Lecithin:cholesterol acyltransferase-induced modifications of liver perfusate discoidal high density lipoproteins from African green monkeys

John Babiak, Hiromitsu Tamachi, Fred L. Johnson, John S. Parks, and Lawrence L. Rudel

Department of Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, 300 South Hawthorne Road, Winston-Salem, NC 27103

Abstract The role of lecithin:cholesterol acyltransferase (LCAT) in the formation of plasma high density lipoproteins (HDL) was studied in a series of in vitro incubations in which perfusates from isolated African green monkey livers were incubated at 37°C with partially purified LCAT for between 1 and 13 hr. The HDL particles isolated from monkey liver perfusate stored at 4°C and not exposed to added LCAT contained apoA-I and apoE, were deficient in neutral lipids, and were observed by electron microscopy as discoidal particles. Particle sizes, measured as Stokes' diameters by gradient gel electrophoresis (GGE), ranged between 7.8 nm and 15.0 nm. The properties of perfusate HDL were unchanged following incubation at 37°C in the presence of an LCAT inhibitor. However, HDL subfractions derived from incubations at 37°C with active LCAT contained apoA-I as the major apoprotein, appeared round by electron microscopy, and possessed chemical compositions similar to plasma HDL. The HDL isolated from perfusate incubations at 37°C with low amounts of LCAT had a particle size and chemical composition similar to plasma HDL_{3a}. In three of four perfusates incubated with higher levels of LCAT activity, the HDL products consisted of two distinct HDL subpopulations when examined by GGE. The major subpopulation was similar in size and composition to plasma HDL_{2a}, while the minor subpopulation demonstrated the characteristics of plasma HDL_{2b}. The data indicate that the discoidal HDL particles secreted by perfused monkey livers can serve as precursors to three of the major HDL subpopulations observed in plasma.—**Babiak, J., H. Tamachi, F. L. Johnson, J. S. Parks, and L. L. Rudel.** Lecithin: cholesterol acyltransferase-induced modifications of liver perfusate discoidal high density lipoproteins from African green monkeys. *J. Lipid Res.* 1986. **27:** 1304–1317.

Supplementary key words liver perfusion • lecithin:cholesterol acyltransferase • HDL • nonhuman primates • electron microscopy

Work in our laboratory has demonstrated that a heterogeneous assortment of lipoprotein particles accumulates during recirculating perfusion of isolated African green monkey livers (1). These particles possess excess surface lipids (phospholipid and free cholesterol) relative to their plasma counterparts and, therefore, unusual morphological features are apparent by electron microscopy. In particular, apoB-containing particles of the hydrated densities of both VLDL and LDL are nonspherical and exhibit excess surface tabs or flaps. ApoA-I and apoE-containing HDL particles are almost completely devoid of neutral lipids and are discoidal in structure. Since these characteristics are also common to the lipoproteins of individuals with lecithin:cholesterol acyl transferase (LCAT) deficiency (2–5), we speculated that liver perfusates from African green monkeys are also deficient in LCAT (6). We further hypothesized that the heterogeneous, irregularly shaped lipoprotein particles found in monkey liver perfusates are typical of the nascent particles secreted by the liver, prior to intravascular modification by LCAT. Subsequently, we have found the LCAT activity in African green monkey liver perfusate to be approximately 2% of plasma enzyme activity in this species, an amount sufficient to esterify only a small portion of the free cholesterol present in the hepatic lipoproteins during a 4-hr perfusion (7).

Other investigators (8–12) have demonstrated that LCAT is capable of converting apoA-I-containing discoidal structures to spherical particles regardless of whether the reactant HDL was derived from *1*) plasma of LCAT-deficient individuals, *2*) rat liver perfusates obtained in the presence of an LCAT inhibitor, or *3*) recombination of apoA-I and phosphatidylcholine, *in vitro*. In the present study we characterized LCAT-induced structural and compositional changes of the apoA-I- and apoE-containing HDL present in monkey liver perfusates. All incubations were performed using whole perfusate from which red blood cells had been removed by low speed centrifugation. Therefore, we avoided ultracentrifugal modifications of

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; GGE, gradient gel electrophoresis; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); PCMPS, *p*-chloromercuriphenylsulfonic acid; TLC, thin-layer chromatography; SDS, sodium dodecyl sulfate; SAA, serum amyloid A protein.

reactant lipoproteins prior to incubation and allowed the apoB-containing lipoproteins present in the perfusate to serve as additional sources of substrate for the LCAT reaction, as needed. Our results indicated that LCAT mediated the conversion of discoidal perfusate HDL into three subpopulations of spherical particles with sizes and chemical and apoprotein compositions similar to plasma HDL_{3a}, HDL_{2a}, and HDL_{2b}.

METHODS

Animals and diets

The adult, male African green monkeys (*Cercopithecus aethiops*) used for these studies were feral animals obtained from a primate importer. They weighed 3.5–4.6 kg and were maintained for 2–6 years on semisynthetic diets containing 0.74 mg of cholesterol/kcal and 40% calories as fat with P/S ratios of either 0.3 (animals #233 and #306) or 2.0 (animals #272 and #302). **Table 1** shows the two diets used in these studies. The animals remained healthy on these diets as judged by routine monitoring of body weight, blood urea nitrogen, fasting blood glucose, hemoglobin, hematocrit, CBC, and total serum protein. There were no diet-related differences in experimental results obtained in this study, so, when appropriate, data were pooled without regard to the diet of the monkeys.

Liver perfusion

Perfusion of isolated livers was performed as described previously (1). Animal donors were fed 11–13 h prior to liver perfusion. The perfusion medium consisted of Krebs-Henseleit original Ringer bicarbonate buffer, containing D-glucose, amino acids, insulin, hydrocortisone, streptomycin, penicillin, and washed human erythrocytes at 22% hematocrit, pH 7.4. Recirculating liver perfusion was performed with 280–320 ml of medium for 90 min at 37°C, after which the liver was flushed free of the original perfusate by non-recirculating perfusion. This first perfusion period was performed to remove plasma lipoproteins from the freshly isolated liver. Recirculating perfusion was then resumed for 4 hr at 37°C with 280–320 ml of fresh medium that contained 1 mCi of [^{14}C]leucine. All medium changes were performed without interruption of the perfusion. Liver color, rate of bile production, rate of oxygen consumption, and rate of perfusate cholesterol accumulation were the criteria used to document that each liver was viable, as previously described (1).

TABLE 1. Diets of African green monkeys used for liver perfusion

Composition	Saturated Fat Diet	Polyunsaturated Fat Diet
	g/100 g	
Butter	23.5	0
Safflower oil	0	11
β-Sitosterol	0.06[a]	0
Cholesterol	0.27	0.0086[a]
Dried egg yolk	0	15
Casein	9	9
Lactalbumin	8	5
Wheat flour	33.5	35
Applesauce	0.7	0
Dextrin	10	0
Sucrose	3.6	12
Alphacel	5	7
Mineral mixture	3.8	3.5
Vitamin mixture	2.6	2.5

[a] Added to compensate for the sterol content of the fat in the other diet.

Incubations

After 4 hr of perfusion, the medium was collected on ice and was adjusted to 0.1% EDTA, 0.1% NaN_3, and 0.04% 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), pH 7.4. Erythrocytes were removed by low speed centrifugation at 4°C. An aliquot of each cell-free perfusate was maintained at 4°C and served as the unincubated control for each study. As an additional control, to observe the effects of all factors in the perfusate besides LCAT, incubations of perfusate at 37°C in the presence of the LCAT-inhibitors DTNB (0.04%) or *p*-chloromercuriphenylsulfonic acid (PCMPS) were performed. Each incubated sample was adjusted (unless otherwise noted in the text) to contain 2% human serum albumin (Cutter Laboratories), and 5 mM β-mercaptoethanol was included in all incubations with active LCAT. Partially purified LCAT with activity equivalent to 250–1,000 μg cholesterol esterified/hr was added to perfusate samples containing 850–2,700 μg of cholesterol. Samples were incubated in 50-ml polypropylene serum tubes under nitrogen at 37°C in a shaking water bath. Lipoprotein isolation was begun promptly after each incubation.

