Transfer of human lymph chylomicron constituents to other lipoprotein density fractions during in vitro lipolysis

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Abstract To ascertain whether chylomicron constituents would be transferred to low density lipoprotein (LDL, d 1.019–1.063 g/ml) and high density lipoprotein (HDL, d 1.063–1.21 g/ml) density fractions during lipolysis in the absence of other lipoproteins, the in vitro effect of bovine milk lipoprotein lipase on human thoracic duct lymph chylomicrons in the presence of albumin was examined. In incubations without lipase, over 90% of chylomicron constituents remained in the 1.006 g/ml supernate, and large particles ranging in diameter mainly from 750–6000 Å were observed by electron microscopy. After the addition of lipase, lipolysis ranged from 69.0–94.6% and numerous collapsed particles with redundant surface were seen, as well as smaller particles within the LDL and HDL density region. With lipolysis, the majority of chylomicron cholesterol and phospholipid mass was transferred to LDL and HDL, while chylomicron apolipoprotein (apo) A-I, A-II, and C-II mass was transferred mainly to HDL. Utilizing either radioiodinated apoA-I and apoA-II reassociated with chylomicrons or radiolabeled chylomicrons, a similar redistribution of apoA-I and apoA-II radioactivity was noted with lipolysis. In contrast, chylomicron apoB (mainly B-48) radioactivity was transferred predominantly to LDL with lipolysis. These data are consistent with the concept that during lymph chylomicron triglyceride hydrolysis, chylomicron apolipoproteins, cholesterol, and phospholipid can be transferred to the LDL and HDL density regions in the absence of acceptor particles.—**Schaefer, E. J., M. G. Wetzel, G. Bengtsson, R. O. Scow, H. B. Brewer, Jr., and T. Olivecrona.** Transfer of human lymph chylomicron constituents to other lipoprotein density fractions during in vitro lipolysis. *J. Lipid Res.* 1982. **23:** 1259–1273.

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Following the ingestion of fat and the action of intestinal enzymes, fat absorption takes place in the small intestine (1). Subsequently, triglyceride is combined with phospholipid, cholesterol, and various proteins, and released into mesenteric lymph as lipoprotein particles by intestinal cells (2). These particles are largely chylomicrons (density <1.0 g/ml) (3). Rat intestinal epithelial cells synthesize apolipoproteins apoA-I, B, and A-IV, and incorporate these proteins into lymph chylomicrons as well as into other lymph lipoprotein density fractions (4–7). Lymph chylomicrons pick up significant amounts of apoC-I, apoC-II, and apoC-III presumably by transfer from lipoproteins filtered from plasma into lymph (8–10). ApoC-II activates the enzyme lipoprotein lipase (LPL), which is responsible for triglyceride hydrolysis (11, 12). Human thoracic duct lymph chylomicrons contain apolipoproteins A-I, A-II, A-IV, B, C-I, C-II, C-III, and H (3, 9, 10, 13–15). ApoH (B_2 glycoprotein I) causes a significant activation of apoC-II stimulated lipoprotein lipase (LPL) activity (16).

After thoracic duct lymph enters blood via the left subclavian vein, lymph chylomicron triglyceride is hydrolyzed by LPL, attached to luminal capillary endothelial cells, resulting in the formation of chylomicron remnants (17). Free fatty acids released from chylomicrons during in vivo lipolysis are transferred to parenchymal cells or are bound to albumin (18). The remnants have been reported to be rapidly cleared from plasma via uptake by the liver (19–21). However, not all chylomicron constituents are rapidly removed from plasma when chylomicron triglyceride is hydrolyzed. In man, radiolabeled chylomicron apolipoproteins A-I, A-II, C-II, and C-III are transferred to high density lipoproteins (HDL), and a small fraction of apoB radio-

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoproteins; SDS, sodium dodecyl sulfate; TMU, tetramethylurea; PAGE, polyacrylamide gel electrophoresis.

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activity is transferred to low density lipoproteins (LDL) following injection of radioiodinated lymph chylomicrons into plasma (10). In the rat, chylomicron apoA-I and phospholipid have been reported to be transferred to HDL following chylomicron infusion (22, 23). The hypothesis tested in the present investigation was that chylomicron apolipoprotein and lipid constituents are transferred to LDL and HDL density fractions during lipolysis in the absence of acceptor lipoproteins. The data presented support this hypothesis.

