

Very Low Density Lipoprotein: *METABOLISM OF PHOSPHOLIPIDS, CHOLESTEROL, AND APOLIPOPROTEIN C IN THE ISOLATED PERFUSED RAT HEART*

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VLDL triglycerides were progressively hydrolyzed to fatty acids (10-120-min perfusions). Concomitantly, phospholipids, cholesterol (predominantly unesterified), and apoC were removed from the VLDL to all other fractions. About 30-35% of the phosphatidylcholine was hydrolyzed to lysophosphatidylcholine and was recovered at *d* > 1.21 g/ml. The phosphatidylcholine- and triglyceride-hydrolyzing activities were confined to membrane supported enzyme(s). The other 60-65% of the phosphatidylcholine was removed unhydrolyzed and was found in fractions of *d* 1.019-1.04 (10-15%), *d* 1.04-1.21 (25-30%), and *d* > 1.21 g/ml (15-20%). [³²P]Sphingomyelin accumulated at the fraction of *d* 1.04-1.21 g/ml. Unesterified cholesterol was found in the fraction of *d* 1.04-1.21 g/ml. ApoC was recovered predominantly in fractions of *d* 1.04-1.21 (50-60%) and *d* > 1.21 g/ml (30-40%). Cholesteryl esters were associated with VLDL during the hydrolysis of 50-70% of the triglycerides, but with advanced lipolysis, appeared in higher densities, mainly *d* 1.019-1.04 g/ml.

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ABSTRACT The fate of rat plasma very low density lipoprotein (VLDL) constituents was determined in the isolated perfused rat heart. VLDL was labeled with [¹⁴C]palmitate, ³²P-phospholipids, [³H] cholesterol, and ¹²⁵I-apolipoprotein C (apoC). Perfusions were performed with an albumin-containing buffer and without plasma. Radioactivity was followed in fractions of $d < 1.019$, $d 1.019-1.04$, $d 1.04-1.21$, and $d > 1.21$ g/ml, prepared by ultracentrifugation.

VLDL triglycerides were progressively hydrolyzed to fatty acids (10-120-min perfusions). Concomitantly, phospholipids, cholesterol (predominantly unesterified), and apoC were removed from the VLDL to all other fractions. About 30-35% of the phosphatidylcholine was hydrolyzed to lysophosphatidylcholine and was recovered at $d > 1.21$ g/ml. The phosphatidylcholine- and triglyceride-hydrolyzing activities were confined to membrane supported enzyme(s). The other 60-65% of the phosphatidylcholine was removed unhydrolyzed and was found in fractions of $d 1.019-1.04$ (10-15%), $d 1.04-1.21$ (25-30%), and $d > 1.21$ g/ml (15-20%). [³²P]Sphingomyelin accumulated at the fraction of $d 1.04-1.21$ g/ml. Unesterified cholesterol was found in the fraction of $d 1.04-1.21$ g/ml. ApoC was recovered predominantly in fractions of $d 1.04-1.21$ (50-60%) and $d > 1.21$ g/ml (30-40%). Cholesteryl esters were associated with VLDL during the hydrolysis of 50-70% of the triglycerides, but with advanced lipolysis, appeared in higher densities, mainly $d 1.019-1.04$ g/ml.

The fraction of $d 1.04-1.21$ g/ml, (containing phosphatidylcholine, sphingomyelin, unesterified cholesterol,

and apoC) contained by negative staining, many disk-like structures.

The study demonstrated that removal of surface constituents (phospholipids, unesterified cholesterol, and apoC) during lipolysis of VLDL is an intrinsic feature of the lipolytic process, and is independent of the presence of plasma. It also indicated that surface constituents may be removed in a particulated form.

INTRODUCTION

Triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins [VLDL]¹) are metabolized by lipoprotein lipases, enzymes situated at endothelial surfaces of capillaries, in muscles, adipose tissue, lung, and other tissues (1-6). During catabolism of the lipoproteins, a triglyceride-depleted ("remnant") particle is formed (7). In experiments carried out either in vivo (8-12) or in vitro (13), it has been shown that phospholipids, unesterified cholesterol, and apolipoprotein C (apoC) are removed from the lipoproteins concomitantly with triglyceride hydrolysis and can be found associated with other plasma protein and lipoprotein fractions, predominantly high density lipoprotein (HDL). Therefore, it has been suggested that HDL participates in the catabolism of chylomicrons and VLDL and is an acceptor of the phospholipids, cholesterol, and apoC generated during lipolysis. However, when lipolysis of VLDL is carried out in plasma-free incubation mixtures (using a purified lipoprotein lipase), apoC is removed from VLDL and is found in fractions of $d 1.04-1.21$ g/ml and $d > 1.21$ g/ml, even in the absence of HDL (14). In the plasma-devoid system, therefore, apoC removal seems to be dependent upon the features of the postlipolysis VLDL particles rather than the presence of HDL.

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¹Abbreviations used in this paper: ApoC, apolipoprotein C; FFA, free fatty acid; HDL, high density lipoprotein; VLDL, very low density lipoprotein; VLDL-TG, very low density lipoprotein-triglyceride.

Whether this observation is unique for unassociated lipoprotein lipases, or can be observed with a membrane-supported enzyme is yet unknown. Also unknown is the fate in the plasma-free systems of the other VLDL surface constituents, the phospholipids, and unesterified cholesterol. In an effort to study these questions, we have determined the fate of the surface constituents of VLDL (phospholipids, cholesterol, and apoC) during triglyceride hydrolysis by membrane-supported lipoprotein lipase, using labeled VLDL and the perfused rat heart. The results of this study are the subject of the present report.

METHODS

Preparation of lipoproteins and labeled lipoproteins. Rat serum was obtained through the abdominal aorta of ether-anesthetized male rats (body wt = 250–300 g), of the Hebrew University strain, whose diet was supplemented with 10% sucrose drinking water for 48 h before exsanguination. The blood was allowed to clot at 4°C, and serum was separated in the SS-2 centrifuge (Ivan Sorvall, Inc. Norwalk, Conn.) at 5,000 rpm for 20 min. VLDL was isolated at plasma density of 1.006 g/ml in the L₂-65B or L₂-50 ultracentrifuge (Beckman Instruments, Inc. Fullerton, Calif.) using the 50 Ti rotor at 50,000 rpm for 18 h by the tube-slicing technique (15). The VLDL was washed and concentrated by two additional spins at density of 1.006 g/ml. The VLDL obtained was shown to be free of contamination with plasma proteins and other lipoproteins by immunochemical and electrophoretic techniques and polyacrylamide gel electrophoresis of apoproteins as described previously (16, 17). The composition of VLDL by weight percent was: triglycerides, 73.3±1.4; phospholipids, 11.2±0.4; cholesterol, 5.3±0.4; and protein, 10.2±1.3 (values are mean±SD of 15 preparations).

