Characterization of Lipoprotein Fractions Isolated from Plasma of Male Wistar Rats by Gradient Ultracentrifugation (43639)

ALFREDO CANTÀFORA,[1] ELENA BRAVO, AND CHONG CHAO YAN
Istituto Superiore di Sanità, Laboratory of Metabolism and Pathological Biochemistry, Rome, Italy

Abstract. **Both Wistar and Sprague-Dawley rat strains have been used for experimental studies on lipoprotein metabolism, although the lipoprotein characteristics of the former strain are less well known than those of the latter. We have defined more precise conditions for separating by density gradient ultracentrifugation the different lipoprotein classes from plasma of young male Wistar rats. Present results confirm that Wistar rats, like other rat strains, have negligible amounts of low density lipoproteins and levels of very low density lipoproteins much lower than high density lipoproteins. The differences between rat strains appeared in the profile of lipoprotein denser than 1.08 g/ml. In fact, we showed Wistar rats have considerable amounts of high density lipoprotein-3 subfraction with a density range between 1.17 and 1.21 g/ml, a mean diameter of 4.9 nm, and a low cholesterol content. In analogy with the Sprague-Dawley strain, Wistar rats had a small proportion of high density lipoprotein-1 subfraction (about 17% of total lipoproteins) and, at variance, in Wistar rats, we did not observe the presence of the so-called very high density lipoprotein fraction described previously in the Sprague-Dawley strain.** [P.S.E.B.M. 1993, Vol 204]

Part of present knowledge on lipoprotein metabolism, especially that concerning hepatic uptake and secretion of lipoprotein lipids, derives from studies performed with rats (1–6). This explains why, in some cases, results of these studies have been extrapolated to other mammalian species notwithstanding the peculiarity of rat lipoprotein composition and its variability in relation with the animal's age (7, 8), sex (9, 10), and strain (11, 12).

Rat lipoprotein metabolism has been studied prevalently in young male animals of either Wistar or Sprague-Dawley strains. However, information on the rat lipoprotein profile mostly derives from Sprague-Dawley animals. This profile is characterized by the presence of both a very high density lipoprotein (VHDL) fraction and a high density lipoprotein (HDL) fraction rich in apolipoprotein-E, identified as HDL1 fraction (13–15). The presence of an HDL1 fraction has been reported in the Osbourne-Mendel strain (16), while there are conflicting data in previous reports on the presence, quantity, and characteristics of this fraction in Wistar rats (17, 18). Furthermore, in Sprague-Dawley as well as in other strains, some points, e.g., the exact contribution of low density lipoprotein (LDL) and HDL3 fractions, are still controversial (4, 11, 19).

In this study, we examined in detail the lipoprotein composition of 3-month-old male Wistar rats because of our interest in understanding the mechanisms by which the liver metabolizes and secretes into bile the steroids taken up from plasma lipoproteins in the animals (1–3). The method used here for separating homogenous lipoprotein fractions was gradient ultracentrifugation (UC) of male Wistar rat plasma. This gave a density profile of lipoproteins useful for determining density boundaries required for a correct lipoprotein separation by sequential UC, which still remains the most widely used method for routine separation of lipoproteins, particularly when they are required for preparative use. The fractions pooled from the gradient UC have been physically and chemically characterized in this study.

[1] To whom requests for reprints should be addressed at Istituto Superiore Sanità, Viale Regina Elena, 299, 00161 Roma, Italy.

Received September 21, 1992. [P.S.E.B.M. 1993, Vol 204]
Accepted June 2, 1993.

0037-9727/93/2041-0090$3.00/0

Materials and Methods

All the chemicals and solvents were of analytical grade (Farmitalia Carlo Erba, Milano, Italy). Enzymatic kits for the assay of triglycerides and free and total cholesterol were purchased from Boehringer Mannheim Italia (Milano, Italy).

Animals and Plasma Preparation. Male Wistar rats with an average weight of 275 ± 20 g were purchased from Charles River Italia (Calco-Como, Italy). The animals were subjected to 24-hr light cycling (12 hr of light and 12 hr of darkness) and were allowed free access to water and commercial rodent diet (a single batch of Diete Standard by Mucedola Srl, Settimo Milanese, Milano, Italy) for at least 1 month before beginning the study. The distribution of rough protein, fat, and cellulose in the diet was 18.5%, 3%, and 6%, respectively. The animals were fasted for about 1 hr before being sacrificed by decapitation. The blood was collected in plastic tubes containing 3 mg/ml of EDTA as anticoagulant. Plasma was immediately separated by low-speed centrifugation at 4°C.

