1. Plasma lipoprotein patterns separated by density gradient ultracentrifugation and monitored by absorbance at 280 nm for two baboons maintained on high cholesterol and high saturated fat diets and used for metabolic studies. Lipoprotein pattern A is for baboon X-3475, which had high plasma HDL_1, and the lipoprotein pattern B is for baboon X-3566, which had low plasma HDL_1. The numbers 1, 2, 3, 4, and 5 represent VLDL, LDL, HDL_1, HDL_2, and HDL_3, respectively.

TABLE 1. Characteristics of baboons used for metabolic studies

	Serum Cholesterol		Lipoprotein Cholesterol				
			VLDL + LDL		HDL		
Phenotype and Baboon No.	Chow	HCHF[a]	Chow	HCHF	Chow	HCHF	Weight
	mg/dl		*mg/dl serum*				*kg*
High HDL_1[b]							
X-1337	162	220	63	94	99	126	30.6
X-2033	160	265	61	147	99	118	20.7
X-3475	195	398	96	205	99	103	24.3
Low HDL_1							
X-1555	101	175	33	54	68	121	32.7
X-2811	110	214	51	122	59	92	26.8
X-3566	81	236	32	161	49	75	16.6
X-3349	98	135	45	50	53	85	29.0
X-3705	105	220	38	109	67	111	28.4

[a]High cholesterol, high saturated fat diet.
[b]High HDL_1 baboons had significantly ($P < 0.005$) higher cholesterol in plasma and HDL on chow diet. Both groups had a significant ($P < 0.025$) increase in cholesterol in plasma and lipoproteins on the HCHF diet.

TABLE 2. Composition of chow and high-cholesterol, high-fat (HCHF) diets

Nutrients	Chow Diet	HCHF Diet[a]
Carbohydrates (% cal)	62	40
Protein (% cal)	28	20
Fat (% cal)	10	40
Energy (kcal per 100 g diet)	329	377
Cholesterol (mg/kcal)	0.03	1.7

[a]HCHF diet was prepared by mixing 81.4% (dry weight basis) of Purina monkey meal 5-5045-6 (a special mix with no added fat, dehydrated alfalfa, sodium chloride, ascorbic acid, or retinyl acetate) with lard (16.5%), sodium chloride (1.1%), retinyl acetate (0.005%), ascorbic acetate (0.2%), and cholesterol (0.74%).

separated by heparin-Sepharose chromatography as described by Huff and Telford (10) for VLDL. The sample was loaded with 0.15 M NaCl onto a heparin-Sepharose column (1 × 30 cm). After loading the column, the lipoproteins were allowed to interact with the column overnight. The lipoproteins were eluted first with 0.15 M NaCl and then with a 1.0 M NaCl solution. Generally, two peaks were obtained. The first peak had lipoproteins that did not bind to the column. The second peak contained lipoproteins that were bound to the heparin-Sepharose column and were eluted by high salt concentration. Fractions corresponding to these peaks were pooled and lyophilized after dialysis against ammonium bicarbonate buffer (pH 8.0). Fractions were then delipidated with chloroform–methanol 2:1 and separated on 10% SDS polyacrylamide slab gels by the method of Weber and Osborn (11). The gels were stained with Coomassie blue and destained with 5% acetic acid.

Isolation of HDL_1, HDL, and apoA-I

To isolate HDL_1 and HDL for labeling and reinjection, fasting (12–14 h) baboons were immobilized with ketamine hydrochloride (10 mg/kg) and 20 ml blood was collected from each animal into tubes containing EDTA (1 mg/ml). For isolation of HDL_1 and HDL, the blood obtained from high and low HDL_1 animals was pooled separately. Plasma obtained by low speed centrifugation was adjusted to d 1.045 g/ml by adding solid KBr and ultracentrifuged in a Beckman ultracentrifuge L5-50, using a 50 Ti rotor, for 20 h at 44,000 rpm at 7°C. After ultracentrifugation, lipoprotein of $d < 1.045$ g/ml were removed by slicing the top 3-ml layer. Since baboon LDL has a density of 1.019–1.45 g/ml, the lipoproteins of $d < 1.045$ g/ml contain all apoB-containing lipoproteins. The infranatant was ultracentrifuged again at d 1.045 g/ml to remove contaminating lipoproteins. The infranatant after washing was adjusted to d 1.070 g/ml by adding solid KBr and ultracentrifuged at 44,000 rpm and 7°C for 22 h using a 50 Ti rotor in a Beckman L5-50 ultracentrifuge. Following ultracentrifugation, the top 3-ml layer containing HDL_1 was obtained by tube slicing. The HDL_1 fraction (d 1.045–1.070 g/ml) thus obtained was layered with a KBr solution of d 1.070 g/ml, ultracentrifuged, and the top 3-ml layer containing HDL_1 was obtained by tube slicing. The HDL_1 fraction (d 1.045–1.070 g/ml) thus obtained was again layered with KBr solution of d 1.070 g/ml and ultracentrifuged under similar conditions to remove contaminating lipoproteins of $d > 1.070$ g/ml. The tubes were sliced and the top 3-ml layer containing pure HDL_1 was obtained. Since the HDL_1 fraction from low HDL_1 baboons had no detectable protein, it was not used and was discarded. For isolation of HDL, the infranatant ($d > 1.070$ g/ml) after slicing of HDL_1 was used. The density was adjusted to 1.21 g/ml by adding solid KBr and the sample was ultracentrifuged for 22 h at 40,000 rpm. After ultracentrifugation the top 3-ml layer was obtained by slicing. The HDL (d 1.070–1.21 g/ml) was washed once by layering with KBr solution of d 1.21 g/ml and ultracentrifuged under similar conditions to remove contaminating lipoproteins. HDL_1 and HDL fractions were dialyzed to remove salt and analyzed for protein content by the method of Lowry et al. (12).

To isolate apoA-I, HDL was delipidated initially by ethanol–ether 3:1, followed by ethanol–ether with increasing proportions of ether, and finally with ether only. The delipidated HDL was dried under nitrogen. Delipidated HDL was dissolved in 0.01 M Tris buffer (pH 8.0) containing 8 M urea, and was fractionated on a DEAE-Bio-Gel column (2.5 × 7.4 cm) as described by Shore and Shore (13). The purity of apoA-I was verified by SDS polyacrylamide gels (11). The purified apoA-I was then iodinated by both ^{125}I and ^{131}I as described below.