VLDL labeled in the triglyceride moiety was prepared similarly from plasma obtained from rats injected 45 min before exsanguination with 15–25 µCi of [¹⁴C] sodium palmitate prepared in 1% albumin solution (Radiochemical Centre, Amersham, England). VLDL labeled in the phospholipid moiety was prepared from rats injected 16 h before exsanguination with 2–3 mCi of H₃³²PO₄ carrier-free (Nuclear Research Center, Beer-Sheva, Israel) (18). VLDL labeled in the cholesterol moiety was prepared following the procedure of Mjøs et al. (12) from plasma of rats injected with 0.2–0.3 mCi of [1,2-³H] cholesterol (Radiochemical Centre) 6 h before exsanguination. The distribution of radioactivity among labeled lipids in each of the labeled VLDL preparations is shown in Table I. To pre-

pare VLDL labeled with ¹²⁵I-apoC, rat serum VLDL was isolated at density of 1.006 g/ml and ¹²⁵I-apoC (0.1–0.2 mg protein, 1–2 × 10⁶ cpm), was added to 10 mg VLDL protein and was allowed to exchange with unlabeled apoC for 30 min, as described previously (14). The VLDL labeled with ¹²⁵I-apoC was isolated by ultracentrifugation at 50,000 rpm for 8 h in the 50 Ti rotor, followed by an additional spin for 16 h. ¹²⁵I-Na, carrier-free, was obtained from the Radiochemical Centre.

To determine the distribution of labeled lipids and apoproteins among lipoprotein fractions, ultracentrifugation was carried out as follows: a concentrated NaCl solution of density 1.116 g/ml was added to 5 ml of ice-cold perfusate in a quantity sufficient to raise the density to 1.019 g/ml (0.47 M of NaCl), and the sample was centrifuged in the 40.3 rotor at 40,000 rpm for 18 h. Lipoproteins of *d* < 1.019 g/ml were separated by the tube-slicing technique. The fraction of *d* > 1.019 g/ml was then adjusted to density of 1.04 g/ml using solid KBr and was subjected to ultracentrifugation in the 40.3 rotor at 40,000 rpm for 24 h. The fraction *d* 1.019–1.04 g/ml was separated by the tube-slicing technique, the infranate was adjusted with solid KBr to a density of 1.21 g/ml, and fractions of density < and > 1.21 g/ml were separated likewise in the 40.3 rotor at 40,000 rpm for 48 h. All procedures were carried out at 4°C.

Perfusion procedure. Isolated rat hearts were dissected from ether-anesthetized female rats weighing 100–200 g which were deprived of food 16 h before the experiment. Rat heart perfusion with a recirculation perfusion apparatus was carried out as previously described (19, 20). The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4) supplemented with 4 g/dl (wt:vol) bovine serum albumin (Pentex Fraction V powder, fatty acid poor, Miles Laboratory, Kanakakee, Ill.) and 200 mg/dl glucose. Perfusions were carried out at 37°C, at a pressure of 100 cm H₂O and with constant gassing with 95% O₂-5% CO₂. The heart rate was maintained within a range of 160–220/min throughout the perfusion period, and the flow rate was 6–8 ml/min.

The reservoir of the perfusion apparatus contained 40 ml of perfusion fluid. Labeled VLDL was added to the perfusion medium at a final concentration of 184 µM of triglyceride, 32 µM of phospholipids, and 28 µM of cholesterol.

Experimental procedures. Perfusion time varied between 20 and 120 min. During perfusion, several 1- and 5-ml aliquots were drawn for chemical and radiochemical analysis. The 1-ml aliquots were used for the determination of total radioactivity and distribution of radioactivity among lipid classes in the perfusate. 5-ml samples obtained either during or at the end of the perfusion were used to separate fractions of *d* < 1.019, *d* 1.019–1.04, *d* 1.04–1.21, and *d* > 1.21 g/ml as described above. The distribution of radioactive lipids among density fractions and between lipid classes in each fraction was determined on aliquots of the chloroform phase obtained after lipid ex-

TABLE I
Radioactive Lipids of [¹⁴C]Palmitate-, ³²P-Phospholipids-, and [³H]Cholesterol-Labeled VLDL

Label	Radioactive lipids*				
	%				
[¹⁴ C]Palmitate	PL† = 3.7±1.4	MG + DG = 3.0±1.6	FA = 1.7±0.8	TG = 87.8±3.2	CE = 2.4±1.0
³² P-Phospholipids	LPC = 4.9±0.9	SP = 6.6±0.7	PC = 83.8±1.7	PE = 3.7±0.5	
[³ H]Cholesterol	UC = 65.3±6.1	CE = 25.6±5.4			

* Values are means±SD of eight preparations.

† Abbreviations used in these tables: PL, phospholipids; MG, monoglycerides; DG, diglycerides; FA, fatty acids; TG, triglycerides; CE, cholesteryl esters; LPC, lysophosphatidylcholine; SP, sphingomyelin; PC, phosphatidylcholine; PE, combined fractions of phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol; UC, unesterified cholesterol.

traction. When ^{125}I -apoC-labeled VLDL was used, the ^{125}I radioactivity content in the perfusate and in each density fraction was determined before lipid extraction, and the resulting radioactivity counts were corrected for energy absorption by high density solutions (21). In these experiments, an additional 1 ml of perfusion medium was taken at time intervals to insure that free iodide was not generated during the perfusion. During and after perfusion, 1-ml samples were mixed with 5 ml isopropanol:heptane: H_2SO_4 , 40:10:1, (vol/vol/vol) for fatty acid determination.

At the end of the perfusion, the heart was washed with 50 ml of medium, blotted, and homogenized in 15 ml of chloroform:methanol 2:1 (vol/vol). The lower organic phase was analyzed for lipid radioactivity content and lipid composition. When ^{125}I -apoC-labeled VLDL was used, ^{125}I radioactivity in the heart was determined before homogenization.

The percent of lipolysis of very low density lipoprotein-triglyceride (VLDL-TG) was calculated by the formula: $\frac{(\text{FFA})_{\text{final}} - (\text{FFA})_{\text{initial}}}{(\text{triglyceride})_{\text{initial}}} \times 100$, where (FFA) final and (FFA) initial represent the free fatty acid concentration of the perfusate at the end and before the perfusion, and (triglyceride) initial represents the triglyceride fatty acids concentration before the perfusion.

Analytical procedure. Lipoprotein protein was determined by the method of Lowry et al. (22). Lipids were extracted in 20 vol of chloroform:methanol 2:1, (vol/vol), and washed according to Folch et al. (23). Phospholipid phosphorus and cholesterol were determined following the procedures of Bartlett (24) and Chiamori and Henri (25), respectively. Lipoprotein triglyceride was determined by the AutoAnalyzer technique (Technicon Instruments Co., Tarrytown, N. Y.). FFA were determined by the $^{63}\text{NiCl}_2$ method as described by Ho (26). Lipids were separated by thin-layer chromatography using silica gel (Merck-Darmstadt, Germany) layered on glass plates and developed in either one of two solvent systems: petroleum ether (30–60°C):diethyl ether:acetic acid 160:40:2 (vol/vol/vol) for neutral lipids, and chloroform:methanol: H_2O 140:50:8 (vol/vol/vol) for phospholipids. Lipids were visualized by iodine vapors and identified with the help of reference standards. The lipids were scraped off the plate and assessed for radioactivity content. Radioactivity was determined by liquid scintillation counting in a Tri-carb liquid scintillation spectrometer 3380 (Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with an absolute activity analyzer model 544.