Lipoprotein isolation and fractionation

The lipoproteins from all incubated and unincubated samples from each perfusion were isolated at the same time by ultracentrifugation in either a 60 Ti (50,000 rpm) or a 50.2 Ti (45,000 rpm) rotor. Lipoprotein samples were recovered by tube slicing and pipetting. Very low density lipoproteins (VLDL) were isolated at 15°C by ultracentrifugation for 18 hr at perfusate density. The d > 1.006 g/ml fraction was adjusted to a density of 1.225 g/ml with solid KBr and was ultracentrifuged for 40 hr at 15°C. Lipoprotein classes were subsequently isolated and were separated by gel filtration chromatography on 4% agarose (Bio-Rad) as previously described (13). The distribution of lipoprotein material in the eluant from the

gel filtration column was examined both by monitoring absorbance at 280 nm as well as by measuring [^{14}C]leucine counts in each fraction. Lipoprotein fractions were pooled based upon inspection of the column profile and then were dialyzed against 0.01% EDTA, 0.01% NaN_3, pH 7.4, for further analysis.

Analytical methods

Protein mass was estimated on lipoprotein fractions by the method of Lowry et al. (14) using bovine serum albumin (Fraction V, Sigma) as the standard. When necessary, samples were extracted with hexane to remove turbidity. Lipid phosphorus was measured by the method of Fiske and SubbaRow (15). Lipids were extracted from lyophilized lipoproteins according to Folch, Lees, and Sloane Stanley (16) and lipid classes were separated by thin-layer chromatography (TLC) as previously described (17). Cholesterol and cholesteryl esters were eluted from the silica gel with chloroform, and the cholesterol mass within each eluted fraction and in whole lipoprotein fractions was measured according to Rudel and Morris (18). Cholesteryl ester mass was calculated as 1.7× esterified cholesterol mass. Triglycerides were eluted from the silica gel with 2:1 chloroform–methanol and were quantitated according to Sardesai and Manning (19). Recovery of free and esterified cholesterol from TLC plates was 70–100% of that applied, and the percentage recovery values were used to correct free and esterified cholesterol and triglyceride quantities to concentrations in intact lipoprotein samples.

Lipoprotein apoproteins were separated in 4–30% polyacrylamide gradient gels (Pharmacia Fine Chemicals) that were equilibrated for 1 hr at 100 V in the electrophoresis running buffer of 0.2% SDS, 0.05 M Tris, 0.02 M Na acetate, and 0.002 M EDTA, pH 8.4. Lyophilized samples (40 μg of protein) were solubilized in 40 μl of a 2% SDS, 10 mM β-mercaptoethanol solution and were heated at 100°C for 2 min after which 10 μl of a 40% sucrose–0.01% bromphenol blue solution was added. Ten μl of each sample was applied and the gels were electrophoresed for 4–5 hr at 100 V. Gels were fixed and stained in 0.1% Coomassie Blue R-250, 50% methanol, and 10% acetic acid overnight, and were destained first in 50% methanol–10% acetic acid and then in 10% methanol–10% acetic acid. Destained gels were densitometrically scanned using a Zeineh scanning densitometer (Biomed Instruments, Inc., Fullerton, CA) with either the laser or with the tungsten light source and a 610 nm filter. Molecular weights were determined by comparison to low molecular weight protein standards (Pharmacia Fine Chemicals) run in each gel. Apoproteins were identified by their size and comigration with monkey apoprotein standards during SDS- and isoelectric focusing-polyacrylamide gel electrophoresis.

Particle size distributions were determined by electrophoresis of lipoprotein samples in 4–30% polyacrylamide gradient gels essentially as described by Nichols, Blanche, and Gong (20). Electrophoresis was done at 125 V for 24 hr in a buffer of 0.09 M Tris, 0.08 M boric acid, 0.003 M EDTA, pH 8.35. Gels were stained with 0.02% Coomassie G-250 in 3.5% perchloric acid and destained in 5% acetic acid. Gels were then scanned with the Zeineh scanning densitometer in the laser mode, and migration distances of major bands were calculated using software provided by Biomed Instruments. Calculations of Stokes' diameters of particles were made assuming the hydrated Stokes' diameters of high molecular weight protein standards (Pharmacia Fine Chemicals) run in each gel to be 17.0 nm for thyroglobulin, 12.2 nm for ferritin, 8.12 nm for lactate dehydrogenase, and 7.1 nm for bovine albumin (21). Sizes of subpopulations of HDL from African green monkeys (HDL_{2b} = 10.1–12.0 nm, HDL_{2a} = 9.3–10.0 nm, HDL_{3a} = 8.6–9.2 nm) were based upon unpublished observations (J. Babiak and L. L. Rudel) of plasma HDL in this species and are comparable to values published for the corresponding human (21) and baboon (22) HDL subpopulations.

Negative stain electron microscopy was performed as previously described (1) using 2% potassium phosphotungstate, pH 6.5, on Formvar, carbon-coated grids. When necessary, samples were concentrated on the grids to a mass concentration of approximately 2 mg/ml by evaporation under nitrogen.

Enzyme purification and measurement

LCAT was partially purified using modifications of a previously published procedure (23). Briefly, 1 liter of fresh, pooled human plasma was obtained from the Red Cross and lipoproteins were precipitated using dextran sulfate. The LCAT was partially purified by phenyl–Sepharose and DEAE–Sepharose column chromatography. The LCAT that eluted from the DEAE–Sepharose column was frequently contaminated with lipid transfer activity that was subsequently removed by passing the preparation over an Affi-gel blue column (1 × 30 cm; Bio-Rad, Richmond, CA) equilibrated with 20 mM phosphate buffer, pH 7.1. At least 80% of the LCAT activity was eluted from the Affi-gel blue column, while all detectable lipid transfer activity was retained. This combination of procedures resulted in a 5000-fold purification of LCAT and this preparation was used as the source of LCAT activity in all experiments. Each purified LCAT preparation was stored at 4°C in 150 mM NaCl, 10 mM Tris, pH 7.4 buffer, and the activity was assayed before each incubation. LCAT activity stored in this manner had a half-life of roughly 6 weeks.

LCAT activity in samples was assayed by a procedure similar to the method of Chen and Albers (24). We used

an exogenous source of substrate in the form of discoidal complexes of apoA-I, egg yolk phosphatidylcholine, and [^{14}C]cholesterol (molar ratio 0.8:250:12.0) that were made by the cholate dialysis method. Details of assay conditions have been described previously (7).

RESULTS

Properties of HDL in liver perfusate

For all samples the size distribution of perfusate HDL was examined by electrophoresis in 4–30% polyacrylamide gradient gels. **Fig. 1** shows the densitometric scans of the HDL of all four monkey liver perfusates used in these studies. HDL (Fig. 1) from all liver perfusates included a major peak corresponding to material of Stokes' diameter of 7.8–7.9 nm. In addition, diffuse bands of lipoprotein material ranging in particle size up to 15.0 nm were also present. Although the perfusate HDL from all African green monkey livers exhibited these same general features, the mass distribution among these HDL populations differed considerably among animals. Among this small sample of monkey perfusates it was not apparent that HDL distribution was correlated to either perfusate endogenous LCAT activity or dietary fat of the monkey.

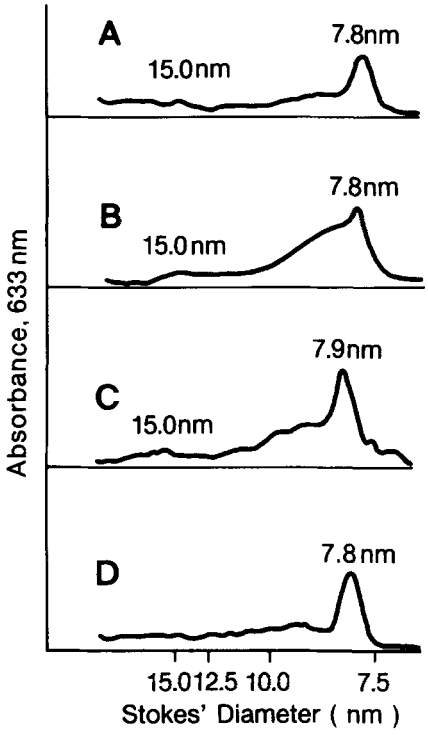

Fig. 1. Densitometric scans of Coomassie blue-stained 4–30% polyacrylamide gradient gels of the HDL from the four African green monkey liver perfusates used for these experiments. Twenty μl of each perfusate ultracentrifugal d 1.006–1.225 g/ml fraction (containing approximately 25 μg of total protein) was electrophoresed and stained as described in Methods. Particle sizes were calculated based on the migration positions of high molecular weight protein standards run in the same gel. All four HDL distributions indicate a major peak of material of Stokes' diameter 7.8–7.9 nm, but differing amounts of larger sized material up to 15.0 nm in size. The four profiles shown are from the liver perfusates of monkeys 306 (A), 272 (B), 233 (C), and 302 (D).

