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### Research Article

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Lipolysis of chylomicrons was associated with a transfer of phospholipid mass into LDL and HDL. At 5 min, 80% of injected triglyceride had been lipolyzed and there was a significant increase in phospholipid mass in LDL and a smaller increase in HDL. At 10 min, the mass of phospholipid in LDL had returned towards control values, and there was a further increase in phospholipid mass in HDL, which suggested phospholipid transfer from LDL to HDL.

In donor lymph [...]

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# Metabolic Fate of Chylomicron Phospholipids and Apoproteins in the Rat

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**ABSTRACT** To study the metabolic fate of chylomicron phospholipid and apoproteins, 15 mg of doubly labeled ( $^3\text{H}$ leu,  $^{32}\text{P}$ phospholipid) rat mesenteric lymph chylomicrons were injected as an intravenous bolus into conscious rats. The specific radioactivity, composition, pool size, and morphology of the plasma lipoproteins were determined after 2–60 min. After injection of chylomicrons, there was a rapid transfer of radioactivity into high density lipoproteins (HDL). At peak specific activity in HDL (2–5 min), 35% of injected apoprotein and 25% of phospholipid radioactivity were recovered in HDL ( $d$  1.063–1.21 g/ml), with smaller recoveries in other lipoproteins and liver. There was an initial rapid rise of  $^{32}\text{P}$  specific activity in HDL and  $d$  1.02–1.063 lipoproteins (low density lipoproteins [LDL]), but whereas LDL specific activity subsequently converged with that of  $d < 1.02$  lipoproteins, HDL specific activity decayed more rapidly than LDL or  $d < 1.02$  lipoproteins.

Lipolysis of chylomicrons was associated with a transfer of phospholipid mass into LDL and HDL. At 5 min, 80% of injected triglyceride had been lipolyzed and there was a significant increase in phospholipid mass in LDL and a smaller increase in HDL. At 10 min, the mass of phospholipid in LDL had returned towards control values, and there was a further increase in phospholipid mass in HDL, which suggested phospholipid transfer from LDL to HDL.

In donor lymph chylomicrons  $^3\text{H}$ -radioactivity was present in apoprotein (apo)B, apoA-I, and apoA-IV, but only radioactivity of apoA-I and apoA-IV were transferred to HDL. Transfer of radioactivity was associated with loss of mass of apoA-I and apoA-IV from the fraction that contained the chylomicron remnants ( $d < 1.02$ ). With injection of 15 mg chylomicron, there was a small but insignificant increase in the relatively large pool of HDL apoA-I. However, 60 min after in-

jection of 250 mg of human or rat intestinal chylomicrons into the rat, there was a significant increase in HDL apoA-I that resulted from acquisition of a major fraction of the chylomicron apoA-I.

After injection of chylomicrons, phospholipid vesicles were observed by negative stain electron microscopy in the LDL and HDL ultracentrifugal fractions, especially in the LDL. Upon addition of an osmotically active compound, cellobiose, vesicles were observed as flattened particles with a double lipid bilayer thickness ( $\approx 100\text{\AA}$ ). To validate further the identity of these particles, chylomicrons were injected into rats with  $^3\text{H}$ glucose, and the recipient rats' plasma was fractionated by chromatography on 6% agarose. Trapping of  $^3\text{H}$ glucose occurred in the void and LDL regions of the column, and vesicular particles were identified in these column fractions by negative stain electron microscopy.

Catabolism of chylomicrons is associated with a rapid transfer of phospholipid, apoA-I, and possibly apoA-IV into HDL. Chylomicron phospholipid appears to give rise to vesicles which are probably incorporated into preexisting HDL. Chylomicron surface components may be an important source of plasma HDL.

## INTRODUCTION

High density lipoproteins (HDL)<sup>1</sup> seem to protect against the development of atherosclerotic cardiovascular disease (1–3), probably by removing cholesterol from the tissues (4). Although the metabolism of HDL is incompletely understood, recent human studies have helped to elucidate HDL apoprotein metabolism (5, 6). Plasma HDL consists of small spherical particles

<sup>1</sup> *Abbreviations used in this paper:* apo, apoprotein; HDL, high density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; VLDL, very low density lipoproteins.

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( $\approx 100$  Å in diameter) in which a core of apolar cholesterol ester and triglyceride is surrounded by a shell of phospholipids and apoproteins (7). The circulating plasma HDL appears to arise from precursor HDL particles of different structure and composition. Hamilton et al. (8) isolated HDL from a recirculating perfused rat liver in the presence of an inhibitor (5,5'-dithionitrobenzoic acid) of the cholesterol-esterifying enzyme, lecithin:cholesterol acyltransferase (LCAT), and showed that the particles were phospholipid/apoprotein bilayer disks, deficient in cholesterol ester and triglyceride. Phospholipid-rich discoidal HDL has also been isolated from rat mesenteric lymph (9). Thus, HDL may be secreted by the rat liver and small intestine as a nascent discoidal particle and converted into spherical HDL by the action of LCAT (10).

In addition to the secretory sources, HDL may also be derived from the catabolism of triglyceride-rich lipoproteins. Chylomicrons and very low density lipoproteins (VLDL) are rapidly degraded by lipoprotein lipase, an enzyme on the capillary endothelium which selectively hydrolyzes the lipoprotein triglyceride (11), forming triglyceride-depleted, cholesterol ester-enriched remnant particles which, in the rat and dog, are primarily taken up by the liver (12, 13). A body of evidence has accumulated which suggests that during lipolysis phospholipids and apoproteins are transferred from chylomicrons and VLDL to HDL (11, 14–19). In the dog, 30% of injected chylomicron  $^{32}\text{P}$  was found in HDL after 20 min (14), and, in man there was an increase in HDL phospholipid and protein mass during alimentary lipemia (15). Chylomicrons injected into functionally eviscerated rats lost phospholipid and tetramethylurea-soluble proteins (16), leading Havel et al. (16, 17) to suggest that phospholipids and C-apoproteins of chylomicrons and VLDL are transferred to HDL during lipolysis. Levy et al. (18) showed a reciprocal relationship between VLDL and HDL levels in human subjects with dietary hypertriglyceridemia; after heparin injection there was a fall in VLDL and a rise in HDL. Recently Chajek and Eisenberg (19) have demonstrated the transfer of a portion of VLDL phospholipid, cholesterol, and apoprotein (apo)C into more dense lipoproteins during circulation through the perfused rat heart (19). In addition, negative stain electron microscopy of the  $d$  1.04- to 1.21-g/ml fraction showed the presence of discoidal lipoproteins, leading these workers to suggest that the surface components of VLDL are removed during lipolysis in particulate form (19).

To investigate the fate of the polar surface constituents of chylomicrons, we prepared chylomicrons radioactively labeled in the apoprotein and phospholipid moieties, and we followed the distribution of radioactivity in the serum lipoproteins as a function of time and dose after injection of chylomicrons into conscious intact rats. Determination of changes in the mass

of lipid and apoprotein in the lipoproteins show that during lipolysis, chylomicron phospholipid, apoA-I, and possibly apoA-IV are transferred into HDL.