Lipoprotein Fractionation. Plasma lipoproteins were separated by gradient ultracentrifugation with the discontinuous gradient described by Chapman *et al.* (20). Briefly, 3 ml of plasma, brought to the density of 1.21 g/ml with NaBr, were layered over 2 ml of a solution at the density of 1.24 g/ml. Next, 2 ml, 2.5 ml, and 3 ml of solutions at densities of 1.21, 1.063, and 1.006 were layered, in that order. The tubes were transferred into the buckets of an SW 41 Ti rotor and were centrifuged for 20 hr at 40,000 rpm at 22°C using a Beckman L-70 ultracentrifuge. After centrifugation, the tube was placed in an apparatus for gradient fractionation produced by Hoefer Scientific Instruments (San Francisco, CA). The gradient was displaced upward by a solution of NaBr with density of 1.34 g/ml pumped into the bottom of the tube at the flow rate of 0.2 ml/min. The gradient passed through the flow cell of a uv detector set at 280 nm (Monitor UV-M; Pharmacia, Uppsala, Sweden) before it was collected in preweighed tubes with a fraction collector.

Experimental Protocol. Each preweighed tube was weighed again after gradient collection. From the weight difference and the volume, an approximate density was calculated for each tube. An aliquot of 130–150 μl was taken from each tube for the determination of protein, free and total cholesterol, and lipid phosphorous. The lipoprotein fractions were collected by pooling the tubes according to the above-mentioned analyses, and their chemical and physical characteristics were determined. Specifically, triglyceride, free and total cholesterol, total protein and apoprotein distribution, and lipid phosphorous concentrations were measured. For the physical determinations, each pooled fraction was extensively dialyzed overnight at 4°C against a solution containing 0.14 *M* NaCl and 0.001 *M* sodium azide. The fractions were submitted to electron microscopy (EM) and gel permeation chromatography (GPC) examinations.

Analytical Procedures. The protein content of plasma and lipoproteins was determined by Bradford's method (21) using bovine serum albumin as standard. Free and total cholesterol and triacylglycerols were estimated with the enzymatic kits according to the instructions given by the manufacturer. The distribution of phospholipid classes and their fatty acid compositions were determined in chloroform-methanol extracts (22) by thin layer chromatography and gas-liquid chromatography, as described previously (23). The percentage of distribution of apoproteins was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the PhastSystem (Pharmacia), as described previously (24). The percentage of distribution of C apoproteins, in a few selected samples, of lipoprotein fractions was determined by isoelectric focusing, as described by Fidge and Nestel (25), with the modifications required to perform the electrophoretic separation with the PhastSystem. Briefly, the PhastGel slab was washed for 10 min in bidistilled water and dried under a gentle air steam. Then, the slab was swollen for 1 hr into 2 m*M* Tris-HCl buffer (pH 7.4) containing 8 *M* urea, 30% glycerol, and 2% ampholine (pH 4–6; Pharmacia-LKB). The delipidated lipoprotein sample was resuspended in 20–40 μl of 10 m*M* Tris-HCl buffer (pH 7.4) containing 6 *M* urea, 1% sodium dodecyl sulfate, and 1.6% ampholine (pH 4–6). The electrophoretic conditions included a prerun (1000 V, 2.0 mA, 15°C, for 75 V-hr) followed by two runs, 200 and 1000 V, lasting 15 and 700 V-hr, respectively. The protein staining was performed with Coomassie blue R350 according to the standard technique for isoelectric focusing with PhastSystem (PhastSystem Development Technique, File No. 200).

A drop of the dialyzed fraction was observed by transmission EM after staining with sodium phosphotungstate (26). Observations were made at 50,000 times magnification with a Zeiss EM 10C electron microscope operating at 60 kV. Particle size was assessed using micrographs in a field of 500–1000 particles counted on a final print at 150,000 times magnification. Finally, each dialyzed fraction was analyzed by GPC with a TSK G3000SW, 600 × 7.5-mm column (Ultropack, by Pharmacia) using a 0.14 *M* sodium phosphate buffer (pH 7.5) as mobile phase at the flow rate of 0.7 ml/min. The eluate was monitored with a refractive index detector (model 156; Beckman).