MATERALS AND METHODS

Lipoprotein lipase

Lipoprotein lipase was purified as previously described from bovine milk by heparin-Sepharose column chromatography (24). The enzyme was prepared in Umea, Sweden, and transported in the frozen state, packed in dry ice, to Bethesda, MD. The activity of the enzyme was 236 units/mg protein (1 unit = 1 μmol of fatty acid released/min), as measured with Intralipid emulsion at pH 8.5 at 25°C. No significant change in the enzyme activity was noted during the course of these experiments. The protein content of the enzyme preparation was 0.3 mg/ml.

Preparation of lymph chylomicrons

Lymph was obtained by cannulation of the thoracic duct of subjects undergoing lymph drainage for purposes of immunosuppression following kidney transplantation at either the University of Colorado Medical Center, Denver, CO, or at the Walter Reed Army Medical Center, Washington, DC. All lymph was collected under sterile conditions following fat-rich meals in 0.1% EDTA, pH 7.4, and shipped or transported on ice (except for lymph utilized in incubations 8 and 9) within a 24-hr time period to Bethesda, MD. In the last two incubations (numbers 8 and 9) the mean polyunsaturated:saturated fat ratio (based on dietary recall and standard food tables (25)) of the meals prior to lymph collection was 0.44, and lymph was collected and maintained at 37°C prior to use. Lymph chylomicrons were isolated utilizing a Beckman SW27 rotor (Beckman Instruments Fullerton, CA). Lymph was overlayered with 0.85% NaCl, 0.01% EDTA solution, and spun at 25,000 rpm for 30 min at 10°C. Chylomicrons utilized for incubations 8 and 9 were isolated at 31°C. The chylomicron supernate was removed, resuspended in normal saline, and reisolated as described above, and this procedure was subsequently carried out a third time. Lymph chylomicrons were radioiodinated with ^{125}I (New England Nuclear, Boston, MA) in 1 M glycine buffer at pH 10 as previously described (10). The amount of lipid labeling was assessed by lipid extraction and trichloroacetic acid precipitation as previously described (10). Free iodine was removed by dialysis against sterile 0.85% NaCl, 0.01 M Tris, pH 7.4. The mean (±SD) efficiency of iodination for chylomicron preparations was 12.9 ± 5.1%, and the mean lipid labeling was 18.3 ± 2.9%. Assuming a molecular weight of 250,000 for chylomicron protein, no more than 1 mol of iodine per mol of protein was incorporated into radiolabeled preparations, and less than 1% of the iodine was in the free form.

Preparation and radioiodination of apolipoproteins

Apolipoproteins A-I, A-II, and C-III_2 were isolated from human plasma high density lipoproteins and very low density lipoproteins by column chromatography as previously described (26, 27). All apolipoprotein preparations did not react with antisera for other apolipoproteins, and they formed discrete bands on sodium dodecyl sulfate (SDS) and tetramethylurea (TMU) polyacrylamide gel electrophoresis (PAGE) (28, 29). Apolipoproteins were radioiodinated with ^{125}I or ^{131}I at pH 8.5 in 1 M glycine buffer by the iodine monochloride method (30). The efficiency of iodination was assessed by precipitation of the protein by 20% trichloroacetic acid, and free iodine was removed by extensive dialysis against sterile 0.85% NaCl, pH 7.4, 0.1 M Tris (10). Assuming molecular weights of 28,000, 18,000, and 10,000, respectively, for apoA-I, apoA-II, and apoC-III_2, no more than 1 mol of iodine per mol of protein was incorporated into radiolabeled preparations. The mean efficiency of iodination was 58.2 ± 6.4% for apoA-I, 52.1 ± 5.2% for apoA-II, and 51.5 ± 9.7% for apoC-III_2. Free iodine accounted for less than 1% of the radioactivity in all preparations. Radiolabeled apolipoproteins were incubated with isolated lymph chylomicrons at 37°C for 30 min, and the chylomicron fraction was then reisolated as previously described.

Analytic procedures

Lipids were extracted by the method of Folch, Lees, and Sloane Stanley (31). In order to measure lipid constituents in lipoprotein fractions in these incubation experiments, a 10- to 100-fold concentration of fractions was generally required, which was carried out in the extraction and resolubilization steps. Phospholipids in lipoprotein fractions were measured by the method of Chalvardjian and Rudnicki (32), and cholesterol and triglyceride were measured by the AutoAnalyzer technique (33) as well as enzymatically (incubations 6–9) (34, 35). Free and esterified cholesterol concentrations in lipoprotein fractions were measured enzymatically as

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previously described (36). Free fatty acids were measured as previously described (37). SDS and TMU polyacrylamide gels were scanned utilizing a Gelman ACD-15 densitometer (Gelman Instrument Co., Ann Arbor, MI) which computes the area under peaks. Identical chromogenicity of apolipoproteins was assumed. Radioactivity was measured in a Packard Model 3375 gamma counter (Packard Instrument Co., Inc., Downers Grove, IL).