Radioactivity associated with ^{125}I -apoC was determined using the Autogamma scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

RESULTS

Hydrolysis of VLDL-TG. Hydrolysis of VLDL-TG by the rat heart lipoprotein lipase was evident during perfusions with medium containing albumin and in the absence of serum (Fig. 1). Labeled fatty acids were the predominant product of the hydrolysis of triglycerides; no accumulation of mono- or diglycerides was observed at any time during the 120-min perfusion. The percent hydrolysis of VLDL-TG calculated from the radioactivity data was 19.0 ± 2.3 after a 10-min perfusion, and 27.5 ± 3.3 , 42.5 ± 3.3 , 53.3 ± 2.9 , and 73.0 ± 6.4 after 20, 40, 60, and 120 min of perfusion, respectively (mean \pm SD of 7–25 experiments). When fatty acid generation was determined chemically, similar degrees of hydrolysis were recorded with correlation coefficient between the

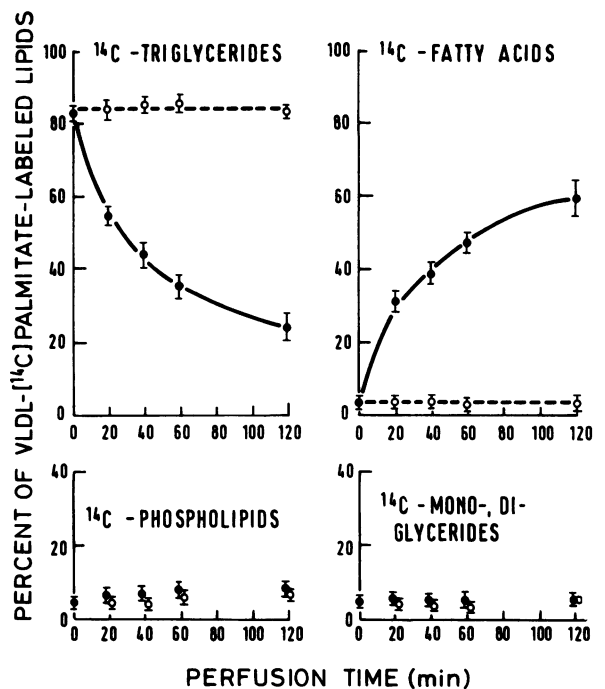


FIGURE 1 ^{14}C Palmitate-labeled lipids in the perfusion medium during isolated rat heart perfusion (●) and recirculation without heart (○) of ^{14}C palmitate-labeled VLDL. Each point is the mean \pm SD of 7–25 isolated rat heart perfusions at a VLDL-TG concentration of $184 \mu\text{M}$.

chemical and radiochemical methods of 0.966 ± 0.06 (mean \pm SD of 38 determinations). Fatty acid generation was 97 ± 13.6 nmol/ml after a 10-min perfusion, and 142 ± 16.6 , 204 ± 33.3 , 288 ± 32.3 , and 414 ± 34 nmol/ml after 20, 40, 60, and 120 min, respectively (values are mean \pm SD of 10–15 experiments). No generation of labeled or unlabeled fatty acids was detected when perfusion medium containing VLDL was allowed to recirculate in a system without a heart for as long as 120 min. Ultracentrifugation of the perfusate revealed that concomitantly with the hydrolysis of VLDL-TG, ^{14}C palmitate-labeled lipids were removed from the fraction of $d < 1.019$ g/ml (containing VLDL and intermediate density lipoprotein) and accumulated predominantly at the fraction of $d > 1.21$ g/ml. An example recorded after a 60-min perfusion is shown in Table II, when 53.3% of the labeled triglycerides was hydrolyzed. At this time point, 42.3% of the total radioactivity present initially in the fraction of $d < 1.019$ g/ml was removed to higher density fractions. This value is in excellent agreement with that of 45.1% calculated from the hydrolysis of VLDL-TG (53.5% hydrolysis of the 85.2% of the VLDL radioactivity associated initially with ^{14}C -labeled triglycerides, Table II). ^{14}C -Labeled triglycerides were the predominant labeled lipid present in the fractions of $d < 1.019$, d 1.019–1.04, and d

TABLE II
Distribution and Composition of [¹⁴C]Palmitate-Labeled Lipids of Lipoprotein Fractions
after 60 min of Rat Heart Perfusion with Labeled VLDL

Density g/ml	¹⁴ C-Labeled lipids* %	¹⁴ C-Labeled lipids in lipoprotein fractions*				
		PL†	MG + DG	FA	TG	EC
Perfusion without heart <1.019 (17)§	96.2±1.0	5.0±0.5	4.2±0.6	1.8±0.3	85.2±1.5	2.5±0.2
Perfusion with heart <1.019 (10)	57.7±2.6	5.1±0.9	6.6±1.2	3.1±0.7	81.6±1.8	2.8±0.5
1.019–1.04 (10)	2.1±0.3	4.8±0.6	5.1±0.7	2.9±0.4	83.3±0.8	5.0±0.8
1.04–1.21 (10)	2.0±0.6	3.6±1.6	7.0±0.6	3.8±1.9	84.0±4.5	2.3±1.0
>1.21 (10)	38.2±3.8	3.5±2.0	4.2±2.0	86.1±7.0	4.5±1.2	0.6±0.5

* Values are mean±SD.

† See Table I for abbreviations.

§ Numbers in parentheses represent the number of experiments.

1.04–1.21 g/ml whereas ¹⁴C-labeled fatty acids were the major labeled lipid of the *d* > 1.21 g/ml fraction.

The fate of phospholipids. To study the fate of phospholipids during lipolysis, VLDL labeled with ³²P-phospholipids and [¹⁴C]palmitate triglycerides was perfused through the rat heart capillaries. Initially, ≈84% of the ³²P-labeled phospholipids in VLDL were in [³²P]phosphatidylcholine and 5% in [³²P]lysophosphatidylcholine (Table I). During perfusion of the rat heart at phospholipid concentrations of either 3.2 or 32 μM, the percent contribution of [³²P]lysophosphatidylcholine to the total radioactivity increased to 25.1±1.3 and 22.0±1.3, respectively, and that of [³²P]phosphatidylcholine decreased to 65.3±1.3 and 64.2±2.7, respectively (Fig. 2). No change was observed in the percent contribution of [³²P]sphingomyelin to the total radioactivity. [³²P]Lysophosphatidylcholine did not accumulate when the ³²P-labeled VLDL was recirculated in a perfusion system without rat heart (Fig. 2, open circles).

To study the properties of the TG-and phosphatidylcholine-hydrolyzing enzymes in the rat heart, a postheparin perfusate obtained during the first 3 min of perfusion of the heart with medium containing 5 U/ml sodium heparin (Pullarin, Evans Medical Corp., Liverpool, England) was collected. Doubly-labeled VLDL was added to 1-ml aliquots of the postheparin perfusate at a final concentration of 32-μM phospholipids and 184-μM triglycerides. After a 30-min incubation, ≈80% of the ¹⁴C-triglycerides were hydrolyzed to ¹⁴C-fatty acids, and ≈20% of the [³²P]phosphatidylcholine was hydrolyzed to [³²P]lysophosphatidylcholine (Table III). Preincubation of the postheparin perfusate with either 1 M NaCl or protamine sulfate (3 mg/ml), resulted in an inhibition of both hydrolytic activities by >80%. Preincubation of the postheparin perfusate with 2 mM of parachloromercuriphenyl sulfonic acid, a known inhibitor of lecithin:cholesterol acyltransferase (27), did

not affect the hydrolysis of either ¹⁴C-triglycerides or [³²P]phosphatidylcholine (Table III).

