Interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma

Shlomo Eisenberg and Daniel Rachmilewitz

Lipid Research Laboratory, Department of Medicine B, Hadassah University Hospital, Jerusalem, Israel

Abstract Incubation of ^{125}I-labeled very low density lipoprotein (VLDL) with lipoprotein lipase-rich (postheparin) plasma obtained from intact or supradiaphragmatic rats resulted in the transfer of more than 80% of apoprotein C from VLDL to high density lipoprotein (HDL), whereas apoprotein B was associated with lipoprotein of density less than 1.019 g/ml (intermediate lipoprotein). The transfer of ^{125}I-labeled apoprotein C from VLDL to HDL increased with time and decreased in proportion to the amount of VLDL in the incubation system. A relationship was established between the content of triglycerides and apoprotein C in VLDL, whereas the amount of apoprotein C in VLDL was independent of that of other apoproteins, especially apoprotein B. The injection of heparin to rats preinjected with ^{125}I-labeled VLDL caused apoprotein interconversions similar to those observed in vitro. The intermediate lipoprotein was relatively rich in apoprotein B, apoprotein VS-2, cholesterol, and phospholipids and poor in triglycerides and apoprotein C. The mean diameter of intermediate lipoprotein was 269 Å (compared with 427 Å), the mean S_f rate was 30.5 (compared with 115), and the mean weight was 7.0 × 10^6 daltons (compared with 23.1 × 10^6). From these data it was possible to calculate the mass of lipids and apoproteins in single lipoprotein particles. The content of apoprotein B in both particles was virtually identical, 0.7 × 10^6 daltons. The relative amount of all other constituents in intermediate lipoprotein was lower than in VLDL: triglycerides, 22%; free cholesterol, 37%; esterified cholesterol, 68%; phospholipids, 41%; apoprotein C, 7%; and VS-2 apoprotein, 60%. The data indicate that (*a*) one and *only one* intermediate lipoprotein is formed from each VLDL particle, and (*b*) during the formation of the intermediate lipoprotein *all* lipid and apoprotein components other than apoprotein B leave the density range of VLDL to a varying degree. Whether these same changes occur during the clearance of VLDL in vivo is yet to be established.

Supplementary key words intermediate lipoprotein · apolipoproteins · iodinated lipoproteins · lipoprotein lipids · gel filtration · electron microscopy · postheparin lipolytic activity

VLDL is the major transport vehicle of triglycerides of endogenous origin in plasma. It is composed of triglycerides (60–70% of total mass), cholesterol and phospholipids (20–30%), and several specific and well-characterized apoproteins (1–7). Similar to chylomicrons, the metabolism of VLDL occurs in close proximity to the plasma compartment and is dependent on triglyceride hydrolysis through the activity of the enzyme system lipoprotein lipase (8–9). The immediate product of triglyceride hydrolysis in vivo is a short-lived lipoprotein of intermediate density (d = 1.006–1.019 g/ml). This lipoprotein, designated "intermediate lipoprotein" (10–11), occupies the density range S_f 12–60 (12, 13), is poor in triglycerides and rich in cholesterol, phospholipid, and apoprotein, and is probably analogous to chylomicron "remnants" (14) or "skeletons" (15).

Recent studies in man (10, 11, 16, 17) and rats (18–22) have indicated that most or all of the protein moiety of plasma LDL originates from VLDL through a stage in formation of the intermediate lipoprotein. LDL particles, however, differ from VLDL not only in triglyceride content but also in protein, cholesterol, and phospholipid content and composition (1, 11). These observations suggest that, concomitantly with triglyceride hydrolysis, VLDL particles undergo additional metabolic changes. The aim of the present investigation was to define the biochemical changes that occur during degradation of VLDL particles in vitro and in vivo. In particular, we aimed at better characterization of the protein and lipid moieties of the intermediate lipoprotein produced after the interaction of VLDL with lipoprotein lipase-rich (postheparin) plasma.

MATERIALS AND METHODS

Preparation of lipoproteins and iodination

Lipoproteins were isolated from 200–300 ml of plasma (containing 0.1% EDTA) from nonfasting rats by prepara-

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Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

tive ultracentrifugation in a Beckman L2-65B ultracentrifuge and a 50.1 rotor as described previously (18, 19). Iodination of VLDL was performed following slight modifications (10, 23) of the McFarlane iodine monochloride method (24). Lipoproteins of $S_f > 400$ (chylomicrons) were removed by a single spin of the ^{125}I-labeled VLDL (18, 19). The chemical, radiochemical, immunological, and physical characteristics of the ^{125}I-labeled VLDL thus obtained were identical with those reported elsewhere (18). All procedures were carried out at 4°C.

Delipidation, polyacrylamide gel electrophoresis, and gel filtration

Prior to delipidation, lipoproteins were dialyzed for 24–48 hr against several changes of 4 l of 0.9% NaCl, 0.01% EDTA (pH 7.45). Delipidation, solubilization of apoproteins, and polyacrylamide gel electrophoresis were performed as described previously (19). Apoproteins were designated according to the A, B, C nomenclature.[1] To determine the distribution of radioactivity among apoproteins, the stained gels were sliced by hand (as demonstrated in Figs. 1 and 2) and assayed for radioactivity. The adequacy of this method for separation of ^{125}I-labeled apoproteins has been discussed previously (19). Apoprotein fractions were separated by gel filtration on Sephadex G-150 using 1.5 × 90 cm columns (Pharmacia, Sweden); the column was eluted with 0.2 M Tris (pH 8.2) containing 0.002 M sodium decyl sulfate and 6 M urea (5, 6, 18, 19). Protein was determined by absorbance at 280 nm and by the method of Lowry et al. (26), and apoproteins were visualized by polyacrylamide gel electrophoresis of every second tube. Tubes containing the same apoprotein were combined, dialyzed against 0.9% NaCl–0.002 M sodium decyl sulfate solution, and assayed for protein and radioactivity. The values thus obtained were within 10% of those calculated from absorbancy and radioactivity determined on individual tubes. The mean of the two values was used to determine the distribution of protein and radioactivity among apoprotein fractions.