## METHODS

### *Experimental procedure*

Doubly labeled ( $^3\text{H}$ ]leu,  $^{32}\text{P}$ ]phospholipid) intestinal chylomicrons were obtained from 275- to 325-g male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.). The main mesenteric lymphatic was cannulated as previously described (20), and the animals were placed in restraining cages with free access to 5% dextrose in 0.9% saline. A corn oil suspension (corn oil, sodium taurocholate [40 mM], and phosphate-buffered saline pH 7.4) (1:4:5, vol/vol per vol) was prepared by brief sonication. 16–18 h after surgery, the animals were infused intraduodenally with unlabeled corn oil emulsion to establish the flow of fatty lymph, then were given a bolus of the corn oil emulsion that contained 200–500  $\mu\text{Ci}$  of  $^3\text{H}$ ]leucine and 20–60  $\mu\text{Ci}$  of  $^{32}\text{P}$ , followed by a 6- to 8-h infusion of corn oil emulsion that contained 1–2 mCi of  $^3\text{H}$ ]leu and 200–400  $\mu\text{Ci}$  of  $^{32}\text{P}$ . Lymph was defibrinated and chylomicrons were isolated by ultracentrifugation at  $3 \times 10^6$  g/min in a SW 27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Chylomicrons were separated from the supernatant fraction by means of a tube slicer and passed through 2% agarose columns to remove residual plasma proteins (20).

Human chylomicrons were obtained from the urine of a patient with chyluria. The patient had chyluria as a result of previous filarial infestation, which had established a chronic fistula between the main mesenteric lymphatics and the urinary tract. Upon ingestion of corn oil, the patient passed large quantities of chylomicrons in his urine. The characteristics of the urinary chylomicrons have been reported elsewhere (21).

A cannula was placed in the femoral vein of 275- to 325-g male Sprague-Dawley rats, and the doubly labeled chylomicrons were administered as an intravenous bolus at the indicated doses 30–60 min after recovery from anesthesia. At various time intervals after injection, animals were exsanguinated from the aorta under ether anesthesia. Blood was collected in syringes that contained 0.25 ml of 0.02 M 5,5'-dithionitrobenzoic acid in 0.75 M phosphate buffer, pH 7.4, and allowed to clot at 4°C. Lipoproteins were isolated by preparative ultracentrifugation in a Beckman 40.3 rotor at 40,000 rpm at  $d = 1.02$  g/ml for 16 h, at  $d = 1.063$  for 20 h, and at  $d = 1.21$  for 36 h (twice). Lipoproteins were kept chilled on ice throughout this procedure. An aliquot of the lipoproteins was removed for liquid scintillation counting to calculate total lipoprotein radioactivity, and the remainder was dialyzed against distilled water, raised to pH 8.5 by addition of  $\text{NH}_4\text{OH}$ . An aliquot was taken for electron microscopy, and the rest was lyophilized. The lyophilized material was delipidated by addition of 5–10 ml of chloroform/methanol (2:1) and washed once with anhydrous diethyl ether at 4°C. The lipid extract was analyzed by quantitative thin-layer chromatography (TLC), and an aliquot was dried under  $\text{N}_2$  for liquid scintillation counting to give phospholipid specific radioactivity. The apoproteins were solubilized in 0.2 M Tris buffer, pH 7.2, which contained 0.1 M decyl sulfate, quantitated by the method of Lowry et al. (22) and analyzed by sodium dodecyl sulfate polyacrylamide gels (20). An aliquot was also used for liquid scintillation counting to determine protein specific radioactivity. Recovery of apoproteins after lyophilization, delipidation, and solubilization was 50–70%.

## Chemical methods

**Lipid analysis.** Lipoprotein lipids were analyzed by TLC with the method of Downing (23) as modified by Katz et al. (24) to quantitate cholesterol, fatty acid, triglyceride, cholesterol ester, lysolecithin, sphingomyelin, and lecithin. The distribution of  $^{32}\text{P}$  in lipoprotein lipids was determined by preparative TLC in chloroform/methanol/acetic acid/water (65:25:4:1). Lipid spots were scraped, and the lipids were removed from the silica by elution with chloroform/methanol (2:1) through a sintered glass funnel. The solvent was dried down under  $\text{N}_2$ , and the lipid was subjected to liquid scintillation counting.

**Apoprotein analysis.** Apoproteins were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis in 5.6% polyacrylamide gels and stained for protein with Coomassie blue (20). The protein bands were identified by comparison of their  $R_f$  values with those of purified rat apoA-IV, apoE, and apoA-I. Apoproteins were quantitated by densitometric scanning of stained gels at 550 nm with a Gilford linear gel scanner (Gilford Instrument Laboratories Inc., Oberlin, Ohio). Apoprotein mass was linearly related to optical density in the range employed (20). For analysis of the distribution of radioactivity in apoprotein samples, duplicate gels were run, one gel stained and the other sliced, dissolved, and counted. In some experiments, apoA-I concentration was measured in chylomicrons and plasma lipoproteins by quantitative immunoelectrophoresis with the rocket technique of Laurell (25).

## Determination of lipid pool sizes

The pools of lipoprotein phospholipid were determined as follows: Mass (milligrams) = total lipoprotein radioactivity (counts per minute)/specific radioactivity (counts per minute per milligram phospholipid). The total lipoprotein radioactivity was corrected for incomplete recovery of serum with published values for serum volume as a percentage of body weight (26). Masses of individual lipids were determined from the percentage of compositions estimated by quantitative TLC.

## Electron microscopy

Negative stain electron microscopy was performed on a Hitachi electron microscope (Hitachi Ltd., Tokyo) operated at 75 kV. Dilute samples ( $\approx 0.1$  mg/ml lipid) were placed on Formvar (Monsanto Co., St. Louis, Mo.)-coated copper grids for 30 s, dried, and stained with 2% Na phosphotungstate (pH 7.4) for 20 s. Lipoproteins were sized from random photographs of grids, using nonconfluent areas where individual particles could be measured. In some experiments twice the volume of 0.5 M cellobiose was added to the sample, incubated for 5 min at 24°C, and then the sample was negatively stained.

All results are given as mean  $\pm$  SEM. The significance of differences between means was determined by Student's  $t$  test, or, when appropriate, by the paired  $t$  test.

## RESULTS

### Characterization of lymph chylomicrons

Nine different preparations of rat mesenteric lymph chylomicrons were used in the experiments. The composition was typical of large chylomicrons, with 91% triglyceride, 4.8% phospholipid, and smaller amounts of other lipids and apoproteins (Table I). The major

apoproteins seen on SDS polyacrylamide gels were apoB, apoA-IV, and apoA-I, whereas apoE and apoC or apoA-II were present in small amounts (Fig. 1). In this gel system, apoC and apoA-II are poorly separated. ApoB, apoA-IV, and apoA-I were also major apoproteins of the human chylomicrons (21). The distribution of  $^{32}\text{P}$  radioactivity in the rat chylomicron phospholipid was lecithin (94%), sphingomyelin (3.6%), and lysolecithin (2.5%). Of the chylomicron apoproteins, only apoB ( $R_f \approx 0.05$ ), apoA-IV ( $R_f = 0.52$ ), and apoA-I ( $R_f = 0.67$ ) were labeled with [ $^3\text{H}$ ]leucine (Fig. 2A), which reflects the pattern of apoprotein synthesis by the rat small intestine (20).

### Fate of chylomicron radioactivity

Serum was collected in 5,5'-dithionitrobenzoic acid from rats sacrificed at 2, 5, 10, 30, and 60 min after intravenous injection of 15 mg of doubly labeled chylomicrons. Table II shows the distribution of phospholipid and apoprotein radioactivity in the serum lipoproteins, expressed as a percentage of the injected chylomicron radioactivity, and Fig. 3 shows the specific activity/time curves. There was a rapid transfer of phospholipid and apoprotein radioactivity and specific radioactivity into HDL between 2 and 5 min after injection of chylomicrons.

