Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity

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Abstract Plasma lipoproteins from male rats were isolated by rate zonal centrifugation. Four lipoproteins were identified: VLDL, LDL, HDL_1, and HDL_2. LDL, HLD_1, and some HDL_2 distributed within the salt density interval of 1.006–1.085 g/ml, while HDL_2 was found in the 1.063–1.21 g/ml interval. HDL_3 was not identified in the rat. Rat VLDL is poor in cholesteryl esters (1.5–3.0% of total mass) and nearly lacks the smaller and denser particle subpopulation which is predominant in humans. Rat LDL, containing a relatively large amount of triglyceride (20.2% of total mass) and a small amount of cholesteryl ester (27.5%), could be isolated free of apoproteins other than apoB. HDL_1 is a cholesteryl ester-rich lipoprotein that occupies a density interval overlapping both LDL and HDL_2. ApoE is the major protein constituent of HDL_1; apoA-I, A-IV, and C are also present. ApoA-I-rich HDL_2 is the only human-like HDL subpopulation found in rats. Lipoproteins from fasted and non-fasted rats were essentially similar. Arachidonic acid contributed 56.7% and 72.3% of total cholesteryl ester fatty acids in HDL_1 and HDL_2, respectively, but only 7.9% and 27.3% in VLDL and LDL, respectively. Palmitic, palmitoleic, and oleic acids were the major cholesteryl ester fatty acids in VLDL and LDL. In vitro incubation of biosynthetically labeled HDL_2 cholesteryl ester with rat plasma demonstrated minimal transfer of the labeled cholesteryl ester to VLDL and LDL. These results indicate biological immiscibility of HDL cholesteryl esters with those of lower density lipoproteins. The finding of cholesteryl ester-poor VLDL and LDL and the presence of HDL as larger and less dense subpopulations is compatible with the absence of cholesteryl ester transfer activity in an animal with pronounced LCAT activity.—**Oschry, Y., and S. Eisenberg.** Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *J. Lipid Res.* 1982. **23:** 1099–1106.

Supplementary key words very low density lipoprotein • low density lipoprotein • high density lipoprotein • plasma cholesterol • lipoprotein lipids • cholesteryl ester fatty acids • cholesteryl ester exchange

The rat is the most commonly used experimental animal in lipoprotein research. Data obtained in rats are frequently extrapolated to the lipoprotein systems of other mammals, including humans. However, lipoprotein metabolism in rats differs from that in humans in several respects. Two of the most important species differences are the remnant removal pathway and the cholesteryl ester transfer reaction. The rat is an animal with an especially efficient mechanism for clearance of chylomicron and VLDL remnants from the circulation (1–4). This mechanism is found predominantly in the liver and is due to interactions of remnants with specific hepatic receptors (5–7). This pathway is believed to cause the low LDL levels of the rat. The second important difference is the presence of cholesteryl ester exchange protein(s) in human (and rabbit) plasma (8–12) and their absence in the rat (13). In humans, as much as 80% of the LCAT-derived cholesteryl esters is transferred to lower density lipoproteins by the action of this protein (14). Its absence presumably will cause accumulation of cholesteryl esters in HDL and a relative paucity of these molecules in other lipoproteins.

In view of the important role that cholesteryl ester plays in the structure and metabolism of lipoproteins, we have decided to re-evaluate rat plasma lipoproteins as an example of a system devoid of cholesteryl ester transfer activity. Our results indeed demonstrate substantial differences between human and rat lipoproteins that can be ascribed, at least in part, to the absence of cholesteryl ester transport and to the pronounced remnant removal pathway in the rat.

METHODS

Preparation of lipoproteins and ultracentrifugation in a zonal system

Blood plasma was obtained from fasted (14 hr) or non-fasted male rats (3–4 months old; 250–300 g body weight) of the Hebrew University strain while on ad lib

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase.

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rat chow diet (2, 3). The plasma lipoproteins were separated into density fractions by ultracentrifugal flotation at various densities between 1.006 and 1.21 g/ml (15) using a Beckman 50-Ti rotor (Spinco Div., Palo Alto, CA) at 45,000 rpm in a L5-50 ultracentrifuge. Density was adjusted with KBr and KBr solutions of known density. Fractions of d < 1.085 g/ml were centrifuged 16–24 hr while fractions of d > 1.085 g/ml were centrifuged for 48 hr.