Analytical ultracentrifugation and electron microscopy

Analytical ultracentrifugation was performed in a Spinco model E ultracentrifuge with schlieren optics and double-sectored analytical cells. The runs were made at 20°C at 30,000 rpm. Sodium chloride solution of density 1.063 g/ml prepared as described by Ewing, Freeman, and Lindgren (27) was used. Standard equations (28) were used to calculate sedimentation values at salt density of 1.063 g/ml (S_f rates).

Negative staining of lipoproteins for electron microscopy was performed as described previously (29). Electron micrographs were obtained with a Philips 300 electron microscope at 60 kV and instrument magnification of 58,000.

Analytical procedures and determination of radioactivity

Lipoprotein protein was determined by the method of Lowry et al. (26). Phospholipids were determined following the procedure of Bartlett (30), and triglycerides by the AutoAnalyzer method (31). Free and esterified cholesterol were separated by thin-layer chromatography on silica gel G using a solvent system of light petroleum ether–diethyl ether–acetic acid 90:10:1; the lipid spots were made visible by iodine vapor. The lipid-containing areas were scraped off the plate, and cholesterol was determined as described by Chiamori and Henry (32). Lipoprotein lipids were extracted with chloroform–methanol 2:1 (v/v) (33). Radioactivity was determined using an Auto-Gamma scintillation spectrometer (Packard, La Grange, Ill.). Radioactive iodine (Na^{125}I, carrier free) was obtained from the Radiochemical Centre, Amersham, England. Sodium heparin (Pularin) was purchased from Evans Medical Ltd., Liverpool, England.

Experimental procedures

The rats used throughout the study were of the Hebrew University strain. This strain of rats has been characterized previously (18, 19).

To prepare lipoprotein lipase-rich (postheparin) plasma, rats under ether anesthesia were injected intravenously with 0.2–0.3 ml of sodium heparin solution (100 units/kg body wt) and were exsanguinated through the abdominal aorta 10 min later. Control (normal) plasma was collected likewise from noninjected rats. Plasma was separated using a Sorval SS-3 centrifuge at 15,000 rpm, 4°C, for 20 min and was used within 60 min of exsanguination. Incubations were carried out in either 6.5-ml or 13.5-ml Beckman ultracentrifuge polyallomer tubes placed in a thermostated bath at 37°C with frequent shaking. This incubation mixtures contained 4 ml of plasma, 0.5–1.0 ml of 20% fatty acid-poor bovine serum albumin, and aliquots of ^{125}I-labeled VLDL. In some experiments these amounts were doubled. Incubations were terminated by the addition of concentrated NaCl solution (density 1.114 g/ml), necessary to bring the density of the incubation mixture to 1.019 g/ml, and the tubes were immediately placed on crushed ice.

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[1] No general agreement exists as yet for the nomenclature of apoproteins. The A, B, C nomenclature (25) is used predominantly in the present study. According to this nomenclature, apoprotein B is the major protein of plasma low density lipoprotein and is also present in very low density lipoprotein. Apoprotein C denotes the group of small molecular weight apoproteins of plasma very low and high density lipoprotein and apoprotein A represents the major apoproteins of high density lipoprotein. Because of the similarities of rat and human plasma lipoprotein apoproteins, this nomenclature is valid for the two species. In the present study, apoprotein B denotes the protein moiety isolated in the first Sephadex fraction or at the origin of polyacrylamide gels (zone 2) (Figs. 1 and 2). Apoprotein C is the group of proteins isolated with the Sephadex fraction III or zone 7 of polyacrylamide gels (Figs. 1 and 2). The recent report by Herbert et al. (7) demonstrated that this zone contains three of the components of rat apoprotein C (C-II, C-III-0, and C-III-3).

Lipoprotein lipase-rich plasma of extrahepatic origin was prepared by the method of Bezman-Tarcher and Robinson (34). Male rats (250 g) were anesthetized, the aorta was ligated at the level of the diaphragm, and a polyethylene cannula was placed in the inferior vena cava and introduced into the right heart. The inferior vena cava was then ligated proximal to the orifices of the hepatic veins and distal to the diaphragm. Heparin solution (100 units/kg body wt) was introduced through the cannula in the inferior vena cava and the rats were exsanguinated 5 min later through the aorta.

To determine the effect of activation of lipoprotein lipase on the metabolism of ^{125}I-labeled VLDL in vivo, rats were injected intravenously with ^{125}I-labeled VLDL and 10 min later with sodium heparin (100 units/kg body wt). Control rats were administered the radioactive VLDL and an equivalent volume of 0.9% NaCl solution without heparin. Groups of rats were killed 5 and 20 min after the injection of heparin or 0.9% NaCl, blood was collected in 0.1% EDTA, and 1 mg of protamine sulfate was added to each 5–7 ml of blood prior to the separation of plasma.

RESULTS

A. Incubation of ^{125}I-labeled VLDL with lipoprotein lipase-rich plasma in vitro

The distribution of radioactivity among lipoproteins was determined after incubation of trace amounts of ^{125}I-labeled VLDL (0.1 mg of protein, 0.5–0.6 mg of triglyceride) with 4 ml of 0.9% NaCl, normal rat plasma, or postheparin plasma. More than 78% of the radioactivity was recovered with lipoproteins of density less than 1.019 g/ml (predominantly VLDL) after incubation of ^{125}I-labeled VLDL with 0.9% NaCl, normal plasma, heat-inactivated postheparin plasma (56°C for 30 min) and nonincubated samples (**Table 1**). The main effect of postheparin (lipoprotein lipase-rich) plasma on the distribution of radioactivity among lipoproteins was a pronounced decrease of lipid- and protein-bound radioactivity associated with lipoproteins of density less than 1.019 g/ml. Polyacrylamide gel electrophoresis of apoproteins isolated from lipoproteins of density less than 1.019 g/ml revealed a complete absence of apoprotein C (zone 7) from samples incubated with postheparin plasma (**Fig. 1**).