Electron microscopy

Chylomicron preparations were diluted to a concentration of 425–850 mg/dl triglyceride, and other lipoprotein fractions were diluted to an appropriate concentration (usually 100–250 μg of protein/ml). All lipoprotein fractions were dialyzed against 0.01 M ammonium bicarbonate, pH 8.5, 0.01% EDTA, 0.002 M sodium azide solution.

Samples were negatively stained as follows. Five-μl aliquots of sample were mixed with a equal volume of 2% sodium phosphotungstate (PTA), adjusted to pH 7.2 with NaOH, and immediately placed on a formvar-coated copper grid. The grid was dried after 1 min by touching the edge of the grid with filter paper. In some cases, a drop of distilled water was subsequently placed on the grid and removed immediately with filter paper as previously described. Grids were examined with a Philips EM 300 electron microscope at magnifications between ×4,000 and ×107,000.

Apolipoprotein A-I, A-II, and C-II assays

Following delipidation of lipoprotein fractions by the method of Folch et al. (31), the lyophilized protein was reconstituted with 0.05 M veronal buffer. Electroimmunoassay plates were prepared with 1.5% agarose (Bio-Rad Laboratories, Richmond, CA), 5% Dextran T10 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.05 M veronal buffer, pH 8.4, 0.05% NaN_3 (Fisher Scientific) (38). Plate size was 100 × 200 mm with 1.5-mm-thick agarose matrix. Following electrophoresis, plates were dialyzed in saline, air dried, and stained with Coomassie Blue; quantitation was determined by peak height measurement. Each plate contained 6 standards, 2 controls, and 15 samples in 2-mm wells. Utilizing this technique for normal human plasma samples (n = 50) gave values of 117.2 ± 17.0 mg/dl for apoA-I concentration, 27.1 ± 4.2 for apoA-II concentration, and 3.1 ± 1.1 for apoC-II concentration. To determine apolipoprotein levels in lipoprotein fractions required a 10- to 100-fold concentration which was carried out in the delipidation and resolubilization steps. The lower limits of detection of apolipoproteins in these assays following a 10-fold concentration (run undiluted) was 0.01 mg/ml.

Fatty acid analysis

Fatty acid analysis of human lymph chylomicron triglyceride was performed by gas–liquid chromatography by Dr. William E. Connor, Department of Medicine, University of Oregon Health Sciences Center, Portland, OR, as previously described (39).

Experimental procedures

All incubations were carried out for 60 min at 37°C in 0.1 M Tris, pH 8.5. A mean of 10.6 ± 1.2 μg of lipoprotein lipase was added to each ml of incubation mixture. Experimental conditions for all incubations are given in **Table 1.** The albumin utilized in incubations 1–7 was lot B 2411 albumin powder, fraction V from bovine plasma (Armour Pharmaceutical Co., Kankakee, IL). Trace amounts of phospholipid were noted in the albumin preparation (in a 16% albumin solution the mean (±SD) phospholipid concentration was 1.2 ± 0.4 mg/dl); cholesterol or triglyceride were not detected. In addition, 16% albumin was incubated with egg yolk phosphatidylcholine (Lipid Products) at 37°C in a shaking water bath for 30 min. Thereafter the incubation mixture was subjected to ultracentrifugation at 1.21 g/ml. No apolipoproteins were detected by SDS PAGE in the 1.21 g/ml supernate. For incubations 8 and 9, Lot 15M human fatty acid-free fraction of albumin (Miles Laboratories, Elkhart, IN) was used. The latter albumin preparation was found to contain no detectable lipid or apolipoproteins. The conditions for all incubations are given in Table 1. In all experiments, control samples were incubated without lipoprotein lipase and subsequent lipoprotein fractions were isolated and analyzed in identical fashion as in incubations with lipase. Lymph chylomicrons were freshly isolated from human thoracic duct lymph as previously described. All preparations were used within 1 week of isolation from freshly harvested human lymph. The amount of lipolysis in incubation experiments was assessed by the decrease in triglyceride as well as by the increase in free fatty acids.