The various density fractions were subjected to zonal ultracentrifugation as described by Patsch et al. (16, 17). All runs were carried out in a Beckman Spinco L5-50 ultracentrifuge equipped to accept the Ti-14 zonal rotor. Gradients and solutions were introduced into the rotor by a Beckman model 141 piston gradient pump. The stock solutions for forming the gradient (distilled water; NaBr, d 1.15 g/ml; NaBr, d 1.30 g/ml; and NaBr, d 1.40 g/ml) were prepared containing 350 μM disodium EDTA. Densities were adjusted with reference to hydrometers and the pH was brought to 7.6 using 1 N NaOH.

The lipoproteins of the density fractions were separated by zonal ultracentrifugation at 15°C on 665-ml gradients of density ranges *a*) 1.0–1.15 g/ml, *b*) 1.0–1.3 g/ml, and *c*) 1.0–1.4 g/ml. The gradients were pumped in from the periphery at a rate of 50 ml/min with the rotor spinning at 3,000 rpm. The density of each sample was adjusted to that of the heaviest part of the gradient with solid NaBr, brought to 30 ml with the heavy stock solution, and injected to the rotor periphery. This was followed by a 20-ml cushion of heavy solution to ensure that the entire sample was inside the rotor. The rotor was then sealed, accelerated, and spun at 42,000 rpm for 45 min (1.0–1.15 g/ml gradient), 170 min (1.0–1.3 g/ml gradient), or for 22 hr at 41,000 rpm (1.0–1.4 g/ml gradient). At the end of the run the rotor was decelerated to 3,000 rpm and its contents were displaced by pumping heavy solution into the rotor from the periphery at a rate of 50 ml/min. The rotor effluent was monitored by continuous measurement of absorbance at 280 nm by an ISCO model UA-5 Absorbance Monitor equipped with a quartz flow-through cell. Fractions of 25 ml were collected. Fractions belonging to one lipoprotein peak were pooled and dialyzed for 24–48 hr against several changes of 3 liters of 0.9% NaCl containing 0.01% EDTA (pH 7.6) and 0.01% NaN_3. The pooled fractions were then concentrated to a volume of 3–6 ml by reverse dialysis. The concentrated fractions were further dialyzed against three changes of 1 liter of dialyzate (as above) and used for further studies. Identical procedures were used to prepare analogous lipoproteins from normolipemic human subjects.

Chemical methods

Lipoprotein protein was determined by the method of Lowry et al. (18) using bovine albumin as a reference standard. Triglycerides were determined by the Autoanalyzer method. Total lipoprotein cholesterol and total lipoprotein unesterified cholesterol were measured by a cholesterol oxidase-cholesterol esterase procedure (19) using commercial kits (Boehringer, Mannheim, Germany). Lipoprotein cholesteryl ester content was calculated by difference. Total phospholipids were determined by the Bartlett method (20). To determine phospholipid classes, lipoprotein-lipids were extracted with chloroform–methanol 2:1 (v/v) and washed according to Folch, Lees, and Sloane Stanley (21). Phospholipids were separated by thin-layer chromatography on HR silica gel coated plates and were identified by use of purified reference compounds using a solvent mixture of chloroform–methanol–water 70:25:4 (v/v/v). The lecithin and sphingomyelin spots were identified, scraped off the plate, and their phosphorus content was determined.

Apoproteins were separated on a SDS-polyacrylamide gel electrophoresis (SDS-PAGE) system using 15% acrylamide (22). Apoproteins were stained with Coomassie blue and identified by use of purified apoprotein standards for apoA-I, apoA-II, apoA-IV, apoB, apoC, and apoE.

Negative staining for electron microscopy

One drop of lipoprotein suspension (1–2 mg protein/ml) was applied to collodion–carbon coated grids and then displaced by several drops of 2% potassium phosphotungstate, pH 7.0. Remaining fluid was withdrawn with filter paper. Electron micrographs were obtained with a Philips 300 electron microscope at 60 kV, at instrument magnification of 90,000 diameters. For measurement of the size distribution, negatives were enlarged to 225,000 and the diameters were measured on the prints.

Gas–liquid chromatography of cholesteryl ester fatty acids

Cholesteryl ester fatty acid profiles were determined on lipoproteins isolated from the plasma of fasted rats. Lipoprotein lipids were extracted with chloroform–methanol 2:1 (v/v) as described above. Cholesteryl esters were separated by thin-layer chromatography with hexane–chloroform 140:60 (v/v) and were extracted with 10 ml of chloroform. Fatty acids were separated on an automated gas–liquid chromatography apparatus (Packard Gas Chromatograph System) after transesterification with boron trifluoride methanol (23).