The HDL were preparatively subfractionated into two size populations by agarose column chromatography, as shown in **Fig. 2**. The distribution of [^{14}C]leucine label is shown because this is indicative of protein synthesized by the liver during the perfusion period. The majority of the HDL label was present in a single peak (designated IV_B) that corresponded to the 7.8–7.9 nm peak observed by gradient gel electrophoresis. A second population that appeared as a shoulder on the main HDL peak (designated IV_A) contained the larger size HDL particles, and this material was pooled separately from the IV_B material.

The chemical compositions of the two HDL fractions isolated by column chromatography are given in **Table 2**. On a percentage basis, the large IV_A particles contained more free cholesterol, while the small IV_B particles were enriched in protein. In each fraction, neutral lipids (cholesteryl ester and triglyceride) accounted for less than 8% of the total mass. When examined by electron microscopy (**Fig. 3**), the IV_A particles (Fig. 3A) were discoidal in appearance and frequently formed rouleaux. The IV_B particles (Fig. 3B) were considerably smaller and both round and discoidal particles that did not form rouleaux were apparent.

The apoprotein content of the IV_A and IV_B particles differed considerably as analyzed by SDS-polyacrylamide gel electrophoresis. **Fig. 4** shows the densitometric scans of the apoproteins within the IV_A (A) and IV_B (B) fractions from one perfusate and is representative of all four perfusates used in these experiments. ApoA-I was the major apoprotein of the IV_B particles, while the IV_A particles contained mainly apoE and lesser amounts of apoA-I. Both fractions exhibited low amounts of serum amyloid A protein (SAA), apoA-II, and apoCs, though the latter two apoproteins are not well resolved by this gel system. In addition, minor amounts of unidentified apoproteins of approximately 40,000 and 70,000 molecular weight were seen in the HDL.

Effects of incubation with and without partially purified LCAT

Control incubations were performed to assess modification of perfusate HDL that can occur independent of LCAT activity. After incubation of perfusate for up to 18 hr at 37°C in the presence of either of the LCAT inhibitors, DTNB or PCMPS, no change in the HDL size distribution was observed by GGE or by gel filtration chromatography. In additional control experiments monkey liver perfusate was incubated at 37°C without

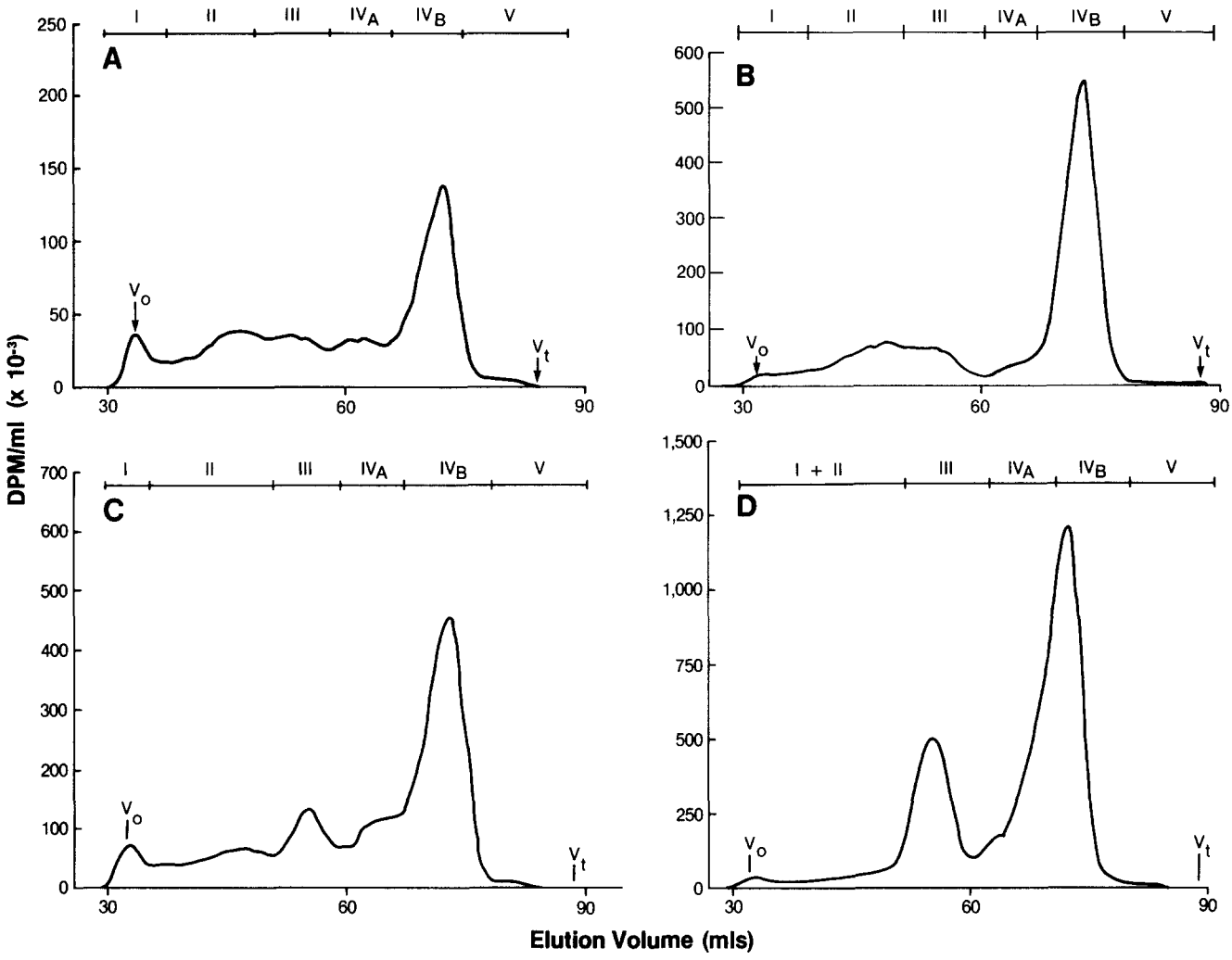

Fig. 2. Agarose column chromatographic profiles of the ultracentrifugal d 1.006–1.225 g/ml fraction of the four African green monkey liver perfusates used in these experiments. Fractions of approximately 1 ml in volume were collected and the ^{14}C radioactivity within each fraction was measured by liquid scintillation counting. Regions I, II, and III represent apoB-containing lipoprotein material. The HDL of each perfusate consisted of a shoulder (the IV_A region) and a major peak (IV_B). Prior to analysis, the HDL were subfractionated into IV_A and IV_B regions, as indicated, based upon visual inspection of the profiles. The four profiles shown are of the liver perfusates of monkeys 306 (A), 272 (B), 233 (C), and 302 (D).

added LCAT to observe the effects of the low levels of LCAT and any other factors already present in the perfusate. After incubation of perfusate for 13 hr at 37°C with and without 2% human serum albumin, the HDL distribution was altered (**Fig. 5**). The major HDL species of both incubations was a population of particles 8.6 nm

TABLE 2. Chemical compositions of HDL subfractions of liver perfusates used in experiments

HDL Subfraction		Chemical Composition					Stokes' Diameter[b]
		Protein	Phospholipid	Free Cholesterol	Cholesteryl Ester	Triglyceride	
		weight %					*nm*
IV_A	n = 3	34.4 ± 6.6[a]	47.1 ± 7.0	10.7 ± 2.6	3.7 ± 2.5	4.1 ± 2.0	
IV_B	n = 4	44.8 ± 2.9	46.4 ± 4.7	3.7 ± 0.6	3.4 ± 1.2	1.8 ± 1.4	7.8 ± 0.05

[a] All values, mean ± SD.
[b] Measured at the center of the peak by calibrated gradient gel electrophoresis.