To ascertain that the hydrolysis of the VLDL, ¹⁴C-triglycerides and [³²P]phosphatidylcholine was confined to the membrane-supported enzyme, samples of rat heart perfusate containing doubly-labeled VLDL (32-μM phospholipid and 184-μM triglycerides) were taken 10 min after initiation of the perfusion and were incubated for an additional 30 min at 37°C in a shaking incubator. As shown in Table IV, no significant increase in the percent hydrolysis of VLDL ¹⁴C-triglyceride or percent generation of [³²P]lysophosphatidylcholine occurred during a 30-min incubation. In contrast, continuing hydrolysis of both ¹⁴C-triglycerides and [³²P]phosphatidylcholine was observed when perfusion of the hearts

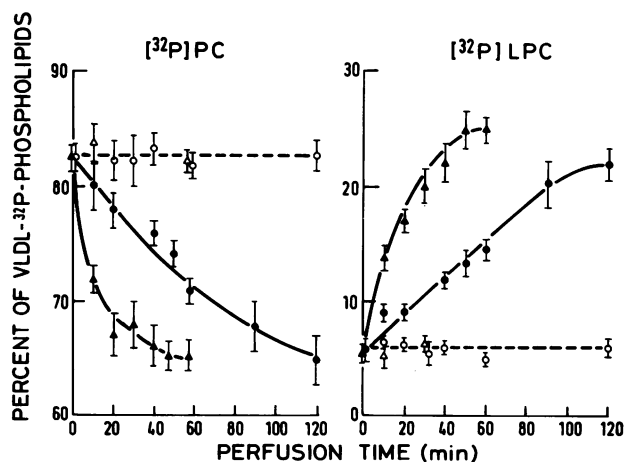


FIGURE 2 ³²P-Phospholipids in the perfusion medium during isolated rat heart perfusion (●,▲) and recirculation without heart (○,△) of ³²P-labeled VLDL. Each point is the mean ±SD of 4–10 isolated rat heart perfusions at VLDL-phospholipid concentration of either 3.1 μM (▲,△) or 31 μM (●,○).

TABLE III
Effects of 1 M NaCl, Protamine Sulfate, and PCMPS* on the Hydrolytic Activity of Rat Heart Postheparin Perfusate Towards ¹⁴C-Labeled Triglycerides and ³²P-Labeled Phosphatidylcholine in VLDL

Treatment	¹⁴ C-Palmitate-labeled lipids†					³² P-Labeled lipids‡		
	PL	MG + DG	FA	TG	EC	LPC	SP	PC
	%					%		
KR Alb§	4.0±0.4	4.6±0.4	2.7±1.0	85.0±1.2	2.5±1.0	4.4±0.3	6.3±0.2	84.8±1.4
PHP	5.9±0.6	3.2±0.3	77.8±1.3	11.5±0.6	1.4±0.3	17.6±0.9	8.9±0.7	70.6±1.4
PHP + 1 M NaCl	3.4±0.5	4.4±0.3	8.7±0.6	79.4±1.0	2.3±0.6	3.3±0.6	6.4±1.4	84.6±1.4
PHP + protamine sulfate	4.1±0.5	4.7±0.7	22.9±2.2	64.8±2.5	2.4±0.5	6.6±0.8	6.5±0.3	81.1±1.0
PHP + PCMPS	5.7±0.5	3.8±1.0	76.1±1.0	8.9±2.0	2.2±0.2	19.0±1.3	8.0±0.5	68.6±1.6

* Abbreviations: PCMPS, parachloromercuriphenyl sulfonic acid; PHP, postheparin perfusate; See Table I for other abbreviations.

† Values are means±SD of five experiments.

§ Krebs-Henseleit bicarbonate buffer containing 4 g/dl albumin (wt/vol) and doubly-labeled VLDL.

with the same doubly-labeled VLDL continued for additional 30 min (Table IV). All samples were subjected to ultracentrifugation at a density of 1.019 g/ml. About 90% of the ³²P-phospholipids were associated with the fraction of *d* < 1.019 g/ml at the end of a 10-min perfusion and 84% after the additional 30-min in vitro incubation. At the end of a 40-min perfusion, only 62.3% of the total ³²P-phospholipids were recovered in this fraction. Although the experiment provided evidence that in our system the heart lipoprotein lipase is bound to the capillary's membrane and is not released into the perfusate, they also indicated that the removal of ³²P-labeled phospholipids during hydrolysis of VLDL is a complex process and cannot be explained solely by generation of [³²P]lysophosphatidylcholine. Therefore, in all subsequent experiments, the amount of ³²P-phos-

pholipids present in ultracentrifugally separated density fractions was determined.

Rat heart perfusions were carried out with doubly-labeled VLDL, and samples obtained after 20-, 40-, 60-, and 120-min periods were separated by sequential ultracentrifugation to fractions of *d* < 1.019, *d* 1.019–1.04, *d* 1.04–1.21, and *d* > 1.21 g/ml. Each fraction was analyzed for total radioactivity and distribution of radioactivity among phospholipid classes. The results obtained from 36 experiments are presented in Table V. Recirculation of doubly-labeled VLDL in the perfusion system without heart did not change the distribution of ³²P-phospholipids among density fractions (Table V). During rat heart perfusion, however, the ³²P-phospholipid content of the fraction of *d* < 1.019 g/ml decreased and that associated with the fractions *d* 1.019–1.04, *d* 1.04–1.21, and *d* > 1.21 g/ml increased (Table V). The composition of ³²P-labeled phospholipid was determined in each lipoprotein fraction. Data representing the 60-min perfusion period is shown in Table VI. More than 80% of the radioactivity in the fractions of *d* < 1.019 and *d* 1.019–1.04 g/ml was in [³²P]phosphatidylcholine. The fraction of *d* 1.04–1.21 also contained predominantly [³²P]phosphatidylcholine (≈70% of the radioactivity), but unexpectedly was found to be the richest in [³²P]sphingomyelin. The composition of ³²P-labeled phospholipids in the fraction of *d* > 1.21 g/ml revealed ≈50% [³²P]lysophosphatidylcholine and 40% [³²P]phosphatidylcholine.

Based on the data described above, it was possible to determine the fate of [³²P]phosphatidylcholine in VLDL as a function of hydrolysis of the VLDL-TG in 46 experiments (Fig. 3). It is evident that concomitantly with triglyceride hydrolysis, [³²P]phosphatidylcholine is removed from VLDL by more than one mechanism. 30–35% of the [³²P]phosphatidylcholine removed from VLDL was hydrolyzed to [³²P]lysophosphatidylcholine

TABLE IV
Comparison between Hydrolysis of VLDL-TG,* Generation of LPC, and Removal of Labeled PC from VLDL during Rat Heart Perfusion with Doubly-Labeled VLDL

Perfusion time	Hydrolysis of VLDL-TG†§	[³² P]LPC of total ³² P-PL§	³² P-Labeled PC <i>d</i> < 1.019 g/ml§
min	%	%	%
0		5.9±0.2	100
10	16.1±2.4	8.7±0.8	90.1±1.0
10 + 30	17.2±3.5	9.3±1.4	84.0±1.3
40	40.4±2.8	12.6±1.2	62.3±1.9

* See Table I for abbreviations.

† Calculated as percent of fatty acids generated out of original VLDL triglycerides (Methods).

§ Values are means±SD of 12 experiments.

^{||} Samples of rat heart perfusate were taken after a 10-min perfusion and incubated for an additional 30 min at 37°C (see text).