Transfer of cholesteryl esters from HDL_2 to other lipoproteins

Rat plasma supplemented with [^{3}H]cholesterol-biosynthetically-labeled HDL_2, and unlabeled rat plasma VLDL was used. [^{3}H]Cholesterol-labeled HDL_2 was isolated by zonal ultracentrifugation from the plasma of rats injected with 0.2–0.3 mCi [7(N)-^{3}H]cholesterol (Amersham Radiochemical Centre, England) 6 hr prior to killing. Incubation mixtures contained 3 ml of rat plasma (0.5 mg of VLDL protein) and 100,000 dpm of [^{3}H]cholesterol-labeled HDL_2 and were carried out at 37°C for 0, 8, and 24 hr. To inhibit LCAT activity, parachloromercuriphenyl sulfonic acid (PCMPS) was included at a final concentration of 2 mM. Similar mixtures containing 1 ml of human plasma fraction of d > 1.21 g/ml were incubated in parallel to those containing rat plasma alone. After the incubation, lipoprotein fractions of d < 1.006 g/ml, d 1.006–1.063 g/ml, and d 1.063–1.21 g/ml were isolated by sequential ultracentrifugation and assessed for [^{3}H]cholesteryl ester contents.

RESULTS

Zonal elution profiles of rat plasma lipoproteins present in density intervals of d < 1.006 g/ml, d 1.006–1.063 g/ml, d 1.006–1.085 g/ml, d 1.063–1.21 g/ml, and d 1.085–1.21 g/ml are shown in Figs. 1–3. Elution profiles of human VLDL (d < 1.006 g/ml), LDL (d 1.019–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) are included for comparison. Nearly all rat plasma VLDL eluted in the first 200 ml of the rotor effluent (**Fig. 1**). There was almost complete absence of heavier VLDL particles (rotor effluent volume of 200–360 ml), which contribute about half of human VLDL.[2] The elution profile of VLDL from fasted and non-fasted rats differed at the first 50–75 ml of the rotor effluent, but coincided from 100 ml on. The difference was due to pronounced turbidity in the initial fractions of VLDL from non-fasted rats, indicating the presence of large light-scattering particles in this sample. Four lipoproteins were observed in rat plasma fraction of d 1.006–1.085 g/ml (**Fig. 2A**). The lightest lipoprotein was present in small amounts and was not further characterized. The position and amount of this lipoprotein fraction varied and in some samples it was barely evident. The heaviest lipoprotein was identified as rat plasma HDL_2 (compare Fig. 2A with Fig. 2C). HDL_2 was excluded

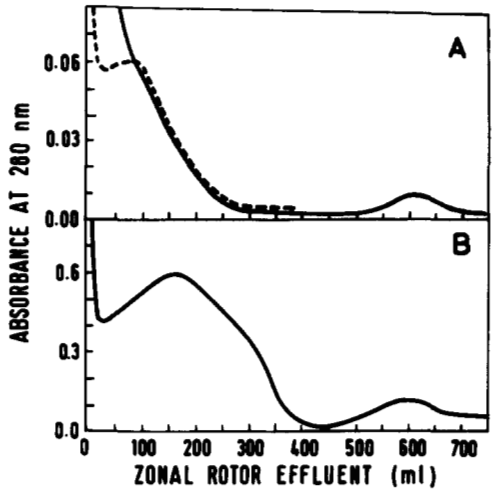

Fig. 1. Rate zonal ultracentrifugation of plasma lipoproteins of d < 1.006 g/ml. Frame A shows elution profile of lipoproteins from fasted (broken line) and non-fasted (solid line) rats. Frame B shows elution profile from fasted normolipemic human subject (plasma triglyceride 133 mg/dl and plasma cholesterol 191 mg/dl). Elution profiles were monitored at 280 nm. Lipoproteins were separated in the 1.0–1.15 g/ml NaBr gradient. The absorbance in non-fasted rat plasma samples was affected by marked turbidity in the first 75 ml effluent volume, and should not be taken as indicative of protein concentration.

