Fate of apolipoproteins C-II, C-III, and E during lipolysis of human very low density lipoproteins in vitro

Shui Pang Tam, Ladislav Dory, and David Rubinstein[1]

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7

Abstract The apoprotein and lipid composition of HDL-like products arising from lipolysis of human VLDL was studied. The VLDL was perfused through beating rat hearts in the absence of serum to avoid possible alteration of the primary products of lipolysis due to apoprotein exchange with serum lipoproteins. The lipolytic products were separated by gel filtration to obviate possible losses of apoproteins from the lipoproteins during ultracentrifugation. Apoprotein B, E, C-II, and C-III were quantitated by electroimmunoassay and lipids were determined by chemical methods. During perfusion, a 50% hydrolysis of the VLDL triacylglycerols was associated with the appearance of 11% of the apoC-II, 30% of the apoC-III, and 20% of apoE of the VLDL in HDL-like particles as isolated by agarose gel filtration. The shift of the apoproteins to these particles was associated with a similar redistribution of cholesterol, phospholipid, and cholesteryl ester. The lipid composition of the HDL-like particles was cholesterol (15.1 ± 4.0%), phospholipid (37.8 ± 3.2%), cholesteryl ester (34.2 ± 2.6%), and triglyceride (12.9 ± 3.2%). The particles possessed a hydrated density of 1.063–1.21 g/ml and were spherical, with particle diameters (mean 124 ± 36 Å, range 50–160 Å) that were comparable to the diameter (140 Å) estimated from calculations of the surface to volume ratio, assuming a spherical particle consisting of a neutral lipid core surrounded by cholesterol, phospholipid, and protein. No discoidal forms or rouleau structures were observed in the HDL-sized fraction isolated by gel filtration. The HDL-like fraction could be resolved further by heparin-Sepharose chromatography into an unretained fraction containing predominantly apoC-III with apoE and apoC-II, and a retained fraction containing apoC-III and apoE. Small amounts of apoE were also recovered in extremely small particles that are normally observed in the d > 1.21 g/ml fraction after ultracentrifugation. No apoC-II or C-III was observed in this fraction. Incubation of VLDL with lipoprotein lipase, mobilized from hearts by heparin perfusion, yielded results that were similar to those with the perfused heart. Hearts perfused with VLDL removed apoB but not apoC-II, C-III, E, or phospholipids from the perfusate. It is concluded that the initial products of VLDL catabolism include spherical particles, similar in size to HDL, that contain apoC-II, C-III, E, cholesterol, cholesteryl esters, and phospholipids. ApoE is also released as a small extensively delipidated particle.—**Tam, S. P., L. Dory, and D. Rubinstein.** Fate of apolipoproteins C-II, C-III, and E during lipolysis of human very low density lipoproteins in vitro. *J. Lipid Res.* 1981. **22:** 641–651.

Supplementary key words lipoprotein lipase · apolipoproteins B, C, and E · perfused heart · cholesterol · triacylglycerols

The catabolism of very low density lipoproteins in vivo results in the appearance of some of their apoproteins in the high density lipoprotein fraction of serum (1, 2). Using a number of in vivo and in vitro models, it has been demonstrated that lipolysis of the triacylglycerols of VLDL and chylomicrons is accompanied by a loss of apoproteins, especially apoC, which is recovered in the HDL and the d > 1.21 g/ml infranatant (3–7). However, due to several complications inherent in the experimental procedures utilized to date, it is difficult to determine unequivocally the distribution and composition of the apoprotein-containing particles initially produced during VLDL catabolism. For example, during studies of the breakdown of isotopically labeled VLDL by post-heparin plasmas (3) or in models such as hepatectomized (8) or supradiaphragmatic rats (4), the appearance of apoproteins in HDL may be the result of an exchange of labeled apoC, which is known to occur between VLDL and HDL (9, 10). In addition, the presence of HDL may provide an acceptor upon which the initial catabolic products are adsorbed (8, 11). While some studies of the lipolysis of VLDL have utilized either purified bovine milk lipoprotein lipase or perfused hearts in the absence of plasma, these involved VLDL upon which purified iodinated apoC had been adsorbed (12, 13), leaving some uncertainty as to whether this substrate represented the natural location and configuration of the apoprotein. Last, most studies have utilized ultracentrifugation for the

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

[1] Deceased, February 5, 1980.

separation of the products of VLDL catabolism, introducing a possible artifact due to the dissociation of apoproteins (14).

We have tried to avoid these problems by studying the catabolism of human VLDL by perfused beating rat hearts, which serve as a source of immobilized lipoprotein lipase, in the absence of plasma. The perfusate lipoproteins were then separated by agarose gel filtration and the apoprotein contents of various sized particles were determined by electroimmunoassay. These studies have provided some insight into the composition of the smaller sized particles produced during VLDL catabolism prior to their possible interaction with existing circulating lipoproteins or removal from the circulation. A preliminary account of portions of this work has been presented (15).

METHODS

Heart perfusions

Hearts, obtained from male hooded rats weighing 250 g and fasted overnight, were perfused as previously described (6). The hearts were cannulated through the aorta and flushed with perfusion solution to remove any residual blood prior to their installation in the perfusion apparatus. The perfusate was 60 ml of a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.22 mM $CaCl_2$ and 5.5 mM glucose, but no albumin. The gas phase was 95% O_2, 5% CO_2. The omission of albumin resulted in a more rapid and regular heart-beat without an extensive decrease in hydrolysis of the triacylglycerols of the VLDL. The resulting free fatty acids were taken up by the heart rather than accumulating in the perfusate. The hearts beat steadily (>175 beats/min) and were changed every 30 min throughout the experiment. Isolated human VLDL was added to the circulating perfusate. Controls consisted of recirculating the perfusate containing the isolated VLDL through the apparatus in the absence of the hearts. All perfusions were of 120-min duration.

Separation of lipoproteins

VLDL was isolated from freshly drawn citrated human blood. Disodium EDTA, pH 7.3, and NaN_3 were added to the plasma for a final concentration of 0.01 and 0.02%, respectively, and the chylomicrons were removed by ultracentrifugation at 27,000 rpm for 45 min in a Beckman ultracentrifuge using an SW-27 rotor. The VLDL was then isolated by a modification (9) of the method of Havel, Eder, and Bragdon (16). The isolated VLDL was washed twice to remove contamination by other plasma protein. The VLDL was iodinated by a modification (17) of the method of McFarlane (18). Approximately 80% of the ^{125}I was bound to the protein moiety. The ^{125}I-labeled VLDL was diluted ten-fold with unlabeled VLDL and the solution was filtered through a 1.5 × 25 cm column of Sephadex G-50, then dialyzed overnight against normal saline.