Results

The typical elution profile, by uv monitoring at 280 nm, of male Wistar rat plasma lipoproteins separated with gradient UC is shown in Figure 1A. In Figure 1A, the line extrapolated through the density values

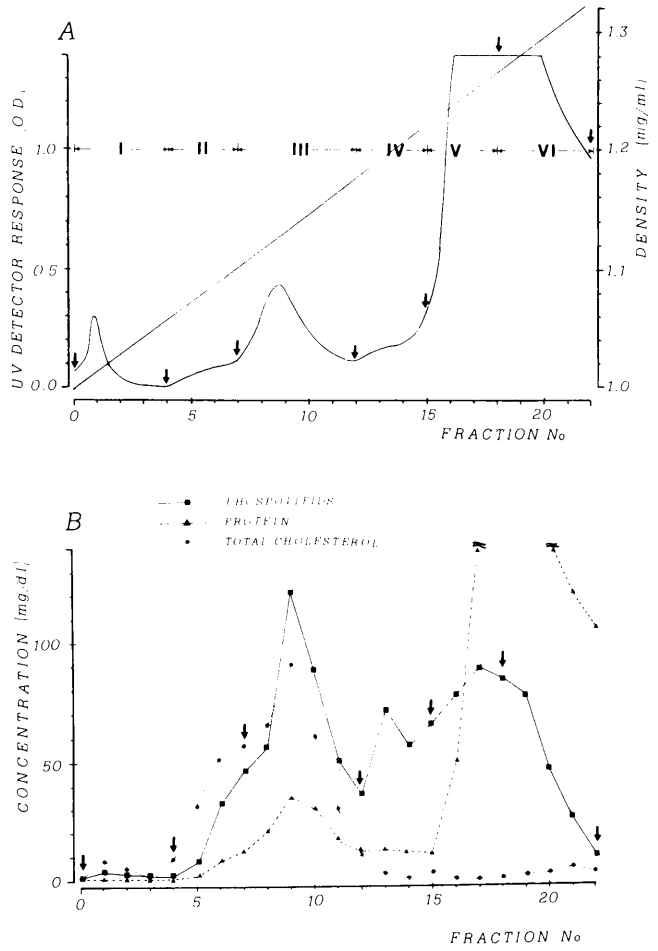

Figure 1. Typical fractionation of lipoproteins from plasma of young male Wistar rat by ultracentrifugation with a discontinuous gradient of sodium bromide. (A) Profile of gradient elution obtained with a uv detector set at 280 nm. The straight line superimposed on the elution profile indicates the variation of density (measured at 20°C) as results from the extrapolation of density values obtained in each tube. The six pooled fractions are indicated by roman numbers. (B) Profile of gradient elution obtained by the determination of protein, total cholesterol, and lipid phosphorous (calculated as phosphatidylcholine) in each tube. In A and B, arrows evidence the limits of the different pools created for further analyses.

found for each tube is also shown. Figure 1B shows the elution profiles obtained by individual measurements of protein, total cholesterol, and phospholipid (calculated from lipid phosphorous assay) concentrations in each tube. In Figure 1, A and B, arrows indicate the limits used for pooling the six different fractions whose physical and chemical characteristics have been determined and described below.

The density intervals of collections, the percentage of distribution, and the total concentration of the major components measured in each of the six pools collected after gradient UC are shown in Table I. The results of apoprotein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in each of these pools are reported separately in Table II. Table I reports the distribution of the apoprotein C group determined by isoelectric focusing electrophoresis in the pooled fractions rich in apoproteins. In a few cases, we also analyzed the distribution of phospholipid classes and their fatty acid distribution (based on the degree of unsaturation) in each of these pools (Table III).

The relevant differences in the composition of each pool are clearly shown by the molar ratios reported in Table IV. Specifically, the molar ratio between the major surface lipids (i.e., free cholesterol and phospholipids) and the molar ratio between core lipids (i.e., cholesteryl ester plus triglyceride) and surface lipids (i.e., phospholipids) indicate a parallel decrease in both free cholesterol and core lipid proportions with increasing density of the pooled fraction. The last two pooled fractions (i.e., Pools V and VI) contain mainly proteins. Phospholipids are the only lipid components present in an appreciable amount.

The pooled fractions were analyzed for their homogeneity and dimensions by GPC and EM. Figure 2 shows the chromatographic patterns obtained by GPC with the lipoprotein-containing pools (Figure 2, A–D, for the lipoprotein pools from I to IV). The chromatograms of protein-rich Pools V and VI are not shown in Figure 2. The GPC analysis demonstrates that the lipoproteins in each pool differ from one another, and have some contamination by either larger or smaller particles or protein. The apparent molecular weights of peaks evidenced in each fraction analyzed were determined by calibrating the column with high molecular weight standards.

The EM images and the statistical evaluation of particle size of each pooled lipoprotein fraction are shown separately in Figure 3 (Pools I–IV). The only lipoprotein fraction that did not show a homogenous distribution of sizes was Pool I, which contained both VLDL and LDL plus a very small proportion of chylomicron remnants. The protein pools V and VI were excluded from this type of examination.