TABLE V
Distribution of ³²P-Labeled Phospholipids among Lipoprotein Fractions during Rat Heart Perfusion with Doubly-Labeled VLDL

Perfu- sion time	Hydrolysis of VLDL-TG	[³² P]Phospholipid as percent of total [³² P]*							
		Perfusion with heart				Perfusion without heart			
		<i>d</i> < 1.019	1.019–1.04	1.04–1.21	>1.21	<1.019	1.019–1.04	1.04–1.21	>1.21
min	%								
0	—(35)†					88.1±1.1	2.3±0.3	1.8±0.3	7.8±0.9
20	31.2±2.4 (9)	69.0±3.8	4.9±1.5	10.2±2.6	15.9±3.4	83.0±2.3	3.4±0.6	3.2±0.6	10.4±2.1
40	42.1±3.5 (9)	62.9±3.3	6.0±0.4	11.0±2.9	20.1±4.9	80.9±4.0	2.8±0.8	3.0±1.5	13.3±3.0
60	57.2±2.9 (14)	55.0±2.9	7.0±1.0	10.9±0.7	27.1±5.3	85.4±1.3	1.5±0.2	1.8±0.5	11.3±1.3
120	84.0±3.7 (4)	33.5±12.7	10.0±2.8	18.4±3.9	38.1±6.8	82.5±3.7	2.3±0.3	5.3±2.0	9.9±2.1

* Values are mean±SD.

† Numbers in parentheses represent number of experiments.

and was found with the fraction of *d* > 1.21 g/ml. The remaining 65–70% appeared as [³²P]phosphatidylcholine in all lipoprotein fractions, 20–25% in *d* > 1.21 g/ml, 25–30% in *d* 1.04–1.21 g/ml, and 10–15% in *d* 1.019–1.04 g/ml.

The fate of cholesterol. In these experiments, VLDL labeled with [³H]cholesterol and [¹⁴C]palmitate was used. When the VLDL labeled with [³H]cholesterol was mixed with the albumin-containing perfusion medium and was separated to density fractions by ultracentrifugation, 80.7% of the radioactive cholesterol was recovered with the fraction of *d* < 1.019 g/ml, whereas 13% of the [³H] was associated with the fraction of *d* 1.04–1.21 g/ml (Table VII).²

During recirculation of [³H]cholesterol-labeled VLDL in a perfusion system without rat heart, additional radioactivity was removed from the fraction of *d* < 1.019 g/ml to the fractions of *d* 1.019–1.04 and *d* 1.04–1.21 g/ml (Table VII). The removal of [³H]cholesterol from the fraction of *d* < 1.019 g/ml was, however, significantly higher when doubly-labeled VLDL was perfused through the rat heart capillaries and VLDL triglycerides were hydrolyzed (Table VII). It appeared predominantly in the fraction of *d* 1.04–1.21 g/ml. [³H]Cholesterol accumulated also in the fraction *d* 1.019–1.04 g/ml especially with advanced lipolysis. The distribution of radioactivity between unesterified [³H] cholesterol and

³H-cholesteryl esters in the perfusion medium either during recirculation or rat heart perfusion remained constant and was about two-thirds in ³H-unesterified cholesterol and one-fourth ³H-cholesteryl esters. It however, differed markedly among ultracentrifugally separated density fractions. An example is shown in Table VIII for samples obtained after 60 min of rat heart perfusion. Density fractions of *d* < 1.019 and *d* 1.019–1.04 g/ml were enriched with ³H-cholesteryl esters whereas unesterified [³H]cholesterol was the predominant labeled constituent in the fraction of *d* 1.04–1.21 g/ml.

On the basis of the data shown in Tables VII and VIII, it was possible to calculate the removal of labeled

TABLE VI
Composition of ³²P-Phospholipids of Lipoprotein Fractions after a 60-min Rat Heart Perfusion with Doubly-Labeled VLDL

Density	[³² P]Phospholipids*		
	LPC†	SP	PC
<i>g/ml</i>	%		
Perfusion Without Heart (9)§			
<1.019	3.3±0.8	7.4±3.7	82.0±4.3
1.019–1.04	3.8±0.3	11.6±1.8	78.1±0.5
1.04–1.21	4.9±2.1	16.1±0.9	70.8±4.3
>1.21	51.5±1.9	4.4±0.4	40.6±0.9
Perfusion With Heart (10)§			
<1.019	3.5±0.4	8.8±1.8	82.2±1.9
1.019–1.04	5.5±1.0	9.9±0.9	81.4±1.4
1.04–1.21	5.6±0.6	12.7±0.9	76.7±0.8
>1.21	49.8±7.4	5.6±0.6	41.6±5.6

* Values are mean±SD.

† See Table I for abbreviations.

§ Numbers in parentheses represent number of experiments.

² The displacement of cholesterol and apoC from VLDL by albumin preparations was studied in detail by one of the investigators and was shown to vary with different albumin preparations. With the specific albumin preparation used here (Methods), displacement of unesterified cholesterol and apoC, but not triglycerides and phospholipids, was demonstrated when rat plasma VLDL was added to albumin solutions. It was dependent on the ratio of VLDL to albumin and on the time and temperature of the incubation. With any albumin preparation, the displacement of unesterified cholesterol and apoC was remarkably constant and reproducible.³

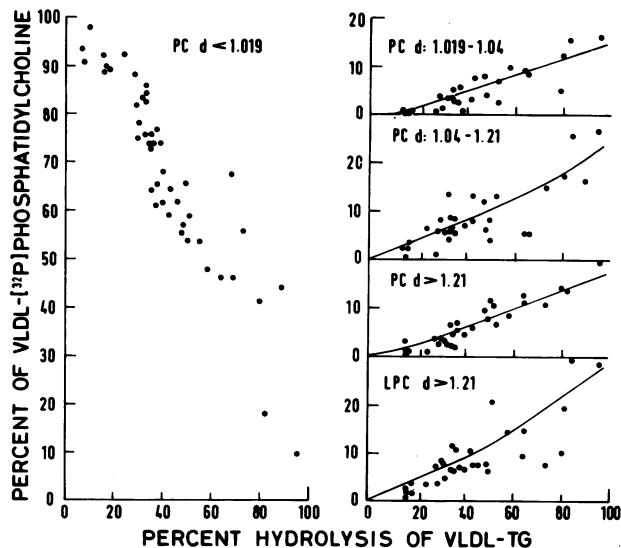


FIGURE 3 The effect of VLDL-TG hydrolysis on the distribution of [³²P]phosphatidylcholine in four density fractions, and generation of [³²P]lysophosphatidylcholine into the fraction of *d* > 1.21 g/ml, during perfusion of isolated rat hearts with doubly-labeled ([¹⁴C]palmitate and [³²P]phospholipid) VLDL. The distribution of ³²P-radioactivity among ultracentrifugally separated density fractions is expressed in percents of the original amount of [³²P]phosphatidylcholine in VLDL. Each point represents an individual experiment and is calculated from the measured distribution of radioactivity among density fractions and among phospholipids in each fraction. Adjustments were made for values obtained during perfusions without heart as carried out in the same experiment and for the same period of time. The effects shown in the figure are therefore due to lipolysis only. Data are from 46 heart perfusions and are derived from the 36 experiments summarized in Table V, and 10 additional perfusions carried out for 10, 30, or 90 min. PC, phosphatidylcholine; LPC, lysophosphatidylcholine.

cholesterol (unesterified and ester) as a function of triglyceride hydrolysis, in 33 rat heart perfusion experiments (Fig. 4). Unesterified [³H]cholesterol was removed from the fraction of *d* < 1.019 g/ml throughout

the whole range of triglyceride hydrolysis although the rate of removal seemed to be slower at low degrees of hydrolysis as compared to high degrees of lipolysis. It appeared predominantly (80–90%) in the fraction of *d* 1.04–1.21 g/ml (Fig. 4). In contrast, the content of ³H-cholesteryl esters in the fraction of *d* < 1.019 did not change much even when about one-half of the triglycerides was hydrolyzed. With more advanced triglyceride hydrolysis, however, ³H-cholesteryl esters disappeared from the fraction of *d* < 1.019 g/ml and appeared in both the fractions of *d* 1.019–1.04 and 1.04–1.21 g/ml, (more so in the former) (Fig. 4).