In these experiments, chylomicrons were isolated from incubation mixtures as previously described. All other density fractions, namely d < 1.006 g/ml, IDL (d 1.006–1.019 g/ml), LDL (d 1.019–1.063 g/ml), HDL_{2b} (d 1.063–1.10 g/ml), HDL_2 (d 1.063–1.125 g/ml), HDL_{2a+3} (d 1.10–1.21 g/ml), and HDL_3 (d 1.125–1.21 g/ml) were isolated by sequential ultracentrifugation utilizing either Beckman 40.3 (39,000 rpm) or 60 Ti (59,000 rpm) rotors in Beckman L265B ultracentrifuges (Beckman Instruments, Fullerton, Ca) (40). Solid KBr or KBr solution was utilized for density adjustments. All lipoprotein isolations for each incubation are listed in Table 1 and were carried out at 4°C, except for incubations 8 and 9 which were carried out at 31°C

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TABLE 1. Conditions of incubation experiments[a]

Incubation Number	Albumin Concentration	Triglyceride Concentration	Incubation Volume	Percent Lipolysis	Label Utilized	Lipoproteins Isolated
	%	mg/dl	ml	%		
1	15.5	991	15	69.0 ± 1.5	^{131}I-apoA-I, ^{125}I-apoA-II	C, V, I + L, H[b]
2	19.5	417	37	92.1 ± 0.7	^{131}-apoA-I, ^{125}I-apoA-II	C, V, I + L, HDL$_2$, HDL$_3$[c]
3	19.5	194	37	85.2 ± 2.1	^{131}I-apoA-I, ^{125}I-apoA-II	C, V, I + L, HDL$_2$, HDL$_3$[c]
4	19.5	285	45	87.5 ± 1.9	^{125}I-apoC-III$_2$	C, V, I + L, HDL$_2$, HDL$_3$[c]
5	16.0	350	15	94.1 ± 0.9	^{125}I-chylomicrons	C, V, L, H[b]
6	16.0	367	15	94.0 ± 1.9	^{125}I-apoA-I, ^{131}I-apoA-II	C; V, I, L, H[b]
7	16.0	383	148	94.6 ± 2.3	^{125}I-chylomicrons	V, I, L, H[b,c]
8	16.0	421	38	79.2 ± 1.4	^{125}I-chylomicrons	V, I, L, HDL$_{2b}$, HDL$_{2a+3}$[b]
9	16.0	405	38	85.4 ± 1.9	^{125}I-apoA-I, ^{131}I-apoA-II	V, I, L, HDL$_{2b}$, HDL$_{2a+3}$[b]

[a] All incubations were carried out for 60 min at 37°C, in 0.1 M Tris, pH 8.5, in the presence and absence of lipase.
[b] Isolated in triplicate utilizing a 40.3 rotor at 39,000 rpm.
[c] Isolated utilizing a 60 Ti rotor at 59,000 rpm.
C, chylomicrons; V, VLDL, d < 1.006 g/ml; I, IDL, d 1.006–1.019 g/ml; L, LDL, d 1.019–01.063 g/ml; H, HDL, d 7t063–1.21 g/ml; HDL$_{2b}$, d 1.063–1.10 g/ml; HDL$_2$, d 1.063–1.125 g/ml; HDL$_{2a+3}$, d 1.10–1.21 g/ml; and HDL$_3$, d 1.125–1.21 g/ml.

to minimize possible triglyceride crystallization (41). In these latter experiments lipoprotein fractions never were brought below 31°C until electron microscopy and compositional analysis were begun.

In all experiments (except 8, 9) aliquots of the incubation mixture were taken at 0, 10, 20, 30, and 60 min for free fatty acid measurements. Lipoprotein fractions isolated from all experiments were subjected to lipid analysis (cholesterol, phospholipid, and triglyceride). Aliquots of the incubation mixture were taken in experiments #1, 2, 8, and 9, and from lipoprotein fractions in incubations 1, 3, 4–6, 8, and 9 for electron microscopy. Aliquots of lipoprotein fractions were taken from all experiments for apolipoprotein determinations; however, apolipoprotein levels could only be determined in incubations 1, 8, and 9; in all other experiments the albumin in isolated lipoprotein fractions resulted in interference with the assay so that apolipoproteins could not be reliably measured. In incubations 5, 7, and 8, lymph chylomicrons, as well as lipoprotein fractions including the 1.21 g/ml infranate were subjected to TMU PAGE (29) and SDS PAGE utilizing a modification of the method of Weber and Osborn (28) as previously described (42). In incubations 7 and 8, SDS slab gel PAGE was also used with 10% acrylamide, 1% bis, utilizing the slab gel technique as described by Laemmli (43). Molecular weight standards, (albumin, 67,000; ovalbumin, 43,000; carbonic anhy-