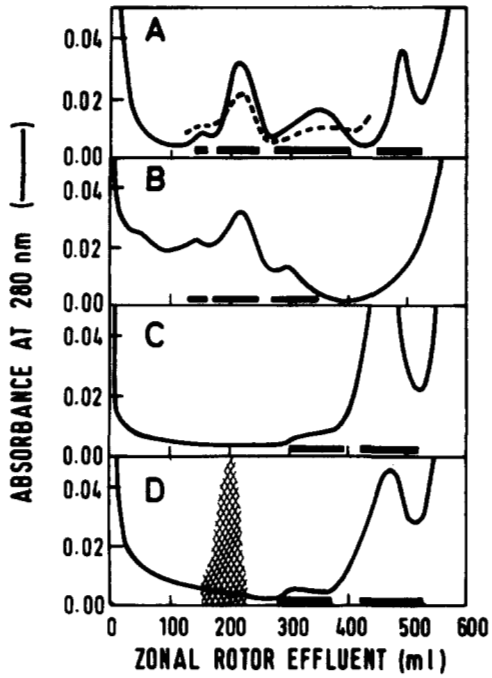

Fig. 2. Rate zonal ultracentrifugation of rat plasma lipoproteins of various densities above 1.006 g/ml, separated in the 1.0–1.3 g/ml NaBr gradient. Lipoproteins of the following density intervals isolated from 60–80 ml rat plasma are presented: d 1.006–1.085 g/ml from fasted (broken line) and non-fasted (solid line) rats (frame A); d 1.006–1.063 g/ml (frame B); d 1.085–1.21 g/ml (frame C); and d 1.063–1.21 g/ml (frame D). Solid bars indicate lipoprotein fractions isolated for further analysis. Cross-hatched area represents elution profile of human plasma LDL (d 1.019–1.063 g/ml).

[2] Oschry, Y., and S. Eisenberg. Unpublished observations.

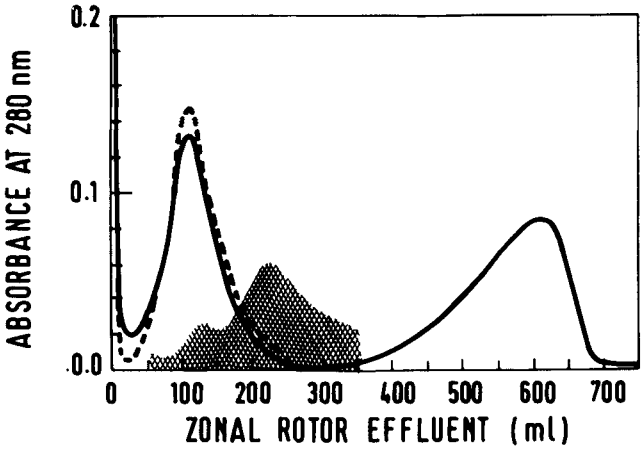

Fig. 3. Rate zonal ultracentrifugation of rat plasma lipoproteins of d 1.085–1.21 g/ml separated in the 1.0–1.4 g/ml NaBr gradient. Almost identical profiles were obtained with rat plasma lipoproteins of density 1.063–1.21 g/ml. Cross-hatched area represents the elution profile of human HDL (d 1.063–1.21 g/ml). Broken line, plasma from fasted rats; solid line, plasma from non-fasted rats.

when a lower density cut of d 1.006–1.063 g/ml was similarly applied (Fig. 2B). Two discernible lipoproteins were identified at the zonal effluent volume of 175–425 ml. The lighter lipoprotein (elution volume (175–275 ml) occupied a position similar to, but slightly denser than, human LDL and is designated rat LDL. The heavier lipoprotein (elution volume 275–425 ml) was not detected in human plasma. It is designated rat HDL_1. Analysis of plasma fractions from fasted rats yielded virtually identical results (Fig. 2A, broken line). Only one lipoprotein was observed when rat plasma fraction of d 1.085–1.21 g/ml from fasted or non-fasted rats was applied to a 1.0–1.4 g/ml gradient and eluted after 22 hr of centrifugation at 41,000 rpm (**Fig. 3**). The elution profiles of these lipoproteins were similar to each other and to human HDL_2. This rat lipoprotein is designated HDL_2. HDL_3 was not observed in rat plasma. In LDL runs (170 min centrifugation at 42,000 rpm and a 1.0–1.3 g/ml NaBr gradient), small amounts of HDL_1 were found in the rat plasma fraction of d 1.085–1.21 g/ml (Fig. 2C). More HDL_1 was identified at plasma density fractions of d 1.063–1.21 g/ml (Fig. 2D).

The composition, size distribution, and SDS-PAGE of isolated rat plasma, VLDL, LDL, HDL_1, and HDL_2 are shown in **Table 1, Fig. 4,** and **Fig. 5.** No significant differences were detected between lipoproteins from fasted and non-fasted rats. Triglycerides were the predominant lipid constituent of VLDL, but were also present in appreciable amounts in LDL (20.2% of mass). Apoproteins, cholesteryl esters, and phospholipids were the main constituents of HDL_1 and HDL_2. The molar ratio of lecithin to sphingomyelin was highest in HDL_2 and lowest in LDL. The diameter of VLDL particles varied considerably, from 311 Å to 578 Å with a mean diameter of 433 Å. The mean diameters were: LDL, 219 Å (range 178 Å to 267 Å); HDL_1, 127 Å (range 89 Å to 178 Å); and HDL_2, 92 Å (range 70 Å to 118 Å). Apoproteins B, A-IV, E, and C were present in VLDL. Apoprotein B was the only protein constituent of LDL, especially when the descending part of the LDL peak was excluded. In HDL_1, apolipoprotein E was the major protein constituent. It is estimated that apoE contributes 40–60% of the total protein mass of this lipoprotein. ApoA-I, apoA-IV, and apoC were also identified in HDL_1. ApoA-I was the major apoprotein in HDL_2. ApoA-IV, apoC, and apoE were also present.