Labeling of HDL, HDL_1, apoE-poor HDL_1 and apoA-I

Purified apoA-I was iodinated by the iodine monochloride method of McFarlane (14) as modified by Bilheimer, Eisenberg, and Levy (15) and described by us for rabbit (16), monkey (17), and human (18) HDL. ApoA-I labeled with ^{125}I was incubated with HDL_1 (isolated from HDL from high HDL_1 animals) and the ^{131}I-labeled apoA-I was incubated with HDL (separately from high and low HDL_1 baboons). The lipoproteins were adjusted to their respective densities and ultracentrifuged and dialyzed prior to injection into baboons. In another experiment, apoE-poor HDL_1 and HDL with labeled apoA-I were used for turnover studies. For the preparation of apoE-poor HDL_1, the apoA-I labeled with ^{125}I was incubated with HDL_1 isolated from high HDL_1 baboons. The HDL_1 was reisolated by ultracentrifugation to remove the apoA-I that was not associated with lipoprotein particles. The HDL_1 was dialyzed to remove salt and separated by heparin-Sepharose chromatography as described above. The unbound fraction, which had no detectable apoE, was pooled, dialyzed, and used for reinjection. The HDL was

incubated with apoA-I labeled with ^{131}I and reisolated and dialyzed prior to reinjection.

To characterize the radioactivity in HDL_1 and HDL incubated with labeled apoA-I, the fractions were precipitated with trichloroacetic acid (TCA) (12% final concentration) and centrifuged to remove the soluble fraction. The precipitate was washed with 5% TCA and extracted with acetone–ether 1:7 and dried in a tube in boiling water. The apoproteins were separated on 10% SDS polyacrylamide slab gels by the method of Laemmli (19). The gels were exposed to X-ray film and kept at −80°F for a few days. Afterwards, the gels were stained and the radioactivity in the apoprotein bands was compared. All the radioactivity (>95%) was associated with the apoA-I band. The radioactivity in apoE-poor (unbound) and apoE-rich (bound) fractions was determined by counting small aliquots. Most of the radioactivity (70%) was present in the unbound or apoE-poor fraction.

Turnover procedures

Autologous (their own HDL_1 and HDL) and heterologous (HDL_1 from high HDL_1 animals injected into low HDL_1 animals) fractions (HDL_1 or HDL) were coinjected into baboons with high or low HDL_1 via the femoral vein. The animals were maintained on a tether system to facilitate bleeding without the use of anesthesia. Prior to the injection of iodinated lipoproteins, animals were given potassium iodide in their drinking water (0.1%) for 3 days to avoid thyroid uptake of ^{125}I and ^{131}I. HDL_1 or apoE-poor HDL_1 from high HDL_1 animals had ^{125}I-labeled apoA-I, whereas the HDL from either high HDL_1 or low HDL_1 animals had apoA-I labeled with ^{131}I. ^{125}I-Labeled HDL_1 (from high HDL_1 baboons) and ^{131}I-labeled HDL (their own) were coinjected into both high and low HDL_1 animals. Each animal was injected with 0.2–0.5 mg of HDL_1 or HDL protein containing 20–50 μCi of radioactivity. After the injections, blood samples (6 ml) were drawn at 5 min, 0.5, 1, 3, 6, 10, 24, 48, 72, 96, 120, and 144 h from the femoral vein via the tether system.

Blood samples from each time were centrifuged to obtain plasma. A small sample of plasma (100 μl) was used to count the total radioactivity using a Searle gamma counter (Nuclear-Chicago, Des Plaines, IL). Lipoproteins were separated by density gradient ultracentrifugation using an SW 41 Ti rotor in Beckman L5-50 and L8-70 ultracentrifuges. The density gradient procedure was a modification of the method of Redgrave et al. (9). The refractive index was measured and the fractions were pooled on the basis of density as described above. After the separation of lipoproteins, the radioactivity in each lipoprotein fraction and in the d > 1.21 g/ml fraction was measured using a small sample (100 μl). To avoid quenching due to high salt concentration, the lipoprotein samples of d > 1.02 g/ml were diluted with water tenfold. The raw radioactivity counts were corrected for overlap of ^{131}I counts into the ^{125}I channel and for isotopic decay.

In vitro experiments

To determine the exchange of labeled apoA-I from HDL_1 and HDL into the plasma, HDL_1 and HDL fractions were incubated with plasma from both high and low HDL_1 animals for 0.5 h. After incubation, the lipoproteins were separated by density gradient ultracentrifugation and the radioactivity in each fraction (0.4 ml) was counted.

Lipid, lipoprotein, and apolipoprotein analyses

Serum cholesterol concentrations were measured by an enzymatic method (20) using a BMC-Autoflo reagent (Boehringer-Mannheim, Indianapolis, IN) with an ABA 100 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, CA). The cholesterol in VLDL + LDL and HDL was measured by the Lipid Research Clinics procedure (21). ApoA-I was measured by electroimmunoassay (22) using monospecific antibody aganst baboon apoA-I prepared in rabbits and purified apoA-I as standards. Total protein in lipoproteins was measured by the method of Lowry et al. (12).

The presence of HDL_1 in the plasma of baboons was detected by a combination of density gradient ultracentrifugation as described above and high performance gel exclusion chromatography (HPLC). The HPLC was performed using a Waters Associates HPLC model 204 with pump (model 6000A) and UV detector (model 440) with automatic injector as described earlier (2). A combination of gel permeation columns, TSK4000PW and TSK3000PW (600 × 7.5 mm), was used with a flow rate of 0.2 ml/min (2).

Kinetic analysis

The turnover data were analyzed using the SAAM computer program (23) on a MicroVAX-II (Digital Equipment Corporation) and the multicompartmental model (24) shown in **Fig. 2**. Since HDL_1 and HDL were coinjected, the data were analyzed simultaneously. In the development of the model, the HDL data were analyzed first. It was found that the two-pool model shown in Fig. 2, consisting of a plasma compartment, C(2), exchanging with an extravascular compartment, C(3), was sufficient to describe the HDL turnover data. All loss of labeled material occurred from C(2).

To analyze the HDL_1 turnover data, the rate constants for the HDL portion of the model were fixed equal to those values found for the HDL turnover studies. Since radioactivity appeared rapidly in HDL when HDL_1 was injected, C(10) was introduced as a rapidly turning over compartment which contained all initial label and from

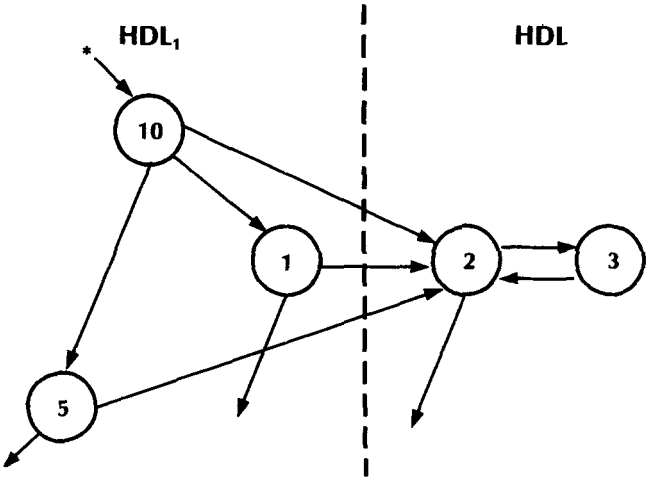

Fig. 2. The multicompartmental model used to analyze the turnover data. The compartments are drawn in circles and the arrows show the direction of movement of labeled materials. The arrows represent the rate constants of the model as summarized in Table 4. Thus, the arrow from compartment C(1), to C(2) is denoted L(2,1). Compartments (10), (1), and (5) sum to give the model-predicted radioactivity in HDL_1 while compartment (2) is the model-predicted radioactivity in HDL. Compartment (3) is an extravascular compartment exchanging with compartment (2). When HDL_1 is injected, all initial radioactivity is assigned to compartment (10); when HDL is injected, all initial radioactivity is assigned to compartment (2). See text for additional explanation.

which radioactivity was rapidly transferred to C(2) and to the two compartments describing the remainder of HDL_1 kinetics, C(1), C(5), or both. No transfer of radioactivity from HDL to HDL_1 was observed. The radioactivity isolated in HDL_1 when HDL was injected paralleled the HDL decay curve and could be described as approximately 5% contamination of HDL in HDL_1.