A ratio of 1.0 mg of VLDL protein to 10 ml of incubation mixture and 60 min of incubation were chosen for further characterization of the labeled proteins in plasma lipoproteins. Four lipoprotein families were isolated: lipoproteins of density less than 1.019 (predominantly VLDL) and lipoproteins of densities 1.019–1.040 g/ml, 1.040–1.085 g/ml, and 1.085–1.21 g/ml. Apoproteins were separated by polyacrylamide gel electrophoresis and sliced into zones as shown in **Fig. 2**. The distribution of radioactivity among apoproteins is presented in **Table 2.** In VLDL ($d < 1.019$ g/ml) incubated with postheparin plasma, the relative contribution of ^{125}I-labeled apoprotein B to the total radioactivity increased and that of ^{125}I-labeled apoprotein C decreased. Apoprotein C constituted the major labeled apoprotein of lipoproteins of densities 1.04–1.085 and 1.085–1.21 g/ml after incubation of ^{125}I-labeled VLDL with either normal or postheparin plasma. The ^{125}I-labeled apoprotein pattern of low density lipoprotein (d =

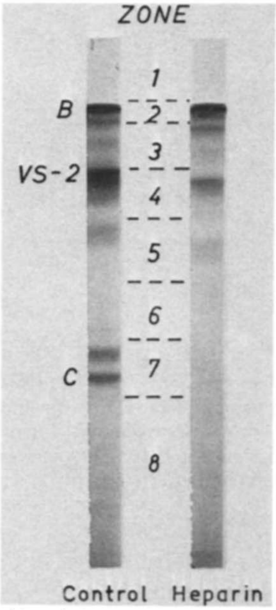

Fig. 1. Polyacrylamide gel electrophoresis of apoproteins of lipoproteins of density less than 1.019 g/ml (VLDL) isolated after incubation of ^{125}I-labeled VLDL with either normal plasma (*left*) or plasma obtained from rats injected with heparin (*right*). Conditions of incubation as described in Table 1.

TABLE 1. Radioactivity of plasma lipoproteins after incubation of ^{125}I-labeled VLDL with plasma obtained from normal rats and from rats injected with heparin

Incubation Mixture	Time	Distribution of Radioactivity among Lipoproteins		
		$d < 1.019$	$d = 1.019–1.21$	$d > 1.21$
	min		%	
0.9% NaCl	30	89.7 ± 1.0	4.1 ± 0.5	6.2 ± 0.6
Normal plasma	0	86.1 ± 1.7	8.3 ± 1.6	5.6 ± 0.6
	30	78.8 ± 1.6	12.9 ± 1.5	8.3 ± 0.5
Postheparin plasma, heated[a]	30	77.4 ± 2.5	14.8 ± 2.2	7.8 ± 0.3
Postheparin plasma	30	46.8 ± 2.5	40.7 ± 3.0	12.5 ± 1.3

Conditions of incubation: 0.1–0.2 ml of ^{125}I-labeled VLDL (0.1 mg of protein, 0.5–0.6 mg of triglyceride, [5–10] × 10^5 cpm) was incubated at 37°C with either 0.9% NaCl, normal plasma (40–60 mg/100 ml triglyceride), or postheparin plasma (15–25 mg/100 ml triglyceride). Lipoproteins were separated and assayed for radioactivity as described in the text. Labeled lipids constituted 20–30% of the radioactivity of lipoproteins of density less than 1.019 g/ml and 10–20% of that of lipoproteins of density higher than 1.019 g/ml in all samples. Results are means ± SE of three to five experiments.

[a] Sample heated at 56°C for 30 min prior to incubation.

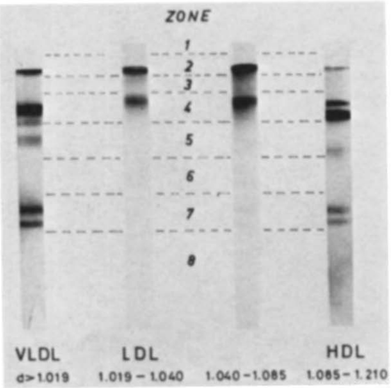

Fig. 2. Apoprotein pattern of rat plasma lipoprotein families as separated by polyacrylamide gel electrophoresis. Lipoprotein isolation, delipidation, and polyacrylamide gel electrophoresis were performed as described in Materials and Methods.

1.019–1.04 g/ml) resembled that of lipoproteins of density less than 1.019 g/ml (Table 2).

Because postheparin plasma contains lipoprotein lipase of hepatic and extrahepatic origin, an attempt was made to isolate lipoprotein lipase-rich plasma free from the hepatic enzyme. The supradiaphragmatic rat was used for this purpose. **Table** 3 shows the results of an experiment in which comparisons were made of the effects of postheparin plasma obtained from supradiaphragmatic rats and of plasma from intact rats on the apoprotein composition of ^{125}I-labeled VLDL and its distribution among lipoproteins. The difference between the two plasma samples was rather small, especially in the composition of ^{125}I-labeled apoprotein of VLDL. Hence, lipoprotein lipase-rich plasma obtained from intact rats was used in all subsequent experiments.