### Phospholipid radioactivity

From 5 to 60 min the majority of serum phospholipid radioactivity was recovered in HDL, accounting for 24% of the injected dose at 5 min and decreasing rapidly to 9% at 60 min (Table II). At the 2-min point, 33% of injected phospholipid radioactivity was

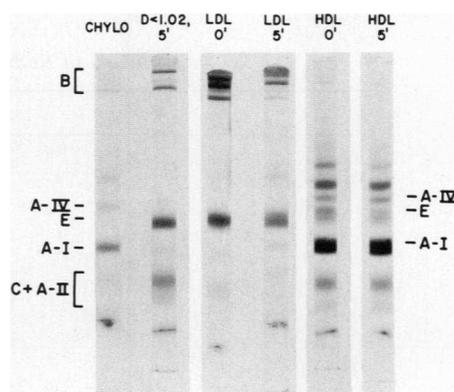


FIGURE 1 SDS-polyacrylamide gels of the apolipoproteins of rat lipoproteins. From left to right the gels are from mesenteric lymph chylomicrons,  $d < 1.02$  lipoproteins 5 min (') after injection of 15 mg of chylomicrons(chylo), control LDL, LDL 5 min after injection of 15 mg of chylomicrons, control HDL, and HDL 5 min after injection of 15 mg of chylomicrons.

TABLE I  
Lipid Composition of Serum Lipoproteins\*

	Cholesterol	Fatty acid	Triglyceride	Cholesterol ester	Lysolecithin	Sphingomyelin	Lecithin	Percentage of protein†
	%							
Intestinal chylomicrons (n = 7)	0.9±0.37	0.54±0.19	91±1.5	1.4±0.24	0.23±0.13	0.23±18	4.3±0.95	1.6±0.31
<i>d</i> < 1.02 lipoproteins								
0 min (n = 3)	10.7±1.6	2.0±0.11	58±1.3	5.5±0.71	1.5±0.32	3.0±0.41	18±2.6	8.2±1.0
2 min (n = 3)	8.0±1.2	2.7±2.2	71±5.5	3.3±0.33	0.63±0.03	1.0±0.58	13±2.4	ND‡
5 min (n = 5)	6.9±1.5	0	65±6.3	5.0±0.97	2.9±1.6	1.6±0.47	14±4.4	10.1±4.0
10 min (n = 5)	8.7±2.0	4.9±2.2	61±4.1	7.8±2.5	0.7±0.19	1.8±0.37	16±4.7	ND
30 min (n = 5)	9.0±3.4	4.7±3.0	53±9.5	8.4±1.0	0.32±0.19	6.7±4.8	14±2.0	ND
60 min (n = 7)	7.1±1.6	1.1±0.6	52±6.6	11±3.0	3±1.2	3.2±0.71	22±3.9	9.4±2.3
LDL ( <i>d</i> 1.02–1.063)								
0 min (n = 6)	9.7±2.5	3±1.5	4.5±1.2	38±4.0	0.16±0.08	10±1.86	32±1.8	36±6.6
2 min (n = 3)	13±3.2	2±0	14±2.6	27±1.0	0.53±0.27	6.7±0.88	37±6.2	ND
5 min (n = 7)	12±0.52	1.6±0.47	4.7±1.4	36±2.7	0.51±0.28	9.6±0.39	36±2.7	30±4.0
10 min (n = 5)	13±0.6	3.0±0.71	7.8±2.5	31±5.1	0.94±0.32	9.3±1.6	36±6.9	ND
30 min (n = 4)	17±1.7	3.4±0.96	7.8±1.8	27±1.8	2.2±1.6	8.4±2.8	35±5.9	ND
60 min (n = 6)	14±1.3	1.2±0.54	10±3.8	37±2.1	1.2±0.60	7.0±0.71	30±4.0	38±3.0
HDL ( <i>d</i> 1.063–1.21)								
0 min (n = 12)	7.0±1.1	0.78±0.36	1.0±0.63	38±1.8	0.88±0.42	7.9±0.44	42±2.0	40±5.3
2 min (n = 5)	8.0±1.3	1.1±0.24	0	32±2.5	0.44±0.13	3.8±0.8	54±1.0	40
5 min (n = 6)	10.6±2.4	1.4±0.79	0.6±0.4	29±2.8	1.2±0.41	9.6±1.1	48±1.9	43±3.2
10 min (n = 5)	9.3±1.2	0.6±0.25	0	29±1.4	0.58±0.37	5.6±1.0	55±2.8	54±5.4
30 min (n = 4)	10±3.6	2.3±0.85	0.80±0.75	35±3.0	1.0±0.3	5.3±1.2	43±1.2	49±15
60 min (n = 5)	6.8±0.21	0.68±0.21	3.5±3.2	35±3.2	0.92±1.2	6.4±1.2	48±3.5	63±5.1

\* Percentage of composition estimated by quantitative TLC. Values given are mean±SEM.

† Protein/(lipid + protein) × 100.

‡ ND indicates not done.

found in the *d* < 1.02 fraction, declining to 3% at 60 min. <sup>32</sup>P radioactivity recovered in low density lipoproteins (LDL) reached a peak of 10% at 5 min and decreased gradually to 4% at 60 min. Because only ≈70–80% of serum apoprotein or lipid radioactivity

was recovered in lipoproteins after preparative ultracentrifugation, the incorporation of radioactivity into lipoproteins shown in Table II and the masses of lipids and apoproteins shown below are slightly underestimated.

TABLE II  
Distribution of Radioactivity after Chylomicron Injection\*

	2 min (3)	5 min (5)	10 min (5)	30 min (5)	60 min (7)
	%				
<sup>32</sup> P radioactivity					
Serum	74±5.9	74±11	54±5.4	42±5.2	31±7.0
<i>d</i> < 1.02	33±4.3	11±1.4	12±5.5	6.2±2.3	3.1±1.1
LDL	6.3±0.33	10±1.5	5.4±1.3	6.2±1.3	4.3±4.2
HDL	22±0.33	24±2.2	19±1.2	14±2.1	8.7±2.6
<i>d</i> > 1.21	3±0.58	3.6±1.1	2.6±1.2	2.4±1.0	2±0.49
Liver	5	12±3.0	20±5.5	26±6.9	18±2.8
<sup>3</sup> H radioactivity					
Serum	53±2.1	62±6.7	53±10	44±7.8	41±5.0
<i>d</i> < 1.02	11±1.2	6.4±2.4	2.4±1.1	1.2±0.73	0.8±0.48
LDL	1±0	4±1.8	2.6±1.4	0.2±0.20	0.9±0.33
HDL	34±0.33	35±1.4	29±2.0	26±3.5	25±1.3
<i>d</i> > 1.21	4±2.3	8.4±4.1	4±2.2	2.3±0.97	2.2±0.79

Numbers in parentheses are the number of rats in the experiments.

\* Results are given as a percentage of the total radioactivity injected in the chylomicrons.