At the end of each 2-hr perfusion, the perfusate was concentrated by dialysis against Aquacide II-A and the lipoprotein particles were separated according to size on a 0.8% agarose gel column. For this purpose two columns, 2.6 × 60 cm and 2.6 × 100 cm, were used in series and packed with Biogel A-1.5 m agarose gel (200–400 mesh). The concentrated perfusate was applied to the column and eluted at a flow rate of 50 ml/hr with a solution of 0.15 M NaCl containing 0.01% disodium EDTA, pH 7.3, and 0.02% NaN_3. The void volume of the column was approximately 220 ml and the bed volume was approximately 660 ml. When ^{125}I-labeled VLDL was applied to the column, a 0.2-ml aliquot was drawn from every second 5-ml elution fraction and counted. Elution fractions were pooled according to different size ranges based on the profile obtained with the isolated lipoproteins and standard proteins (Fig. 1), dialyzed against 5 mM $NaHCO_3$, lyophilized, and dissolved in 1 ml of 0.06 M barbital buffer, pH 8.6, containing 0.016% Triton X-100. Some apoE-containing lipoproteins were separated by affinity chromatography on heparin-Sepharose columns. The heparin was linked to Sepharose 6B by the use of cyanogen bromide according to the method of March, Parikh, and Cuatrecasas (19).

Preparation of antigens and antibodies

Isolated human VLDL was delipidated with ethanol–ether 3:1 (v/v) (20). The apoproteins were dissolved in 4 M guanidine hydrochloride and separated on a BioGel A-1.5 m column as described for rat apo VLDL by Wong and Rubinstein (21). The apoC polypeptides (apoC-I, apoC-II, and apoC-III) which co-elute from the column were further separated by preparative isoelectric focusing according to Radola (22) as described by Marcel, Bergseth, and Nestruck (23). The purity of all of the isolated apoproteins was checked by polyacrylamide urea gel electrophoresis and analytical gel isoelectric focusing. Antisera to apoB, apoE, apoC-II, and apoC-III_2 were prepared in New Zealand white rabbits by sequential bi-weekly intradermal injections of the purified antigens, emulsified with complete Freund's adjuvant. One week after the third injection, the rabbits were bled via the ear vein and the γ-globulins were iso-

lated by $(NH_4)_2SO_4$ precipitation (24). Antibody specificity of the isolated γ-globulin fraction was checked by Ochterloney double diffusion analysis (25) using human albumin and isolated VLDL apoproteins. Anti apoC-II only reacted with apoC-II and gave no immune precipitate when diffused against apoC-II-deficient serum (supplied by Dr. W. C. Breckenridge, University of Toronto). Anti apoC-III_2 reacted identically with all apoC-III isomorphs but showed no reactivity against apoC-II, apoE, or apoB. Anti apoE was specific for apoE and showed no reactivity against apoB, apoA-I, or the low molecular weight polypeptides. Anti apoB exhibited similarly high specificity for apoB and possessed no reactivity against other apoproteins.

Analysis of apoproteins

The levels of apoB, apoC-II, apoC-III, and apoE were determined by modifications of the rocket immunoelectrophoresis assay of Laurell (26). The following conditions were used. For apoB, a 1% agarose gel was prepared in 0.06 M barbital buffer, pH 8.6, containing 0.016% Triton X-100. For apoC-II and apoC-III, a 1.3% agarose gel was prepared in the same buffer with 7% dextran T-10, while a 2% agarose gel in the barbital buffer with 5% dextran T-10 was used for apoE. In all cases the immunoelectrophoresis was run at 2 v/cm for 18 hr. The plates were successively soaked in 0.15 M NaCl for 1 hr and in distilled water for 15 min, covered with filter paper, dried at 60°C and stained as described by Crowle and Cline (27). The electroimmunoassays were initially standardized with isolated LDL (d 1.04–1.05 g/ml) for apoB, and pure apoproteins for apoE, C-II, and C-III. Subsequently, a preparation of lyophilized VLDL was used as the standard for each determination. The electroimmunoassays for apoB and apoE were validated by comparison with human VLDL. For this purpose totally delipidated VLDL was dissolved in 4 M guanidine hydrochloride and separated on Biogel A-1.5 m columns (21). The protein content of the peaks containing apoB (corrected for undissolved apoB) and apoE eluted from the column, determined by the procedure of Lowry et al. (28), was compared to the values obtained by electroimmunoassay of an aliquot of the same VLDL preparation. These agreed within 5% for each apoprotein. The electroimmunoassays for apoC-II and apoC-III were cross-validated in a similar fashion. The apoC-II and C-III content of VLDL was determined in the assays using pure apoC-II and C-III as a standard. The same VLDL was then totally delipidated, solubilized, and chromatographed on BioGel A-1.5 m; the apoC peak (apoC-I, C-II, and C-III) was then re-chromatographed on DEAE cellulose (29, 30) and the protein content of the individual C apoprotein peaks was determined by the method of Lowry et al. (28). The protein values for the C-II and C-III content of the VLDL preparation were within 6% of the apoC-II electroimmunoassay determination and 5% of the original C-III determination.

Analytical procedures

Lipids were extracted for analysis from the VLDL and various perfusate fractions by the method of Folch, Lees, and Sloane Stanley (31) and were separated by thin-layer chromatography on silica gel G with a solvent system of petroleum ether–diethyl ether–acetic acid 90:10:1 (v/v). The various lipids were eluted from the plates and estimated as previously described (32). Protein was determined by the method of Lowry et al. (28). The lipoproteins were visualized in the electron microscope after negative staining with phosphotungstic acid at pH 7.2 (33).

Materials

Rats and rabbits were obtained from Canadian Breeding Farms, St. Constant, Quebec and $Na^{125}I$ was from Charles E. Frosst and Co., Montreal, Quebec. Biogel A-1.5 m agarose gel and dextran T-10 were purchased from BioRad Laboratories, Richmond CA; Sea Kem LE agarose powder for immunoelectrophoresis was from Marine Colloids, Inc., Rockland ME; Sepharose CL-4B and Sepharose-linked heparin were from Pharmacia (Canada) Ltd., Montreal, Quebec; Triton X-100 was from Rohm and Haas, Philadelphia, PA; Aquacide II-A was from Calbiochem Behring Corp., La Jolla, CA; silica gel G was from Brinkmann Co., Missisauga, Ontario; and acrylamide, bisacrylamide, cyanogen bromide, and SDS were from Eastman Kodak Co., Rochester, NY. All other analytical grade reagents were purchased from Canlab Scientific Co., Dartmouth, N.S. Human citrated blood was generously supplied by the Nova Scotia Transfusion Centre of the Canadian Red Cross.