The disappearance of ^{125}I-labeled apoprotein C from lipoproteins of density less than 1.019 g/ml after incubation of 1 mg of ^{125}I-labeled VLDL with 4 ml of postheparin plasma was graded. The ^{125}I-labeled apoprotein C content in lipoproteins of density less than 1.019 g/ml decreased with time of incubation from 35.0% of total protein-bound radioactivity at time zero (unincubated sample) to 26.8%, 18.9%, 11.2%, and 8.0% at the end of 2, 10, 30, and 60 min of incubation, respectively. During these periods of incubation, 4.5%, 23.6%, 44.4%, and 79.9%, respectively, of the VLDL triglyceride was hydrolyzed. Similar data were recorded when the amounts of VLDL introduced to the incubation mixture (10 min) were 0.1, 0.2, 0.4, 1.0, and 2.0 mg. Labeled apoprotein C constituted 5.7%, 7.5%, 9.9%, 18.9%, and 27.6%, respectively, of the protein-bound radioactivity of these samples when 67.6%, 57.5%, 44.9%, 23.6%, and 19.5%, respectively, of the triglycerides was hydrolyzed. The data from these two experiments were used to determine the relationship between the amount of triglyceride hydrolyzed and the content of ^{125}I-labeled apoprotein C in VLDL (**Fig.** 3). An exponential relationship was found between the percentage of triglyceride hydrolyzed and the percentage of ^{125}I-labeled apoprotein C leaving the VLDL density range ($d < 1.019$ g/ml).

B. Effects of heparin injection on labeled apoproteins of ^{125}I-labeled VLDL in vivo

Groups of three or four rats were injected with ^{125}I-labeled VLDL, and 10 min later they were injected with either heparin (100 units/kg) or an equivalent volume of 0.9% NaCl. Rats were killed 5 and 20 min after the injection of heparin (15 and 30 min after the injection of ^{125}I-labeled VLDL), and their plasma was used for lipoprotein

TABLE 2. Distribution of radioactivity among lipoproteins and apoproteins after incubation of ^{125}I-labeled VLDL with normal and postheparin plasma

Incubation Mixture	Lipoprotein Density	^{125}I-labeled Lipoprotein	^{125}I-labeled Lipid	^{125}I-labeled Apoproteins[a]							
				1	2	3	4	5	6	7	8
	g/ml	*% of dose*	*% of ^{125}I-labeled lipoprotein*	*% of protein-bound radioactivity*[b]							
None			24.6 ± 1.4	4.2	26.5	9.4	10.2	6.2	5.2	36.5	1.3
Normal plasma	<1.019	84.1 ± 1.2	26.7 ± 2.2	5.9	27.5	13.6	13.8	6.5	4.3	25.6	2.8
	1.019–1.040	3.7 ± 0.6	7.5 ± 0.9	6.7	22.3	6.9	9.1	5.7	5.8	39.5	5.0
	1.040–1.085	3.9 ± 0.6	9.4 ± 0.4	4.0	9.6	5.1	7.8	2.4	3.5	65.1	2.5
	1.085–1.21	4.0 ± 0.5	8.8 ± 0.4	2.2	7.7	9.0	6.9	3.4	3.1	64.5	3.2
	>1.21	4.3 ± 0.7									
Postheparin plasma	<1.019	52.7 ± 1.3	29.1 ± 2.5	7.5	42.4	13.8	14.8	7.6	4.3	7.7	1.8
	1.019–1.040	8.7 ± 1.3	23.3 ± 1.2	12.5	43.2	10.9	10.5	3.9	2.5	12.6	3.9
	1.040–1.085	14.2 ± 1.6	17.2 ± 1.4	4.1	18.4	5.5	6.0	2.2	2.8	58.8	2.2
	1.085–1.21	18.4 ± 0.9	12.6 ± 0.6	0.6	2.4	8.6	4.9	4.9	8.9	68.3	2.4
	>1.21	7.0 ± 0.9									

Values are means ± SE of six experiments. 1 mg of ^{125}I-labeled VLDL (6–9 mg of triglyceride, [20–50] × 10^6 cpm) was incubated for 60 min at 37°C with either normal or postheparin rat plasma. The distribution of radioactivity among lipoproteins and apoproteins and the percentages of ^{125}I-labeled lipids (chloroform–methanol-extractable radioactivity) were determined after lipoprotein isolation, lipid extraction, and polyacrylamide gel electrophoresis as described in Materials and Methods.

[a] Zones 1–8 from polyacrylamide gel electrophoresis.

[b] Means of six experiments. Standard error of the mean ranged between 5% and 15% of the mean for all values.

isolation and determination of labeled lipids and labeled apoproteins. Compared with saline-treated animals, heparin caused a reduction of the plasma triglyceride levels from 61.6 ± 7.9 to 20.3 ± 7.5 and from 53.7 ± 7.9 to 11.3 ± 3.7 mg/100 ml, respectively. Concomitantly, radioactivity associated with VLDL ($d < 1.019$ g/ml) decreased from 59.4 ± 2.9 to 47.6 ± 3.2% and from 48.8 ± 1.9 to 37.5 ± 2.4% of plasma radioactivity, and that associated with HDL ($d = 1.063–1.21$) increased from 23.7 ± 1.9 to 31.7 ± 2.8% and from 31.7 ± 2.1 to 39.9 ± 2.1%, respectively. The two groups of animals did not differ in plasma cholesterol levels, in percentage of injected radioactivity remaining in the plasma or recovered in the liver, or in percentage of labeled lipids in lipoproteins.

The pattern of labeled apoproteins in lipoproteins of density less than 1.019 g/ml isolated from heparin-injected rats (**Table 4**) was indistinguishable from that observed after in vitro incubation of ^{125}I-labeled VLDL with postheparin plasma (as shown in Tables 2 and 3). After heparin administration, the VLDL was rich in labeled apoprotein B and poor in apoprotein C content (Table 4). Most of the apoprotein C that disappeared from lipoproteins of density less than 1.019 g/ml was recovered in circulation together with HDL.