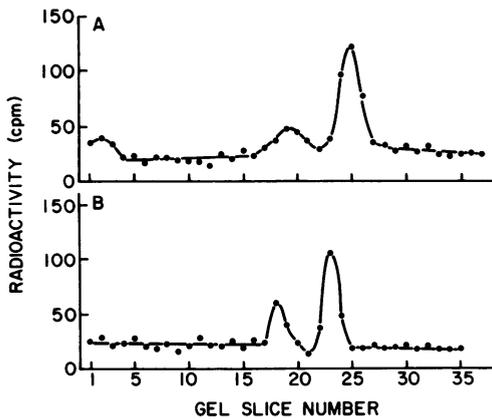


FIGURE 2 Distribution of  $^3\text{H}$ -radioactivity in apoproteins of (A) doubly labeled ( $^{32}\text{P}$ ,  $^3\text{H}$ ]leu) mesenteric lymph chylomicrons and (B) HDL 5 min after injection of 15 mg of chylomicrons. Duplicate gels were stained, showing that the peaks of radioactivity (A) corresponded to apoB, apoA-IV, and apoA-I; and (B) to apoA-IV and apoA-I. There was no radioactive label in the bands corresponding to apoE and apoC plus apoA-II. Although the gel is shorter in (B), the  $R_f$  values of apoA-IV and apoA-I are identical to the corresponding peaks in (A).

The decline in serum phospholipid radioactivity was partly a result of uptake by the liver, increasing from 5% of injected chylomicron radioactivity at 2 min to 26% at 30 min (Table II). The difference between injected radioactivity minus (radioactivity in plasma and liver) increased with time. At 60 min, the spleen had acquired 0.7% and the erythrocytes  $\cong 1\%$  of the phospholipid radioactivity. Thus, at 60 min, 50% of phospholipid radioactivity was unaccounted for, which indicates that it was present in tissues other than the blood, liver, and spleen.

#### Apoprotein radioactivity

2 min after injection of chylomicrons, 53% of injected  $^3\text{H}$  radioactivity was recovered in serum, and, after 5 min, 62% (Table II). Loss of apoprotein radioactivity from serum presumably reflected hepatic uptake of chylomicron remnants and possibly transient sequestration of chylomicrons in capillaries during lipolysis. The transfer of chylomicron apoprotein radioactivity into HDL was extensive. A maximum of 35% of injected chylomicron radioactivity was transferred into HDL at 2–5 min with a subsequent gradual decline to 25% at 60 min. The  $d < 1.02$  fraction only accounted for 11% of injected apoprotein radioactivity at 2 min, decreasing to 1% at 60 min. The LDL contained small amounts of protein radioactivity. Expressed as a percentage of radioactivity recovered in serum, HDL accounted for  $66 \pm 5.6\%$  of apoprotein radioactivity at 2 min, and increased to  $83 \pm 4.4\%$  at 60 min. Between 2 and 8% of chylomicron apoprotein

radioactivity was recovered at  $d > 1.21$ , which represents  $\cong 10\%$  of the total serum radioactivity at all times.

To determine the effect of dose, animals were given 3–25 mg of chylomicrons and sacrificed after 5 min. In the animals receiving the different doses, there was no major difference in the transfer of phospholipid and apoprotein radioactivity into HDL.

#### Specific activity of lipoproteins

**Phospholipids.** There was a rapid fall in the specific radioactivity of the phospholipid of  $d < 1.02$  lipoproteins, from 27% of the specific activity of the injected chylomicrons at 2 min to 4% at 60 min (Fig. 3A). Simultaneous with the fall in  $d < 1.02$  radioactivity there was a rapid rise in LDL and HDL phospholipid specific

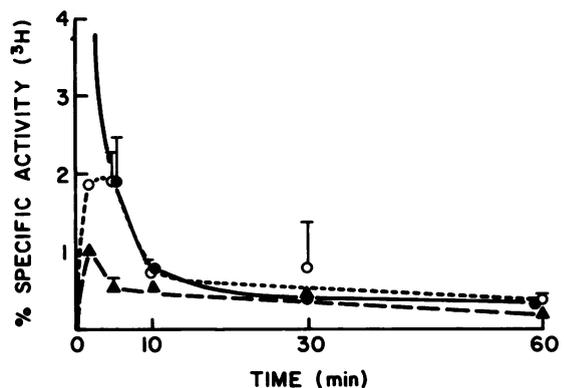
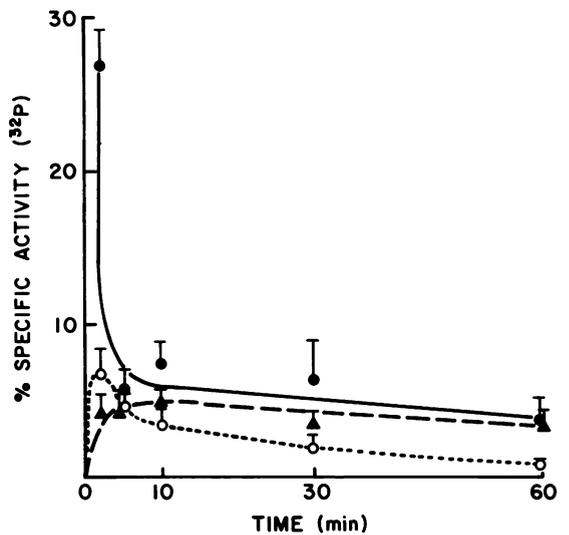


FIGURE 3 Specific radioactivity of (A) phospholipid and (B) total apoprotein of serum lipoproteins after injection of 15 mg of chylomicrons. The  $y$ -axis is expressed as a percentage of specific activity of phospholipid or apoproteins in the injected chylomicrons. Closed circles represent  $d < 1.02$  lipoproteins, open circles represent HDL, and triangles represent LDL. The points shown are the mean  $\pm$  SEM values.

activity, which reached peak values of 5–6% at 2–5 min. At 2–10 min, the specific activities of LDL, HDL, and  $d < 1.02$  lipoproteins were not significantly different. The specific activity of LDL phospholipid decayed slowly between 10 and 60 min. By contrast, the decay of HDL phospholipid specific activity was significantly more rapid than that of LDL, with  $0.83 \pm 0.17\%$  and  $3.4 \pm 0.93\%$ , respectively, remaining at 60 min ( $P < 0.05$ ).

**Apoproteins.** The specific activity of the total apoprotein of HDL rose rapidly, reaching a value similar to that of  $d < 1.02$  lipoproteins at 5 min, with a subsequent slow decay of both in parallel (Fig. 3B). The specific activity of LDL total protein was significantly less than that of HDL ( $P < 0.05$  at 5 min). This reflects the high content of unlabeled apoE in LDL (Fig. 1).

### Lipoprotein composition

The lipid composition of the plasma lipoproteins at different time points after injection of chylomicrons is shown in Table I. Compared with the injected chylomicrons, the lipoproteins of  $d < 1.02$  showed a progressive decrease in triglyceride content from 71 to 52%, accompanied by increases in content of cholesterol ester, lecithin, and sphingomyelin. At 30 and 60 min, the composition of the fraction was similar to that of plasma VLDL (time = 0), which indicates complete removal of chylomicron remnants. At the early time points, LDL was somewhat enriched in phospholipid and depleted in apoprotein. The peak phospholipid:cholesterol ester ratio in LDL occurred at 2 min.

After injection of chylomicrons, the relative lipid composition of HDL showed an enrichment in phospholipid and unesterified cholesterol and a decrease in cholesterol ester (Table I). This resulted in a peak in the ratio of phospholipid to cholesterol ester at 10 min with a gradual return towards the control value at 60 min. The ratio of phospholipid:cholesterol ester at 5 min ( $2.06 \pm 0.11$ ) and 10 min ( $2.14 \pm 0.19$ ) were significantly greater ( $P < 0.01$ ) than the ratio at 60 min ( $1.63 \pm 0.23$ ) or control ( $1.37 \pm 0.09$ ).