Two experiments were performed to determine the miscibility of cholesteryl esters from different lipoproteins. In the first experiment, the fatty acid composition of cholesteryl esters in VLDL, LDL, HDL_1, and HDL_2 from fasted rats was determined by gas–liquid chromatography (**Table 2**). Cholesteryl ester fatty acid profiles of HDL_1 and HDL_2 were distinctly different from those of VLDL and LDL. In the two HDL species, we

TABLE 1. Lipid and protein composition of rat plasma lipoproteins

		Protein	TG	CE	UC	PL	LE/SP
		mg/100 mg lipoprotein					
VLDL	Non-fasted	11.9 ± 0.3[a]	63.3 ± 0.7	2.5 ± 0.3	4.0 ± 0.4	18.3 ± 0.9	5.0 ± 0.4
	Fasted	12.5 ± 1.3	65.1 ± 1.9	2.5 ± 0.3	3.1 ± 0.3	16.7 ± 0.6	N.D.[b]
LDL	Non-fasted	23.6 ± 1.3	20.2 ± 1.6	27.5 ± 1.4	7.8 ± 0.7	21.0 ± 1.2	3.4 ± 0.3
	Fasted	22.1 ± 0.5	20.5 ± 0.3	27.0 ± 1.2	7.3 ± 0.1	22.5 ± 0.4	N.D.
HDL_1	Non-fasted	28.3 ± 1.0	6.2 ± 0.5	25.6 ± 1.0	7.9 ± 0.6	32.0 ± 0.9	4.9 ± 0.3
	Fasted	26.1 ± 1.7	4.1 ± 0.5	28.9 ± 0.5	7.1 ± 0.1	33.2 ± 1.9	N.D.
HDL_2	Non-fasted	40.5 ± 1.5	2.7 ± 0.6	25.9 ± 1.8	3.6 ± 0.4	27.1 ± 0.7	8.0 ± 0.7
	Fasted	41.6 ± 1.7	1.2 ± 0.3	25.8 ± 0.4	3.2 ± 0.2	28.1 ± 0.9	N.D.

[a] Data are means ± S.E. of three to seven determinations. The molecular weight of cholesteryl esters was taken as 650.
[b] N.D. = Not determined.