The fate of apoC. Mixing ¹²⁵I-apoC-labeled VLDL with perfusion medium containing 4% fatty acid poor albumin resulted in a distribution of radioactivity among density fractions, similar to that described previously (14). Recirculation of the labeled VLDL in the perfusion apparatus without heart resulted in further removal of ¹²⁵I-apoC from the fraction of *d* < 1.019 g/ml (Table IX). It was transferred predominantly to the fraction of *d* > 1.21 g/ml where the amount of ¹²⁵I-apoC increased from 9.8% at time zero to 21% after 120 min of recirculation. Rat heart perfusion with doubly-labeled VLDL ([¹⁴C]triglyceride and ¹²⁵I-apoC) revealed further removal of ¹²⁵I-apoC from the fraction of *d* < 1.019 g/ml and a different pattern of distribution of the ¹²⁵I-apoC among density fractions. After 120 min of rat heart perfusion, only 38% of the ¹²⁵I-apoC remained in the fraction of *d* < 1.019 g/ml. The radioactivity removed from this fraction appeared predominantly in the fraction of *d* 1.04–1.21 g/ml, although some ¹²⁵I-apoC was transferred also to the fraction of *d* > 1.21 (Table IX). The effect of the degree of triglyceride hydrolysis on the distribution of ¹²⁵I-apoC among the different densities in 39 individual rat heart perfusions is shown in Fig. 5. It is evident that concomitantly with lipolysis, ¹²⁵I-apoC is progressively and linearly removed from VLDL (*d* < 1.019 g/ml) and appears predominantly in the fractions of *d* 1.04–1.21 g/ml (50–55% of the radioactivity

TABLE VII
Distribution of ³H-Labeled Cholesterol among Lipoprotein Fractions during Rat Heart Perfusion with Doubly-Labeled VLDL*

Perfu- sion time	Hydrolysis of VLDL-TG	[³ H]Cholesterol as percent of total ³ H†							
		Perfusion with heart				Perfusion without heart			
		<i>d</i> < 1.019	1.019–1.04	1.04–1.21	> 1.21	< 1.019	1.019–1.04	1.04–1.21	> 1.21
min	%								
0	—(9)§	62.5±4.4	4.3±1.0	27.5±5.0	5.7±0.7	80.7±4.9	2.0±0.5	13.0±4.6	4.3±1.2
20	27.8±4.6 (8)	55.4±5.3	6.0±1.8	32.3±6.9	6.3±1.1	73.2±10.5	3.3±1.3	19.4±9.8	4.1±1.7
40	32.9±5.1 (8)	56.3±3.6	6.1±1.3	30.3±4.6	7.3±0.5	69.2±11.9	3.2±1.1	22.7±11.4	4.9±0.9
60	40.6±10.0 (9)	48.1±1.4	9.3±3.0	35.0±4.5	7.6±1.7	71.8±10.0	2.9±1.1	20.4±10.3	4.9±0.8
120	52.4±12.8 (8)					66.0±13.3	3.4±1.2	25.0±11.9	5.6±0.5

* Labeled VLDL contained both unesterified and esterified [³H]cholesterol, (see Table VIII).

† Values are mean±SD.

§ Numbers in parentheses represent the number of experiments.

TABLE VIII
Distribution and Composition of Radioactivity in [³H]Cholesterol of Lipoprotein Fractions after a 60-min Rat Heart Perfusion with Doubly-Labeled VLDL

Density	Percent of [³ H]cholesterol*					
	Perfusion with heart		Perfusion without heart		K-R albumin,† nonperfused	
	UC‡	CE	UC	CE	UC	CE
<i>g/ml</i>						
Perfusate	68.4±1.3	26.9±1.1	64.2±2.5	29.5±4.0	68.8±1.1	22.6±2.1
<1.019	48.1±5.6	48.0±4.6	56.2±5.9	37.5±5.1	61.5±2.2	30.5±3.1
1.019–1.04	43.5±3.8	38.7±3.3	—	—	—	—
1.04–1.21	88.8±3.0	5.6±2.9	92.6±1.7	1.5±1.1	92.0±3.7	2.3±1.5

* Values are mean±SD of nine experiments.

† Perfusion medium Krebs-Henseleit bicarbonate buffer contained 4 g/dl albumin (wt/vol).

‡ See Table I for abbreviations.

removed) and $d > 1.21$ g/ml (30–40% of the radioactivity removed).

Radioactivity in the perfusate and in the rat heart. Labeled phospholipids, cholesterol, and apoC were quantitatively recovered in the perfusate throughout the 120-min rat heart perfusion. Values for the 60-min time interval are shown in Table X, and demonstrate that a mean of 96.1% of the ³²P-phospholipids, 98.8% of the [³H]cholesterol, and 98.6% of the ¹²⁵I-apoC were recovered in the perfusate. Only minimal amounts of these labeled constituents were found in the heart. No generation of free iodide (10% trichloroacetate non-precipitable radioactivity) was found during the perfusion. About 25% of the [¹⁴C]palmitate-labeled lipid however has disappeared from the perfusion medium during the 60-min rat heart perfusion (Table X). More than one-half of the [¹⁴C]palmitate that disappeared from the medium was found in the heart, predominantly with triglycerides and phospholipids. Since labeled phospholipids, cholesterol, and apoC were detected in the heart only in trace amounts, we suggest that the [¹⁴C]palmitate found in the heart tissue represents uptake and esterification of fatty acids generated during lipolysis rather than uptake or binding of VLDL particles to or by the heart.

DISCUSSION

Triglyceride-rich lipoproteins, chylomicrons, and VLDL, are composed of nonpolar core containing triglycerides and cholesteryl esters, and a polar or amphipathic surface containing phospholipids, unesterified cholesterol, and apoproteins (28). Triglyceride hydrolysis by lipoprotein lipases therefore must induce a reduction of the core volume of the lipoproteins. Concomitantly, surface constituents are removed from the lipoproteins, resulting in a similar ratio of core-volume and surface-area of intact and post-lipolysis particles. A similar surface to the volume ratio of polar to apolar constit-

uents was indeed demonstrated for chylomicrons and VLDL particles of different diameters isolated from human plasma (29, 30) or produced during lipolysis either in vitro (13, 31) in the supradiaphragmatic portion of the rat (12), or in the isolated perfused rat heart (2). Whenever studied, the phospholipids, unesterified cholesterol, and apoproteins (mainly apoC) removed from the triglyceride-rich lipoproteins were found to be transferred to HDL. It has been therefore suggested that HDL serves as an acceptor for the surface con-