RESULTS

In order to avoid the possible dissociation of apoproteins due to high ultracentrifugal forces and/or salt concentrations during the fractionation of the lipoprotein, VLDL and their lower molecular weight derivatives were separated by agarose gel filtration chromatography. The elution pattern of a mixture of individually isolated VLDL, LDL, HDL, and purified albumin and cytochrome c used to standardize the column, is shown in **Fig. 1.** The column was de-

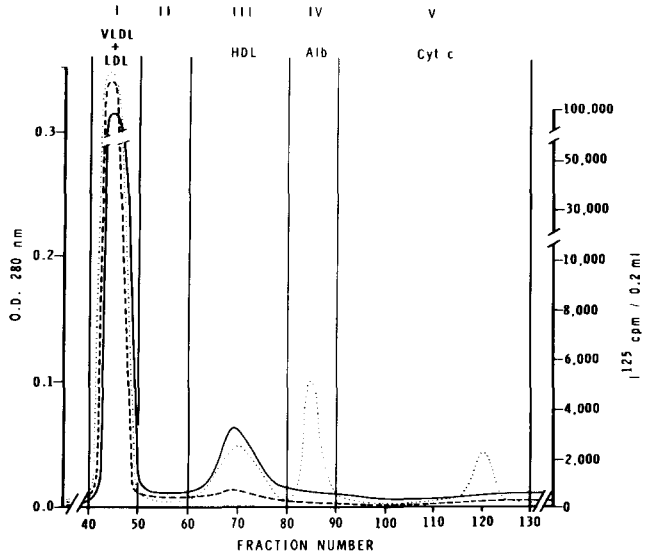

Fig. 1. Gel filtration of lipoproteins, (....), Mixture of isolated proteins used to standardize the column, measured as absorbance at 280 nm; (– – –), concentrated perfusate containing ^{125}I-labeled VLDL recirculated through the perfusion apparatus in absence of hearts; (——), concentrated perfusate after perfusion of hearts with ^{125}I-labeled VLDL. The eluates collected between each set of vertical lines were pooled for subsequent analyses. The absence of a shoulder representing LDL on fraction I when the standard protein mixture was applied is probably due to the limited sensitivity of the light absorption readings compared to the isotope determinations of 0.2-ml aliquots of each fraction with ^{125}I used subsequently.

signed to separate the lower molecular weight fractions from the large particles. Discrimination between VLDL and LDL was sacrificed in order to get a definitive separation of HDL from the larger lipoproteins on one side and from albumin and smaller particles on the other. It will be noted that the elution pattern of a concentrated perfusate containing ^{125}I-labeled VLDL which had been recirculated through the perfusion apparatus in the absence of hearts was indistinguishable from the VLDL in the mixture of proteins used to standardize the column, except for a slight contamination eluting in the HDL area. However recirculation of the ^{125}I-labeled VLDL through the beating rat hearts for 2 hr resulted in a number of changes in the elution pattern of the concentrated perfusate. The radioactivity of the VLDL fraction as defined by peak height has diminished by 8% and shifted slightly to the right, indicating a reduction in size due to the formation of VLDL remnants. In addition, a significant peak was now found in fraction III (Fig. 1) containing particles corresponding in size to the HDL. Fraction III was further analyzed in the ultracentrifuge to determine the flotation characteristics of the ^{125}I-labeled lipoprotein particles at various salt densities. Only 1% of the total fraction ^{125}I-labeled lipoprotein radioactivity was isolated at $d < 1.063$ g/ml, the majority (91%) in the density region = 1.063–1.21 g/ml, and only 8% was present in the $d > 1.21$ g/ml infranatant. Furthermore the fraction III lipoproteins were found to have α mobility comparable to isolated serum HDL upon agarose gel electrophoresis (results not shown).

In order to determine the distribution of apoproteins among the particles of various sizes, the eluates were pooled into the five fractions shown in Fig. 1. The distribution of apoC-II, apoC-III, apoE, and apoB among these fractions, as determined by electroimmunoassay, is shown in **Table 1.** In the initial experiments, ^{125}I-labeled VLDL served as a substrate for the heart lipoprotein lipase. Subsequently, unlabeled VLDL was used with no difference in the distribution of the apoproteins. In control experiments, i.e., VLDL recirculated in the absence of the heart, apoC-II and C-III were virtually restricted to fraction I with a slight overlap into fraction II. On the other hand, after the VLDL had been recirculated through the heart, 11% of the apoC-II and 30% of the apoC-III shifted into fraction III, which corresponds in size to HDL. However, no apoC-II and apoC-III were detected in the smaller particles (Fractions IV and V) which upon ultracentrifugation and isolation would normally be recovered in the $d > 1.21$ g/ml infranatant. ApoE was also primarily restricted to fraction I in the control experiments with some spillage into fraction II, but none was detected in the HDL or smaller particles. Following heart perfusion a considerable portion of apoE was recovered in the HDL-sized fraction. In addition apoE was consistently observed in fractions IV and V. The latter approaches the size of the delipidated apoprotein, which has a molecular weight of 35,000 daltons (34). The distribution of apoB in the perfusate was also measured to establish that the appearance of apoC-II, apoC-III, and apoE in the smaller fractions was not due to a random redistribution or disintegration of the VLDL or its remnants. It will be noted that the apoB remained restricted to the VLDL plus LDL fraction I following both control and experimental perfusions.

Although the perfused rat heart was initially used as a source of immobilized lipoprotein lipase, the possibility that the initial lipolytic products of VLDL catabolism might be altered by selective removal of lipids or apoprotein by the heart was considered. Therefore eight hearts each were perfused for 3 min with 30 ml of 0.15 M NaCl containing 100 units of heparin to release the lipoprotein lipase. The resulting perfusate was divided into two aliquots, one being

TABLE 1. Distribution of apoproteins of human VLDL following rat heart perfusions

	Total Apoprotein[a]	Column Fractions I	II	III	IV	V
	μg	*percentage*				
ApoC-II						
Control	193 ± 17	94 ± 4	5 ± 3	1 ± 1	0	0
+ Heart	188 ± 15	84 ± 2	5 ± 3	11 ± 4	0	0
ApoC-III						
Control	612 ± 15	96 ± 2	3 ± 2	1 ± 1	0	0
+ Heart	600 ± 10	62 ± 3	8 ± 1	30 ± 2	0	0
ApoE						
Control	153 ± 21	91 ± 5	9 ± 4	0	0	0
+ Heart	150 ± 24	62 ± 4	7 ± 1	22 ± 2	4 ± 1	5 ± 1
ApoB[b]						
Control	915 ± 21	99 ± 1	1 ± 1	0	0	0
+ Heart	665 ± 33	96 ± 1	4 ± 1	0	0	0

[a] The total apoprotein represents the sum of the immunoassayable apoprotein recovered in each column fraction after perfusion.