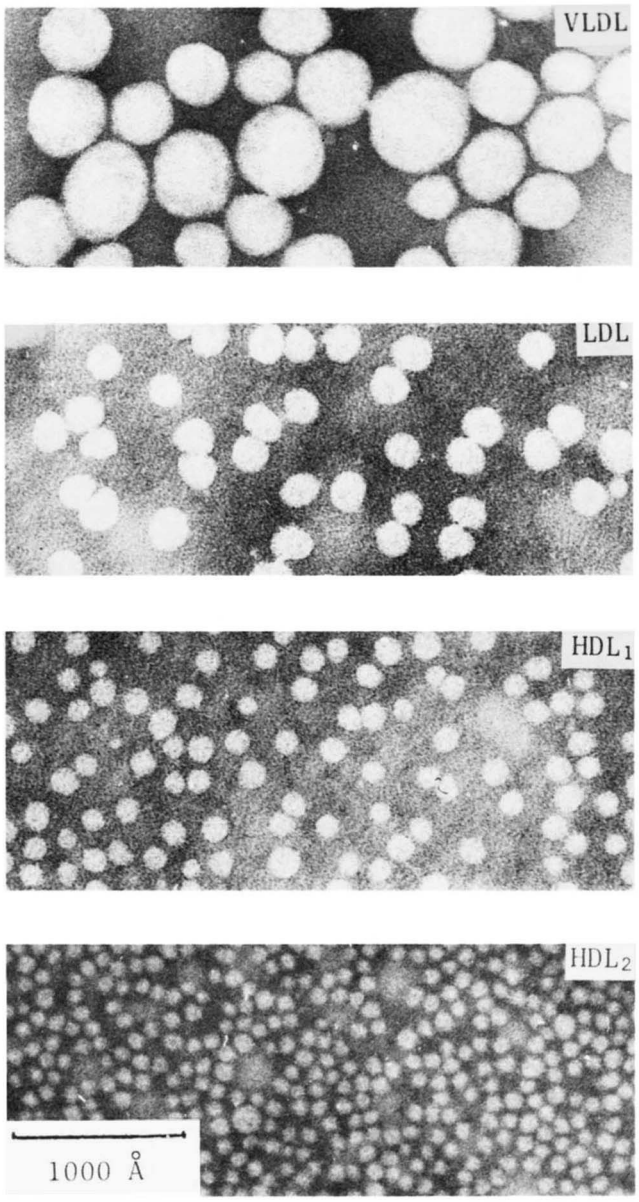

Fig. 4. Electron micrographs of rat plasma VLDL, LDL, HDL_1, and HDL_2. Original magnification ×225,000.

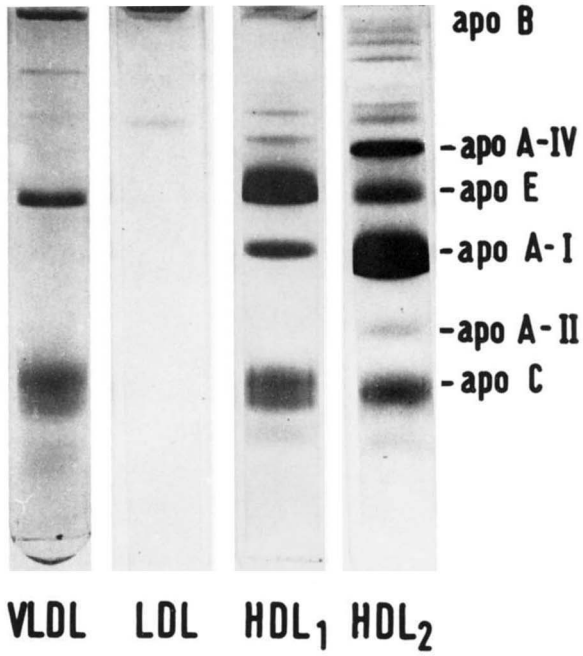

Fig. 5. SDS-PAGE of apoproteins of rat plasma VLDL, LDL, HDL_1, and HDL_2. Gels (15% acrylamide) were loaded with 50 μg of lipoprotein-protein.

observed preponderance of arachidonic acid and paucity of palmitic, palmitoleic, and especially oleic acids. Cholesteryl ester fatty acid profiles of VLDL and LDL were generally similar, except for increased proportion of arachidonic acid in LDL. Notwithstanding, palmitic, palmitoleic, and oleic acids contributed 62.9% of the VLDL and 46.2% of the LDL cholesteryl ester fatty acids, distinguishing them from the HDL species. These results indicated immiscibility of HDL_1 and HDL_2 cholesteryl esters with those of VLDL and LDL. Further evidence that appreciable amounts of cholesteryl esters do not move from HDL_2 to other lipoproteins was obtained when rat plasma containing [^{3}H]cholesterol-labeled HDL_2 was incubated in vitro for 24 hr (**Fig. 6**, solid lines). In these experiments, lipoproteins were separated by sequential ultracentrifugation. As some HDL_2 floats at density of 1.085 g/ml, this lipoprotein was isolated at the density interval of 1.063–1.21 g/ml. After 8 and 24 hr of incubation, minimal decrease of [^{3}H]cholesteryl ester content in HDL_2 was observed, from 97.8% of total radioactivity to 95.6% and 95.0% (Fig. 6). There was almost no change of [^{3}H]cholesteryl ester content in VLDL, but in the fraction of d 1.006–1.063 g/ml, it increased slightly, from 1.8% to 2.6% and 3.3% of total radioactivity (Fig. 6). This increase might account for the amount of arachidonic acid in the LDL cholesteryl esters or might be due to an increase in the [^{3}H]cholesteryl ester content in the HDL_1 pres-

TABLE 2. Cholesteryl ester fatty acid profiles of rat plasma lipoproteins

Fatty Acid	VLDL	LDL	HDL_1	HDL_2
	mole/100 mole fatty acids			
16:0	18.6 (2.9)[a]	12.6 (0.3)	10.7 (0)	6.1 ± 3.2
16:1	16.4 (1.7)	9.3 (4.3)	6.1 (1.2)	3.5 ± 1.2
18:1	28.1 (3.3)	24.3 (1.9)	5.9 (1.0)	3.3 ± 0.7
18:2	23.9 (1.7)	24.5 (3.2)	19.0 (0.3)	14.0 ± 1.1
20:4	7.9 (0.9)	27.3 (1.6)	56.7 (0.2)	72.3 ± 4.3

[a] Results are mean of two experiments (VLDL, LDL, and HDL_1) or mean ± S.D. of four experiments (HDL_2). Numbers in parentheses are range of the two experiments.