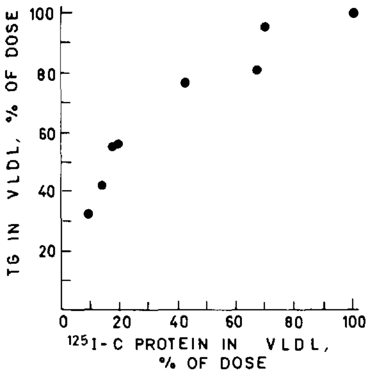

Fig. 3. Relationship between the percentage of triglyceride hydrolyzed and the percentage decrease of ^{125}I-labeled apoprotein C of lipoproteins of density less than 1.019 g/ml after incubation of ^{125}I-labeled VLDL with postheparin plasma. Individual values were compiled from the data presented in the text. Triglyceride and ^{125}I-labeled apoprotein C contents of lipoproteins of density less than 1.019 g/ml immediately after mixing ^{125}I-labeled VLDL with postheparin plasma were taken as the 100% values.

C. Characterization of the intermediate lipoprotein

The protein content and lipid composition of lipoproteins of density less than 1.019 g/ml isolated after incubation of VLDL with normal plasma were found to be indistinguishable from those of nonincubated VLDL (**Table 5**). Incubation of VLDL with postheparin plasma resulted in a lipoprotein of a different composition. Compared with VLDL, it contained less triglyceride and more protein, cholesterol, and phospholipids. The molar ratio of free to esterified cholesterol in this lipoprotein was found to decrease from 2.5 to 1.3.

3–6 mg of protein obtained after delipidation of VLDL incubated with normal or postheparin plasma was further fractionated by gel filtration on Sephadex G-150. Three fractions were obtained. Fraction I contained only the apoprotein B band (see Fig. 1); fraction II contained the VS-2 apoprotein, and fraction III, apoprotein C. About 90% of the total gel radioactivity was found in association with the stained bands in fractions I (apoprotein B, slice 2) and III (apoprotein C, slice 7). Fraction II was more heterogeneous, 82% of the gel radioactivity being associated with gel

TABLE 3. ^{125}I-labeled apoproteins of lipoproteins isolated after incubation of ^{125}I-labeled VLDL with normal and postheparin plasma obtained from intact or supradiaphragmatic rats

Source of Plasma	Lipoprotein Density	^{125}I-labeled Apoproteins	Distribution of ^{125}I-labeled Apoproteins[a] 1	2	3	4	5	6	7	8
	g/ml	*% of dose*	*% of protein-bound ^{125}I*							
VLDL, original			6.7	25.5	9.8	11.9	6.4	2.2	35.1	2.4
Intact rats, normal	<1.019[b]	86.0 ± 1.24	4.6	39.5	8.0	10.0	3.7	2.0	24.4	2.9
	1.019–1.04	0.7 ± 0.22	10.0	39.6	12.2	8.0	5.0	5.9	16.8	1.7
	1.04–1.21	13.3 ± 1.44	2.6	11.1	16.7	13.1	5.0	2.0	48.0	1.5
Intact rats, post-heparin	<1.019[b]	53.0 ± 5.47	13.3	56.3	14.8	3.4	3.2	2.3	6.0	0.6
	1.019–1.04	11.6 ± 2.10	11.2	49.5	11.3	8.6	4.9	3.3	8.5	1.7
	1.04–1.21	35.5 ± 3.58	1.5	6.2	17.5	13.9	5.0	3.0	51.9	1.0
Supradiaphragmatic rats, postheparin	<1.019[b]	65.6 ± 1.91	11.4	54.8	14.7	6.8	3.0	1.7	6.9	0.7
	1.019–1.04	4.3 ± 0.56	17.1	58.3	7.1	6.5	2.3	2.3	4.6	1.8
	1.04–1.21	30.1 ± 2.33	4.9	4.9	17.5	11.9	2.3	1.2	55.4	1.9

3.5-ml samples of plasma obtained from intact normal rats, intact rats injected with heparin, and supradiaphragmatic rats injected with heparin were incubated with ^{125}I-labeled VLDL (0.4 mg of protein, 16×10^6 cpm) at 37°C for 30 min. Lipoproteins were isolated by flotation and delipidated, and apoproteins were separated by polyacrylamide gel electrophoresis. To determine the distribution of radioactivity among apoproteins, stained protein bands were sliced off the gels (as shown in Figs. 1 and 2) and counted. Values are means ± SE of three to five experiments.

[a] Zones 1–8 from polyacrylamide gel electrophoresis.

[b] Protein contents in lipoproteins of density less than 1.019 g/ml of the three samples were 0.78 ± 0.12, 0.19 ± 0.01, and 0.19 ± 0.04 mg, respectively. Triglycerides: 3.85 ± 0.45, 0.65 ± 0.19, and 0.65 ± 0.16, respectively. Cholesterol: 0.34 ± 0.04, 0.13 ± 0.02, and 0.19 ± 0.04 mg, respectively.

TABLE 4. ^{125}I-labeled apoproteins of plasma lipoproteins of rats injected with ^{125}I-labeled VLDL and heparin

Lipoprotein Fraction	Injected Solution	Time after Injection	^{125}I in Apoproteins	Distribution of ^{125}I among Apoproteins[a] 1	2	3	4	5	6	7	8
		min	*% injected dose/ml plasma*	*% of total ^{125}I*							
VLDL, original				4.1	26.8	6.6	11.3	7.4	5.6	37.9	1.0
d < 1.019	0.9% NaCl	5	2.543	8.3	35.5	9.5	7.8	4.0	3.6	29.8	1.5
	Heparin	5	2.074	9.6	50.6	16.0	9.8	5.4	3.2	4.6	0.8
	0.9% NaCl	20	1.618	10.6	30.0	9.6	7.0	2.3	3.7	32.9	3.9
	Heparin	20	1.163	7.8	63.9	13.1	5.1	2.9	2.1	3.6	1.5
d = 1.019–1.063	0.9% NaCl	5	0.383	16.4	26.7	9.1	5.5	2.6	2.0	36.0	1.7
	Heparin	5	0.569	7.8	43.6	18.1	9.5	3.3	2.3	15.1	0.3
	0.9% NaCl	20	0.380	16.0	24.5	8.0	6.7	2.6	2.1	38.9	1.2
	Heparin	20	0.500	16.3	47.2	11.0	5.1	2.6	1.7	13.3	2.8
d = 1.063–1.21	0.9% NaCl	5	1.152	3.8	6.1	17.7	9.8	2.1	1.7	56.8	2.0
	Heparin	5	1.572	2.5	3.1	20.1	8.4	1.0	1.4	61.2	2.3
	0.9% NaCl	20	1.159	2.2	4.1	17.6	8.7	1.3	1.6	62.3	2.2
	Heparin	20	1.465	2.3	3.6	15.2	8.4	1.2	1.3	66.0	2.0

Distribution of radioactivity among apoproteins was determined after isolation of lipoproteins, delipidation, and polyacrylamide gel electrophoresis as described in Materials and Methods. Values are means of the experiments described in the text. SEM ranged between 5% and 20% of the mean for all values.