Analysis of the distribution of  $^{32}\text{P}$ -label in the serum lipoprotein lipids showed that most of the radioactivity was in phosphatidylcholine, as in the injected chylomicrons. However, the  $d > 1.21$  fraction showed an enrichment of radioactivity in lysolecithin. At 5 min, the distribution of phospholipid radioactivity in the  $d > 1.21$  fraction was lecithin (16%), sphingomyelin (17%), and lysolecithin (67%), whereas at 60 min the respective values were 52, 22, and 26%.

The apoprotein composition of the serum lipoproteins after injection of chylomicrons is shown in Fig. 1. Compared with the injected chylomicrons, the  $d < 1.02$  fraction showed absent or markedly diminished apoA-I and apoA-IV, and a marked enrichment with

apoE and C-apoproteins (Fig. 1). ApoE was a major apoprotein of LDL at all time points. At 5 min in HDL there was a slight depletion of apoE. As determined by scanning the SDS gels, there was a significant increase in the ratio of apoA-I:apoE in HDL at 5 min ( $3.0 \pm 0.52$ ), compared with control HDL ( $2.1 \pm 0.48$ ) or 60-min HDL ( $1.9 \pm 0.68$ ) (Table III).

Analysis of the distribution of radioactivity in the SDS gels of HDL apoproteins showed two peaks, in contrast with the three peaks of radioactivity observed in the chylomicron apoprotein (Fig. 2). The peaks of radioactivity in HDL corresponded to the  $R_f$  values of apoA-I and apoA-IV in duplicate SDS gels. The peak of radioactivity corresponding to apoB in the chylomicron apoprotein was absent in HDL. The distribution of radioactivity in the apoproteins of HDL was similar at all time points.

### Changes in lipoprotein lipid mass

Whereas the above data are consistent with a transfer of chylomicron surface components (protein and phospholipid) to other plasma lipoprotein fractions during catabolism, this conclusion is only justified if there are accompanying changes in the mass of these constituents.

TABLE III  
Changes in HDL Apoproteins after Injection of Chylomicrons

	Mass apoA-I*	ApoA-I:apoE†
	mg	
Control	$0.93 \pm 0.80$ ( $n = 4$ )	$2.1 \pm 0.48$ ( $n = 6$ )
5 min, 15 mg rat chylomicrons	$1.0 \pm 0.15$ ( $n = 4$ )	$3.0 \pm 0.52$ ( $n = 7$ ) <sup>‡</sup>
60 min, 15 mg rat chylomicrons	—	$1.9 \pm 0.68$ ( $n = 7$ )
60 min, 250 mg human chylomicrons	$1.33 \pm 0.15$ ( $n = 4$ ) <sup>§</sup>	$>20.0$ ( $n = 4$ )
60 min, 250 mg rat chylomicrons	1.30 ( $n = 2$ )	5.3 ( $n = 2$ )

\* Mean  $\pm$  SEM determined by quantitative immunoelectrophoresis (25).

† Mean  $\pm$  SEM determined by scanning SDS polyacrylamide gels.

§ Total mass of apoA-I (human and rat) in HDL. Human and rat apoA-I were determined separately by quantitative immunoelectrophoresis, with monospecific antisera. Rat apoA-I represented  $0.96 \pm 0.15$  mg and human apoA-I  $0.34 \pm 0.07$  mg of the total. Total mass of apoA-I was significantly increased ( $P < 0.05$ ) compared with controls.

<sup>‡</sup> Ratio significantly increased ( $P < 0.05$ ) compared with controls.

After injection of chylomicrons, there was a progressive decrease in the mass of triglyceride recovered in the  $d < 1.02$  lipoproteins, with  $10 \pm 3$  mg triglyceride at 2 min,  $3.5 \pm 5$  mg at 5 min, and base-line values ( $\approx 2$  mg) at later time points (Fig. 4). Immediately after the rapid phase of lipolysis (5 min), there was a statistically significant ( $P < 0.05$ ) increase in the mass of phospholipid in LDL and a smaller, statistically insignificant increase in the mass of phospholipid in HDL. At 10 min, the mass of phospholipid in LDL decreased, whereas in HDL phospholipid mass rose significantly above control values ( $P < 0.05$ ). The increased mass of HDL phospholipid was sustained at 60 min.

In addition to phospholipid, injection of chylomicrons was followed by changes in mass of lipoprotein cholesterol ester. At 5 min, cholesterol ester disappeared from HDL and increased in LDL by a corresponding amount ( $\approx 0.5$  mg), thus indicating transfer. Between 5 and 60 min there was a progressive increase in mass of cholesterol ester in HDL and an apparent small decrease in LDL. These results show that changes in the relative lipid composition of HDL (phospholipid:cholesterol ester ratio) reflect simultaneous changes in mass of more than one component.

The pattern of changes in the lipoprotein lipids noted at 5 min was dependent upon the dose of injected chylomicrons (Fig. 5). In these experiments, animals were given 3–25 mg of chylomicrons and sacrificed 5 min later. In a dose of up to 10 mg of chylomicrons, all of the injected chylomicron triglyceride had been removed after 5 min of circulation (Fig. 5). With increasing dose there was a pronounced increment of LDL phospholipid and a smaller increase in HDL phospholipid. Also cholesterol ester appeared to be transferred from HDL to LDL in proportion to the injected dose.

#### Changes in lipoprotein apoprotein mass

SDS gels of the chylomicron remnant-containing fraction ( $d < 1.02$ ) showed a marked decrease or disappearance of apoA-I and apoA-IV from the chylomicron remnant-containing  $d < 1.02$  fraction, compared with the injected chylomicrons (Fig. 1), which indicates loss of mass of apoA-I and apoA-IV from the chylomicron remnants. This was verified by rocket immunoelectrophoresis of the  $d < 1.02$  fraction, which showed no detectable apoA-I at 5 min. In these experiments ( $n = 4$ ), the injected chylomicrons contained  $\approx 0.08$  mg apoA-I whereas the  $d < 1.02$  fraction contained  $< 0.02$  mg apoA-I, given the limits of detection of apoA-I in the standard curve. There was a corresponding small, but statistically insignificant, increase in the mass of apoA-I in HDL (Table III).

To evaluate changes in the mass of apoA-I in HDL, we injected a large dose (250 mg) of human or rat in-

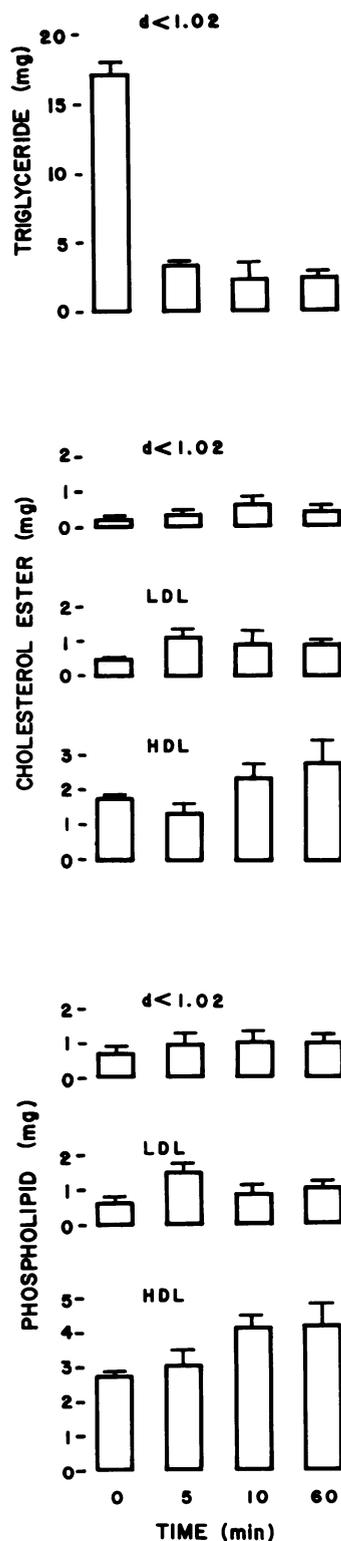


FIGURE 4 Changes in mass of lipids in the serum lipoproteins as a function of time after injection of 15 mg of chylomicrons. The calculation of lipid masses is described in Methods.