Fig. 1. The chylomicron fraction isolated at 31°C prior to the addition of albumin and lipoprotein lipase is shown. Chylomicrons exhibited rounded profiles with diameters mainly ranging from 750 to 6,000 Å, ×35,000. **Fig. 2.** The incubation mixture with lipoprotein lipase for 1 min is shown. Note irregular finger-like projections extending from several particles and areas of collapsed surface film at arrows. ×39,000 (incubation 1, limited albumin). **Fig. 3.** The incubation mixture with lipoprotein lipase for 10 min is shown. Note particles with a range of polymorphic profiles due to indentation of the particle surfaces reflecting the changing ratio of surface components to the volume of the triglyceride core. ×35,000. **Fig. 4.** The incubation mixture with lipoprotein lipase for 30 min is shown. Note collapsed chylomicron profiles composed primarily of large areas of surface film with variable amounts of core lipid still present. ×40,000. **Fig. 5.** The fraction of density <1.006 g/ml (VLDL and chylomicrons) is shown. Incubation time was 60 min. Large, irregularly-shaped surface fragments containing variable amounts of lipid may be seen, in addition to numerous smaller round lipoprotein particles 200–400 Å in diameter. ×107,000. All specimens were diluted to an appropriate concentration and negatively stained with 2% sodium phosphotungstate. The bar marker represents 1,000 Å for all figures, and samples were taken from incubation 9 unless otherwise indicated.

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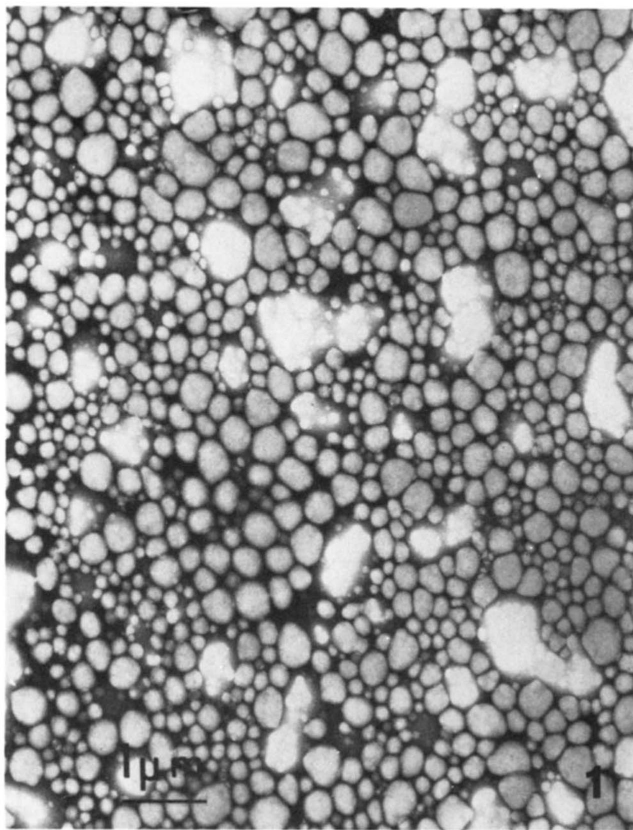