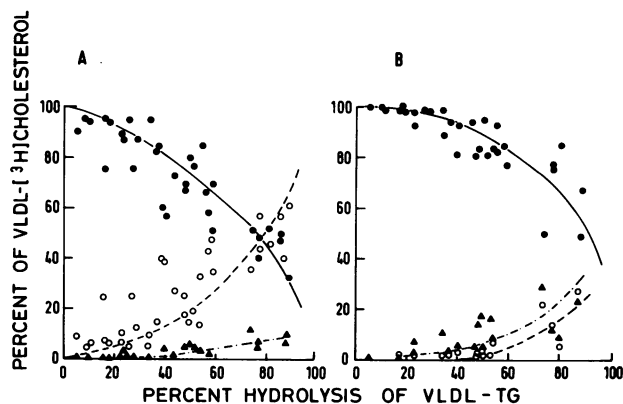


FIGURE 4 The effect of VLDL-TG hydrolysis on the distribution of (A) unesterified [³H] cholesterol and (B) ³H-cholesteryl esters among density fractions during perfusion of isolated rat hearts with doubly-labeled ([¹⁴C]palmitate and [³H]cholesterol) VLDL. The distribution of [³H]cholesterol among ultracentrifugally separated density fractions is expressed in percents of the [³H]cholesterol isolated with VLDL ($d < 1.019$ g/ml) at the end of recirculation without heart. Unesterified [³H] cholesterol and ³H-cholesteryl esters were separated in each fraction by thin-layer chromatography as described in Methods. Radioactivity in VLDL ($d < 1.019$ g/ml) (●); in the fraction of d 1.019–1.04 g/ml (▲); in the fraction of d 1.04–1.21 g/ml (○). Data are from 33 isolated rat heart perfusions at VLDL cholesterol and triglycerides concentrations of 28 and 184 μ M, respectively, as shown in Table VII.

TABLE IX

Distribution of ¹²⁵I-Labeled ApoC among Lipoprotein Fractions during Rat Heart Perfusion with Doubly-Labeled VLDL

Perfu- sion time min	Hydrolysis of VLDL-TG %	[¹²⁵ I]ApoC as percent of total [¹²⁵ I]*							
		Perfusion with heart				Perfusion without heart			
		d < 1.019	1.019-1.04	1.04-1.21	>1.21	<1.019	1.019-1.04	1.04-1.21	>1.21
0	—(8)†					79.7±2.3	2.8±1.4	7.7±1.1	9.8±1.7
20	36.0±2.7 (10)	56.1±3.2	4.8±0.5	19.7±2.4	19.4±1.7	73.8±5.1	2.6±0.4	10.1±3.0	13.5±2.7
40	38.1±3.4 (10)	55.0±2.7	6.5±0.6	21.4±2.3	17.1±1.1	70.6±5.8	2.8±0.3	11.0±3.4	15.6±2.7
60	47.0±4.6 (11)	46.7±3.5	6.3±0.4	23.5±2.4	23.5±2.1	69.3±4.8	3.3±0.7	9.9±3.2	17.5±2.8
120	64.8±6.5 (8)	37.8±4.2	8.1±0.7	30.0±3.8	24.1±2.7	61.9±8.2	5.3±1.0	11.5±3.0	21.3±4.4

* Values are mean±SD.

† Numbers in parentheses represent number of experiments.

stituents generated during the lipolytic process (32, 33). The exact pathways for the removal of surface constituents during lipolysis of triglyceride-rich lipoproteins however have not been yet fully elucidated. Thus, whether the presence of HDL is obligatory for the removal of surface constituents from VLDL is unknown, as is the fate of individual lipid and apoprotein constituents. To study these questions, we have used here the isolated perfused rat heart, quadruply-labeled VLDL, and a buffered albumin perfusion medium. Using this

system, we have observed a continuous hydrolysis of VLDL triglycerides by the membrane-supported rat heart lipoprotein lipase, (similar to that reported by Fielding et al. [34], while using plasma or plasma fractions containing perfusion medium) and progressive removal of surface constituents from the VLDL and IDL density range.

Two different pathways were responsible for removal of phospholipids from VLDL: hydrolysis of glycerophosphatides to lyso-compounds (predominantly lysophosphatidylcholine), and removal of intact molecules. The generation of lysophosphatidylcholine was (a) confined to a membrane-supported enzyme, (b) could

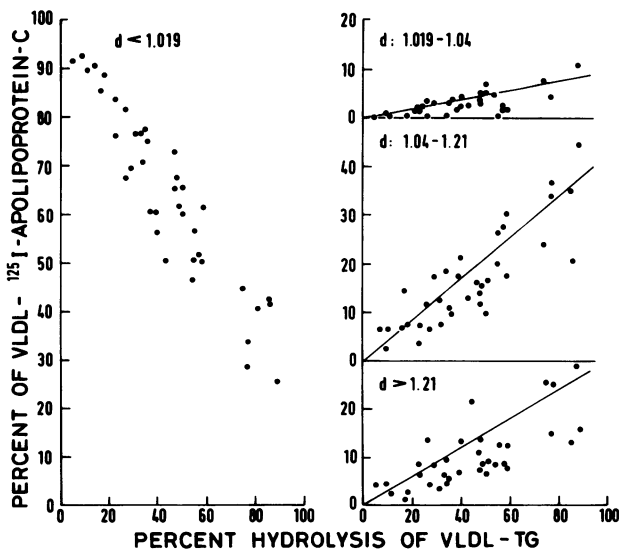


FIGURE 5 The effect of VLDL-TG hydrolysis on the distribution of [¹²⁵I]apoC among four density fractions during perfusion of isolated rat hearts with doubly-labeled ([¹⁴C]-palmitate and [¹²⁵I]apoC) VLDL. The distribution of [¹²⁵I]-apoC among ultracentrifugally separated density fractions is expressed as percent of [¹²⁵I]-apoC isolated with VLDL (d < 1.019 g/ml) at the end of recirculation without heart. Data are from 39 isolated rat heart perfusions at VLDL protein and triglyceride concentrations of 22.5 mg/liter and 184 μM, respectively, as shown in Table IX.

TABLE X

Radioactivity in Perfusate and in Heart Homogenates after a 60-min Rat Heart Perfusion with [¹⁴C]Palmitate, ³²P-Phospholipids-, [³H]Cholesterol-, and [¹²⁵I]-ApoC-Labeled VLDL

Label	Radioactivity perfusate* %	Radioactivity in heart homogenate* %	Distribution of radioactive lipids* %
[¹⁴ C]Palmitate	75.4±10.1	13.1±3.4	PL‡ = 18.4±4.1 MG + DG = 6.3±1.2 FA = 3.3±1.6 TG = 66.0±7.0 CE = 2.6±0.8
³² P-Phos- pholipids	94.1±5.2	1.1±0.2	LPC = 7.7±1.8 SP = 18.1±4.2 PC = 58.3±7.7
[³ H]Cholesterol	98.8±2.0	2.4±1.2	UC = 86.1±7.8 CE = 10.2±4.3
¹²⁵ I-ApoC	98.6±0.5	0.5±0.1	

* Values are means±SD of 8-12 experiments.

‡ See Table I for abbreviations.

be released into the perfusion medium by heparin, (c) occurred in parallel to triglyceride hydrolysis, and (d) was inhibited by preincubation of a lipoprotein lipase-rich (postheparin) perfusate with 1 M NaCl or protamine sulfate. It therefore most probably represented a phospholipase activity of the rat heart lipoprotein lipase, similar to that described previously in rat tissues (35, 36), postheparin plasma derived from supradiaphragmatic rats (18) or human plasma (37), and in bovine milk lipoprotein lipase (38).