[b] Following recirculation of VLDL through the apparatus, it was found that 20–30% of the apoB was trapped in the apparatus, probably adhering to the silastic tubing lung. This loss was similar whether intact VLDL or remnants (prepared by submitting the VLDL to the action of lipoprotein lipase mobilized from the heart) were perfused. The total level of apoB shown in the table has been corrected for this loss.

Each figure represents the mean ± SEM of six experiments, except for the total apoB content, which represents four experiments. Additional experiments were performed using tetramethylurea to precipitate apoB in each fraction. No difference in distribution was noted and these are included in the percentage distribution, but not the total. Each experiment consisted of a control (no hearts in the apparatus) and an experiment perfusion. To each perfusate was added 2.4 mg of VLDL protein.

heated at 56°C for 30 min to inactivate the lipoprotein lipase and serve as a control. To each was then added VLDL (2.4 mg protein), and the volume was adjusted to obtain a final concentration of 5 mM $CaCl_2$ and 1.25% defatted bovine serum albumin in 0.1 M Tris-HCl buffer, pH 8.0. The mixture was then incubated for 2 hr at 37°C. The distribution of the apoproteins after incubation and separation of lipoproteins by gel filtration is shown in **Table 2.** The results are similar to those found when the perfused hearts were used, except that the difference in total apoB between the control and experi-

TABLE 2. Distribution of apoproteins of human VLDL following incubation with mobilized rat heart lipoprotein lipase

	Total Apoprotein	Column Fractions I	II	III	IV	V
	μg	*percentage*				
ApoC-II (4)						
Control	188 ± 12	96 ± 1	3 ± 1	1 ± 1	0	0
Experimental	184 ± 10	85 ± 2	4 ± 1	11 ± 2	0	0
ApoC-III (4)						
Control	610 ± 10	97 ± 1	2 ± 1	1 ± 1	0	0
Experimental	600 ± 15	64 ± 2	6 ± 1	30 ± 2	0	0
ApoE (5)						
Control	168 ± 15	93 ± 1	7 ± 1	0	0	0
Experimental	170 ± 18	70 ± 2	6 ± 1	18 ± 4	2 ± 1	5 ± 1
ApoB (3)						
Control	875 ± 38	100 ± 1	0	0	0	0
Experimental	850 ± 44	100 ± 1	0	0	0	0

Each figure is the mean ± SEM of the number of experiments indicated in parentheses, each consisting of a control (heat-inactivated lipoprotein lipase) and an experimental (active enzyme) flask. Incubation conditions are described in the text.

mental perfusions (cf. Table 1) did not occur. ApoC-II and apoC-III were transferred from VLDL to the HDL size fraction but not to fractions IV and V, while apoE was recovered as HDL and, to a lesser extent, as smaller sized particles in fraction V. In parallel with the perfusion experiments, apoB remained in the void volume after incubation of the VLDL with either the active or inactivated lipoprotein lipase. Similar results, not shown here, were also obtained with crude and purified rat adipose tissue lipoprotein lipase prepared as previously described (35), but the degree of lipolysis and the redistribution of apoproteins was considerably less.

The fate of the lipid moieties of VLDL is shown in **Table 3.** It will be seen that during the heart perfusion or incubation with the mobilized lipoprotein lipase about 50% of the triacylglycerols was hydrolyzed. In the perfusion experiment there was a small, though not statistically significant, uptake of free cholesterol and cholesteryl esters by the heart. As expected, following lipolysis by mobilized lipoprotein lipase, no change occurred in the total levels of any lipid except the triacylglycerols. Most of the lipid in each class was retained in fraction I but there was a significant redistribution of cholesteryl esters to fraction III and of phospholipid to fractions III and IV. These changes, also shown in Table 3, occurred in both the perfused heart and mobilized lipase incubation experiments. While the increment of cholesterol in fraction III was not statistically significant, the lipid composition of fraction III was characterized by a high relative content of unesterified cholesterol (15.1 ± 4.0%), cholesteryl esters (34.2 ± 2.6%), and phospholipid (37.8 ± 3.2%) with small amounts of triglyceride (12.9 ± 3.6%).

Since albumin was not present in the perfusion it was considered possible that small amounts of free fatty acid or lysophosphatidylcholine normally bound by albumin might influence the distribution of the apoproteins. Although the studies with mobilized lipase, where albumin was included, indicated no difference in apoprotein distribution, perfusions were conducted in the presence of 0.5 or 4.0% albumin in the perfusion medium. At 0.5% albumin there was no effect on lipolysis or apolipoprotein distribution. With 4% albumin there was an increase in lipolysis of triacylglycerols from 50% to 70%. The amounts of apoC-II (15–16%) and apoC-III (30–31%) in fraction III were similar to previous experiments, while the amount of apoE in fraction III (24–28%) and fraction IV and V (11%) was slightly higher than that observed without albumin. Thus the data indicate that the observed distributions of apoproteins were not a consequence of the accumulation of free fatty acids or lysophosphatidylcholine in the medium or on the lipoproteins.

The question arises whether at least some of the particles found in the HDL-sized fraction III contain both apoC and apoE. In an attempt to solve this problem, advantage was taken of the observation that apoE-containing lipoproteins bind to heparin-Sepharose columns (36, 37). Fraction III of the concentrated perfusate eluate was applied to a 2.5 × 20 cm column containing the heparin-Sepharose and eluted successively with 0.12 M NaCl and 1.2 M NaCl, both in 0.005 M sodium phosphate buffer, pH