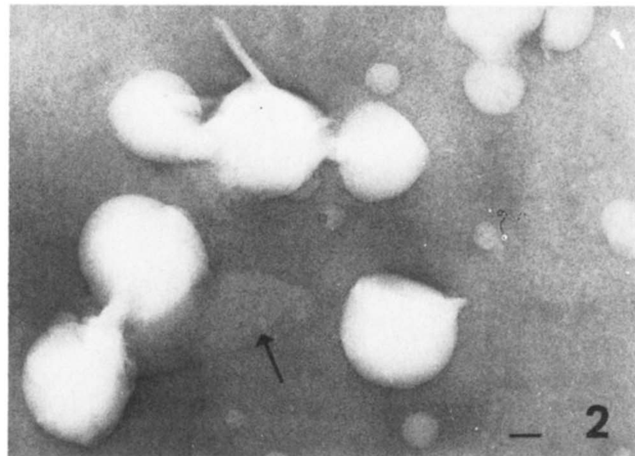

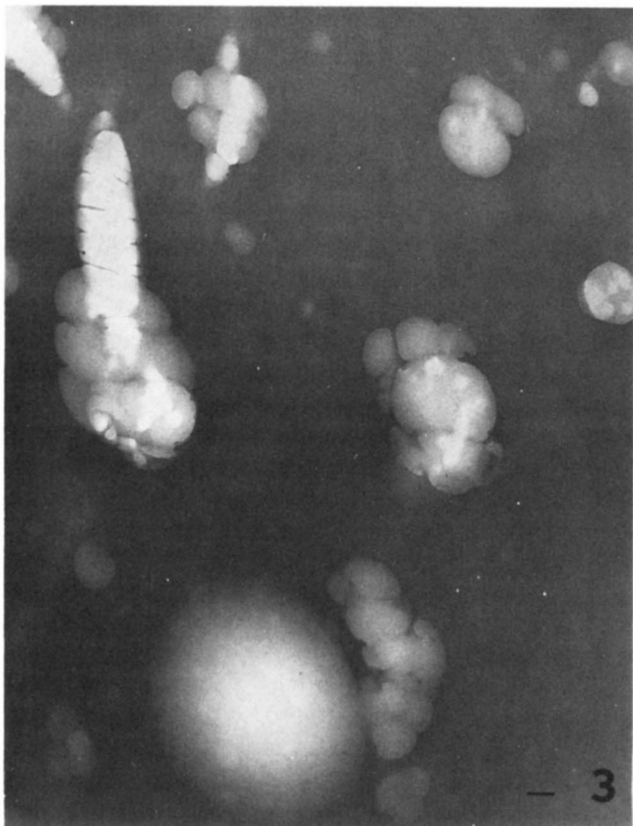

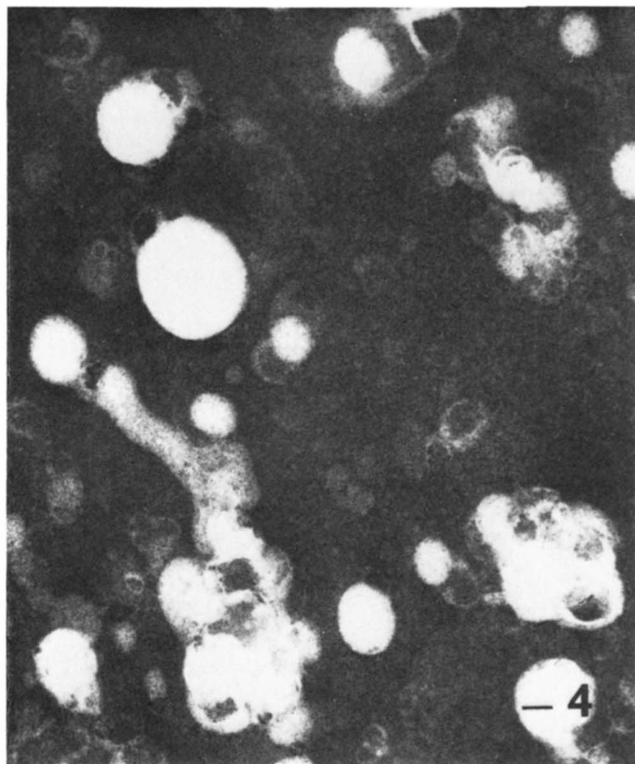