With the HDL_1 structure as given in Fig. 2 added to the HDL structure, all data were analyzed simultaneously using this model and the rate constants were allowed to adjust to obtain the best fit of the data. Residence times of ^{125}I- and ^{131}I-labeled lipoproteins in the HDL_1 region and HDL_1 in the plasma were estimated from the model described in Fig. 2. The steady state input into C(10) was estimated for unit mass in the HDL_1 region. The inverse of this gave the residence times. For HDL, the residence times were calculated from the equation given below:

Residence Time = IC(2)/L(0,2) + IC(4)/L(0,4), where IC(X) is the initial radioactivity in compartment X and L (0,X) is the rate constant describing radioactivity leaving compartment X.

The model described in Fig. 2 was also used to analyze the data for the turnover studies of apoE-poor HDL_1 (labeled with ^{125}I-labeled apoA-I). There was very little radioactivity transferred from HDL to HDL_1, and therefore these data were not analyzed.

Statistical analyses

Metabolic variables from the kinetic analysis were expressed as mean and standard deviation when appropriate. The kinetic variables of high and low HDL_1 animals were compared using the *t*-test.

RESULTS

Heterogeneity of HDL_1

To determine the apoprotein heterogeneity of HDL_1, plasma samples from six animals with high HDL_1 were separated by density gradient ultracentrifugation. Four

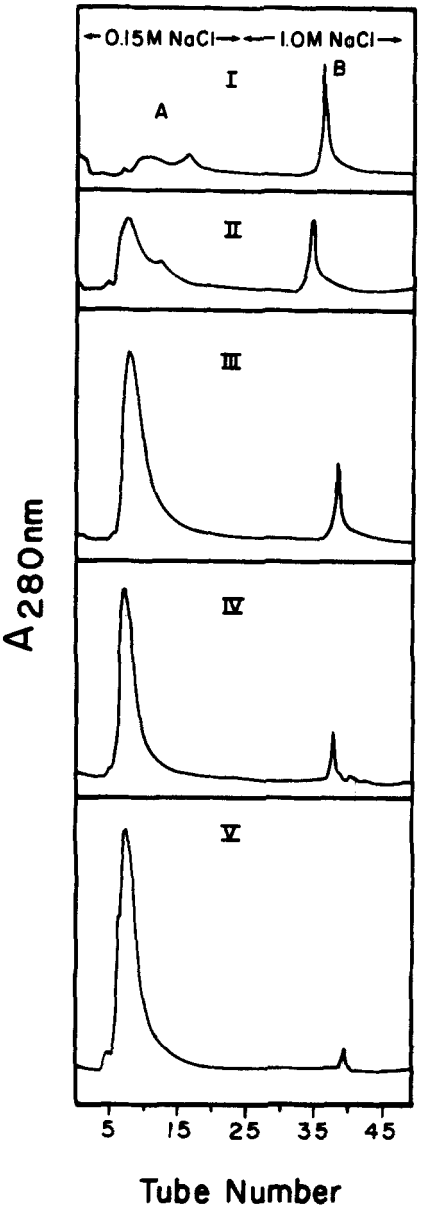

Fig. 3. Heparin-Sepharose fractionation of five fractions (I–V) of plasma lipoproteins between d 1.045 and 1.070 g/ml from a representative baboon with high HDL_1 fed a high cholesterol and high saturated fat diet. The samples were loaded with 0.15 M saline. Peak A shows the lipoproteins that did not bind to the column. Peak B shows the bound lipoproteins after elution by 1.0 M saline. The absorbance at 280 nm was recorded.

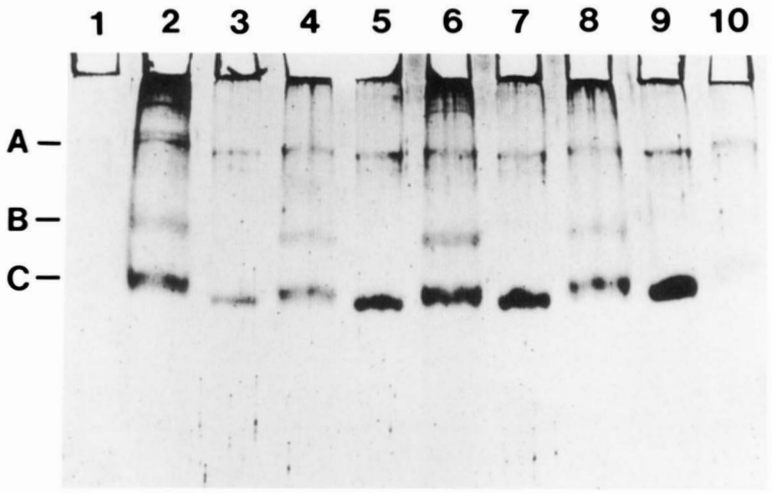

Fig. 4. SDS-electrophoresis of apoproteins from HDL_1 subfractions separated by heparin-Sepharose chromatography. Lanes 1, 3, 5, 7, and 9 represent HDL_1 subfractions that were not bound to the heparin-Sepharose column for fractions 1, 2, 3, 4, and 5, respectively. Lanes 2, 4, 6, 8, and 10 represent HDL_1 subfractions that were bound to the heparin-Sepharose column for fractions 1, 2, 3, 4, and 5, respectively. A, B, and C bands correspond to albumin, apoE, and apoA-I, respectively. ApoA-I and apoE were recognized by antibodies against these apoproteins on similar gels and albumin was detected by its comigration with pure standard of baboon serum albumin.

fractions of HDL_1 between LDL and HDL (d 1.045–1.070 g/ml) and one fraction corresponding to lighter HDL_2 (d < 1.080 g/ml) were collected. Each fraction from two of the animals having higher amounts of HDL_1 was further fractionated by heparin-Sepharose chromatography. As shown in **Fig. 3**, most of the lipoprotein from the first fraction was bound to the column and eluted with 1.0 M NaCl. A small amount of this fraction was not bound to the heparin-Sepharose. As the density of the fraction increased, the proportion of lipoproteins bound to the column decreased. The fourth fraction of HDL_1 had only a small amount of lipoprotein bound to the column. The HDL fraction had virtually no binding to the column. Both bound and unbound fractions were subjected to SDS polyacrylamide gel electrophoresis. As shown in **Fig. 4**, the bound fractions had both apoA-I and apoE. The unbound fractions, on the other hand, had no detectable apoE and had apoA-I as their major apoprotein. The bound fractions had apoA-I and apoE in approximately equal proportions as judged by the uptake of Coomassie blue by the protein bands. Thus, the HDL_1 fraction had apoA-I as its major apoprotein. The lighter HDL_1 particles included apoE-containing particles, which also contain apoA-I. It seems unlikely that HDL_1 from high HDL_1 baboons had any appreciable subpopulation with only apoE as its apoprotein. Although apoA-I is the major apoprotein of HDL_1, the relative proportions of apoE and apoA-I vary with the size of the HDL_1 particles.