The results of all physical evaluations made on the lipoproteins pooled from gradient UC are summarized in Table V. These include the mean density of the fraction, the value of appearent molecular weights determined by GPC, and the sizes determined by EM.

Discussion

Despite the fact that the lipoprotein characteristics of Wistar rats are not well defined, this strain has been used frequently in metabolic studies. This may have affected the results obtained, because the lipoprotein fractions were prepared by sequential UC with density boundaries derived from other strains and species. Moreover, reports on the effects of both dietary and drug treatments on cholesterol biosynthesis in rats have suggested that the use of different rats strains may be the cause of some of the inconsistencies found in the literature (12, 27).

Table I. Density and Chemical Composition of the Plasma Fractions Isolated by Elution from the Discontinuous Ultracentrifugation Gradients[a]

Pool	Density interval (g/ml)	Protein (%)	Cholesteryl ester (%)	Free cholesterol (%)	Triglyceride (%)	Phospholipid (%)	Total concentration (mg/dl)
I	<1.05	6.3 ± 0.54	5.0 ± 1.51	5.4 ± 0.65	66.6 ± 3.30	16.5 ± 2.57	63.0 ± 9.23
II	1.05–1.08	28.9 ± 1.35	23.0 ± 1.27	12.1 ± 0.96	5.9 ± 0.33	30.1 ± 1.61	88.0 ± 8.86
III	1.10–1.16	34.3 ± 3.82	28.3 ± 5.54	3.7 ± 0.94	1.1 ± 0.15	32.6 ± 1.81	208.3 ± 15.50
IV	1.17–1.21	41.7 ± 5.41	1.3 ± 0.89	0.1 ± 0.04	4.8 ± 1.14	52.2 ± 7.35	73.9 ± 8.28
V	1.22–1.24	87.1 ± 6.27	0.2 ± 0.01	0.0 ± 0.00	0.3 ± 0.06	12.3 ± 2.13	1096.5 ± 122.70
VI	>1.25	96.3 ± 4.34	0.1 ± 0.01	0.0 ± 0.00	0.1 ± 0.01	3.4 ± 0.61	933.3 ± 77.31

[a] The results reported are means ± SD of five different preparations.

Table II. Percentage of Distribution of Apoproteins Obtained by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of the Six Pooled Fractions Collected after Elution of the Discontinuous Ultracentrifugation Gradients[a]

Pool	Apo-AI (%)	Apo-AII (%)	Apo-AIV (%)	Apo-E (%)	Apo-C (%)	Albumin (%)
I	ND	ND	9 ± 1.1	34 ± 3.0	57 ± 3.2	ND
II	11 ± 1.2	1 ± 0.1	2 ± 0.2	78 ± 2.5	7 ± 0.9	ND
III	56 ± 5.9	6 ± 0.6	4 ± 1.8	26 ± 1.7	8 ± 1.6	Traces
IV[b]	64 ± 2.6	2 ± 0.3	19 ± 2.1	5 ± 0.7	9 ± 1.4	1 ± 0.2
V	1 ± 0.4	ND	2 ± 0.5	ND	ND	97 ± 1.0
VI	ND	ND	Traces	ND	ND	100 ± 0.5

Pool	Apo-$CIII_3$ (%)	Apo-$CIII_0$ (%)	Apo-CII (%)	Apo-CI (%)
I	12 ± 1.8	50 ± 3.8	28 ± 2.7	ND
II	40 ± 5.1	25 ± 1.9	35 ± 3.0	ND
III	30 ± 4.0	42 ± 2.6	28 ± 2.9	ND
IV	48 ± 5.2	27 ± 1.6	25 ± 2.6	ND

[a] The percentage of distribution of the apolipoprotein C group obtained by isoelectric focusing in the four pooled fractions rich in apoproteins (Pools I–IV) is also reported. These results represent the means ± SD of three different determinations. ND, not detectable; traces, below 1%.
[b] In Pool IV, a band appeared with an approximate mol wt of 20,000 that accounted for 6 ± 0.8%.

In this study, the distribution, composition, and size of the lipoprotein classes in young male Wistar rat were roughly similar to those reported by other authors (11). Specifically, we have confirmed (by EM and the composition of Pool I) that the plasma of Wistar rats contains both VLDL and LDL fractions in a proportion comprising between 10% and 15% of total plasma lipoproteins. These fractions were pooled so that enough material for further analyses and manipulations was obtained. However, it is clear that it would be possible to isolate pure VLDL by using the proper density limits when preparing the pools.