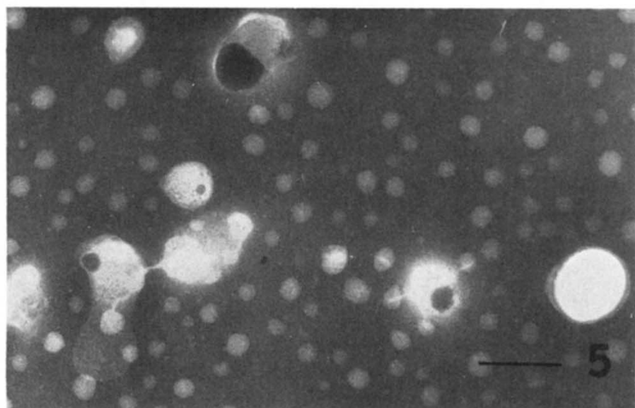

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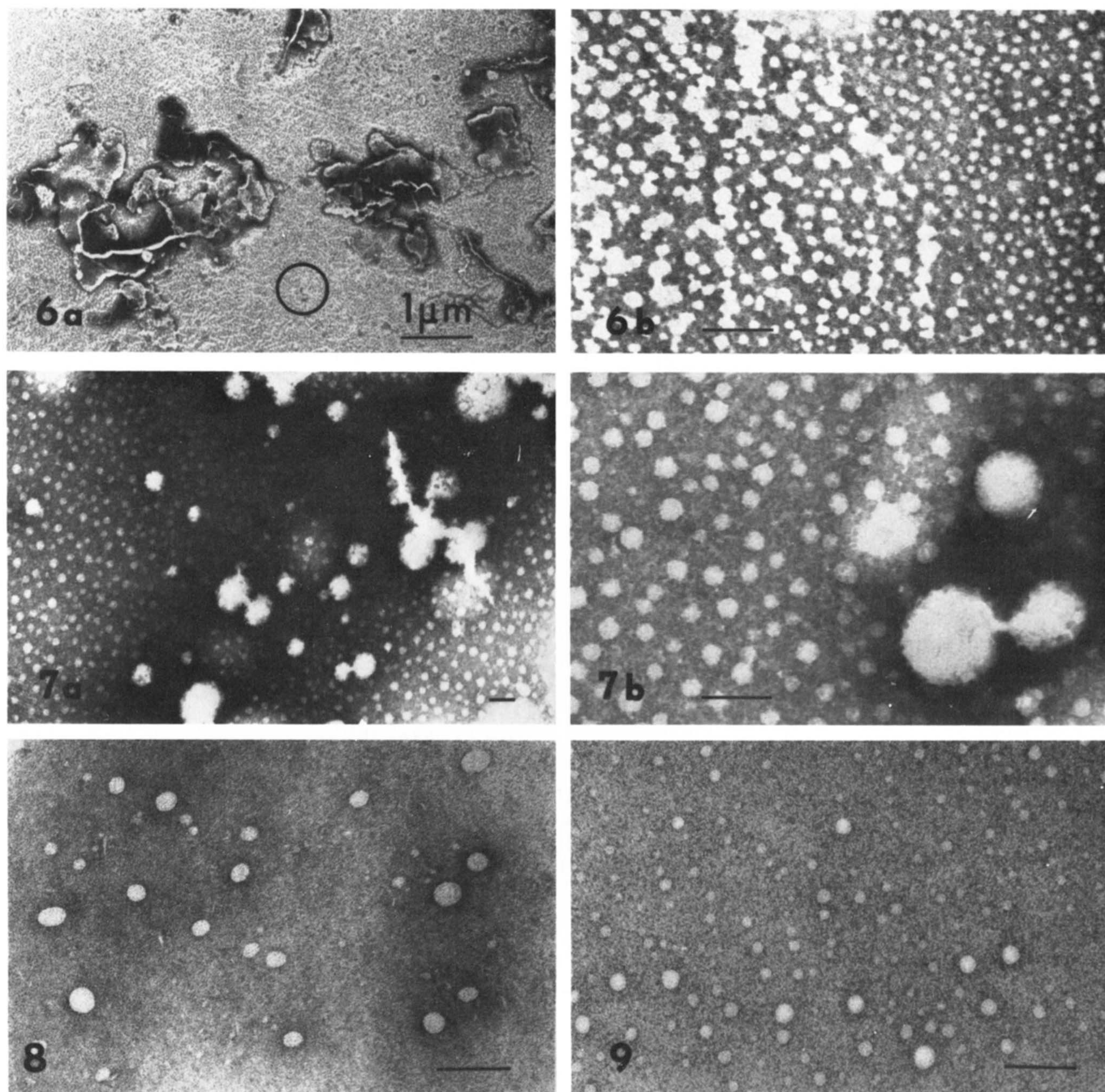