[a] Zones 1–8 from polyacrylamide gel electrophoresis.

slices 3, 4, and 5. Contamination of any gel zone with apoproteins of the other fractions was negligible.

The relative contents of protein and radioactivity among the apoprotein fractions of all samples are presented in **Table 6.** Apoprotein composition of VLDL incubated with normal plasma was indistinguishable from that of nonincubated VLDL. The apoprotein composition of VLDL incubated with postheparin plasma was, however, very different from that of the other samples. Apoprotein fraction III, the main apoprotein fraction of VLDL (53-60% of total protein), constituted less than 10% of the total apoproteins of postheparin samples. Apoprotein fraction I constituted about 57% of total protein of VLDL incubated with postheparin plasma compared with only 21% in the other samples. There was also an increase in the proportion of fraction II protein after incubation of VLDL with postheparin plasma, to 34% of the total protein. The percentage distribution of radioactivity among the three apoprotein fractions differed among all samples. Apoprotein fraction III contained less radioactivity relative to all other fractions and apoprotein fraction I contained more radioactivity than other fractions when comparing VLDL incubated with normal plasma with that of the nonincubated sample and that incubated with postheparin with that incubated with normal plasma (Table 6). In view of the changes in both protein and radioactivity content of each fraction in the various samples, the specific activity of apoprotein fraction III relative to that of fraction I (determined independently, and on samples at the peak height of protein and radioactivity) was about one-half in incubated compared with nonincubated samples (Table 6).

The electron microscopic appearance of particles isolated at density less than 1.019 g/ml from VLDL incubated with either normal or postheparin plasma and their diameters are shown in **Fig. 4.** In general, the particles isolated after incubation with normal plasma were of larger diameter and more heterogeneous with regard to their diameters than those isolated after incubation with postheparin plasma (Fig. 4). The mean diameter of the former particles was 427 Å and of the latter particles 269 Å. Assuming that

TABLE 5. Lipid and protein composition of VLDL after incubation with normal and postheparin plasma

Incubation Mixture	Protein	Triglyceride	Phospholipids	Cholesterol[a]	Ratio of Free to Esterified Cholesterol[b]
	mg/100 mg lipoprotein[c]				
None	16.2 ± 0.3	60.5 ± 3.1	14.6 ± 2.1	8.7 ± 0.3	Not determined
Normal plasma	14.3 ± 1.4	62.0 ± 1.0	14.5 ± 1.2	9.2 ± 0.7	2.45 ± 0.2
Postheparin plasma	19.0 ± 2.4	46.4 ± 3.6	20.1 ± 3.8	14.5 ± 1.2	1.34 ± 0.2

[a] Free and esterified cholesterol determined as cholesterol (mol wt, 400).
[b] Means ± SE of three determinations.
[c] Means ± SE of six determinations.

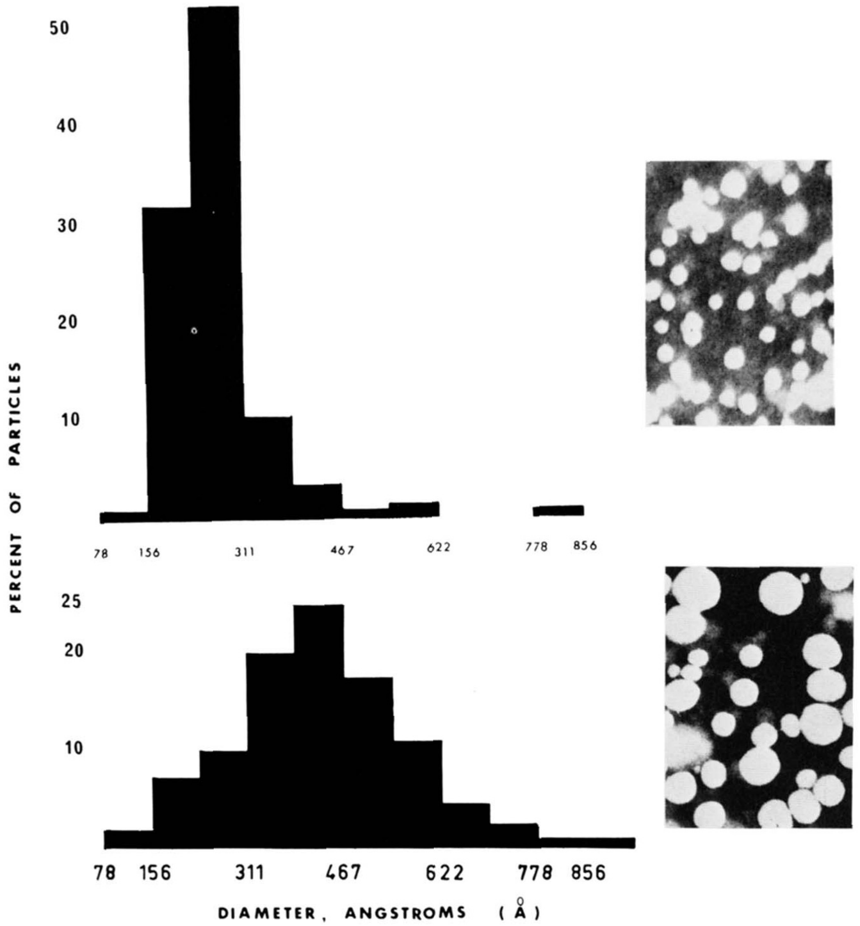

Fig. 4. Frequency distribution of diameters of VLDL particles after incubation with normal plasma (*bottom*) and postheparin plasma (*top*). Negatively stained electron micrographs of the two preparations are shown; magnification, ×126,000.

both particles are spherical, their volumes are 40.1 and 10.0 × 10^6 $Å^3$, or the volume of normal VLDL is four times greater than that of postheparin VLDL.