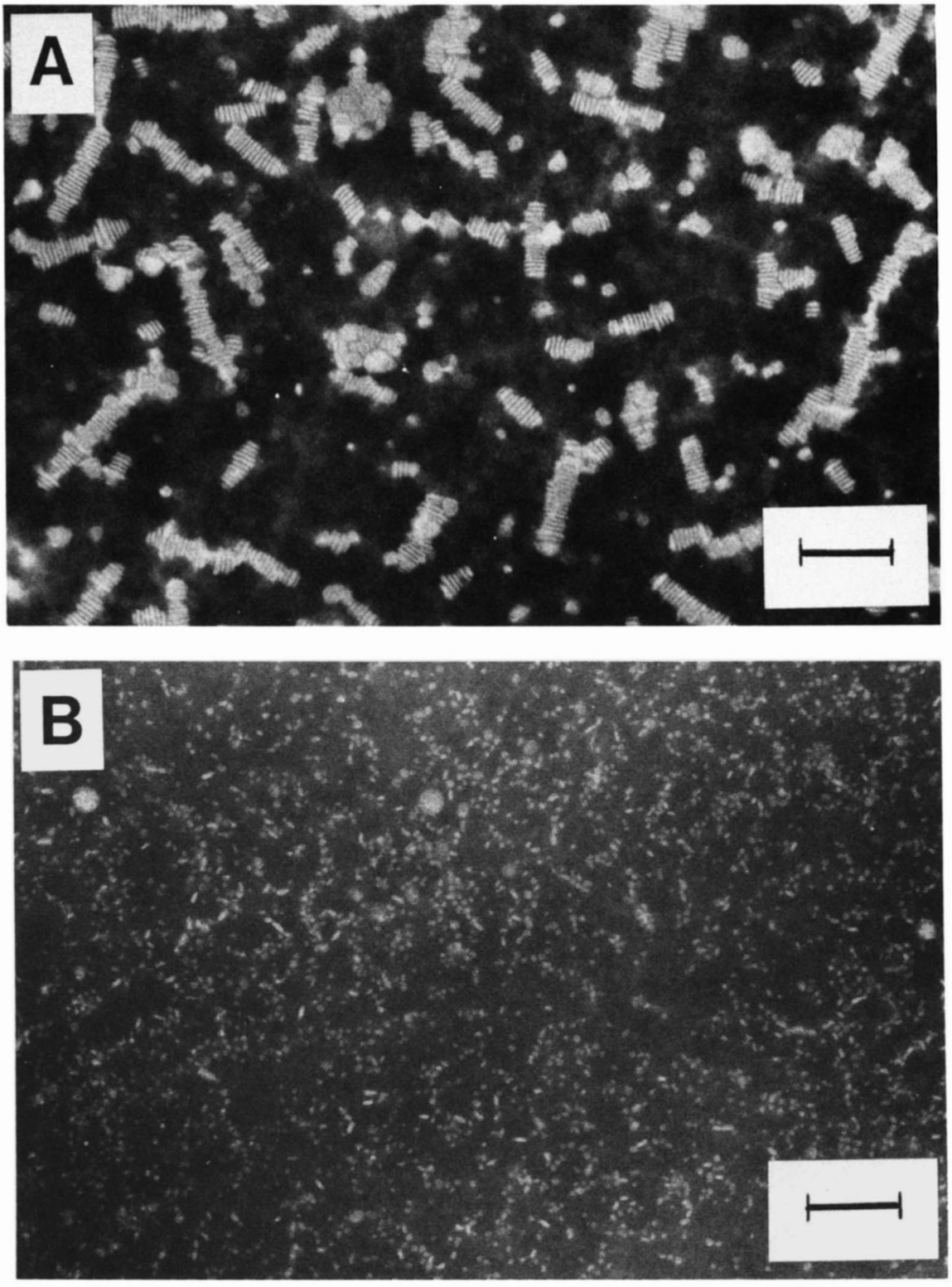

Fig. 3. Negative stain electron micrographs of representative perfusate HDL subfractions isolated from agarose column regions IV_A (A) and IV_B (B) from monkey 233. The IV_A fraction (A) contained clearly discoidal particles with minor axes of approximately 4.5 nm and long axes up to 20.0 nm. The IV_B fraction (B) contained both round and elliptical particles that were less than 10 nm in diameter. The bar markers indicate 100 nm.

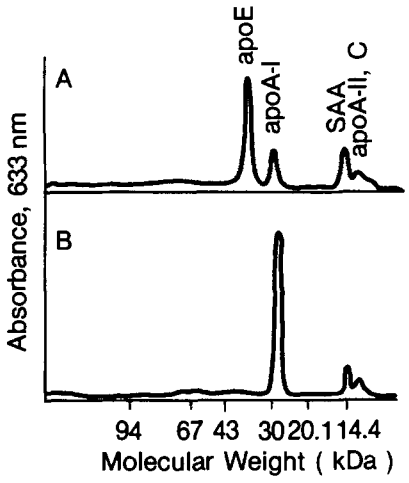

Fig. 4. Densitometric scans of Coomassie blue-stained 4–30% SDS-polyacrylamide gradient gels of apoproteins from representative perfusate HDL subfractions isolated from agarose column regions IV_A (A) and IV_B (B) from monkey 272. Approximately 10 μg of total protein was applied to each lane and the gels were electrophoresed and stained as described in Methods. The migration position of molecular weight protein standards run in the same gel are indicated.

in size (Fig. 5B, 5C), which is within the size range of plasma HDL_{3a} (21, 22). When the same perfusate was incubated for 13 hr at 37°C with added partially purified LCAT, a major peak of particle size of plasma HDL_{2a} (9.7 nm) and a minor peak of particle size of plasma HDL_{2b} (11.1 nm) were observed (Fig. 5D). **Fig. 6** illustrates the effect of the time of incubation at 37°C upon the size of the HDL. In this example, the perfusate HDL (Fig. 6A) was converted to larger particle size (Fig. 6B) after only 3 hr at 37°C with added LCAT. Incubation at 37°C for a longer period of time (12 hr, in Fig. 6C) resulted in a slight increase in the HDL particle size. In total, perfusates from four livers were incubated at 37°C for different times or with different amounts of added LCAT activity. In all incubations, the major HDL species was equivalent in size to either plasma HDL_{3a} or HDL_{2a}. In three of the four perfusates, a minor product of HDL_{2b} size was formed during incubation with added LCAT.

After incubation, HDL were isolated and subfractionated by ultracentrifugation and agarose column chromatography. **Fig. 7** compares the column profiles of each of two liver perfusate lipoprotein preparations to the profiles after incubation at 37°C with added LCAT. Incubation with LCAT caused a shift in the LDL (column regions I, II, and III) to smaller particle size and a shift in HDL (column regions IV_A and IV_B) to larger size. For further characterization, the material in the HDL was pooled as two separate fractions as before. The major peak, containing either the HDL_{3a} or HDL_{2a} species, was within the pool designated IV_B, while IV_A contained the leading edge of the peak (HDL_{2b} material).

Fig. 8 shows representative electron micrographs of HDL isolated from agarose column regions IV_A (Fig. 8A) and IV_B (Fig. 8B). HDL subfractions from all incubations at 37°C appeared round by electron microscopy. Discoidal particles were not found in any incubated samples.

The chemical composition of each isolated HDL subfraction was determined. As stated above, after incubation at 37°C with active LCAT, the major HDL species isolated within fraction IV_B was equivalent in size to HDL_{3a} (8.6–9.2 nm) in some instances (e.g., Figs. 5B and 5C) and to HDL_{2a} (9.3–10.0 nm) in other cases (e.g., Figs. 5D, 6B, and 6C). Thus, HDL isolated within the same region of the agarose column elution profile (that is, IV_B) consisted of particles that differed measurably in size, (HDL_{3a} or HDL_{2a}) as determined by gradient gel electrophoresis. HDL_{2b}-size particles (10.1–12.0 nm), when present, were the predominant species isolated within

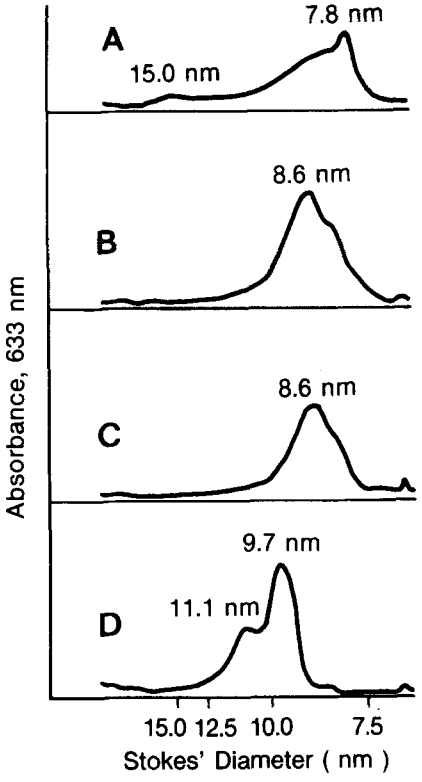

Fig. 5. Densitometric scans of Coomassie blue-stained 4–30% polyacrylamide gradient gels from one experiment showing the effects of LCAT activity on the size distribution of perfusate HDL. Samples were electrophoresed, stained, and scanned and particle sizes were determined as for Fig. 1. All scans shown are from experiments performed on the liver perfusate from monkey 272. Aliquots of perfusate contained 1420 μg of total cholesterol and were maintained at either 4°C or 37°C for 13 hr in the presence of 5 mM β-mercaptoethanol. (A) HDL distribution of liver perfusate stored at 4°C throughout the experiment. (B, C) HDL distribution after incubation at 37°C of the same liver perfusate without (B), and with (C) human serum albumin added to a final concentration of 2% (w/v). (D) Perfusate HDL distribution after incubation at 37°C for 13 hr with 250 μg of cholesterol esterified/hr of exogenous LCAT activity.