Catabolism of apoA-I-labeled HDL_1 and HDL

The decay of ^{125}I-labeled apoA-I from HDL_1 and its appearance in HDL, and the decay of ^{131}I-labeled apoA-I from HDL in a representative high HDL_1 animal are given in **Fig. 5** and in a low HDL_1 animal in **Fig. 6**. Whereas in both types of animal the decay of ^{125}I-labeled HDL was similar, the decay of HDL_1 from the low HDL_1 animal was much faster than in the high HDL_1 animal. In both there was a substantial amount of radioactivity in HDL appearing from HDL_1 at the early time points; this is material that arrives from C(10).

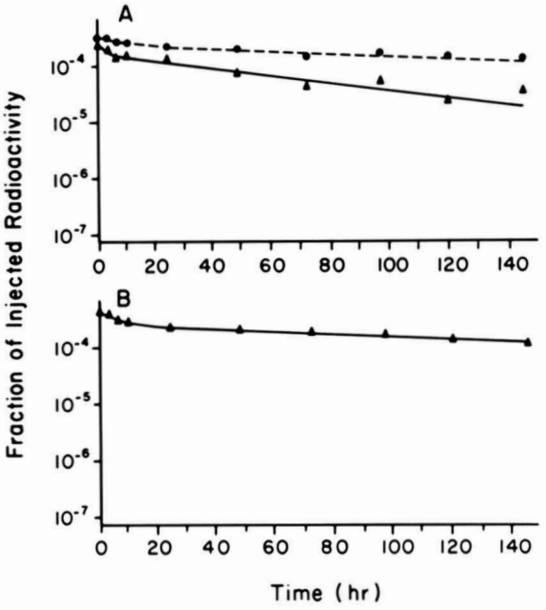

Fig. 5. Decay of radioactivity from HDL_1 (A) and HDL (B) when apoA-I labeled fractions were injected into an animal (X-2033) with high HDL_1. The radioactivity from HDL_1 (▲—▲) decays rapidly and appears in HDL (●---●).

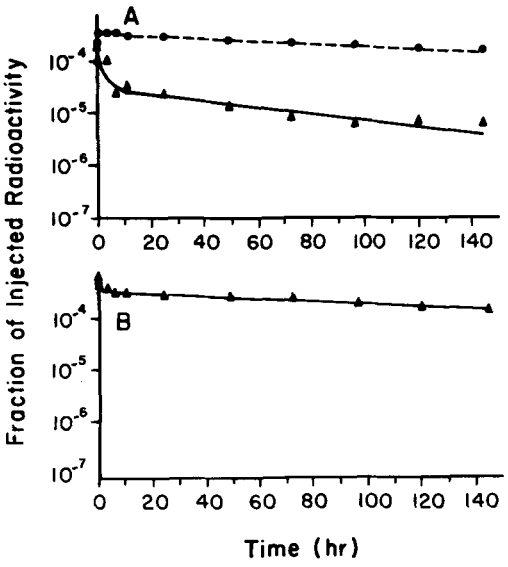

Fig. 6. Decay of radioactivity from HDL_1 (A) and HDL (B) when apoA-I-labeled fractions were injected into an animal (X−2811) with low HDL_1. The radioactivity from HDL_1 (▲—▲) decays rapidly and appears in HDL (●---●).

The kinetic parameters estimated from the model are summarized in Tables 3–6. The estimated residence times are given in **Table 3**, the rate constants for each animal are given in **Table 4**, and the relative rates of transfer from HDL_1 to HDL are summarized in **Table 5**. The percent transfer from C(10) to C(1), C(2), and C(5) for each animal is summarized in **Table 6**. In Table 4, L(2,1) was zero for some animals, while in others (L(2,5) was zero. These were dictated by the nature of the three curves being analyzed simultaneously.

Both high and low HDL_1 animals catabolized HDL_1 in a similar way. The HDL_1 had a very short residence time as compared to HDL (Table 3). High HDL_1 baboons had longer residence time for HDL_1 (20.1 ± 2.6 h) than that for low HDL_1 baboons (13.6 ± 9.8 h). Most of the radioactivity from HDL_1 was transferred to HDL (Table 5) in both high (76.6 ± 3.0%) and low (89.4 ± 18.3%) HDL_1 baboons. There was no difference in the catabolism of HDL (either from HDL_1 or injected as HDL) between high and low HDL_1 baboons.

Catabolism of apoA-I labeled apoE-poor HDL_1 and HDL

The decay of ^{125}I-labeled apoA-I-labeled apoE-poor HDL_1 and ^{131}I-labeled apoA-I-labeled HDL in high and low HDL_1 animals was similar to that in Figs. 5 and 6. The decay of ^{131}I-labeled apoA-I-labeled HDL was described by C(2) and C(3), the extravascular compartment. The decay of ^{125}I-labeled apoA-I-labeled HDL_1 was described by C(10), C(1) and C(5). A large fraction of the injected apoE-poor HDL_1 was rapidly transferred from C(10) to (C(2) (HDL). In the model in Fig. 2, C(1) is a fast compartment and C(5) is a more slowly turning over compartment. From these data, only the total turnover rate could be determined for C(1) and therefore, as a constraint, L(0,1) was set to zero, and all the radioactivity from C(10) was transferred to the HDL [C(2)]. The decay of ^{125}I-labeled apoA-I in HDL was similar to that of ^{131}I-labeled apoA-I-labeled HDL as for the previous experiment.