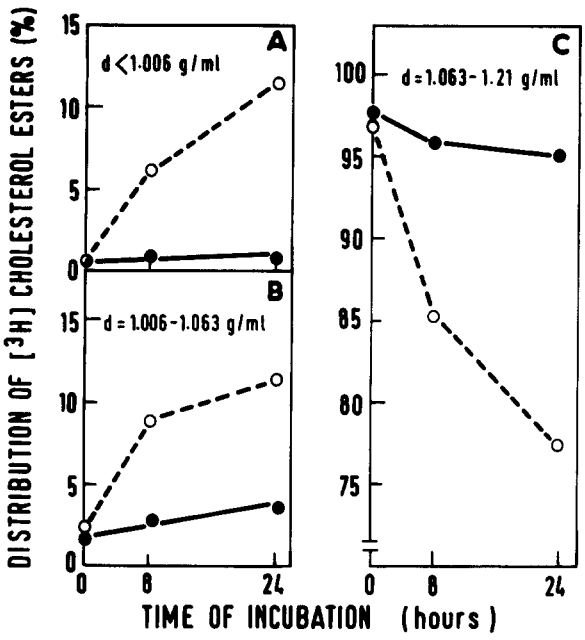

Fig. 6. Distribution of [^{3}H]cholesteryl ester from biosynthetically labeled HDL_2 to lower density rat lipoproteins. Incubation mixtures contained 3 ml of rat plasma, 100,000 dpm [^{3}H]cholesterol-labeled HDL_2 (70–75% of radioactivity in [^{3}H]cholesteryl esters), and 2 mM PCMPS without (● —— ●) or with (○ - - - ○) 1 ml of human plasma protein fraction of d > 1.21 g/ml. Incubations were carried out at 37°C for 0, 8, and 24 hr. Lipoproteins were isolated at densities of <1.006 g/ml, 1.006–1.063 g/ml, and 1.063–1.21 g/ml and assessed for [^{3}H]cholesteryl ester content. Data show percent distribution of [^{3}H]cholesteryl esters among the lipoproteins and are means of three experiments.

ent in this fraction. In contrast, considerable exchange and/or transfer of [^{3}H]cholesteryl ester from HDL_2 to other lipoproteins, especially to VLDL, was observed when human plasma fraction of d > 1.21 g/ml was added to the incubation mixture (Fig. 6, broken lines).

DISCUSSION

Rat lipoproteins are usually separated by sequential ultracentrifugation at fixed salt density intervals (24–26). Relatively few studies using other methods to separate rat plasma lipoproteins have been published (27–29). The accuracy of results obtained by fixed density centrifugation of the lipoproteins of various species depends on the assumption that those lipoproteins are analogous to the ones for which the method was developed (15). This assumption is evidently incorrect for rat lipoproteins.

Rate zonal ultracentrifugation separates lipoproteins in a continuous density gradient and is therefore especially suited for lipoproteins that overlap the "classic" salt boundaries. Indeed, two recent studies using rate zonal ultracentrifugation have indicated the presence of more than one lipoprotein in the rat plasma fraction of density either above or below 1.063 g/ml (30, 31). The present study reports separation of all rat plasma lipoproteins in a zonal rotor and demonstrates significant differences between rat and human lipoproteins.

Rat plasma lipoproteins from fasted and non-fasted rats are strikingly similar. No differences were detected in LDL, HDL, and HDL_2. The only dissimilarity was observed in the profiles of VLDL, where the presence of larger light-scattering particles was observed in non-fasted samples. These are presumably of dietary origin, i.e., chylomicrons and chylomicron remnants. Yet, the contribution of chylomicrons to the total lipoproteins of d < 1.006 g/ml was very small as we have not found compositional differences between samples from fasted and non-fasted rats. Furthermore, larger particles were rare in electron micrographs from non-fasted rats. The registered absorbance in the initial fractions of non-fasted samples is mainly the result of light-scattering rather than the presence of significant amounts of chylomicron protein or lipids.