The use of density limits derived from either humans or other mammals (1.006 or 1.019–1.063 g/ml) for isolation of the LDL fraction would lead to substantial contamination with HDL1, which has been found in Wistar rats by us and in other strains by other authors (13, 16, 28). The use of proper density boundaries allowed the isolation of purer fractions, but not complete separation of LDL and HDL1. In fact, the upper limit of the HDL1 fraction density overlapped the density range of the HDL2 class. The latter fraction, which is the main lipoprotein in Wistar rats, showed a density range (1.08–1.16 g/ml) quite different from that reported for HDL2 either in human or in other rat strains (4, 10, 13, 16, 28). The uv absorption profile (Fig. 1) of ultracentrifugation gradients showed, at the beginning of the protein peak, a shoulder with a density of 1.17–1.21 g/ml. This fraction showed physical and chemical characteristics suggesting that HDL3 particles are present in male Wistar rats, as reported previously by other authors (18) and by ourselves (1, 3). The size of this fraction was in agreement with data in the literature (18, 28). However, its composition characteristics were quite different from those of HDL3 isolated from humans and other mammals, being lower in both free and esterified cholesterol content. This probably caused the differences observed between rats and other species in the density limits of this fraction (i.e., 1.17–1.21 g/ml and 1.12–1.21 g/ml, respectively). The fact that Oschry and Eisenberg (4) did not observe the presence of HDL3 in the Hebrew University strain is

Table III. Percentage of Distribution of Phospholipid Classes and Their Fatty Acid Composition in the Fractions Pooled after Elution from Ultracentrifugation Gradient[a]

Pool	Phospholipid		Fatty acid distribution in each phospholipid class		
	Class	Distribution	Saturated	Monounsaturated	Polyunsaturated
I	LPC	42.4 (3.8)	55.5 (1.8)	39.8 (2.1)	4.7 (0.2)
	SM	6.8 (0.4)	81.7 (2.7)	18.1 (1.4)	0.2 (0.2)
	PC	43.5 (5.2)	71.3 (3.3)	14.3 (1.3)	14.4 (1.1)
	PS	2.6 (0.5)	89.8 (1.8)	9.2 (1.4)	1.0 (0.3)
	PE	4.7 (0.8)	55.8 (2.8)	6.0 (0.3)	38.2 (2.1)
II	LPC	36.0 (4.9)	59.7 (3.4)	8.0 (0.4)	32.4 (2.8)
	SM	17.5 (0.6)	84.3 (4.0)	15.0 (2.2)	0.6 (0.4)
	PC	41.3 (3.9)	62.6 (2.4)	10.1 (1.0)	27.3 (1.4)
	PS	2.2 (0.8)	97.7 (0.6)	2.3 (0.4)	0.5 (0.2)
	PE	3.0 (0.6)	57.4 (1.4)	8.3 (0.6)	34.3 (1.8)
III	LPC	5.0 (3.2)	86.9 (2.2)	8.0 (1.8)	5.1 (0.6)
	SM	13.6 (0.7)	72.3 (1.4)	27.5 (1.0)	0.2 (0.1)
	PC	72.4 (6.1)	61.1 (1.8)	9.5 (0.8)	28.4 (1.2)
	PS	4.9 (1.2)	92.5 (0.8)	6.7 (0.3)	0.8 (0.1)
	PE	4.1 (0.9)	56.4 (2.7)	8.9 (0.9)	34.7 (1.8)
IV	LPC	14.4 (3.6)	90.5 (2.0)	6.6 (0.6)	2.9 (0.7)
	SM	12.8 (0.8)	90.9 (1.5)	8.9 (0.5)	0.2 (0.0)
	PC	60.0 (5.6)	75.8 (1.8)	9.5 (0.8)	14.7 (1.0)
	PS	7.7 (2.4)	92.7 (0.9)	6.8 (0.5)	0.4 (0.3)
	PE	5.1 (0.8)	58.2 (0.6)	9.0 (0.3)	32.8 (1.4)
V+VI	LPC	41.5 (2.7)	76.5 (1.8)	6.3 (0.7)	17.2 (0.9)
	SM	4.4 (0.6)	79.6 (1.9)	20.1 (1.3)	0.3 (0.2)
	PC	30.5 (2.6)	65.5 (0.9)	9.8 (0.5)	24.7 (1.2)
	PS	4.8 (2.3)	93.0 (1.2)	6.5 (0.5)	0.5 (0.2)
	PE	18.8 (3.6)	57.1 (1.3)	13.8 (0.7)	29.1 (1.7)

[a] Fatty acid composition is based on the degree of unsaturation. Results reported are the means of two determinations. Numbers in parentheses are the range of these two experiments.