The S_f rates of VLDL and the intermediate lipoprotein were determined by analytical ultracentrifugation. The mean S_f rate of VLDL incubated with normal plasma was 115 and that of VLDL incubated with postheparin plasma was 30.5. Using Dr. Lindgren's nomogram on the relationship of S_f rates to molecular weights (35), the molecular weights of the two lipoproteins are estimated to be 23.1 and 7.0 × 10^6 daltons, respectively. The ratio of these molecular weights is 3.3, not unlike the ratio of the calculated volumes of the two lipoproteins. Using molecular weights derived from S_f rates (which take into account differences in density between the two lipoproteins) and knowing the relative contributions of lipids and apoproteins to each lipo-

TABLE 6. Protein and radioactivity content of apoprotein fractions obtained by gel filtration on Sephadex G-150

Incubation Medium	Protein			Radioactivity			Relative Specific Activity		
	I	II	III	I	II	III	I	II	III
		% of total			*% of total*				
None	21.0 ± 4.0	22.4 ± 4.6	56.6 ± 8.0	24.0 ± 0.8	41.9 ± 2.7	35.7 ± 2.7	1.00	1.77 ± 0.34	0.82 ± 0.11
Normal plasma	21.5 ± 2.0	22.7 ± 3.9	55.7 ± 4.3	28.7 ± 1.7	42.8 ± 4.2	28.4 ± 4.1	1.00	1.46 ± 0.31	0.44 ± 0.07
Postheparin plasma	57.0 ± 4.1	33.8 ± 4.6	9.2 ± 2.7	46.1 ± 5.0	48.7 ± 4.0	5.2 ± 2.0	1.00	1.46 ± 0.31	0.35 ± 0.15

Means ± SE of three experiments. 2–4 mg of apoproteins obtained from nonincubated VLDL and VLDL incubated with either normal or lipoprotein lipase-rich (postheparin) plasma was applied to 90 × 1.5 cm columns. Apoproteins were eluted with 0.2 M Tris–6 M urea–2 mM sodium decyl sulfate buffer (pH 8.2). The three protein fractions obtained (I, II, and III) were assessed for protein and radioactivity content as described in Materials and Methods.

TABLE 7. Lipid and apoprotein mass in VLDL and intermediate lipoprotein particles

Lipoprotein	Contributions of Apoproteins and Lipids[a] to Single Lipoprotein Particles						
	Apoprotein B	Apoprotein VS-2	Apoprotein C	TG	PL	FC	EC
	10^6 daltons/particle						
VLDL	0.70	0.73	1.80	14.10	3.28	1.48	1.04
Intermediate lipoprotein	0.73	0.44	0.12	3.10	1.36	0.55	0.71

TG, triglyceride; PL, phospholipid; FC, free cholesterol; EC, esterified cholesterol.
[a] The lipid values represent an apparent aggregate molecular weight contribution.

protein mass (Tables 5 and 6), it was possible to calculate the actual mass of each component in a single lipoprotein particle[2] (Table 7). The data demonstrate that the content of all lipids and apoproteins in an intermediate lipoprotein particle is decreased compared with a VLDL particle. The only exception is apoprotein B, the absolute contributions of which to single VLDL and intermediate lipoprotein particles were almost identical, 0.7 × 10^6 daltons.

DISCUSSION

The pathways of catabolism of chylomicrons and VLDL, the two triglyceride-rich lipoproteins, have been partially elucidated during the last decade. The first stage of degradation is lipoprotein lipase-mediated triglyceride hydrolysis resulting in the formation of a triglyceride-poor, cholesterol-rich particle (12, 13). The nature and fate of this particle, designated variously as "remnant" (14), "intermediate lipoprotein" (10, 11), or "skeleton" (15), are obscure. Yet, in both humans (10, 11) and rats (18-22), some or most of plasma LDL originates in the circulation from VLDL through an intermediate lipoprotein stage. LDL particles, however, differ markedly from VLDL in apoprotein and lipid content and composition (1, 11). Thus, mechanisms responsible for these changes must operate concomitantly with triglyceride hydrolysis. The present investigation was undertaken to study the fate of apoprotein and lipid constituents of VLDL during its interaction with the lipoprotein lipase system. The use of lipoprotein lipase-rich plasma obtained from rats injected with heparin was found to be a highly reproducible system for these studies. This system has advantages over that of isolated enzymes in that it contains the two major lipoprotein lipase species (of hepatic and extrahepatic origin [36-41]) and is carried in the natural milieu of VLDL catabolism, i.e., whole plasma. To further validate the system, we have compared the apoprotein composition of ^{125}I-labeled VLDL incubated with postheparin plasma (containing both the hepatic and the extrahepatic enzymes) with samples incubated with postheparin plasma obtained from supradiaphragmatic rats (containing only the extrahepatic enzyme [38]), and VLDL isolated from rats injected with ^{125}I-labeled VLDL and heparin (Tables 1-4). The patterns of ^{125}I-labeled apoproteins in VLDL isolated in the three experiments were almost identical, as were the protein and lipid compositions of the first two samples.