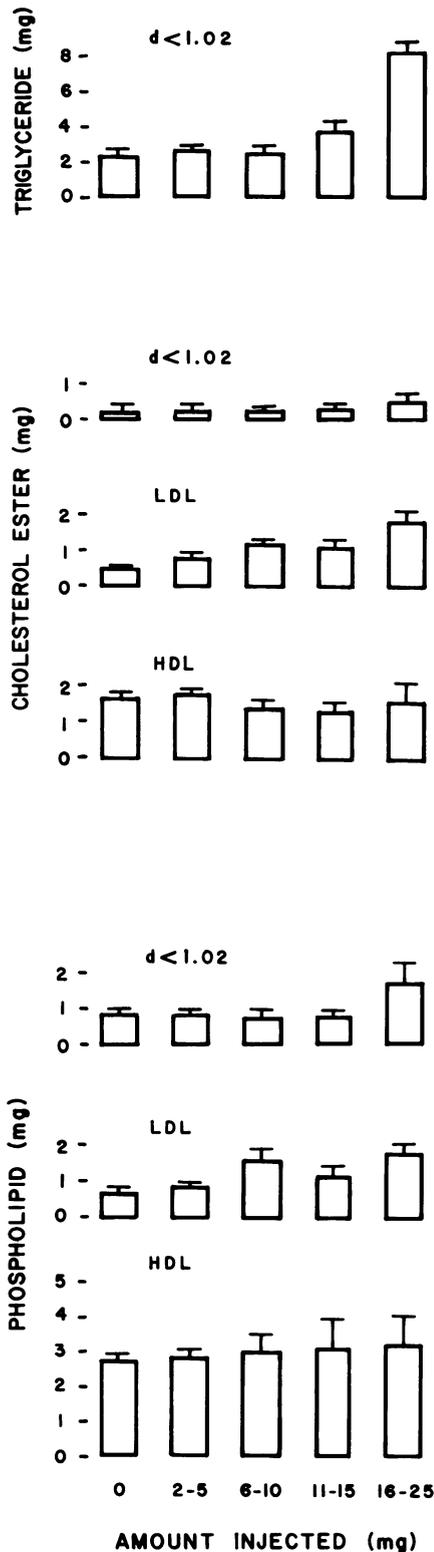


FIGURE 5 Changes in mass of lipids in the serum lipoproteins as a function of the dose of injected chylomicrons. For all doses of chylomicrons the circulation time was 5 min.

testinal chylomicrons and allowed them to circulate for 60 min. SDS gels of the  $d < 1.02$  fraction showed disappearance of apoA-I and apoA-IV (Fig. 6). There was a marked increase in the ratio of apoA-I to apoE in HDL, for both rat and human chylomicrons, and an increase in mass of apoA-I in HDL of  $\cong 0.4$  mg (Fig. 6, Table III). In these experiments, the injected chylomicrons contained  $\cong 1$  mg apoA-I. Because there was no cross-reaction between human apoA-I and rat anti-serum, or vice versa, after injection of human chylomicrons, we were able to determine the relative contributions of human and rat apoprotein in HDL. With the human chylomicron experiments, the recipient HDL contained a similar amount of rat apoA-I to controls ( $0.96 \pm 0.15$  mg) and an additional increment of  $0.34 \pm 0.07$  mg human apoA-I. After injection of human chylomicrons, apoA-IV was absent from HDL by SDS gels (not shown). After injection of rat chylomicrons, there were no clear changes in the relative amounts of apoA-IV in HDL (Fig. 6). After injection of the large dose of chylomicrons, there was an increased amount of apoA-I present in the  $d 1.02-1.063$  fraction (Fig. 6).

#### Incubation of chylomicrons in serum

Mesenteric lymph chylomicrons were incubated for 0, 5, or 60 min in fresh rat serum at  $37^\circ\text{C}$  with the same ratio of chylomicrons:serum as used in the chylomicron injection studies (Table IV). This resulted in a partial transfer of apoprotein and phospholipid radioactivity into HDL. The transfer of radioactivity into HDL was slower and less extensive than after chylomicron injection (Table IV). In the incubation studies, there was no major alteration in HDL lipid composition, which shows that transfer of radioactivity from chylomicrons

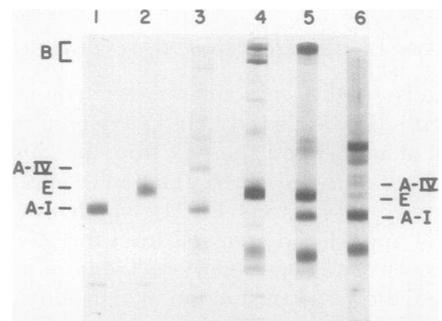


FIGURE 6 SDS-polyacrylamide gels of the apolipoproteins of rat serum lipoproteins, 60 min after injection of 250 mg of rat mesenteric lymph chylomicrons. The gels are: 1, rat apoA-I; 2, rat apoE; 3, rat mesenteric lymph chylomicrons injected in this experiment; 4,  $d < 1.02$  fraction at 60 min; and 5,  $d 1.02-1.063$  fraction at 60 min; and 6,  $d 1.063-1.21$  fraction at 60 min. Apoproteins were identified by their  $R_f$  values, compared with immunologically pure standards of apoA-I, apoE, and apoA-IV.

TABLE IV

Redistribution of Radioactivity and Relative Lipid Mass of Serum Lipoproteins after Injection or Incubation of Chylomicrons\*

	<sup>32</sup> P				<sup>3</sup> H				Phospholipid/cholesterol ester HDL†
	<i>d</i> < 1.02	LDL	HDL	<i>d</i> > 1.21	<i>d</i> < 1.02	LDL	HDL	<i>d</i> > 1.21	
Incubation									
0 min	88±0.67	4.3±0.32	2.7±0.33	4.3±0.33	65±1.5	7±1.2	13±0.33	15±0.58	1.54±0.24 (n = 3)
5 min	73±1.0	4.3±0.33	16±0.67	7.0±1.5	45±1.5	8±0	30±0.67	17±2.0	1.58±0.18 (n = 3)
60 min	34±1.0	12±1.0	38±0	17±1.5	33±2.7	9.3±0.33	38±1.5	20±3.8	1.61±0.13 (n = 3)
Injection									
0 min	—	—	—	—	—	—	—	—	1.30±0.15 (n = 12)
5 min	33±8.3	18±3.9	43±7.0	6±1.6	11±3.8	7.4±2.9	68±8.0	14±6.4	2.06±0.19 (n = 6)
60 min	13±2.7	23±2.2	49±4.5	15±2.7	2±1.2	5±2.3	84±4.4	8±2.7	1.63±0.23 (n = 7)

\* Serum lipoproteins were isolated by preparative ultracentrifugation after injection of 15 mg of doubly labeled (<sup>3</sup>H]leu, <sup>32</sup>P) rat mesenteric lipoproteins into 250-g rats (with 8–9 ml serum [26]). In the incubations, 1.7 mg chylomicrons were incubated with 1 ml of fresh rat serum at 37°C, then lipoproteins were isolated by preparative ultracentrifugation.