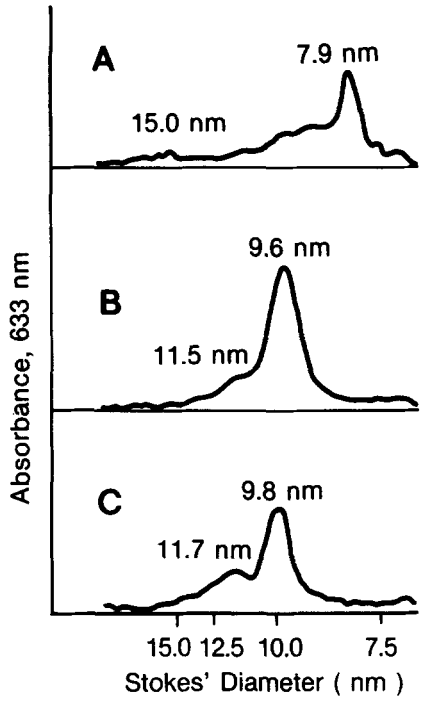

Fig. 6. Densitometric scans of Coomassie blue-stained 4–30% polyacrylamide gradient gels from an experiment showing the effects of the time of incubation (in the presence of exogenous LCAT activity) on the size distribution of perfusate HDL. Electrophoresis conditions were the same as described for Fig. 1. All scans shown are from experiments performed on the liver perfusate from monkey 233. (A) HDL distribution of the perfusate that was stored at 4°C throughout the experiment. (B,C) Perfusate HDL distribution after incubation of perfusate containing 2,700 μg of total cholesterol with 380 μg of cholesterol esterified/hr added LCAT activity, 5 mM β-mercaptoethanol, and 2% human serum albumin at 37°C for 3 hr (B) and 12 hr (C).

column region IV_A. The average composition of each subpopulation is presented in **Table 3**. With increasing particle size the HDL contained a progressively lower average percentage of protein (49% for HDL_{3a} to 38% for HDL_{2b}), but a progressively higher average percentage of neutral lipids (cholesteryl ester + triglyceride = 17% for HDL_{3a} to 25% for HDL_{2b}).

After incubation at 37°C, apoA-I was the major apoprotein of all HDL subfractions as analyzed by SDS-polyacrylamide gel electrophoresis (**Fig. 9**). All HDL subfractions also contained low amounts of SAA, apoA-II, and apoCs. ApoE was barely detectable in the large size HDL subfractions in only one of the four perfusates studied. To assess whether apoE accumulated in other lipoprotein fractions, the apoE/apoB ratios of perfusate VLDL and LDL were estimated by densitometer scanning of SDS polyacrylamide gels. The apoE/apoB ratio of VLDL was unchanged (mean ± SEM, 0.125 ± 0.029 for perfusate maintained at 4°C versus 0.155 ± 0.047 for perfusate incubated at 37°C with active LCAT, n = 4) while the apoE/apoB ratio of LDL decreased following incubation with active LCAT (0.446 ± 0.190 versus 0.087 ± 0.031, n = 3).

Table 4 shows the molar distribution of lipid components among the VLDL (d < 1.006 g/ml fraction), LDL (agarose column regions I, II, and III of the d 1.006–1.225 g/ml fraction) and HDL (agarose column regions IV_A and IV_B) of one liver perfusate after 0, 3, and 12 hr of incubation at 37°C with added LCAT. Also indicated in parentheses are the differences between each of the 3-hr and 12-hr time points and the 0-hr material. All lipoprotein fractions contained higher levels of cholesteryl

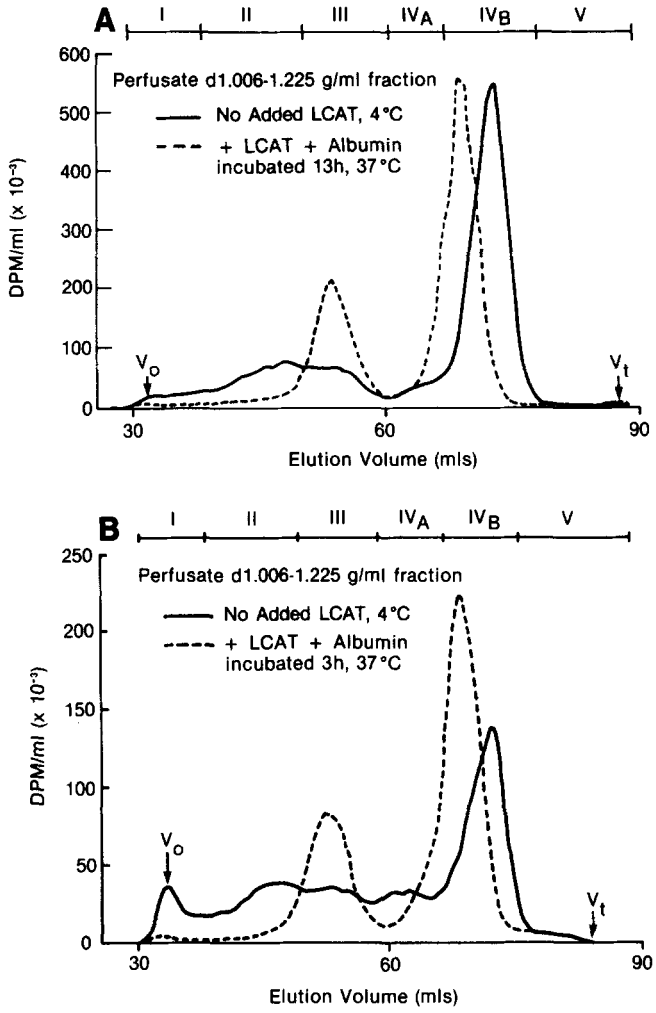

Fig. 7. Agarose column chromatographic profiles of the d 1.006–1.225 g/ml fractions from two separate experiments showing the effects of incubation with exogenous LCAT activity on the distribution of mass among apoB-containing lipoproteins (column regions I, II, and III) and HDL (column regions IV_A and IV_B). Radioactivity distributions were determined as described for Fig. 2 and in Methods. Representative results of experiments with the liver perfusate from monkeys 272 (A, 1,420 μg of total cholesterol and 250 μg of cholesterol esterified/hr added LCAT activity) and 306 (B, 1930 μg of total cholesterol and 1000 μg of cholesterol esterified/hr added LCAT activity). Incubations with exogenous LCAT activity included 2% human serum albumin and 5 mM β-mercaptoethanol.