Metabolic fate of HDL_1

Table 5 summarizes the metabolic fate of HDL_1 from high HDL_1 animals when injected into both high and low HDL_1 animals. A small amount of radioactivity was transferred to C(5) which decayed rapidly without going into HDL to a great extent. There was a slightly higher radioactivity transfer to C(5) in high HDL_1 animals than that in low HDL_1 when apoA-I-labeled HDL_1 was injected (9.1 ± 4.7% in low HDL_1 vs. 21.7 ± 8.3% in high HDL_1 animals). The radioactivity transferred to C(1) (from where the radioactivity was either transferred to HDL or decayed directly) was similar in both high and low HDL animals when apoA-I-labeled HDL_1 was injected. The radioactivity transferred to HDL C(2) was higher than that transferred to either C(1) or C(5) in both high and low HDL_1 baboons. ApoE-poor HDL_1 turnover studies suggest that most (>70%) of the radioactivity was

TABLE 3. Residence times of apoA-I in HDL_1 and HDL after injection of iodinated apoA-I-labeled HDL_1, and apoA-I-labeled HDL

Phenotype and Baboon No.	Residence Time (h)	
	HDL	HDL_1
	ApoA-I-Labeled HDL_1	
High HDL_1		
X-1337	106.5 (14)[a]	19.4 (7)
X-2033	89.1 (11)	22.9 (5)
X-3475	103.2 (12)	17.9 (15)
Low HDL		
X-1555	114.8 (11)	5.0 (6)
X-2811	81.3 (9)	11.4 (7)
X-3566	74.3 (7)	24.3 (20)
	ApoA-I-Labeled/ApoE-Poor HDL_1	
High HDL_1		
X-2033	62.7 (5)	4.4 (6)
X-3475	83.3 (5)	14.1 (4)
Low HDL_1		
X-3349	102.3 (7)	3.5 (8)
X-3705	133.7 (72)	5.2 (6)

[a]Standard deviation in percent.

TABLE 4. Rate constants for the model

Rate Constants	Baboon Number									
	1337[a]	2033[a]	3475[a]	1555[b]	2811[b]	3566[b]	2033[a,c]	3475[a,c]	3705[b]	3349[b]
	pools/h									
L(1,10)	8.11(27)[d]	11.9(12)	24.3(19)	20.4(7)	18.1(8)	11.7(17)	5.89(20)	5.11(17)	2.87(27)	4.85(32)
L(5,10)	13.4(12)	13.1(7)	6.04(13)	2.66(10)	7.19(9)	3.85(8)	4.11(32)	9.94(7)	3.73(20)	5.79(7)
L(2,10)	28.5(6)	25.0(5)	19.6(24)	26.9(5)	24.7(6)	34.4(5)	40.00(3)	34.96(2)	43.4(1)	39.4(4)
L(0,1)	0.12(144)	0.26(61)	0.29(123)	0(-)	0(-)	0.79(29)	1.70(32)	0.20(41)	0.17(22)	2.75(55)
L(2,1)	0.14(118)	0.16(79)	0.83(47)	0.55(29)	0.46(30)	0(-)	0(-)	0(-)	0(-)	0(-)
L(0,5)	0.006(78)	0.004(73)	0.007(13)	0(-)	0(-)	0.003(23)	0.019(5)	0.015(5)	0.02(12)	0.022(3)
L(2,5)	0.008(61)	0.007(44)	0(-)	0.013(9)	0.014(6)	0(-)	0(-)	0(-)	0(-)	0(-)
L(0,2)	0.009(14)	0.01(11)	0.01(12)	0.009(11)	0.01(9)	0.013(7)	0.016(5)	0.10(5)	0.012(7)	0.007(72)
L(3,2)	0.05(52)	0.46(47)	0.05(56)	0.07(99)	0.10(56)	0.14(21)	0.04(42)	0.03(55)	0.142(66)	0.01(31)
L(2,3)	0.08(42)	0.95(39)	0.09(46)	0.20(86)	0.04(60)	0.45(19)	0.081(41)	0.10(54)	0.37(58)	0.011(110)

[a]High HDL_1 baboon.
[b]Low HDL_1 baboon.
[c]High HDL_1 baboons were used again for apoE-poor HDL_1 turnover studies.
[d]Standard deviation in percent.

transferred to C(2) or HDL. Since most of the radioactivity appeared in HDL, especially from the apoE-poor HDL_1, the results suggest that apoE-poor HDL_1 is a precursor of HDL.

In vitro experiments

To determine whether HDL_1 and HDL (which were labeled with iodinated apoA-I) were contaminated with other lipoproteins, HDL_1 and HDL (100 μl with a radioactivity of 0.15–0.5 μCi) were incubated with 2 ml of plasma for 0.5 h. After incubation, the plasma lipoproteins were separated by density gradient ultracentrifugation and the absorbance at 280 nm was monitored. Each fraction was counted to determine the radioactivity. As shown in **Fig. 7**, most of the radioactivity of HDL_1 was recovered in the HDL_1 region in the plasma of both high and low HDL_1 animals. Some of the radioactivity was

TABLE 5. Rates of transfer of HDL_1 when apoA-I-labeled HDL_1 from high HDL_1 animals was injected

Phenotype and Baboon No.	Rate of Transfer (mass/h)				
	R(2,10)	R(2,1)	R(2,5)	U(10)	$\frac{R(2,1) + R(2,5) + R(2,10)}{U(10)} \times 100$
	ApoA-I-Labeled HDL_1				
High HDL_1					
X-1337	0.03(10)[a]	0.004(100)	0.008(62)	0.052(7)	80.18%
X-2033	0.022(8)	0.004(74)	0.007(44)	0.044(5)	75.00%
X-3475	0.022(28)	0.020(41)	0(-)	0.056(15)	75.00%
Low HDL_1					
X-1555	0.11(9)	0.082(30)	0.011(9)	0.203(6)	100.00%
X-3566	0.028(20)	0(-)	0(-)	0.041(21)	68.30%
X-2811	0.044(8)	0.032(25)	0.013(6)	0.089(6)	100.00%
	ApoA-I-Labeled/ApoE-Poor HDL_1				
High HDL_1					
X-2033	0.18(6)	0(-)	0(-)	0.23(6)	78.30%
X-3475	0.05(4)	0(-)	0(-)	0.072(4)	69.40%
Low HDL_1					
X-3349	0.24(10)	0(-)	0(-)	0.282(10)	85.10%
X-3705	0.15(6)	0(-)	0(-)	0.19(6)	78.90%

[a]Standard deviation in percent.

TABLE 6. Metabolic fate of HDL_1

Phenotype and Baboon No.	Transfer (%) To C(5)	To C(1)	To C(2)
	ApoA-I-Labeled HDL_1		
High HDL_1			
X-1337	26.8	16.2[a]	57.0[b]
X-2033	26.2	23.8	50.0
X-3475	12.1	48.7	39.3
Low HDL_1			
X-1555	5.3	40.9	53.9
X-2811	14.4	36.2	49.4
X-3566	7.7	23.4	68.9
	ApoA-I-Labeled/ApoE-Poor HDL_1		
High HDL_1			
X-2033	8.2	11.8	80.0
X-3475	19.9	10.2	69.9
Low HDL_1			
X-3499	11.6	9.7	78.8
X-3705	7.5	5.7	86.8

[a]Values are significantly higher ($P < 0.01$) for apoA-I-labeled HDL_1 than those for apoA-I-labeled/apoE-poor HDL_1 (both high and low HDL_1 animals were grouped together).