Table IV. Molar Ratios of Free Cholesterol to Phospholipids (CH/PL) and Cholesteryl Ester Plus Triglyceride to Phospholipids (CE+TG/PL) in the Pooled Fractions[a]

Pool	FC/PL	CE+TG/PL
I	0.68 ± 0.04	6.14 ± 0.45
II	0.80 ± 0.05	1.56 ± 0.06
III	0.24 ± 0.02	1.09 ± 0.03
IV	0.01 ± 0.00	0.11 ± 0.02
V	0.01 ± 0.01	0.04 ± 0.00
VI	0.00 ± 0.00	0.04 ± 0.00

[a] The results are reported as means ± SD of determinations made in five different samples.

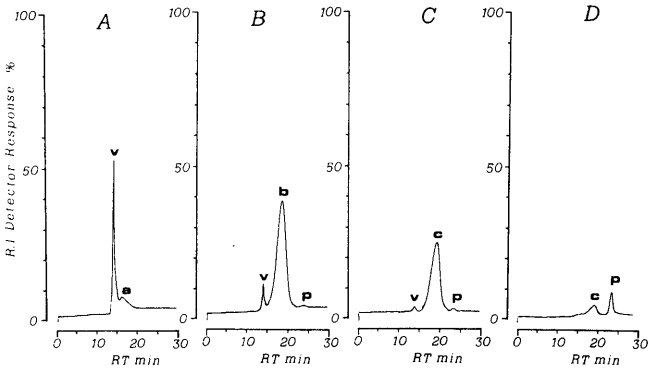

Figure 2. Gel permeation chromatography of the pooled lipoprotein fractions. (A) Pool I: Large aggregates, i.e., chylomicron remnants and VLDL, elute with the void volume of the column (v). A lipoprotein identified as LDL elutes later (a). (B) Pool II: The column separates small amounts of large aggregates (v), a lipoprotein identified as HDL1 (b), and traces of protein (p). (C) Pool III: This pool contains mostly the HDL2 fraction (c) plus traces of large aggregates and protein (v, p). (D) Pool IV: This pool contains a mixture of small lipoproteins (c), identified as HDL2 and HDL3, and proteins (p). The molecular weights determined by GPC in each pool are reported in Table V. The chromatographic conditions are reported in Materials and Methods Analytical Procedures.

not surprising, considering the reported interstrain differences (11, 12, 27).

The fraction collected as Pool V with an average density of 1.23 g/ml was composed mainly of protein (87.1 ± 6.27%) and phospholipids (12.3 ± 2.13%), whereas apolipoproteins and lipids were found in only negligible amounts (Tables I, II, and IV). This excludes the possibility that Pool V is similar to the VHDL fraction reported by Darr *et al.* (19) with Sprague-Dawley rats. Pool V had a high proportion of lysophosphatidylcholine (i.e., lysophosphatidylcholine repre-