† Relative lipid composition determined by quantitative TLC.

microns to HDL occurs by exchange mechanisms as well as by mass transfer.

### Electron microscopy

5 min after injection of 15 mg of chylomicrons, the *d* 1.02–1.063 and HDL fractions (Fig. 7A–C) were more heterogeneous in morphology than equivalent control lipoprotein fractions (not shown). They contained a population of particles that were identical in appearance to single-walled phospholipid vesicles produced by sonication, except they were more heterogeneous in size. To demonstrate the bilamellar morphology of these particles, lipoproteins were treated with an osmotically active compound, 0.5 M cellobiose, just before negative staining. Under these conditions, some of the vesicles were seen as collapsed particles, of variable diameter (300–650 Å), and with the thickness of two phospholipid bilayers (90–100 Å, Fig. 7C). Under observer-blinded conditions, vesicular particles were detected in seven of seven LDL and HDL fractions from injected rats, and in zero of seven controls. In the *d* 1.02–1.063 fraction, vesicles viewed on edge represented 4% of all particles (*n* = 650) (Fig. 7A) and 1% of particles in HDL (*n* = 700) (Fig. 7B). However, these percentages underestimate the number of vesicular particles present in the sample, because in control vesicle preparations of similar lipid concentration a minority of particles were seen on edge. 60 min after chylomicron injection, the LDL and HDL fractions had returned to normal morphology (e.g., Fig. 7D).

### [<sup>3</sup>H]Glucose trapping experiments

In further experiments rat mesenteric lymph chylomicrons (200 mg) were injected in a bolus with 100

μCi of [<sup>3</sup>H]glucose and allowed to circulate for 40 min. The recipients rats' plasma was immediately passed over a 120 × 1.0-cm column of cross-linked 6% agarose. [<sup>3</sup>H]Glucose radioactivity formed peaks in the void, LDL (or small vesicle), plasma protein, and total column volumes. These represented, respectively, 0.75, 0.35, 0.58, and 98.3% of the recovered radioactivity (means of duplicate experiment with ≈800,000 cpm representing 100% radioactivity). Upon prior incubation of plasma with 0.5 M cellobiose (plasma: cellobiose—1:1, vol/vol), 60 and 50% of the [<sup>3</sup>H]glucose radioactivity was released from the void and small vesicle regions, respectively, whereas 105% of radioactivity remained in the plasma protein region. These results suggest trapping of [<sup>3</sup>H]glucose within the internal aqueous compartment of phospholipid vesicles (void and LDL regions) and nonspecific binding to plasma protein. Furthermore, examination of the fractions by negative stain electron microscopy showed phospholipid vesicles, identical in morphology to those shown in Fig. 7, only in fractions corresponding to the peaks of releasable [<sup>3</sup>H]glucose radioactivity (in the void and LDL regions). In control experiments, releasable [<sup>3</sup>H]glucose radioactivity was absent from the void and LDL regions of plasma from rats injected with saline plus [<sup>3</sup>H]glucose and of chylomicrons incubated *in vitro* with [<sup>3</sup>H]glucose. SDS gels of apoproteins (20 μg protein) extracted from the void volume showed an identical apoprotein pattern to the equivalent ultracentrifugal fraction (Fig. 6 [4]). Thus, loss of apoA-I from chylomicron remnants was not a result of ultracentrifugation. In the LDL or small vesicle region there were major bands corresponding to apoE and apoA-IV and several higher molecular-weight-identified proteins. In contrast with the LDL ultracentrifugal fraction (Fig. 6 [5]), apoA-I was present in only trace amounts in the LDL or small vesicle region.

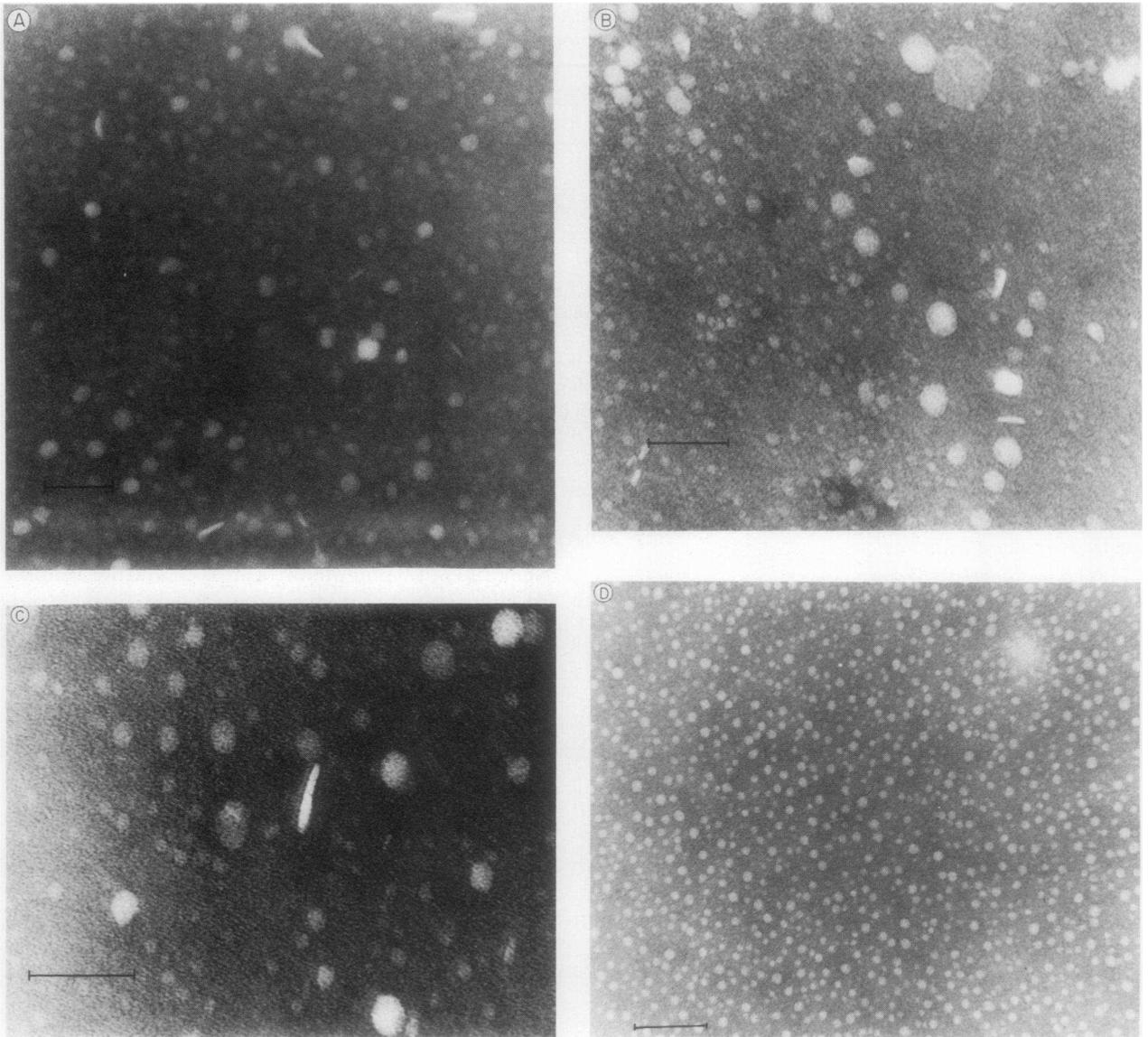


FIGURE 7 Negative stain electron micrographs of serum lipoproteins after injection of rat mesenteric lymph chylomicrons. (A) Lipoproteins of  $d$  1.02–1.063 5 min after chylomicron injection. (B)  $d$  1.063–1.21 fraction 5 min after chylomicron injection. (C) High magnification view of  $d$  1.063–1.21 fraction showing phospholipid vesicle (center field). (D)  $d$  1.063–1.21 fraction showing phospholipid vesicle (center field) 60 min after chylomicron injection. To bring out morphology of collapsed vesicles, samples were mixed with double volume cellobiose just before negative staining. The bars represent 1,000 Å.