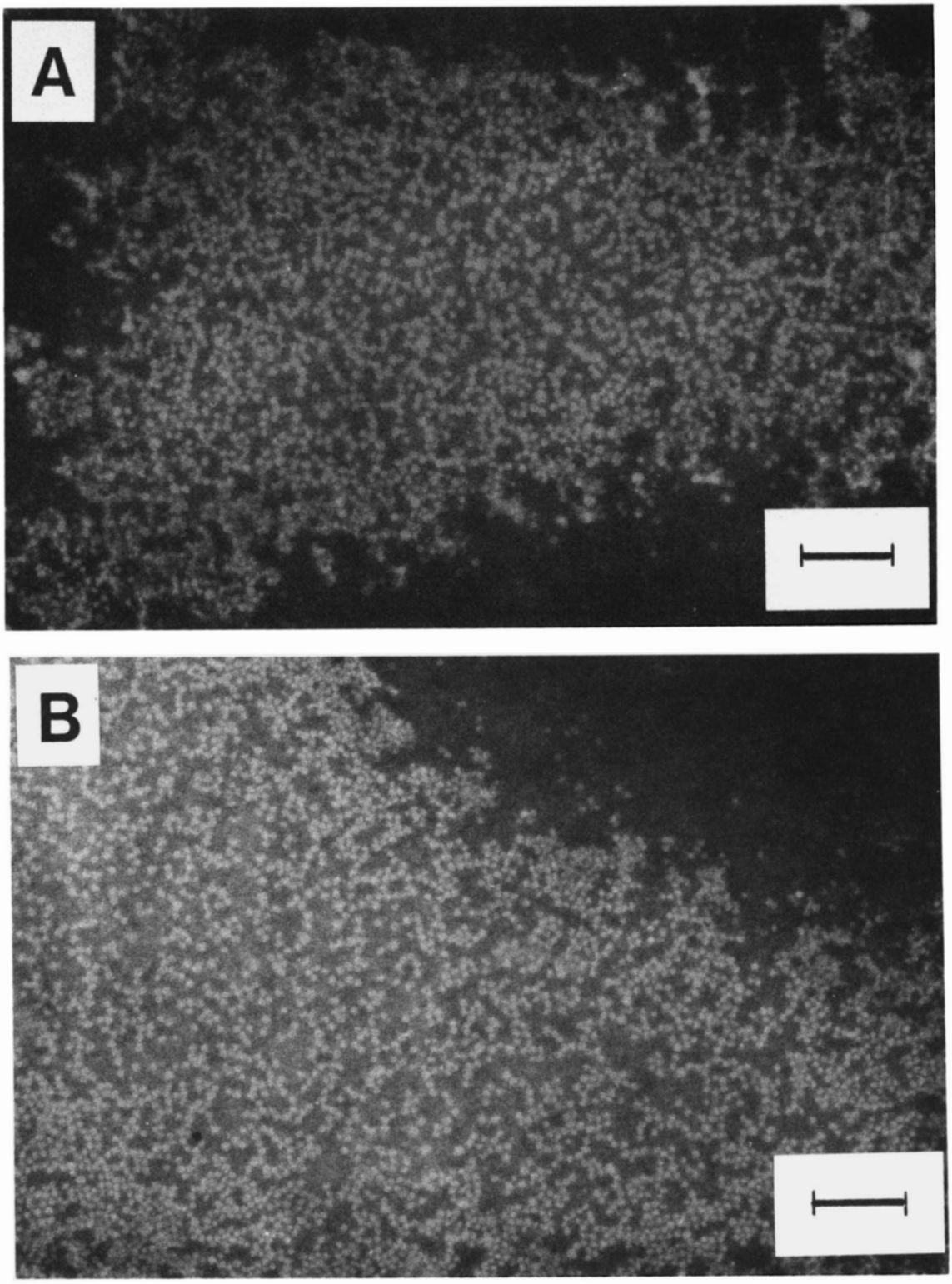

Fig. 8. Negative stain electron micrographs of HDL isolated from agarose column regions IV_A (A) and IV_B (B) after incubation of perfusate from monkey 272 with exogenous LCAT. Incubation conditions are the same as described in Fig. 7. The bar markers represent 100 nm.

ester and lower levels of free cholesterol and phospholipid after incubation with added LCAT. A slight decrease in VLDL triglyceride and slight increases in LDL and HDL triglyceride were also apparent. It is of interest that the HDL fraction did not accumulate cholesteryl ester beyond 3 hr of incubation, but continued to lose free cholesterol

TABLE 3. Chemical composition of HDL subfractions of liver perfusates after incubation at 37°C with active LCAT

HDL Subfraction		Chemical Composition: Protein	Phospholipid	Free Cholesterol	Cholesteryl Ester	Triglyceride	Stokes' Diameter[b]
				weight %			*nm*
HDL_{3a}	n = 5	48.8 ± 1.9[a]	32.0 ± 5.6	2.0 ± 0.6	12.2 ± 5.0	5.1 ± 1.4	8.8 ± 0.3
HDL_{2a}	n = 5	46.6 ± 3.4	31.9 ± 3.7	2.2 ± 0.7	14.8 ± 1.9	4.6 ± 1.8	9.7 ± 0.1
HDL_{2b}	n = 7	38.5 ± 2.9	33.5 ± 5.2	3.3 ± 0.9	14.3 ± 4.9	10.5 ± 3.6	11.3 ± 0.3

Perfusates from all four monkeys were incubated at 37°C with between 0–1000 μg/hr exogenous LCAT activity for between 1 and 13 hr. Following each incubation, the HDL were isolated and separated into subfractions of two sizes as described in the text. The HDL within each subfraction were defined on the basis of particle size as HDL_{3a}, HDL_{2a}, or HDL_{2b}. Since each perfusate was incubated under several conditions, n is greater than 4 for each subfraction.

[a] All values, mean ± SD.

[b] Measured at the center of the peak by calibrated gradient gel electrophoresis.

and phospholipid and gain low amounts of triglyceride during this period. In addition, during the first 3 hr of incubation, the HDL fraction accumulated 1.04 μmol of cholesteryl ester, but lost only 0.57 μmol of free cholesterol and 0.91 μmol of phospholipid. The LDL fraction, on the other hand, lost 1.39 μmol of free cholesterol and 1.97 μmol of phospholipid, but gained only 0.92 μmol of cholesteryl ester during the first 3 hr of incubation. It is also apparent from Table 4 that the molar decrease in total phospholipid was 35% greater at the 3-hr and 12-hr time points than the molar changes in either free cholesterol or cholesteryl ester, suggesting the presence of phospholipase activity in the incubation medium.

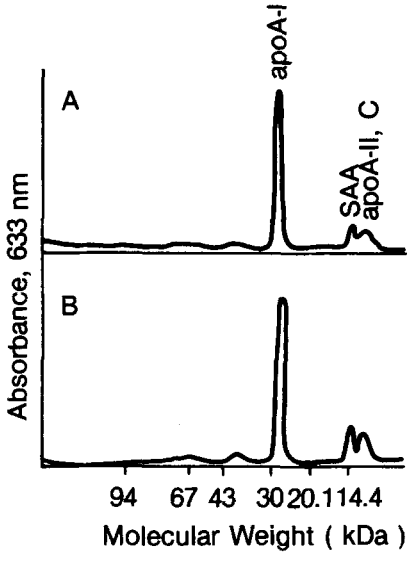

Fig. 9. Densitometric scans of Coomassie blue-stained 4–30% SDS-polyacrylamide gradient gels of HDL isolated from agarose column regions IV_A (A) and IV_B (B) after incubation of the liver perfusate from monkey 272 with exogenous LCAT. Incubation conditions are the same as described in Fig. 7. Electrophoresis was performed as described for Fig. 4 and in Methods.

DISCUSSION

Plasma HDL from humans (21,25), baboons (22), and African green monkeys (J. Babiak and L. L. Rudel, unpublished results), when examined by gradient gel electrophoresis, demonstrate up to five distinct subpopulations. The mean particle size of each subpopulation is fairly consistent within a single animal species, but the relative amount of mass within each subpopulation may be modified by factors such as diet (26, 27), genetic background (22), and exercise (28). The metabolic basis of distinct HDL subpopulations and their physiological significance is unknown. HDL particles are thought to appear in the circulation as neutral lipid-poor discoidal structures containing predominantly apoA-I and phospholipid (29). These particles are converted to cholesteryl ester-containing spherical HDL by the action of LCAT using free cholesterol provided by other lipoprotein particles and cells. Several other investigators have observed such an LCAT-induced conversion of the HDL isolated from the plasma of LCAT-deficient individuals (8, 9) or of apoA-I:phospholipid recombinant particles (11, 12). The primary product, as suggested by other studies as well as our incubations with low levels of LCAT (Fig. 5B, 5C), is an HDL_{3a} particle (8, 12). In contrast, when we incubated liver perfusates with higher levels of LCAT, the HDL usually consisted of two distinct HDL subpopulations when examined by gradient gel electrophoresis (Fig. 5D). The predominant subpopulation in all instances was equivalent in particle size and composition to plasma HDL_{2a}, while the minor subpopulation had the characteristics of plasma HDL_{2b}. Our data show, therefore, that the discoidal HDL particles that accumulate during perfusion of isolated African green monkey livers have the potential to be converted to spherical HDL particles with the properties and particle size heterogeneity typical of plasma HDL.