TABLE 3. Levels and distribution of lipids following lipolysis of VLDL

	Total Lipid		Column Fraction III		Column Fraction IV	
	Control	Experimental	Control	Experimental	Control	Experimental
	mg		*percentage*			
Perfused heart (5)						
Triacylglycerols	11.7 ± 0.3	5.6 ± 0.1	2 ± 1	2 ± 1	3 ± 1	3 ± 1
Free cholesterol	2.8 ± 0.1	2.3 ± 0.3	4 ± 1	6 ± 1	4 ± 1	5 ± 2
Cholesteryl esters	3.6 ± 0.6	2.8 ± 0.4	4 ± 1	12 ± 2	4 ± 1	6 ± 2
Phospholipids	4.7 ± 0.2	4.6 ± 0.2	4 ± 1	8 ± 1	4 ± 1	10 ± 1
Mobilized lipoprotein lipase (4)						
Triacylglycerols	11.6 ± 1.0	5.8 ± 0.2	2 ± 1	5 ± 1	1 ± 1	5 ± 2
Free cholesterol	3.0 ± 0.2	2.8 ± 0.2	2 ± 1	3 ± 1	2 ± 1	4 ± 1
Cholesteryl esters	4.0 ± 0.5	3.9 ± 0.5	2 ± 1	9 ± 2	2 ± 1	5 ± 2
Phospholipids	4.1 ± 0.3	4.0 ± 0.3	3 ± 1	8 ± 1	3 ± 1	10 ± 1

The values represent the means ± SEM of the number of experiments indicated in parentheses. Most of the lipid was retained in fraction I, with no significant differences between the distribution except in fraction I for the cholesteryl esters and phospholipids (83 ± 3 vs 70 ± 6 and 86 ± 1 vs 75 ± 1%, respectively) reflecting the changes shown in fractions III and IV above. The phospholipid values have been corrected for 65% recovery from thin-layer chromatography.

7.4. The elution pattern of serum HDL (noted by the dashed line) as well as the concentrated fraction III (noted by the solid line) is shown in **Fig. 2.** An unbound (A) and a bound (B) fraction was obtained. The majority of serum HDL mass, as measured by absorbance, was in fraction A. Analysis of the apoC-II, apoC-III, and apoE content of the serum HDL revealed that most of the apoC-II and apoC-III were found in peak A (unbound), but the apoE was equally divided between peak A and peak B (bound). This differs from a recent report that most of the apoE of rat HDL appears to be bound on heparin-Sepharose columns (37). To test the specificity and capacity of the heparin affinity column, apoC and apoE were isolated from VLDL by the acetone–isopropanol extraction procedure of Holmquist and Carlson (38) and applied to the heparin-Sepharose column. Under these conditions, the C apoprotein was completely eluted in peak A while the apoE was present only in peak B, as determined by double immunodiffusion and the production of class specific antibodies when the eluates were used as antigens. In addition, the quantity of HDL apoE bound to the column was considerably greater than the total found in fraction III. The ratios of apoC-II and apoC-III to apoE among the bound and unbound fraction III particles represented in Fig. 2, were 2.0 and 5.7, respectively. The apoC-II and C-III/apoE ratios for bound and unbound serum HDL were 2.6 and 6.2, respectively. Thus it appears that fraction III contains both relatively apoE-rich (bound) and apoE-poor (unbound) HDL-sized particles. However, the apo E-rich particles also contain a great deal of apoC-III which itself does not bind to the apoE affinity column, suggesting that the two apoproteins are present at least in part as a single particle in this fraction. On the other hand a portion of the apoE, eluted in peak A, appears to be positioned within the particle so as to be unavailable for binding to heparin. Peak A also contains the majority of the apoC-III and almost all of the apoC-II present in fraction III.

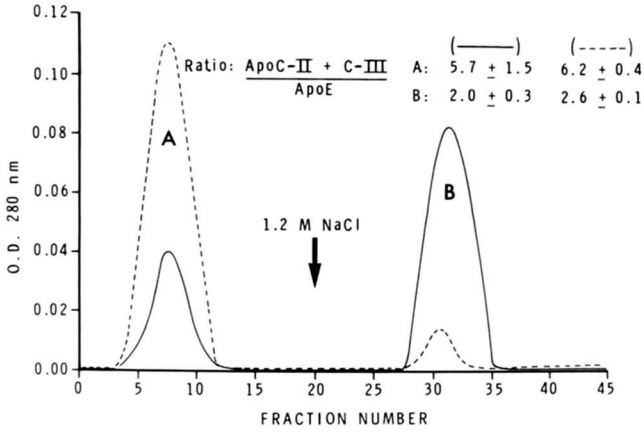

Fig. 2. Separation by heparin-Sepharose affinity chromatography of serum HDL (– – –) and concentrated fraction III lipoproteins (——) derived from VLDL. Peak A was eluted with 0.12 M NaCl, peak B with 1.2 M NaCl, both in 0.005 M phosphate buffer, pH: 7.4. The arrow represents the change in NaCl concentration. The apoC-II + apoC-III:apoE ratio is the mean ± SEM of four experiments with fraction III, two using commercially prepared heparin-Sepharose and two using heparin linked to Sepharose in the laboratory. No difference in the elution patterns or apoC-II + apoC-III:apoE ratio was observed.

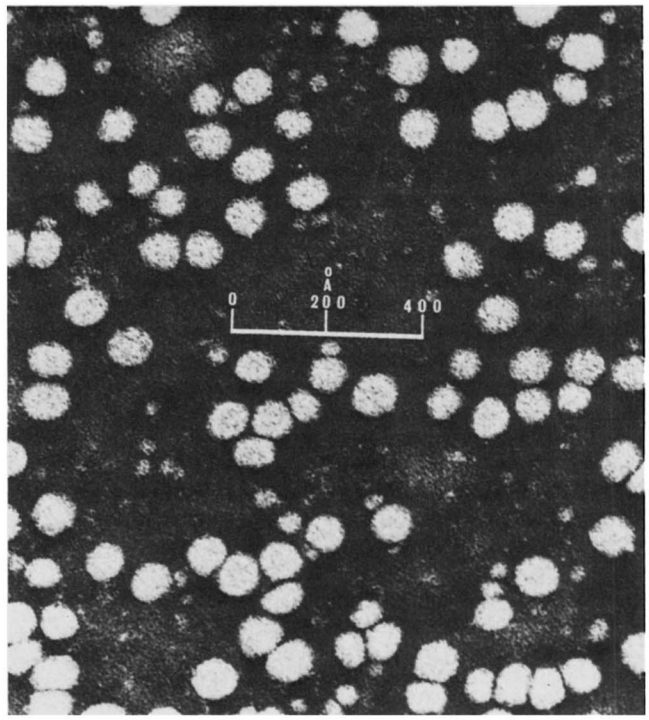

Fig. 3. Electron micrograph of negatively stained particles found in column fraction III after perfusion of VLDL through isolated rat hearts. Magnification: 395,000x. The particles range in size from 50–160 Å with a mean diameter of 124 ± 36 Å.