The protein moiety of rat plasma VLDL is composed of at least three apoprotein fractions (5, 6). Analogous to human VLDL, the fractions may be designated apoprotein B (VS-1, P-I), apoprotein C (VS-3, P-III), and apoprotein VS-2 or P-II. The metabolism of apoprotein B and apoprotein C in human (10, 11, 23) and rat (18-22) plasma VLDL is heterogeneous. Apoprotein B is the precursor of the protein moiety of plasma LDL. Apoprotein C, present predominantly in VLDL and HDL (2, 5, 6), represents a discernible pool of apoproteins that are distributed among lipoproteins in proportion to their concentration in plasma (23). During steady-state conditions, it has been hypothesized (but never proved) that the bidirectional transfer of apoprotein C between lipoproteins represents an exchange reaction. In the present study we were able to show that after incubation of ^{125}I-labeled VLDL with normal plasma no change occurred in the content of apoproteins in VLDL, whereas the specific activity of apoprotein C decreased by about 30-50%. Thus, this type of transfer of apoprotein C between lipoproteins must represent an exchange phenomenon.

A transfer of apoprotein C from VLDL and chylomicrons to HDL has also been observed after the injection of heparin to humans (11, 42) and during clearance of alimentary chylomicronemia (43). These observations were extended here to the heparin-injected rat and were further evaluated in vitro. After the incubation of ^{125}I-labeled VLDL with postheparin plasma, more than 80% of the ^{125}I-labeled apoprotein C introduced as part of the labeled VLDL was recovered with HDL. This marked change of

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[2] An example to illustrate this calculation is the content of apoprotein B in VLDL. The weight of a VLDL particle is 23.1 × 10^6 daltons. Protein constituted 14.3% of total mass, and apoprotein B 21.5% of total protein. Apoprotein B thus constitutes 3.07% of 23.1 × 10^6 daltons, or 0.71 × 10^6 daltons.

distribution of apoprotein C among lipoproteins occurred without further decrease of the specific activity of ^{125}I-labeled apoprotein C in VLDL and hence must represent a net transfer reaction. Whether similar changes occur in VLDL particles interacting with lipoprotein lipase attached to capillary endothelial cells is yet to be established.

The mechanisms of association of apoprotein C with various lipoproteins are poorly understood. Our results demonstrate clearly that the content of apoprotein C in VLDL is related to the content of triglycerides in the VLDL particles and is independent of all other apoproteins, in particular apoprotein B. The graded transfer of apoprotein C from VLDL to HDL observed during the course of the incubation may therefore be explained as a "disintegration" of a complex particle. According to this view, concomitant with triglyceride hydrolysis, apoprotein C units become loosely associated (or dissociated) with the partially degraded VLDL and are then transferred to HDL. In sharp contrast, the association of apoprotein B with VLDL is independent of the degree of lipolysis, and apoprotein B constitutes a major protein moiety of the intermediate lipoprotein.

Compared with VLDL, the intermediate lipoprotein produced in vitro was almost devoid of apoprotein C; it was enriched with apoprotein B and contained some of the VS-2 proteins. The lipid and apoprotein composition of the intermediate lipoprotein more closely resembled a "triglyceride-rich" LDL than a "triglyceride-poor" VLDL. In only one previous study was the composition of an in vitro-produced postlipolysis VLDL reported (12). The study, carried out with human plasma, demonstrated a lipoprotein form similar to that described here. The reasons for the slow hydrolysis of triglycerides in the intermediate lipoprotein produced in vitro (12) or in vivo (13) are unknown. It may be speculated, however, that the removal of one of the components of apoprotein C (C-II, apo-LP-Glu), a documented activator of the lipoprotein lipase system (44–47), may have contributed to this observation.

Based on analysis of lipid and apoprotein composition of VLDL and LDL density subfractions isolated from two hyperlipemic patients, it was previously deduced that during the metabolic conversion of VLDL to LDL one and only one LDL particle is formed from each VLDL particle (11). A similar analysis was carried out here for VLDL and the intermediate lipoprotein isolated after the incubation of VLDL with normal or postheparin plasma. The data demonstrated that the weight contributions of apoprotein B to VLDL and intermediate particles were virtually identical, about 0.7 × 10^6 daltons. An analysis of data published by Koga et al. (6, 48) and by Bersot et al. (5) on the characteristics of rat plasma LDL enabled us to calculate the weight contribution of apoprotein B to one LDL particle, about 0.6 × 10^6 daltons, a value similar to that calculated here for apoprotein B mass in one VLDL and intermediate lipoprotein particle. Thus, one single mechanism of formation of intermediate lipoprotein and LDL seems to operate in humans and rats either in vivo or in vitro.

The number of protein units of apoprotein B in one lipoprotein (of any density) is unknown. Estimates have been published for human LDL and they range from 2 (49) to 20–60 (50–52). Our results are obviously consistent with the possibility that apoprotein B represents a single unit. They indicate that if more than one unit of apoprotein B is present in lipoproteins, most or all of these units are linked together either structurally or functionally. Of the other components in VLDL, about 60–70% of the protein of the second Sephadex fraction (VS-2, P-II) was associated with the intermediate lipoprotein. This fraction may contain predominantly the recently described "arginine-rich" apoprotein (53–55). The fate of phospholipids and cholesterol (especially unesterified cholesterol), 40–60% of which disappears from VLDL during its interaction with lipoprotein lipase-rich plasma, is currently under investigation.

The biological significance of the intermediate lipoprotein is yet to be determined. We have shown previously (18, 19) that about 80% of the ^{125}I-labeled apoprotein B injected into rats as part of ^{125}I-labeled VLDL disappears from circulation within 1–2 hr of the injection, presumably at an intermediate lipoprotein stage. A similar conclusion was reported recently by Faergeman et al. (20, 21) and by Mjøs et al. (22). It is thus possible that the intermediate lipoprotein represents an unstable lipoprotein form that is either removed from circulation (predominantly by the liver) or converted into LDL. With regard to this suggestion, it is interesting to note that intermediate lipoprotein ("remnant") is catabolized by arterial smooth muscle cells in tissue cultures more efficiently than VLDL (56). JLR

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