## DISCUSSION

Our experiments show transfer of a major fraction of chylomicron phospholipid and apoA-I into HDL after injection of intestinal chylomicrons in the rat. The administration of doubly labeled ( $^{32}\text{P}$ )phospholipid, ( $^3\text{H}$ )leu chylomicrons was followed by a rapid increase in HDL and LDL phospholipid radioactivity and specific radioactivity. Measurement of the changes in

phospholipid mass indicates that the transfer of radioactivity was in part a result of mass transfer. Lipolysis of chylomicrons was initially followed by an increase in phospholipid mass in LDL (at 5 min, Fig. 4), then in HDL (at 10 min, Fig. 4). These data suggest that chylomicron phospholipid was initially incorporated into particles of  $d$  1.02–1.063 and then taken up by HDL. Based on the amount of phospholipid contained in the injected chylomicrons (0.5–1.0 mg), the

increment in HDL phospholipid at 10 and 60 min ( $\approx 1$  mg, Fig. 4) indicates that most of the chylomicron phospholipid was transferred into HDL.

Simultaneous with phospholipid, chylomicron apoA-I and possibly apoA-IV were transferred into HDL. This was shown by loss of mass of apoA-I and apoA-IV from chylomicrons and  $d < 1.02$  lipoproteins concomitant with transfer of apoA-I and apoA-IV radioactivity into HDL. In experiments performed with the 15-mg dose of chylomicrons, the amount of injected apoprotein was too small to cause a statistically significant change in the relatively large pool of HDL apoA-I or apoA-IV. To evaluate apoprotein mass changes in HDL, we injected a large dose (250 mg) of human intestinal chylomicrons, obtained from a patient with chyluria. After 60 min of circulation, there was an increment of apoA-I in the HDL fraction that was sufficient in magnitude to account for transfer of a major fraction of the chylomicron apoA-I into HDL (Table III). A similar increase in mass of apoA-I in HDL occurred after injection of a large dose of rat mesenteric lymph chylomicrons, which indicates that the observed apoprotein transfer was not a result of peculiar catabolism of human chylomicrons in the rat. Changes in HDL apoA-IV were inconsistent, possibly reflecting ultracentrifugal losses of this apoprotein.

With incubation of chylomicrons in plasma, changes in the HDL lipid composition were small and statistically insignificant (Table IV), which indicates that *in vivo* lipid transfer occurred secondary to lipolysis. After injection of chylomicrons, a heterogeneous population of abnormal particles was observed by negative stain electron microscopy in the LDL and HDL ultracentrifugal fraction. Some of these particles were positively identified as vesicles by the fact that they formed collapsed structures with a double bilayer thickness upon incubation with cellobiose (Fig. 7C). Phospholipid vesicles were also demonstrated by trapping of [ $^3\text{H}$ ]glucose and by negative stain electron microscopy of recipient rats' plasma fractionated by agarose column chromatography. Therefore, vesicles did not arise as a result of ultracentrifugation. The phospholipid vesicles probably formed from the chylomicron surfaces during triglyceride removal. *In vitro* lipolysis of chylomicrons gives rise to phospholipid lamellae at the chylomicron surface (11, 27). Presumably, the rapid hydrolysis of triglyceride causes shrinkage of the chylomicron core, leaving the chylomicron surface relatively intact. The redundant surface may form lamellae which gives rise to phospholipid vesicles. During *in vitro* lipolysis of VLDL, phospholipid, cholesterol, and C-apoprotein have been shown to be removed as discoidal HDL particles (19), a mechanism that is similar in principle to that suggested here.

The uptake of chylomicron phospholipid into HDL might occur by mechanisms similar to those proposed

for lecithin vesicles or multilamellar liposomes (28–31). Upon incubation of egg yolk lecithin vesicles with a several-fold excess of plasma HDL, phospholipid mass was incorporated into the HDL fraction, forming particles which appeared homogeneous by negative stain electron microscopy and equilibrium density ultracentrifugation (31) which suggests direct uptake by pre-existing HDL. The transfer of cholesterol ester from HDL to LDL at 5 min (Figs. 4 and 5) could conceivably reflect transient formation of a collision complex between vesicles and HDL. The resulting phospholipid-rich spherical HDL would probably be suitable substrates for LCAT *in vivo*.

The surface of the nascent chylomicron is deficient in unesterified cholesterol. The increase in content of unesterified cholesterol in HDL after chylomicron injection (Table I) suggests that the phospholipid transferred into HDL may act as a receptor for unesterified cholesterol derived from other lipoproteins and cell membranes. Between 5 and 60 min there was a major increase in mass of cholesterol ester in HDL (Fig. 4), replacement of vesicles with HDL of normal morphology (Fig. 7D), and a high content of  $^{32}\text{P}$  in lysolecithin in the  $d > 1.21$  fraction. These findings strongly suggest that the unesterified cholesterol transferred into HDL from other sources is subsequently esterified by the action by LCAT. The rapid clearance of lysolecithin by many tissues including arteries and a variety of vascular organs (32, 33) might account for the fact that at 60 min 50% of phospholipid radioactivity was present in tissues other than blood, liver, or spleen.

The small intestine is a source of apoA-I in humans (20, 34) as well as the rat (35–37). Studies in chyluric subjects show that the human small intestine is a quantitatively significant source of phospholipid and apoA-I (21). After ingestion of fat, normal subjects showed a 13% rise in plasma apoA-I (34). However, in plasma, the apoA-I was no longer associated with chylomicrons (34). Also, after injection of radioactively labeled chylomicrons into normal humans, there was a rapid transfer of chylomicron apoA-I and apoA-II radioactivity into HDL (38). Thus, in addition to phospholipid (15), there is transfer of human A-apoproteins from chylomicrons to HDL upon entry into the circulation. Although mechanisms of transfer of human chylomicron surface constituents into HDL have not been elucidated, studies in a number of pathological conditions suggest that the mechanisms might resemble those shown in our study. Phospholipid vesicles arise in the plasma of hypertriglyceridemic subjects after heparin injection (39), and in patients with LCAT deficiency (40) or Tangier disease (41) after ingestion of fat. Thus, in humans, chylomicron phospholipid may dissociate in vesicle form, at least in situations of rapid lipolysis or deficiency of HDL or LCAT. Such a mechanism could also contribute to the vesicular

particles accumulating in the plasma of patients with acquired LCAT deficiency (cholestatic jaundice [42] or alcoholic hepatitis [43]). The well-known inverse correlation between plasma triglyceride and HDL levels (44–47) and the low levels of HDL occurring in states of impaired lipolysis (48, 49) indicate that lipolysis of chylomicrons or VLDL may make an important contribution to normal human HDL.

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