TABLE 4. Molar amounts of each indicated lipid constituent found within the liver perfusate VLDL, LDL, and HDL fractions of monkey 233 after 0 hr, 3 hr, and 12 hr of incubation

Lipoprotein Fraction	Amounts of lipid constituents											
	Cholesteryl Ester			Free Cholesterol			Phospholipid			Triglycerides		
	0 hr	3 hr	12 hr	0 hr	3 hr	12 hr	0 hr	3 hr	12 hr	0 hr	3 hr	12 hr
							μmol					
VLDL	0.74	1.07 (0.33)	1.78 (1.04)	1.13	0.82 (−0.31)	0.50 (−0.63)	1.81	1.64 (−0.17)	1.37 (−0.44)	2.28	2.02 (−0.26)	2.03 (−0.25)
LDL	1.24	2.16 (0.92)	2.64 (1.40)	2.65	1.26 (−1.39)	0.58 (−2.07)	4.03	2.06 (−1.97)	1.64 (−2.39)	0.94	1.08 (0.14)	1.28 (0.34)
HDL	0.20	1.24 (1.04)	1.24 (1.04)	1.02	0.45 (−0.57)	0.24 (−0.78)	3.49	2.58 (−0.91)	1.63 (−1.86)	0.10	0.22 (0.12)	0.34 (0.24)
Total	2.18	4.47	5.66	4.80	2.53	1.32	9.33	6.28	4.64	3.32	3.32	3.65

Incubations were performed on perfusate containing 2,700 μg of total cholesterol to which LCAT activity of 380 μg cholesterol esterified/hr was added. Differences between the 0 hr and each later time point are indicated in parentheses.

Several features of our incubation system may account for the heterogeneity of the HDL we observe following incubation with LCAT. First, liver perfusate HDL consist of a large variety of discoidal particles with Stokes' diameters ranging between 7.8 and 15.0 nm. The possibility that small, discoidal apoA-I-containing particles are precursors to small, spherical plasma HDL and that large discoidal particles are converted to large plasma HDL is currently under investigation in our laboratory. In addition, the largest discoidal particles found in the liver perfusate are rich in apoE. Apoprotein exchange or whole particle fusion between apoA-I-rich and apoE-rich particles is possible, and may result in the formation of particles with differing lipid and apoprotein compositions. Additional studies are necessary to determine incubation conditions that may modulate such interparticle reactions. Secondly, other as yet undefined factors present in the perfusate may play an important role in the interconversion of HDL subpopulations. One such factor present in the plasma of rats, rabbits, and humans has been reported by Rye and Barter (30) to mediate the conversion of small HDL to large HDL. If this factor is present in our incubation system it is likely to originate in the perfusate. It is not a contaminant in our partially purified LCAT preparation because this factor is reported as not coeluting with LCAT during ion-exchange column chromatography (30). The importance of such a factor in our system can only be determined by additional experiments with purified lipoproteins and enzyme components. The data in this paper do show, however, that LCAT activity is essential for the conversion of discoidal HDL to spherical HDL and that the heterogeneity of the spherical HDL is, in part, related to the amount of LCAT activity present.

A third explanation for the heterogeneity of the HDL products observed is that our incubations included the surface lipid-rich, apoB-containing particles found in the monkey liver perfusates. During the incubations, these particles contributed both free cholesterol and phospholipid to the LCAT reaction (Table 4). An analogous situation may occur *in vivo*. Surface lipid-rich remnants of plasma VLDL and chylomicrons are produced in the circulation by the lipolysis of core triglycerides (31, 32). Such remnant particles have been proposed to contribute to the heterogeneity of plasma HDL by transferring phospholipid and free cholesterol directly to the HDL fraction. Although in the present studies we have not shown that surface lipid-rich, apoB-containing particles interact in the same manner with both spherical and discoidal HDL particles, our results do provide evidence that the transfer of surface lipids to HDL particles is important in the development of HDL particle heterogeneity. Our data also suggest that the excess surface lipid found on apoB-containing lipoprotein particles in monkey liver perfusate may be rapidly transferred to other lipoproteins in the circulation. This process may occur in the intact animal, so apoB-containing particles with excess surface lipids would not be observed in plasma samples.

The redistribution of core lipid molecules during incubation suggests that lipid transfer activity is present in monkey liver perfusate (Table 4). During time-course experiments it was apparent that the HDL fraction accumulated saturating levels of cholesteryl esters relatively rapidly (by 3 hr in the example in Table 4). Essentially all the cholesteryl esters produced by LCAT with further incubation could be accounted for by increased ester mass among the apoB-containing lipoproteins. It is also apparent from Table 4 that the HDL contributed considerable amounts of phospholipid and some free cholesterol as substrates for LCAT even after the HDL stopped accumulating cholesteryl ester. There are two possible explanations for these unusual results. First, in our system LCAT may act directly on the apoB-containing lipoprotein particles that contain apoE (1), a known secondary activator of LCAT (33). In this case, surface lipids are

transferred as needed from the HDL fraction to the apoB-containing particles. However, this possibility is unlikely because in separate experiments (J. Babiak and L. L. Rudel, unpublished observations) we have been unable to detect significant esterification of cholesterol during incubations of liver perfusate apoB-containing particles with partially purified LCAT. A second mechanism that may explain the redistribution of core lipid molecules observed in our incubations is that HDL particles serve as the primary acceptors of LCAT-produced cholesteryl esters. Under these conditions, the HDL would become saturated with core lipids, and lipid transfer proteins in the monkey liver perfusate would then mediate the transfer of cholesteryl ester molecules from core-rich HDL to core-deficient, apoB-containing lipoproteins.

As shown in Table 4, the expected one-for-one molar exchange of cholesteryl ester for triglyceride molecules, as described by Morton and Zilversmit (34), was not observed. That is, the molar amount of triglyceride gained by the HDL fraction during the course of the incubation was less than the amount of cholesteryl ester accumulated by the apoB-containing particles. It is possible that the potential for one-way transfer of neutral lipids from the core-rich HDL (that continue to receive newly synthesized cholesteryl esters from LCAT) to the core-poor LDL and VLDL may predominate over the bidirectional reaction mediated by the lipid transfer protein between typical plasma lipoprotein particles.

It was apparent from compositional analysis of liver perfusate lipoproteins after incubation with LCAT (Table 4) that there was phospholipase activity present in the incubation medium. In the example shown in Table 4, the molar decrease in phospholipid was 35% greater than the molar changes in either free or esterified cholesterol at both the 3-hr and 12-hr time points. A possible explanation for this is that the phospholipase activity of LCAT was greater than its transferase activity. Recent data of Jauhiainen and Dolphin (35) have demonstrated that the phospholipase and transacylase activities of LCAT are separate steps that can, under some circumstances, be independently inhibited. However, the presence of a separate phospholipase activity in the incubation system cannot be ruled out.

In our study, the large discoidal particles present in the unincubated liver perfusates contained apoE as the major protein moiety. After incubation with LCAT, all HDL particles were rich in apoA-I. We observed a loss of apoE from both perfusate HDL and LDL, while the VLDL content of apoE appeared unchanged. Since no lipoproteins functioned as acceptors for apoE during the incubations, it is apparent that some apoE did not co-isolate with lipoprotein particles during ultracentrifugation. We were, however, unable to determine whether apoE remained on the spherical HDL products throughout the incubation and was lost during ultracentrifugation, or was displaced by other apoproteins during the incubation. In the one instance when apoE remained within the HDL fraction, it was found in trace quantities only on the large (HDL_{2b}) product particles. In plasma, apoE is present in the HDL fraction of several animal species (22, 36, 37) and is generally associated only with the largest HDL subpopulations (HDL_{2b} and HDL_1). It has been suggested that these apoE-containing HDL particles are produced by the addition of apoE to pre-existing, apoA-I-containing HDL (38). Our data suggest an alternate pathway. It is possible that apoE-containing plasma HDL may be formed from large, discoidal, apoE-containing particles (2, 39) such as those present in the monkey liver perfusates. Zorich, Jonas, and Pownall (33) have shown that apoE can serve as a cofactor for LCAT when present in apoE-phosphatidylcholine–cholesterol recombinant discoidal complexes. Presumably, such discoidal particles could accumulate LCAT-derived cholesteryl esters and be converted into apoE-containing spherical particles. Hence, the large apoE-containing, discoidal particles present in the unincubated liver perfusates may be converted to spherical apoE-containing particles by the action of LCAT without the addition of exogeneous apoE. In our incubation system, however, very little apoE remained within the HDL fraction following incubation with added LCAT at 37°C. In separate experiments (J. Babiak and L. L. Rudel, unpublished observations) in which apoE-rich perfusate discoidal HDL was incubated in whole plasma, we have observed that African green monkey plasma LDL can serve as an acceptor of the apoE from the discoidal particles. Since African green monkey plasma HDL contain very little apoE, we presume that, *in vivo*, the apoE of nascent HDL particles is efficiently transferred to other lipoprotein fractions or is readily displaced by other apoproteins, such as apoA-I. ApoE-containing HDL may appear in high concentrations in plasma when the production of apoE-containing discoidal HDL exceeds the capacity of other lipoprotein particles to accept apoE from the HDL, rather than when apoA-I-containing HDL are able to acquire large amounts of exogenous apoE.