The particles of fraction III were visualized by electron microscopy following negative staining (**Fig. 3**) and found to be spherical. No discoidal forms or rouleaux were observed in any of the electron microscope fields examined. The majority of particles range in size from 120–160Å with a minor subgroup with diameters of approximately 50 Å. Human HDL, prepared at the same time, was found to be more uniform in size with diameters of about 130 Å. The size and structure of the particles were not influenced by the addition of albumin to the perfusion medium. The diameter of the particles was estimated from the lipid composition as described by Shen, Scanu, and Kezdy (39), assuming a neutral lipid core of cholesteryl ester and triglyceride surrounded by a

TABLE 4. Relative removal of VLDL components during heart perfusion

	Percentage of Control	P
Apo B (6)	67 ± 4	0.005
Apo C-II & Apo C-III (6)	98 ± 3	n.s.
Apo E (6)	96 ± 3	n.s.
Triacylglycerols (5)	48 ± 2	0.001
Cholesterol (5)	83 ± 6	0.05
Cholesteryl esters (5)	81 ± 8	0.05
Phospholipids	98 ± 2	n.s.

The sum of each lipoprotein moiety recovered in each column fraction after heart perfusion was calculated as a percent of that recovered in the control perfusion. The figures represent the mean ± SEM of the number of experiments indicated in parentheses.

monolayer of cholesterol and phospholipid. The radius of the neutral lipid core was determined by the ratio of the area of surface constituents and the volume of core constituents. A value of 20.5 Å was added to the radius to account for the monolayer of cholesterol, phospholipid, and protein. The diameter (140 Å) was in good agreement with the mean value (124 ± 36 Å) value observed by electron microscopy. It should be noted that the molar amounts of cholesterol in these particles are considerably higher than those observed or predicted by the studies of Shen et al. (39) for serum lipoproteins.

An examination of the changes in the quantities in apoproteins and lipids of VLDL following lipolysis (Tables 1–3) suggests that the perfused heart selectively removes apoB and possible free and esterified cholesterol. However, comparisons are obscured by the variations in lipid and apoprotein content among the different VLDL preparations used in the repetitive experiments. To minimize this effect, the quantity of each VLDL constituent following the heart perfusion has been expressed in **Table 4** as a percentage of that found after the control perfusion. There was a significant uptake of one-third of the apoB during perfusion, accompanied by a lesser and statistically less significant removal of free and esterified cholesterol. Recent studies (40) of chylomicron metabolism in perfused rat heart have also indicated an uptake of cholesteryl ester but no removal of apoB. The apoB retention observed in this study may be due to differences in particle size of chylomicron and VLDL remnants or a function of the amount or origin (41) of apoB in chylomicrons compared to VLDL. The retention of apoB is selective, since other apoproteins are not lost, and may be a function of VLDL remnant formation and filtration into the heart lymphatic system. The decrease in triacylglycerols is attributable to lipolysis and occurred with both perfusion and incubations with the mobilized lipoprotein lipase. There was however no loss of any other components in the latter (cf. Tables 2 and 3).

DISCUSSION

The perfused rat heart is an excellent model of immobilized lipoprotein lipase. It hydrolyzes the triacylglycerols of human VLDL, leading to a loss of apoC from the lipoprotein and an accumulation of LDL (6). The lipoprotein lipase has a high affinity for the substrate and is not affected by binding to the cardiac capillary endothelium (42). In the experiments cited here, about 50% of the VLDL-triacylglycerols was consistently hydrolyzed. This degree of hydrolysis was not significantly exceeded in any of the experiments although the hearts were changed every 30 min during the perfusion, while, in the incubation experiments, lipoprotein lipase mobilized from four hearts was used in each incubation flask. This is consistent with the observation that the rate of hydrolysis of VLDL-triacylglycerols diminishes as remnants are formed (6, 43).

The present data clearly indicate that apoC-II and apoC-III initially released from the VLDL during lipolysis are found in HDL as part of a spherical particle, but not in the low molecular weight fractions IV and V, corresponding to the $d > 1.21$ g/ml fraction of serum. While Eisenberg and his collaborators (12, 13) recovered apoC in the $d > 1.21$ g/ml fraction after lipolysis of VLDL, their results may be attributable to their technique of adsorbing purified ^{125}I-labeled apoC onto the intact VLDL, which is then released as the triacylglycerols are hydrolyzed. Furthermore, in their experiments the adsorbed ^{125}I-labeled apoC is found in the $d > 1.21$ g/ml fraction even when the VLDL upon which it adsorbed is recirculated in the absence of the heart (12).

Recently Eisenberg et al. (44) in a further study using iodinated apoC-II and apoC-III$_1$ which had been allowed to adsorb onto human VLDL in the presence of serum, showed that as a result of addition of purified lipoprotein lipase, radioactive C-II and C-III$_1$ were lost from the VLDL to the HDL density range upon ultracentrifugal isolation. The ratio of radioactive C-II to C-III$_1$ in the VLDL and HDL changed little during the course of lipolysis (0–48% of VLDL triglyceride). These results are difficult to interpret in terms of apoprotein mass transfer during lipolysis, as no data on specific radioactivity of the iodinated apoproteins were given and the specific radioactivity of the apoC-II and C-III$_1$ in the VLDL after adsorption was apparently not

measured. However if one assumes that the quantity of labeled apoC-II and C-III_1 binding to the VLDL was sufficiently small so as not to perturb the existing mass ratio of apoC-II to apoC-III_1 within the VLDL, and using the normalized value of one for the ratio of radioactive C-II to C-III_1 (44, Fig. 3), then, as human VLDL ($d < 1.006$ g/ml) contains more apoC-III_1 than apoC-II (45), the specific radioactivity of the VLDL apoC-II would be higher than that of the VLDL apoC-III_1. Thus the loss of equal amounts of radioactivity from the labeled VLDL during triglyceride lipolysis reported by Eisenberg et al. (44) would, in mass terms, reflect a greater loss of apoC-III_1 than of apoC-II. This preferential loss of apoC-III would be further amplified if the behavior of apoC-III_2 mimicked that of radioactive C-III_1. The radioactive data of Eisenberg et al. (44) could thus be construed as being consistent with the data contained in this report which demonstrates, in mass units, the preferential loss of apoC-III as compared to apoC-II from human VLDL as a result of exposure to lipoprotein lipase. The preferential loss of significant proportions of VLDL apoC-III during moderate lipolysis may explain the ability of the perfused liver to clear VLDL remnants that still contain a high proportion of triglycerides (46).