The generation of [32 P]lysophosphatidylcholine accounted for about one-third of the total [32 P]phosphatidylcholine removed from the VLDL. The other two-thirds were recovered to a varying degree in all other density fractions, predominantly that of d 1.04–1.21 g/ml. That fraction of density similar to HDL was the richest in [32 P]sphingomyelin with a phosphatidylcholine to sphingomyelin radioactivity ratio of 5.0–6.0 (Table VI) as compared to 12.7 in the original 32 P-labeled VLDL, and 9.0–11.0 in the VLDL isolated during the perfusion. In a separate experiment (data not shown), we have found that the [32 P]phosphatidylcholine isolated with the fraction of d 1.04–1.21 g/ml was almost not susceptible to the phospholipase activity of a lipoprotein lipase-rich (postheparin) rat heart perfusate. Similarly, HDL triglyceride (39) and phospholipids³ are not susceptible to lipoprotein lipases. These observations suggest that the [32 P]lysophosphatidylcholine generated during the perfusion could not originate from the hydrolysis of [32 P]phosphatidylcholine molecules removed from VLDL to the fraction of d 1.04–1.21 g/ml, and that the hydrolysis of the phosphatidylcholine has most probably occurred at the surface of the VLDL. Lysophosphatidylcholine formation is therefore a very early event in VLDL metabolism and precedes the process of removal of unhydrolyzed glycerophosphatides and sphingomyelin from the lipoprotein.

Both unesterified cholesterol and apoC were removed from the VLDL and IDL density range in parallel to the hydrolysis of triglycerides. They were quantitatively recovered with the buffer fractions of d 1.04–1.21 g/ml (80–90% of the unesterified cholesterol and 50–60% of the apoC) and $d > 1.21$ g/ml (apoC), especially when the degree of triglyceride hydrolysis did not exceed 50–60%.

The fate of cholesteryl esters was markedly different from that of unesterified cholesterol, phospholipids, and apoC. In agreement with previous observations (12, 13), we found that significant amounts of cholesteryl esters disappeared from the VLDL and IDL density range only when the degree of triglyceride hydrolysis exceeded 50–70%. Since at these degrees of lipolysis, cholesteryl esters, unesterified cholesterol, and phos-

pholipids were all found at the density interval of 1.019–1.04 g/ml, we suggest that they represent low density lipoprotein-like particles of a hydrated density between 1.019 and 1.04 g/ml. Formation of low density lipoprotein-like particles (d 1.019–1.063 g/ml) *in vitro* was described recently when human plasma VLDL was incubated with a purified lipoprotein lipase (40). The presence of cholesteryl esters at the fraction of d 1.04–1.21 g/ml, observed only with very advanced degrees of lipolysis, may then represent low density lipoprotein-like particles of hydrated density > 1.04 g/ml.

The simultaneous measurements of degrees of triglyceride hydrolysis and removal of surface constituents from VLDL in many experiments have prompted us to test the hypothesis that the removal of surface constituents is related to the decrease of surface area of the lipoprotein as induced by lipolysis (2, 12, 13, 29–31). To this end, percents of VLDL-TG hydrolysis were converted to fractions of the surface area of the lipoprotein assuming that the particles have a perfect spherical shape. The percent of surface constituents remaining with the VLDL at several different degrees of lipolysis were then plotted and compared to a linear relationship curve (Fig. 6). As demonstrated in the figure, phospholipids removal rates were superimposed on the linear line as were those of unesterified cholesterol during hydrolysis of 50–60% of the VLDL-TG. ApoC removal was slightly faster than the linear expected curve, especially at moderate degrees of lipolysis, whereas unesterified cholesterol was slightly slower at advanced degrees of lipolysis. In spite of these small deviations from the linear relationship, the data suggest a strong

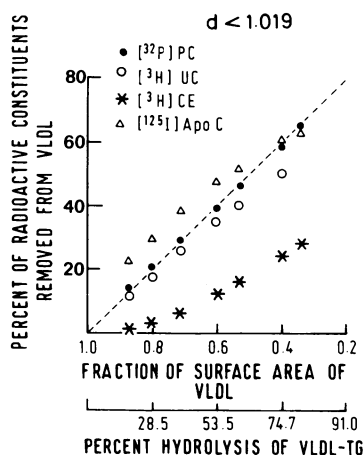


FIGURE 6 The relationships between the calculated decrease of the VLDL surface area and the amounts of [32 P]-phosphatidylcholine (●), unesterified [3 H] cholesterol (○), [3 H]-cholesteryl esters (*), and [125 I]apoC (△), remaining with VLDL during isolated rat heart perfusions. The dotted line represents the theoretical linear relationship curve. Data have been compiled from the experiments shown in Figs. 3–5.

³ Eisenberg, S. Unpublished observations.

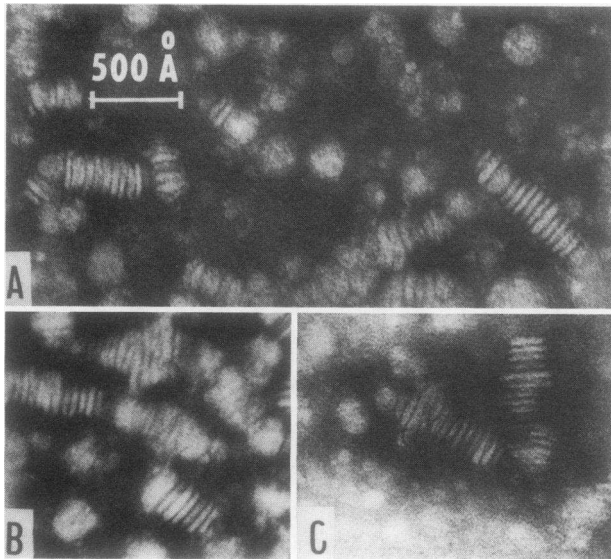


FIGURE 7 Negative stains of particles of the density fraction of 1.04–1.21 g/ml at the end of 120 min of isolated rat heart perfusion. Original VLDL triglyceride concentration was 184 μ M. A, B, and C represent three different fields (original magnification $\times 175,000$).

correlation between the calculated decrease of surface area and the observed degrees of removal of surface constituents. This conclusion indicates that the two phenomena are related, and that no, or very few, surplus surface constituents are associated with VLDL particles when the core volume is progressively decreased. Since no lipoprotein acceptor (i.e. HDL) was present in the perfusion medium, we conclude that the removal of surface constituents must reflect changes of the chemical and (or) physical properties of the VLDL particles themselves, as induced by the lipolysis.

Several different pathways for removal of surface constituents from VLDL during lipolysis were demonstrated in the present study. One path of particular interest to us was the accumulation of lipids and apoC in the density interval of 1.04–1.21 g/ml, suggesting that they may be associated in a particulated form. Indeed, when concentrated and viewed by electron microscopy (Fig. 7), this fraction contained many disk-shaped fragments similar to those described in the plasma of patients with obstructive jaundice (41, 42) or familial deficiency of lecithin:cholesterol acyltransferase (43–45). However, it is unknown whether apoC phospholipids and unesterified cholesterol are removed from VLDL in a particulated form, or whether the disks represent lipid:protein associations formed after the removal of unassociated molecules as seen *in vitro* (46, 47). Yet it is interesting to note that disk-shaped lipoproteins were found in plasma of patients with either familial HDL deficiency (48, 49) or familial lecithin:cholesterol acyltransferase deficiency (43–45), and that

in these conditions the amount of disks seems to vary in proportion to the amount of fat in the diet (45, 49).

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