[b]Values are significantly lower ($P < 0.01$) for apoA-I-labeled HDL_1 than those for apoA-I-labeled/apoE-poor HDL_1.

also recovered in the HDL region. The proportion of radioactivity recovered in HDL from the plasma of the low HDL_1 animal was higher than that recovered from the plasma of the high HDL_1 animal. A similar transfer of radioactivity from HDL_1 (using apoA-I-labeled HDL_1) in the plasma of these animals under in vivo conditions (5 and 30 min) is given in **Fig. 8**. The proportion of radioactivity transferred to HDL in both animals increased with time, but in the low HDL_1 animal it was always higher. Thus, under both in vivo and in vitro conditions, the transfer of HDL_1 radioactivity was faster in low HDL_1 animals than that in high HDL_1 animals. The transfer of HDL_1 radioactivity to HDL was time-dependent, and it appeared that the injected sample had little or no detectable contamination.

The radioactivity of HDL (HDL_2 + HDL_3) stayed within this fraction both during in vitro incubations (Fig. 7) and in vivo injections (Fig. 8) in both high and low HDL_1 animals. The radioactivity in HDL decreased with time (Fig. 8) without undergoing any detectable exchange with other lipoproteins, including HDL_1.

DISCUSSION

Compositional and metabolic heterogeneity of HDL_1

These studies were conducted to determine the metabolic fate of unusual lipoproteins (HDL_1) that are between

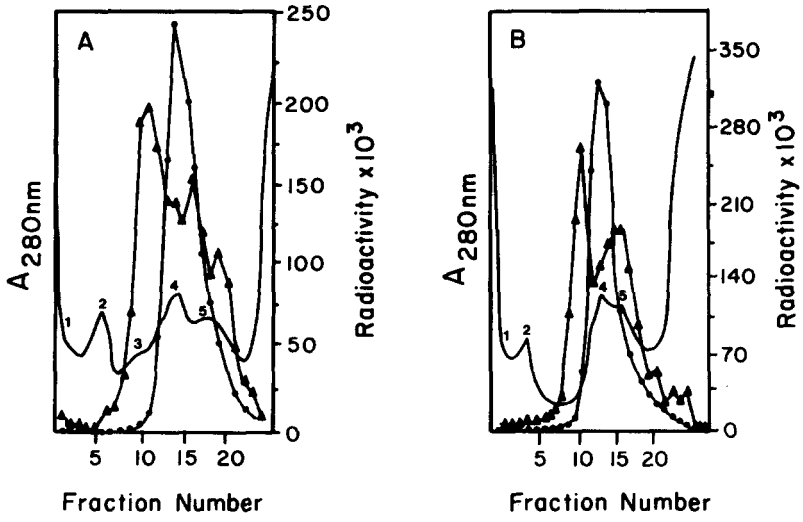

Fig. 7. In vitro distribution of radioactivity from HDL_1 (▲—▲) and HDL (●—●) in plasma lipoproteins of an animal with high (X-2033, A) and another with low (X-2811, B) HDL_1. The plasma samples (2 ml) from these animals were incubated with 100 μl of labeled HDL_1 and HDL with different radioisotopes of iodine for 30 min. The plasma lipoproteins were separated by density gradient ultracentrifugation and 0.4-ml fractions were collected. The radioactivity in these fractions was measured as shown. The absorbance at 280 nm (—) shows the distribution of radioactivity from HDL_1 and HDL into the lipoproteins. Numbers 1, 2, 3, 4, and 5 correspond to VLDL, LDL, HDL_1, HDL_2, and HDL_3, respectively.

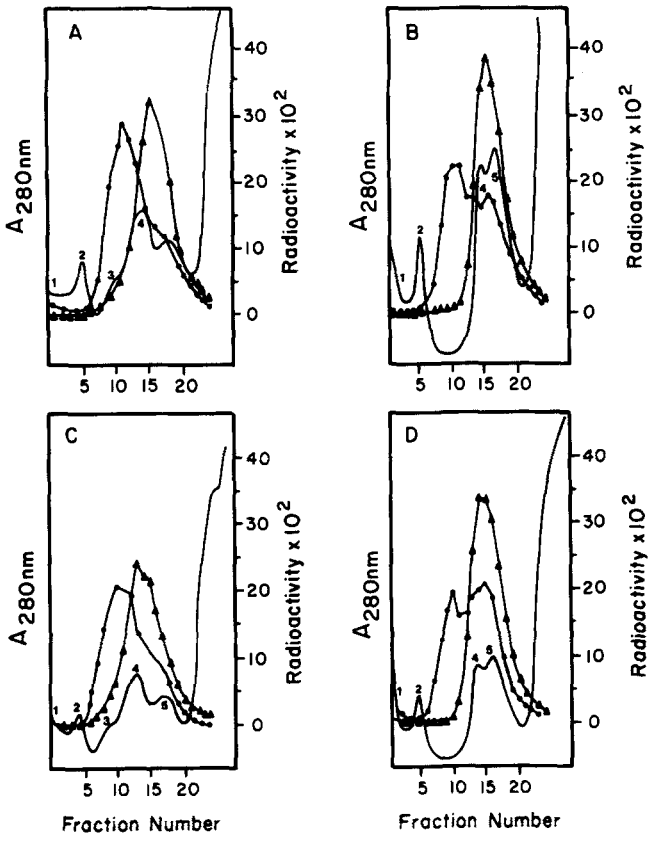

Fig. 8. In vivo distribution of radioactivity from HDL_1 (●—●) and HDL (▲—▲) in plasma lipoproteins following the injection of labeled HDL_1 and HDL into a high (X-2033, A and C) and a low HDL_1 (X-2811, B and D) animal. A and B represent the distribution of radioactivity at 5 min and C and D represent the distribution of radioactivity at 30 min. The lipoproteins were separated and processed as described for those shown in Fig. 7 and the absorbance of 280 nm (—) was monitored. Numbers 1, 2, 3, 4, and 5 correspond to VLDL, LDL, HDL_1, HDL_2, and HDL_3, respectively.

LDL and HDL in size and density and have apoA-I as their major apoprotein. The results show that these lipoproteins are heterogeneous in their composition and in metabolism. HDL_1 particles containing apoA-I with or without apoE were detected. Most particles, however, contain apoA-I without detectable apoE. The HDL_1 particles are either cleared from the plasma directly or converted to HDL. Both high and low HDL_1 animals catabolize HDL_1 by the same routes, but low HDL_1 animals convert the HDL_1 to HDL more rapidly. Most of the radioactivity in HDL_1 was transferred to HDL (compartment 2) either directly or indirectly via intermediate compartment 1. A small amount of radioactivity was removed directly without conversion to HDL. Thus, the HDL_1 accumulating in these animals seems mainly to be a precursor for HDL.