The authors would like to acknowledge the technical contributions of Ramesh Shah and Joy Martin as well as the skillful efforts of Linda Odham in manuscript preparation. This work was supported by National Institutes of Health grants #HL14164, #HL24736 and #HL07115.

Manuscript received 24 April 1986.

REFERENCES

1. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies of the production of low density lipoproteins by

perfused livers from nonhuman primates: effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221–236.
2. Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. 1980. Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **21:** 625–634.
3. Forte, T. A., A. Nichols, J. Glomset, and K. Norum. 1974. The ultrastructure of plasma lioproteins in lecithin:cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab Invest.* **33(Suppl. 137):** 121–132.
4. Soutar, A. K., B. L. Knight, and N. B. Myant. 1982. The characterization of lipoproteins in the high density fraction obtained from patients with familial lecithin:cholesterol acyltransferase deficiency and their interaction with cultured human fibroblasts. *J. Lipid Res.* **23:** 380–390.
5. Forte, T., K. R. Norum, J. A. Glomset, and A. V. Nichols. 1971. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy. *J. Clin. Invest.* **50:** 1141–1148.
6. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1985. Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey. *J. Lipid Res.* **26:** 403–417.
7. Johnson, F. L., J. Babiak, and L. L. Rudel. 1986. High density lipoprotein accumulation in the perfusates of isolated livers of African green monkeys. Effects of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **27:** 537–548.
8. Chen, C., K. Applegate, W. C. King, J. A. Glomset, K. R. Norum, and E. Gjone. 1984. A study of the small spherical high density lipoproteins of patients afflicted with familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **25:** 269–282.
9. Norum, K. A., J. A. Glomset, A. V. Nichols, T. Forte, J. J. Albers, W. C. King, C. D. Mitchell, K. R. Applegate, E. L. Gong, V. Cabana, and E. Gjone. 1975. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: effects of incubation with lecithin:cholesterol acyltransferase in vitro. *Scand. J. Clin. Lab. Invest.* **35(Suppl 42):** 31–55.
10. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
11. Jonas, A., S. A. Sweeny, and P. N. Herbert. 1984. Discoidal complexes of A and C apolipoproteins with lipids and their reactions with lecithin-cholesterol acyltransferase. *J. Biol. Chem.* **10:** 6369–6375.
12. Nichols, A. V., E. L. Gong, P. J. Blanche, T. M. Forte, and V. G. Shore. 1984. Interaction of model discoidal complexes of phosphatidylcholine and apolipoprotein A-I with plasma components—physical and chemical properties of the transformed complexes. *Biochim. Biophys. Acta.* **793:** 325–337.
13. Rudel, L. L., J. A. Lee, M. D. Morris, and J. M. Felts. 1974. Characterization of plasma lipoproteins separated and purified by agarose-column chromatography. *Biochem. J.* **139:** 89–95.
14. 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.
15. Fiske, C. A., and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. *J. Biol Chem.* **66:** 375–400.
16. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
17. Rudel, L. L., L. Pitts, and C. A. Nelson. 1977. Characterization of plasma LDL of nonhuman primates fed dietary cholesterol. *J. Lipid Res.* **18:** 211–222.
18. Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *o*-phthalaldehyde. *J. Lipid Res.* **14:** 364–366.
19. Sardesai, V. M., and J. A. Manning. 1968. The determination of triglycerides in plasma and tissues. *Clin. Chem.* **14:** 156–161.
20. Nichols, A. V., P. J. Blanche, and E. L. Gong. 1983. Gradient gel electrophoresis of human plasma high density lipoproteins. *In* Handbook of Electrophoresis: Lipoprotein Methodology and Human Studies. Volume III. L. Lewis, editor. CRC, Boca Raton, FL. 29–47.
21. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408–419.
22. 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:** 27–45.
23. Chen, C-H., and J. J. Albers. 1985. A rapid large-scale procedure for purification of lecithin:cholesterol acyltransferase from human and animal plasma. *Biochim. Biophys. Acta.* **834:** 188–195.
24. Chen, C-H., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
25. Anderson, D. W., A. V. Nichols, T. M. Forte and F. T. Lindgren. 1977. Particle distribution of human serum high density lipoproteins. *Biochim. Biophys. Acta.* **493:** 55–68.
26. Babiak, J., A. V. Nichols, E. L. Gong, C. A. McMahan, T. J. Kuehl, G. E. Mott, and H. C. McGill, Jr. 1985. Effects of dietary polyunsaturated and saturated fats on lipoproteins in the baboon. *Atherosclerosis.* **57:** 1–17.
27. Krauss, R. M., G. Butterfield, F. T. Lindgren, V. Shore, S. Shatluck, and Y. Deshailez. 1981. Influence of dietary polyunsaturated:saturated fat ratios on serum high density lipoproteins in normal subjects. *Clin. Res.* **29:** 266A.
28. Wood, P. D., W. L. Haskell, S. N. Blair, P. T. Williams, R. M. Krauss, F. T. Lindgren, J. J. Albers, P. H. Ho, and J. W. Farquhar. 1983. Increased exercise level and plasma lipoprotein concentrations: a one-year, randomized, controlled study in sedentary, middle-aged men. *Metabolism.* **32:** 31–39.
29. Tall, A. R., and Small, D. M. 1978. Plasma high-density lipoproteins. *New Engl. J. Med.* **299:** 1232–1236.
30. Rye, K-A., and P. J. Barter. 1986. Changes in the size and density of human high-density lipoproteins promoted by a plasma-conversion factor. *Biochim. Biophys. Acta.* **875:** 429–438.
31. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
32. Patsch, J. R., A. M. Gotto, Jr., T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoproteins in vitro. *Proc. Natl. Acad. Sci. USA.* **75:** 4519–4523.

33. Zorich, N., A. Jonas, and H. J. Pownall. 1985. Activation of lecithin:cholesterol acyltransferase by human apolipoprotein E in discoidal complexes with lipids. *J. Biol. Chem.* **260:** 8831–8837.
34. Morton, R. E., and D. B. Zilversmit. 1983. Inter-relationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751–11757.
35. Jauhiainen, M., and P. J. Dolphin. 1986. Human plasma lecithin:cholesterol acyltransferase. An elucidation of the catalytic mechanism. *J. Biol. Chem.* **261:** 7032–7043.
36. Mahley, R. W. 1978. Alterations in plasma lipoproteins induced by cholesterol feeding in animal including man. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. American Physiological Society, Bethesda, MD. 181–197.
37. Gibson, J. C., A. Rubinstein, P. R. Bukberg, and W. V. Brown. 1983. Apolipoprotein E-enriched lipoprotein subclasses in normolipidemic subjects. *J. Lipid Res.* **24:** 886–898.
38. Koo, C., T. L. Innerarity, and R. W. Mahley. 1985. Obligatory role of cholesterol and apolipoprotein E in the formation of large cholesterol-enriched and receptor-active high density lipoproteins. *J. Biol. Chem.* **260:** 11934–11943.
39. Ragland, J. B., P. D. Bertram, and S. M. Sabesin. 1977. Identification of nascent high density lipoproteins containing arginine-rich protein in human plasma. *Biochem. Biophys. Res. Commun.* **80:** 81–88.