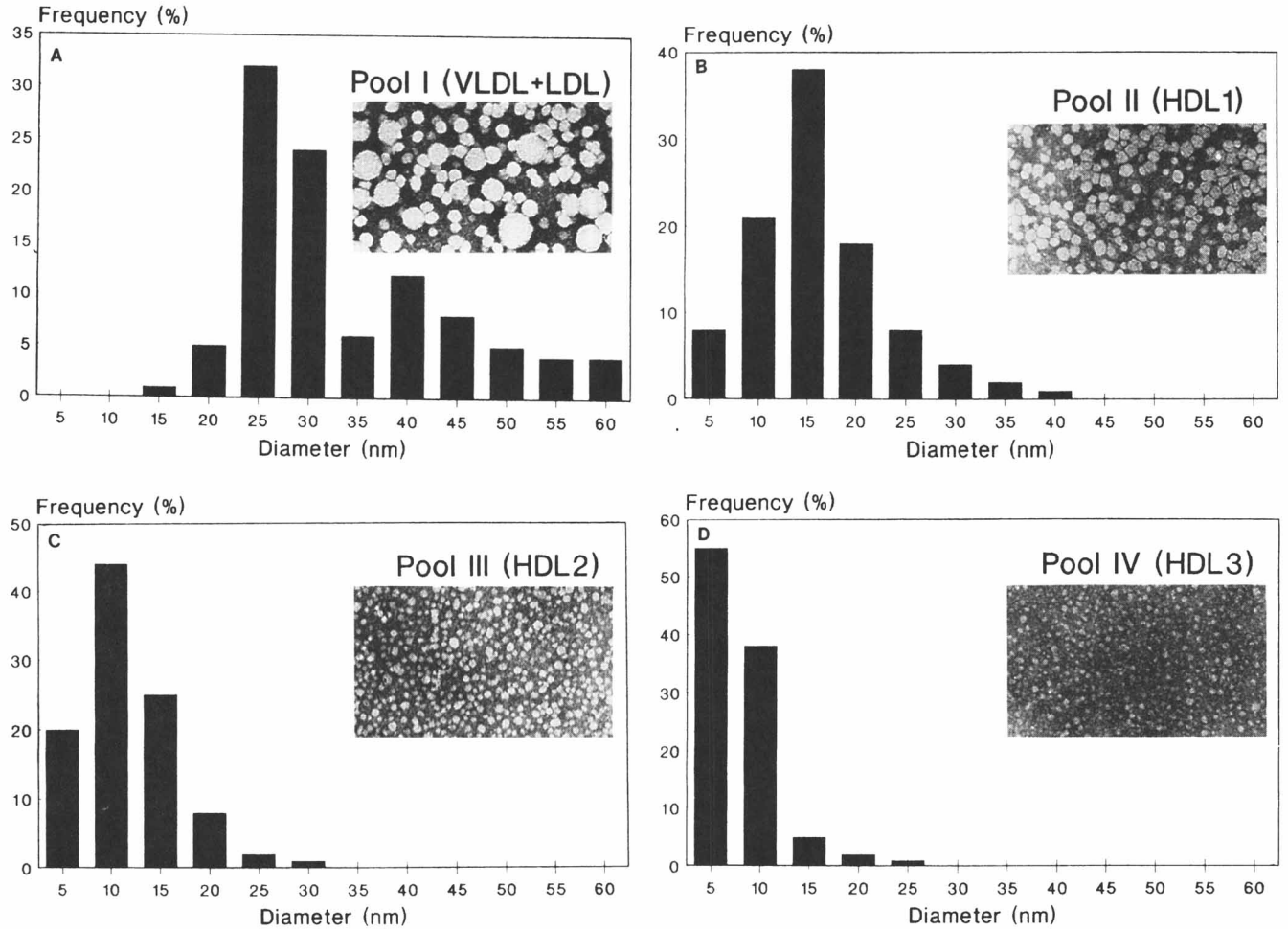

Figure 3. Electron microscopy micrographs of pooled fractions containing lipoprotein aggregates (Pools I-IV) and statistical evaluation of their sizes by frequency analysis.

Table V. Summary of Physical Characteristics of Lipoproteins Pooled after Elution from Gradient Ultracentrifugation of Male Wistar Rat Plasma[a]

Pool	Identification	Density (g/ml)	Size by EM (nm)	Mol wt by GPC
I	VLDL+LDL	1.018 ± 0.006	31.5 ± 11.07	>3,000,000
II	HDL1	1.066 ± 0.008	13.7 ± 6.87	910,000
III	HDL2	1.124 ± 0.006	9.1 ± 5.03	370,000
IV	HDL3	1.189 ± 0.005	4.9 ± 3.03	330,000
V	Protein aggregates	1.230 ± 0.005		520,000
				330,000
				190,000
VI	Albumin	1.260 ± 0.010		68,000

[a] The results reported are means ± SD from five different determinations. In other cases, the average of two or three determinations is reported.

sented more than 40% of phospholipids) and appeared to be the major plasma carrier of this phospholipid class.

The analytical studies on the lipoprotein profile of *Rattus norvegicus* reported here suggest that the various strains of rats used in experimental studies differ among them in HDL composition and profile, i.e., density boundaries among the HDL subclasses (4, 19, 20). This means that in both nutritional and pharmacological studies that involve lipid and lipoprotein metabolism, it is convenient to use rat strains whose lipoprotein specificities are well characterized.

This investigation was partially supported by the Progetto Fin-

alizzato Invecchiamento of the National Research Council of Italy (Grants 92.00399.PF40 and 93.00477.PF.40, Inv. 93.3.000).

The expert technical assistance of Pasqualina Crateri for EM determinations and the valuable help given by Dr. Ida Blotta are gratefully acknowledged.