HDL_1 in other species

A number of animal species accumulate lipoproteins between LDL and HDL that are rich in apoE (25). In dogs, these lipoproteins contain apoE as their sole protein and are removed rapidly by the liver (26). Lp[a] lipoproteins or sinking prebeta lipoproteins (27) in humans and baboons (28) also occur between the LDL and HDL density region. Since HDL_1 lipoproteins have no apoB or apoB-like protein, the HDL_1 lipoproteins differ from Lp[a]. Unlike baboon HDL_1, Lp[a] is larger than LDL and, on gel filtration chromatography, it is separated before LDL (1). HDL_1 is separated between LDL and HDL by both density gradient separation and gel filtration chromatography (2,8). Since the majority of baboon lipoprotein particles that accumulate between LDL and HDL have apoA-I as their major apoprotein without any detectable apoE, these also differ from HDL_c or HDL with apoE (29). However, some particles containing apoA-I and apoE or only apoE may be similar to HDL_c or HDL with apoE (29). Recently, human subjects with hyperalphalipoproteinemia were reported to have a major population of HDL larger than HDL_2 (30,31). Their HDL differed from HDL_c in that it did not inhibit binding of LDL to LDL receptors in cultured human fibroblasts (31). The HDL_1 accumulating in the plasma of high HDL_1 baboons may be similar to these larger HDL particles in hyperalphalipoproteinemic subjects.

Relationship of compositional heterogeneity of HDL_1 to its metabolic heterogeneity

These studies further demonstrate that HDL_1 is as heterogeneous in its metabolism as in its composition. This metabolic heterogeneity may be related to its compositional heterogeneity. The component removed directly without being converted to HDL may be the apoE-rich particles. ApoA-I-rich particles, or those containing apoA-I without apoE, may be converted to HDL directly. Other particles rich in apoA-I but also containing some apoE may form the intermediate pool C(1) which, after reorganization, is either converted to HDL or is directly removed from the circulation. HDL_1 in baboons may thus consist of particles that are similar to HDL_c and are removed directly from the circulation and those that are similar to HDL and are precursors to HDL. Since the majority of the particles represent those that are rich in apoA-I and are eventually converted to HDL, the accumulation of these particles in plasma is likely to be due to a problem in their conversion to HDL. Results of the experiment in which apoA-I-labeled apoE-poor HDL_1 was used for turnover studies suggest that the metabolic heterogeneity is indeed due to heterogeneity in composition of HDL_1. Most of the apoE-poor HDL_1 (>70%) was transferred to HDL [C(2)] as compared to 39–70% when apoA-I-labeled HDL_1 was injected. On the other hand, the transfer of apoE-poor HDL_1 to C(1) was significantly lower than that for HDL_1. This observation suggests that apoE-poor HDL is the precursor to HDL.

Compositional abnormality in HDL_1

The residence times of HDL and HDL_1 in plasma from high and low HDL_1 baboons were similar. The residence times for HDL_1 in low HDL_1 animals were slightly less than those in high HDL_1 animals. These observations suggest that the HDL_1 particle may have abnormal composition and the accumulation of HDL_1 may be related to its altered composition. Thus, a genetic defect related to HDL_1 composition and accentuated by feeding a saturated fat diet seems responsible for the accumulation of HDL_1 in high HDL_1 animals. The physiological mechanism or the genetic defect responsible for the accumulation and slower transfer of HDL_1 to HDL is not obvious from these experiments. These particles are rich in cholesteryl esters (32) and the transfer of cholesteryl esters to other lipoproteins may be related to this delayed conversion of HDL_1 to HDL. Recent studies suggest that cholesteryl ester transfer from HDL to VLDL and LDL is slower in high HDL_1 animals than in low HDL_1 animals (32), although, like human subjects with hyperalphalipoproteinemia (31), animals with high HDL_1 have cholesteryl ester transfer activity in their plasma similar to that from low HDL_1 animals. Thus, the slower transfer of cholesteryl ester from HDL to VLDL and LDL may be related to an apoprotein or HDL particle abnormality, leading to the accumulation of particles, intermediate in size between LDL and HDL, that are precursors to HDL.

Hypothesis

We suggested that a genetic defect in HDL_1 or HDL composition related to cholesteryl ester transfer (but not cholesteryl ester transfer protein) is responsible for accumulation of HDL_1 in high HDL_1 baboons. This is supported by the current results that the HDL_1 accumulated in high HDL_1 baboons is a precursor for HDL and by previous observations that the transfer of cholesteryl ester from HDL to VLDL + LDL is slower in high HDL_1 baboons (32). We propose a hypothesis (**Fig. 9**) for the metabolic mechanism by which these lipoproteins accumulate in the plasma of high HDL_1 baboons. The formation of HDL_1 may be similar to that for HDL_c as postulated by Mahley (29). Like HDL_c, the HDL_1 may be formed as a result of HDL_{2+3} acquiring free cholesterol from peripheral tissues (29). Although the present studies do not show conversion of HDL_{2+3} to HDL_1, it is possible that this process occurs slowly in extracellular spaces (29) and is not detected by kinetic studies. This step in the hypothesis is based on the observations in the literature (29). The free cholesterol in these particles is then esterified by lecithin:cholesterol acyltransferase, and the particles become enriched with cholesteryl esters. These particles can then donate their cholesteryl esters to lower density lipoproteins, mediated by cholesteryl ester transfer protein, and join the pool of HDL_{2+3}. Particles of HDL_1 density also pick up apoE from extrahepatic cells or from other plasma lipoproteins and become enriched with apoE. ApoE-rich particles are rapidly removed from the plasma by receptors that recognize apoE. A genetic defect (possibly the presence of an inhibitor in the HDL particle) in HDL composition affects the cholesteryl ester transfer in high HDL_1 animals. Thus, HDL_1 from high HDL_1 baboons cannot donate its cholesteryl esters to lower density lipoproteins at normal rates and may not be readily converted

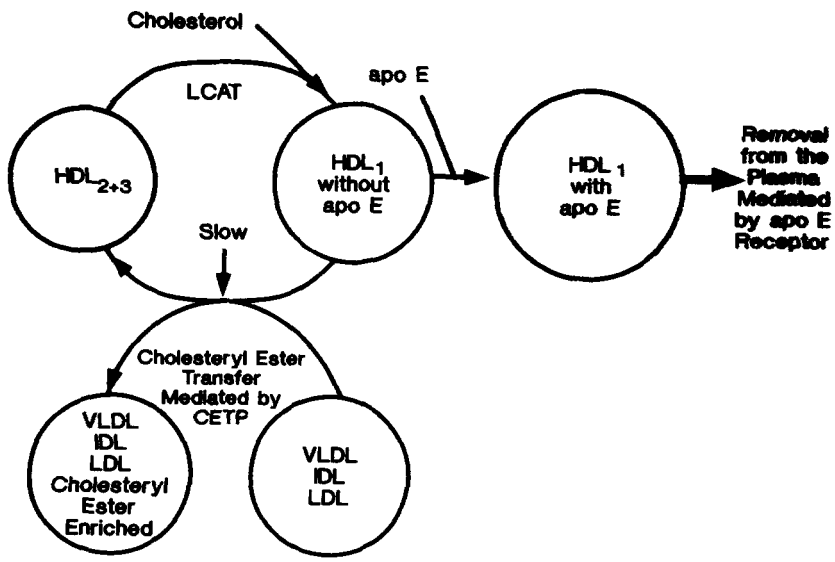

Fig. 9. Proposed hypothesis for the metabolism of HDL_1 in high HDL_1 animals. LCAT, lecithin:cholesteryl acyltransferase enzyme and CETP, cholesteryl ester transfer protein. The transfer of cholesteryl ester from HDL_1 to lower density lipoproteins is slow in high HDL_1 baboons and retards the conversion of HDL_1 to HDL_{2+3} leading to their accumulation in the plasma.

to HDL and, therefore, will accumulate in the plasma. The removal of apoE-rich lipoproteins is not affected by this defect and their removal is similar in both high and low HDL_1 animals. Studies are in progress to determine this genetic defect in HDL composition in high HDL_1 baboons that leads to the accumulation of lipoprotein in HDL_1 density region.