Following lipolysis of VLDL, apoE is also found in the HDL-sized fraction III. This is not surprising since VLDL is catabolized to LDL which contains very little apoE (47, 48). Suri, Tang, and Robinson (7) noted a transfer of labeled apoE from VLDL to HDL following incubation of the VLDL with post-heparin plasma or injection into supradiaphragmatic rats. While such a transfer might be due to the exchange of apoE between the lipoproteins (9, 49), the present data demonstrate the occurrence of an absolute transfer of apoE from VLDL to HDL sized particles during lipolysis. Unlike apoC-II and apoC-III, apoE is also found in smaller particles following lipolysis, some of which approach the delipidated apoprotein in size. The latter cannot be attributed to experimental artifacts since apoE remained bound to the VLDL after the control recirculation and isolation. Such virtually delipidated apoE is also secreted by perfused rat livers, especially when taken from normolipemic animals (32). It therefore seems likely that the distribution of the apoE among the various lipoproteins depends on the amount and type of lipid being carried by the VLDL.

Studies on the lipolysis of the triacylglycerol-rich lipoproteins have suggested that a fragment of the membrane coat is released that is relatively rich in cholesterol, phospholipids, and the soluble apoproteins, and appears discoidal in shape (8, 12, 13). The appearance of some phospholipid in fraction III is consistent with this observation. However, more phospholipid appears in fraction IV, where albumin is normally eluted. While some of this material may be lysophosphatides (not separated from phospholipids by the thin-layer chromatography system used here) carried by albumin, the perfusates, unlike the incubation experiments, contained no albumin. Thus it is probable that the phospholipids in fraction IV largely correspond to the unhydrolyzed phospholipids found in the $d > 1.21$ g/ml infranatant following heart perfusion by Chajek and Eisenberg (12). Although the appearance of the hydrophobic cholesteryl esters in the HDL-sized fraction III was unexpected, it is consistent with the observation that these lipoproteins are spherical.

Several laboratories have reported that a discoidal remnant of the surface coat of triglyceride-rich lipoproteins is formed on exposure to lipoprotein lipase (12, 50). However, preparations of HDL or LDL plus HDL in these studies also suggest the presence of spherical particles in the electron microscopic picture of these fractions. Although the C apoproteins have been shown to transfer to these fractions during lipolysis, there has been no demonstration that the apolipoproteins are associated with the discoidal particles. In the present study, gel filtration chromatography was used to isolate a fraction of HDL-sized spherical particles that contain a considerable portion of the apolipoproteins of the perfusion. The formation of these particles may be a function of several factors. The content of cholesteryl ester in human VLDL is higher compared to rat VLDL. The rate or extent of lipolysis of the VLDL may influence the formation of spherical particles. Finally, the conditions of isolation that used size as opposed to density for fractionation may be more selective than ultracentrifugation. The quantitative factors influencing the formation of spherical particles is under further study. Other serum lipoproteins were not present during these studies in order to facilitate the quantitation of the redistribution of apoproteins. Thus these studies do not eliminate the possibility that these primary particles undergo further transformation through interaction or equilibration with other lipoproteins as has been suggested in other studies (51).

Since at least a portion of HDL-sized particles contain both apoE and apoC-III, it seems likely that the fraction III lipoproteins are formed directly from the catabolism of the VLDL. The division of the HDL-sized lipoproteins into two populations having markedly different apoC-II and apoC-III:

apoE ratios indicates the existence of a spectrum of VLDL products. Furthermore, in view of the relatively wide range of diameters of these particles, it is possible that they might normally be further modified in the circulation.

The mechanism of the selective removal of apoB by the perfused heart is not clear. This uptake appears to be restricted to apoB and thus is not due to binding or trapping of the intact VLDL. Furthermore, the uptake of trichloroacetic acid-precipitable radioactivity from human ^{125}I-labeled VLDL in the perfused rat heart is prevented if the lipoprotein lipase is first removed by perfusion with heparin (6). Both human and rat ^{125}I-labeled LDL are taken up by perfused rat heart.[2] It is therefore tempting to suggest that VLDL is converted to LDL which is then removed by the heart. However, the physiological significance of the uptake of apoB, and free and esterified cholesterol must await correlation with intracellular effects, such as the control of cholesterol metabolism.

This work was supported by grants from the Nova Scotia Heart Foundation and the Medical Research Council of Canada. The technical assistance of Mr. Bruce Rubinstein and the generous cooperation of the Nova Scotia Red Cross Transfusion Service is gratefully acknowledged. Electron microscopy was carried out by Mr. T. Tidball, Dept. of Anatomy, Dalhousie University. The assistance of Drs. W. Carl Breckenridge and P. J. Dolphin in the revision of the text is gratefully acknowledged.

Manuscript received 28 January 1980, in revised form 4 August 1980, and in re-revised form 29 December 1980.

REFERENCES

1. Larosa, J. C., R. I. Levy, W. V. Brown, and D. S. Fredrickson. 1971. Changes in high density lipoprotein composition after heparin-induced lipolysis. *Am. J. Physiol.* **220:** 785–791.
2. Schonfeld, G., C. L. Gulbrandsen, R. B. Wilson, and R. S. Lees. 1972. Catabolism of human very-low-density lipoprotein in monkeys: the appearance of human very-low-density lipoprotein peptides in monkey high-density lipoproteins. *Biochim. Biophys. Acta.* **270:** 426–432.
3. Eisenberg, S., and D. Rachmilewitz. 1975. Interaction of rat plasma very low density lipoproteins with lipoprotein lipase-rich (post-heparin) plasma. *J. Lipid Res.* **16:** 341–351.
4. Mjos, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
5. Glangeaud, M. C., S. Eisenberg, and T. Olivecrona. 1977. Very low density lipoprotein. Dissociation of apolipoprotein C during lipoprotein lipase induced lipolysis. *Biochim. Biophys. Acta.* **486:** 23–25.
6. Dory, L., D. Pocock, and D. Rubinstein. 1978. The catabolism of human and rat very low density lipoproteins by perfused rat hearts. *Biochim. Biophys. Acta.* **528:** 161–175.
7. Suri, B. S., M. E. Tang, and D. S. Robinson. 1979. The metabolic conversion of very low density lipoprotein into low density lipoprotein by the extrahepatic tissues of the rat. *Biochem. J.* **178:** 455–463.
8. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
9. Rubenstein, B., and D. Rubinstein. 1972. Interrelationship between rat serum very low density and high density lipoproteins. *J. Lipid Res.* **13:** 317–324.
10. Kashyap, M. L., L. S. Srivastava, B. A. Hynd, G. Perisutti, D. W. Brady, P. Gartside, and C. J. Glueck. 1978. The role of high density lipoprotein apolipoprotein C-II in triglyceride metabolism. *Lipids.* **13:** 993–942.
11. Patsch, J. R., A. M. Gotto, T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein-like particles during lipolysis of very low density lipoproteins in vitro. *Proc. Natl. Acad. Sci. USA.* **75:** 4519–4523.
12. Chajek, T., and S. Eisenberg. 1978. Very low density lipoproteins. Metabolism of phospholipids cholesterol and apolipoprotein C in the isolated perfused rat heart. *J. Clin. Invest.* **61:** 1654–1665.
13. Eisenberg, S., and T. Olivecrona. 1979. Very low density lipoprotein. Fate of phospholipids, cholesterol, and apolipoprotein C during lipolysis in vitro. *J. Lipid Res.* **20:** 614–623.
14. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. *Biochem. Med.* **17:** 347–355.
15. Rubinstein, D., S. P. Tam, and L. Dory. 1979. Fate of apoC and apoE during lipolysis of human very low density lipoproteins in vitro. *Circulation.* **60:** II–72 (Abstract).
16. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
17. Fidge, N.H., and P. Poulis. 1974. Studies on the radioiodination of very low density lipoproteins obtained from different mammalian species. *Clin. Chim. Acta.* **52:** 15–26.
18. MacFarlane, A. S. 1958. Efficient trace-labelling of protein with iodine. *Nature (London).* **182:** 53.
19. March, S. C., I. Parikh, and P. Cuatrecasas. 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. *Anal. Biochem.* **60:** 149–152.
20. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular

[2] Dory, L. Unpublished results.

weight peptides of the serum VLDL and HDL. *Anal. Biochem.* **44:** 576–588.
21. Wong, L., and D. Rubinstein. 1978. The levels of apolipoprotein-E in hypercholesterolemic rat serum. *Can. J. Biochem.* **56:** 161–166.
22. Radola, B. J. 1974. Isoelectric focusing in layers of granulated gels. *Biochim. Biophys. Acta.* **386:** 181–195.
23. Marcel, Y. L., M. Bergseth, and A. C. Nestruck. 1979. Preparative isoelectric focusing of apolipoproteins C and E from human very low density lipoproteins. *Biochim. Biophys. Acta.* **573:** 175–183.
24. Harboe, N., and A. Ingild. 1973. Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* **2:** 161–164.
25. Dolphin, P. J., L. Wong, and D. Rubinstein. 1978. A comparison of some immunological characteristics of VLDL of normal and hypercholesterolemic rat sera. *Can. J. Biochem.* **56:** 673–683.
26. Laurell, C. B. 1965. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.
27. Crowle, A. J., and L. J. Cline. 1977. An improved stain for immunodiffusion tests. *J. Immunol. Methods.* **17:** 379–381.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
29. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244:** 5687–5694.
30. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1970. Further characterization of apolipoproteins from the human plasma very low density lipoproteins. *J. Biol. Chem.* **245:** 6588–6594.
31. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
32. Noel, S-P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubinstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
33. Blanchette-Mackie, E. J., and R. O. Scow. 1973. Effects of lipoprotein lipase on the structure of chylomicrons. *J. Cell. Biol.* **58:** 689–708.
34. Utermann, G. 1975. Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very low density lipoproteins: apolipoprotein E. *Hoppe-Seyler's Z. Physiol. Chem.* **356:** 1113–1121.
35. Dolphin, P. J., and D. Rubinstein. 1974. The metabolism of very low density lipoprotein and chylomicrons by purified lipoprotein lipase from rat heart and adipose tissue. *Biochem. Biophys. Res. Commun.* **57:** 808–814.
36. Shelburne, F. A., and S. H. Quarfordt. 1977. Interaction of heparin with an apoprotein of human very low density lipoprotein. *J. Clin. Invest.* **60:** 944–950.
37. Quarfordt, S.H., R. S. Jan, L. Jakoi, S. Robinson, and F. Shelburne. 1978. The heterogeneity of rat high density lipoproteins. *Biochem. Biophys. Res. Commun.* **83:** 786–793.
38. Holmquist, L., and K. Carlson. 1977. Selective extraction of human serum very low density apolipoproteins with organic solvents. *Biochim. Biophys. Acta.* **493:** 400–409.
39. Shen, R. W., A. M. Scanu, and F. J. Kezdy. 1977. Structure of human serum lipoproteins inferred from compositional analyses. *Proc. Natl. Acad. Sci. USA.* **74:** 837–841.
40. Fielding, C. J. 1978. Metabolism of cholesterol-rich chylomicrons–mechanism of binding and uptake of cholesterol esters by vascular bed of perfused rat heart. *J. Clin. Invest.* **62:** 141–151.
41. Krishna, K. V., L. F. Walker, J. Borensztein, and G. Schonfeld. 1980. Apolipoprotein B variant derived from rat intestine. *Proc. Natl. Acad. Sci. USA.* **77:** 3806–3810.
42. Fielding, C. J. 1976. Lipoprotein lipase: evidence for high and low affinity enzyme sites. *Biochemistry.* **15:** 879–884.
43. Higgins, J. M., and C. J. Fielding. 1975. Lipoprotein lipase: mechanism for formation of triglyceride-rich remnant particles from very low density lipoproteins and chylomicrons. *Biochemistry.* **14:** 2288–2292.
44. Eisenberg, S., J. R. Patsh, J. T. Sparrow, A. M. Gotto, and T. Olivecrona. 1979. Removal of apolipoproteins C-II and C-III-1 during lipolysis in vitro. *J. Biol. Chem.* **254:** 12603–12608.
45. Kane, J. P., S. Teizo, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
46. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652–658.
47. Rubenstein, B., and G. Steiner. 1975. A comparison of the peptide composition of human serum low and very low density lipoprotein. *Can. J. Biochem.* **53:** 128–134.
48. Lee, D., and P. Alaupovic. 1974. Composition and concentration of apolipoproteins in very low and low density lipoproteins of normal human plasma. *Atherosclerosis.* **19:** 501–520.
49. Weisgraber, K. H., R. W. Mahley, and G. Assmann. 1977. The rat arginine-rich apoprotein and its redistribution following injection of iodinated lipoproteins into normal and hypercholesterolemic rats. *Atherosclerosis.* **28:** 121–140.
50. Deckelbaum, R. J., S. Eisenberg, M. Fainaru, Y. Barenholz, and T. Olivecrona. 1979. In vitro production of human plasma low density lipoprotein-like particles. A model for very low density lipoprotein catabolism. *J. Biol. Chem.* **254:** 6079–6087.
51. Patsch, J. R., A. M. Gotto, Jr., T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein 2-like particles during lipolysis of very low density lipoproteins in vitro. *Proc. Natl. Acad. Sci. USA* **75:** 4519–4523.