1. Bravo E, Cantafora A, Argiolas L. Hepatic uptake and metabolism of free cholesterol from different lipoprotein classes. An in vivo study in the rat. Biochim Biophys Acta **1003**:315–320, 1989.
2. Bravo E, Cantafora A. Hepatic uptake and processing of free cholesterol from different lipoproteins with and without sodium taurocholate administration. An in vivo study in the rat. Biochim Biophys Acta **1045**:74–80, 1990.
3. Bravo E, Guldur T, Botham KM, Mayes PA, Cantafora A. Hepatic uptake and processing of cholesterol and cholesteryl ester from chylomicron remnants: An in vivo study in the rat. Biochim Biophys Acta **1123**:85–91, 1992.
4. Oschry Y, Eisenberg S. Rat plasma lipoproteins: Re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. J Lipid Res **23**:1099–1106, 1982.
5. Windler E, Chao YS, Havel RJ. Determinants of uptake of triglyceride-rich lipoproteins and their remnants in the rat. J Biol Chem **255**:5475–5480, 1980.
6. Spady DK, Turley SD, Dietchy JM. Receptor-independent low density lipoprotein transport in the rat in vivo. Quantitation, characterization and metabolic consequences. J Clin Invest **76**:1113–1122, 1985.
7. Choi Y-S, Goto S, Ikeda I, Sugano M. Age-related changes in lipid metabolism in rats: The consequence of moderate food restriction. Biochim Biophys Acta **963**:237–242, 1988.
8. Cantafora A, Masella R, Pignatelli E, Verna R. Influence of age on the lipoprotein profile of male Wistar rats. Arch Gerontol Geriatr [Suppl] **3**:93–100, 1992.
9. Watkins ML, Fizett N, Heimberg M. Sexual influences on hepatic secretion of triglyceride. Biochim Biophys Acta **481**:280–286, 1972.
10. Patsch W, Kim K, Wiest W, Schonfeld G. Effects of sex hormones on rat lipoproteins. Endocrinology **107**:1085–1094, 1979.
11. Chapman MJ. Comparative analysis of mammalian plasma lipoproteins. Methods Enzymol **128**:70–143, 1986.
12. Kritchevsky D, Tepper SA. Serum cholesterol levels of imbred rats. Am J Physiol **207**:631–633, 1964.
13. Danielsson B, Ekman R, Johansson BG, Nilsson-Ehle P, Petersson BG. Isolation of a high density lipoprotein with high contents of arginine-rich apoprotein (apoE) from rat plasma. FEBS Lett **86**:299–302, 1978.
14. Camejo G. Structural studies in rat plasma lipoproteins. Biochemistry **6**:3228–3241, 1967.
15. Lusk LT, Walker LF, DuBien LH, Getz GS. Isolation and partial characterization of high-density lipoprotein HDL1 from rat plasma by gradient centrifugation. Biochem J **183**:83–90, 1979.
16. Windmueller HG, Levy RI. Total inhibition of hepatic lipoprotein production in the rat by orotic acid. J Biol Chem **242**:2246–2254, 1967.
17. Mills GL, Taylaur CE. The distribution and composition of serum lipoproteins in eighteen animals. Comp Biochem Physiol **40B**:498–501, 1971.
18. Pasquali-Ronchetti I, Calandra S, Baccarani-Contri M, Mantaguti M. The ultrastructure of rat plasma lipoproteins. **53**:180–192, 1975.
19. Darr WH, Windler EET, Stephan KU, Walli AK, Greten H. Characterization and catabolism of rat very high density lipoproteins. J Lipid Res **26**:672–673, 1985.
20. Chapman MJ, Goldstein S, Lagrange D, Laplaud PM. Comparative analysis of mammalian plasma lipoproteins. J Lipid Res **22**:339–358, 1981.
21. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye-binding. Anal Biochem **72**:248–254, 1976.
22. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem **226**:497–509, 1957.
23. Alvaro D, Angelico M, Cantafora A, Di Biase A, Gaeta GB, Ginanni Corradini S, Tripodi MF, Attili AF, Utili R. Influence of tauroursodeoxycholic and taurodeoxycholic acids on hepatic metabolism and biliary secretion of phosphatidylcholine in the isolated rat liver. Biochim Biophys Acta **878**:216–224, 1986.
24. Di Biase A, Bravo E, Puoti C, Patane F, Blotta I, Cantafora A. Changes of lipoprotein composition associated with liver cirrhosis. Ital J Gastroenterol **21**:204–209, 1989.
25. Fidge NH, Nestel PJ. Metabolism of apolipoprotein C. Methods Enzymol **129**:443–457, 1986.
26. Norhausen RW. Electron microscopy of negatively stained lipoproteins. Methods Enzymol **128**:442–457, 1986.
27. Kritchevsky D, Tepper SA. Influence of nicotinic, picolinic and pyridine-3-sulfonic acids on cholesterol metabolism in the rat. J Nutr **82**:157–161, 1964.
28. Skinner ER, Rooke JA. Fractionation of rat plasma lipoproteins by affinity chromatography on heparin-sepharose. Comp Biochem Physiol **65B**:645–650, 1980.