Several differences are evident when comparing the rat lipoproteins studied here with data on "analogous" human lipoproteins (32). The elution profile of rat VLDL indicates nearly complete absence of the smaller and denser particle subpopulation, which in humans with comparable VLDL levels make up most of that fraction.[2] This possibly reflects the rapid clearance of VLDL remnants in the rat (1–7). Also, rat VLDL is very poor in cholesteryl ester content, only 2–3% of total mass, compared to 12–17% in humans (32). Rat LDL is similar to human in apoprotein profile (predominantly apoB). In rats, however, this lipoprotein is relatively rich in triglycerides. In agreement with Lusk et al. (30), we find that it is almost impossible to isolate this apoB-rich LDL free of apoE and other apoproteins when using conventional centrifugation. Striking differences were found between the HDL systems of humans and rats. The rat completely lacks HDL_3 (as a separate lipoprotein subclass), but contains appreciable amounts of a lipoprotein designated HDL_1. This lipoprotein has been previously identified in rat d < 1.063 g/ml (30, 33) or d > 1.063 g/ml fractions (31). Here we show HDL_1 to be a polydisperse population extending both above and below the density 1.063 g/ml. Indeed, trace amounts can be found even at salt density greater than 1.085 g/ml. We have characterized HDL_1 isolated from the d 1.006–1.085 g/ml density fraction, thereby avoiding the exclusion of significant subpopulations of this particle. This HDL_1 can be isolated free of apoB, contains apoE as the predominant apoprotein (40–60% of protein mass), and is a cholesteryl ester-rich lipoprotein.

Some, or even most of the differences between rat

and human lipoproteins can be explained on a metabolic basis, especially by the absence of cholesteryl ester transfer activity. Cholesteryl esters in plasma are formed in high density lipoproteins by the lecithin:cholesterol acyltransfer reaction (34). In human and other species, these cholesteryl esters are transferred to lower density lipoproteins and constitute the major source of cholesteryl esters for all lipoproteins (14, 34). This reaction is catalyzed by a plasma protein factor, the cholesteryl ester transfer/exchange protein (8–12) possibly identical with apolipoprotein D (10). The protein may be part of a specialized high density lipoprotein particle that also includes apoA-I and LCAT (11), as well as the plasma phospholipid exchange protein (12). The protein, or possibly an additional plasma factor, catalyzes another plasma reaction that results in transfer of cholesteryl ester between lipoproteins, the reaction of exchange of triglycerides for cholesteryl esters (35, 36). Rat plasma reportedly lacks both reactions (13).

Our study substantiates this report as we have found HDL_2 cholesterol to be almost immiscible with cholesteryl esters in VLDL and LDL in in vitro experiments (Fig. 6). The fatty acids profiles of cholesteryl esters of the different lipoproteins lends further support to this conclusion. Arachidonic acid is the major fatty acid in HDL_2 cholesteryl esters (72.3%) but contributes only 7.9% to VLDL and 27.3% to LDL cholesteryl ester fatty acids. This predominance of arachidonic acid in rat HDL but not VLDL can also be found with conventional ultracentrifugation (d 1.063–1.21 g/ml) (37). Thus, plasma cholesterol arachidonate, which is formed mainly in HDL by the LCAT reaction (38), does not find its way to VLDL. The general paucity of cholesteryl esters in rat VLDL, and its degradation product, LDL, is compatible with the absence of cholesteryl ester transfer in this species. This lack of cholesteryl ester transfer in rats can be ascribed to either the lack of the protein that catalyzes the reaction or to the inability of the transfer protein to interact with the plasma lipoproteins. Our observation that cholesteryl esters are readily transferred from rat HDL_2 to lower density lipoproteins when human plasma proteins are added indicates that the former view is correct.

The rat HDL system is also compatible with the absence of cholesteryl ester transfer in the face of continued formation of cholesteryl esters. Such a situation will result in accumulation of LCAT-derived cholesteryl esters in the HDL, and conversion of the small and relatively cholesteryl ester-poor HDL_3 particles to HDL_2 (39). Rat HDL_1 represents an HDL population especially enriched with cholesteryl ester whose origin and function are unknown. Similar cholesteryl ester-rich and apoE-rich HDL populations are found in animals during cholesterol feeding (40–42), as well as in abetalipoproteinemia, a human disease where acceptors for LCAT-derived cholesteryl esters are absent (43).

The HDL_1 cholesteryl ester fatty acid profile reported here indicates that HDL_1 is also formed in the plasma compartment. This is implied by the high contribution of arachidonic acid (56.7%), and minimal contribution of palmitic, palmitoleic, and especially oleic acid to the HDL_1 cholesteryl ester fatty acids. This profile is akin to that of HDL_2, suggesting even conversion of one lipoprotein to the other. The presence of large amounts of apoE in HDL_1 together with other HDL apoproteins (A-I, A-IV, and C) may then reflect a simple distribution of apoproteins between two HDL populations (HDL_1 and HDL_2) with different physical properties. Alternatively, HDL_1 may represent a discernible lipoprotein class whose origin and function in a normal animal await evaluation.

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