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REFERENCES

1. Williams, M. C., R. S. Kushwaha, and H. C. McGill, Jr. 1987. Quantitation of baboon lipoproteins by high performance gel exclusion chromatography. *Lipids.* **22:** 366-374.
2. Williams, M. C., J. L. Kelley, and R. S. Kushwaha. 1984. Detection of an abnormal lipoprotein in a large colony of pedigreed baboons using high-performance gel exclusion chromatography. *J. Chromatogr.* **308:** 101-109.
3. Flow, B. L., T. C. Cartwright, T. J. Kuehl, G. E. Mott, D. C. Kraemer, A. W. Kruski, J. D. Williams, and H. C. McGill, Jr. 1981. Genetic effects on serum cholesterol concentrations in baboons. *J. Hered.* **72:** 97-103.
4. Kammerer, C. M., G. E. Mott, K. D. Carey, and H. C. McGill, Jr. 1984. Effects of selection for serum cholesterol concentrations on serum lipid concentrations and body weight in baboons. *Am. J. Genet.* **19:** 333-345.
5. MacCluer, J. W., C. M. Kammerer, J. L. VandeBerg, M-L. Cheng, G. E. Mott, and H. C. McGill, Jr. 1987. Detecting genetic effects on lipoprotein phenotypes in baboons. *Genetica.* **73:** 159-168.
6. Nichols, A. V., T. J. Kuehl, E. L. Gong, H. G. McGill, Jr., P. Blanche, and T. M. Forte. 1981. ApoE containing lipoproteins in the baboons. *Arteriosclerosis.* **1:** 89a.
7. Babiak, J., E. L. Gong, A. V. Nichols, T. M. Forte, T. J. Kuehl, and H. C. McGill, Jr. 1984. Characterization of HDL and lipoproteins intermediate to LDL and HDL in the serum of pedigreed baboons fed an atherogenic diet. *Atherosclerosis.* **52:** 24-45.
8. McGill, H. C., Jr., C. A. McMahan, R. S. Kushwaha, G. E. Mott, and K. D. Carey. 1986. Dietary effects on serum lipoproteins of dyslipoproteinemic baboons with high HDL_1. *Arteriosclerosis.* **6:** 651-663.
9. Redgrave, T. G., D. C. K. Roberts, and C. E. West. 1975. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal. Biochem.* **65:** 42-49.
10. Huff, M. W., and D. E. Telford. 1984. Characterization and metabolic fate of two very-low-density lipoprotein subfractions separated by heparin-Sepharose chromatography. *Biochim. Biophys. Acta.* **796:** 251-261.
11. Weber, K., and J. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulphate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406-4412.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.
13. Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry.* **12:** 502-507.
14. McFarlane, A. S. 1958. Efficient trace labeling of proteins with iodine. *Nature.* **182:** 53-57.
15. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212-221.
16. Kushwaha, R. S., W.R. Hazzard, and J. Engblom. 1978. High density lipoprotein metabolism in normolipidemic and cholesterol-fed rabbits. *Biochim. Biophys. Acta.* **530:** 132-143.
17. Kushwaha, R. S., D. M. Foster, and W. R. Hazzard. 1982. Effect of diet-induced hypercholesterolemia on high density lipoprotein metabolism in pigtail monkeys (*Macaca nemestrina*). *Metabolism.* **31:** 43-51.
18. Haffner, S. M., R. S. Kushwaha, D. M. Foster, D. Applebaum-Bowden, and W. R. Hazzard. 1983. Studies on the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. *Metabolism.* **32:** 413-420.
19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature.* **227:** 680-685.
20. Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470-475.
21. Manual of Laboratory Operations. 1975. Lipid Research Clinics Program, Vol. 1: DHEW Publications No. (NIH) 75-628. U. S. Government Printing Office, Washington, D.C.
22. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45-52.
23. Foster, D. M., and R. C. Boston. 1983. Use of computers and compartment analysis; the SAAM and CONSAM programs. *In* Compartmental Distribution of Radiotracers. J. S. Robertson editor. CRC Press Inc., Boca Raton, FL. 73-142.
24. Zech, L. A., E. J. Schaefer, T. J. Bronzert, R. L. Aamodt, and H. B. Brewer, Jr. 1983. Metabolism of human apolipoproteins A-I and A-II: compartmental models. *J. Lipid Res.* **24:** 60-71.
25. Mahley, R. W. 1978. Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, Jr., and J. A. Ontko, editors. American Physiology Society, Bethesda, MD. 181-197.
26. Sherrill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of the canine lipoproteins containing only apoprotein E by a high affinity receptor. *J. Biol. Chem.* **255:** 1804-1807.
27. Albers, J. J., V. G. Cabana, G. R. Warnick, and W. R. Hazzard. 1975. Lp[a] lipoprotein: relationship to sinking prebeta lipoprotein, hyperlipoproteinemia and apolipoprotein B. *Metabolism.* **24:** 1047-1054.
28. Rainwater, D. L., G.S. Manis, and R. S. Kushwaha. 1986. Characterization of an unusual lipoprotein similar to human lipoprotein[a] isolated from the baboon, *Papio* sp. *Biochim. Biophys. Acta.* **877:** 75-78.
29. Mahley, R. W. 1982. Atherogenic hyperlipoproteinemia. The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. *Med. Clin. North Am.* **66:** 375-402.

30. Koizumi, J., H. Mabuchi, A. Yashimura, I. Michishita, M. Takeda, H. Itoh, Y. Sakai, T. Sakai, K. Neda, and R. Takeda. 1985. Deficiency of serum cholesterol-ester transfer activity in patients with familial alphahyperlipoproteinemia. *Atherosclerosis.* **58:** 175–186.
31. Yokoyama, S., T. Kursawa, O. Mishikawa, and A. Yamamoto. 1986. High density lipoproteins with poor reactivity to cholesteryl ester transfer reaction observed in a homozygote of familial hyperalphalipoproteinemia. *Artery.* **14:** 43–51.
32. Kushwaha, R. S., H. C. McGill, Jr., D. L. Rainwater, and M. C. Williams. 1987. Decreased cholesterol ester transfer among plasma lipoproteins leads to the accumulation of HDL_1 in some families of baboons. *Arteriosclerosis.* **7:** 539a.