Fig. 6a. Fraction of density 1.006–1.019 g/ml (IDL). Low magnification view of specimen to show extensive areas of flattened surface film and surrounding areas containing small particles. ×11,000. **Fig. 6b.** Higher magnification of specimen illustrated in Fig. 6a to show details of an area containing only particles ranging in diameter from 150 to 400 Å. ×107,000. **Fig. 7a.** Fraction of density 1.019–1.063 g/ml (LDL). Note numerous small particles and large polymorphic remnants similar to those noted in the incubation mixture prior to centrifugation (cf. Fig. 4). ×39,000. **Fig. 7b.** Higher magnification of area from specimen illustrated in Fig. 7a, showing many particles 200–400 Å in diameter and several larger (1,000–2,000 Å diameter) remnants. ×107,000. **Fig. 8.** Fraction of density 1.063–1.10 g/ml (HDL_{2b}) showing particles ranging from 100 to 400 Å in diameter. ×107,000. **Fig. 9.** Fraction of density 1.10–1.21 g/ml (HDL_{2a+3}), showing particles ranging from 100 to 350 Å in diameter. Many more particles are in the 100–200 Å size range than noted in the d 1.063–1.10 g/ml fraction. ×107,000. All specimens were diluted to an appropriate concentration and negatively stained with 2% sodium phosphotungstate. The bar marker represents 1,000 Å for all figures, and samples were taken from incubation 9 unless otherwise indicated. Incubation time for all figures was 60 min.

drase, 30,000; trypsin inhibitor, 20,100; and lactalbumin, 14,400) as well as purified apolipoprotein A-I, A-II, and C-III_2, were used to identify gel bands. In incubation 8, the B apoproteins within lipoproteins were also examined utilizing 3.5% acrylamide gels as previously described (44). In order to assess apolipoprotein

TABLE 2. Lymph chylomicron triglyceride fatty acid composition[a]

Fatty Acid	Percent of Total
14:0	1.9
14:1	0.8
16:0	23.4
16:1	3.3
18:0	12.5
18:1	35.3
18:2	13.3
18:3	3.3
20:3	2.2
22:6	0.5
Other	3.5

[a] Percentage composition.

radioactivity in lipoprotein fractions, gel bands were cut out, and the radioactivity was measured. The mean recovery of radioactivity from gels was 94.1 ± 2.2% of amount loaded. In addition, chylomicron preparations were tested for the presence of apoA-I, apoA-II, apoB, apoC-II, and apoC-III with monospecific antisera utilizing the technique of Ouchterlony (45).

RESULTS

Electron microscopy

The initial chylomicron suspension consisted primarily of particles ranging in size from 750–6000 Å in diameter typical of chylomicrons as reported in the literature (46) (**Fig. 1**). A small number of intermediate sized particles 400–750 Å in diameter were also routinely present in our preparations prior to the addition of albumin or lipoprotein lipase.

Samples of the incubation mixture taken as early as 1 min following the addition of lipoprotein lipase to chylomicrons showed finger-like projections extending from the surface of chylomicrons (**Fig. 2**). Further lipolysis increased the number of polymorphic particle profiles and collapsed surface films (**Figs. 3 and 4**). The incubation mixture viewed after significant lipolysis, showed a wide range of particle sizes, with very few typical chylomicrons, and numerous collapsed and contorted profiles, presumably representing the chylomicron "remnants" after removal of core triglyceride (Fig. 4). Occasional profiles from negatively stained fractions

TABLE 3. Lipid concentrations after incubation of lymph chylomicrons with and without lipoprotein lipase[a]

Experiment	Lipoprotein Fraction	Without Lipase: Triglyceride	Without Lipase: Cholesterol	Without Lipase: Phospholipid	With Lipase: Triglyceride	With Lipase: Cholesterol	With Lipase: Phospholipid
		mg/dl					
1	Chylomicrons	920 ± 34 (90.7)	25.6 ± 1.1 (89.8)	53.9 ± 3.1 (87.1)	131 ± 3 (41.9)	11.2 ± 1.1 (41.3)	20.9 ± 1.7 (37.9)
	VLDL	75 ± 2 (7.3)	1.7 ± 0.2 (6.0)	4.6 ± 0.5 (7.4)	99 ± 2 (28.7)	1.1 ± 0.2 (4.1)	1.5 ± 0.4 (2.7)
	IDL + LDL	11 ± 2 (1.1)	0.8 ± 0.1 (2.8)	1.5 ± 0.3 (2.4)	57 ± 1 (18.2)	8.7 ± 1.2 (32.0)	13.6 ± 1.8 (21.5)
	HDL	5 ± 1 (0.5)	0.3 ± 0.1 (1.0)	0.6 ± 0.2 (0.9)	22 ± 3 (7.0)	6.1 ± 0.9 (22.6)	11.8 ± 0.3 (21.5)
	1.21 B[b]	3 ± 0 (0.3)	0.1 ± 0.1 (0.4)	1.3 ± 0.3 (2.1)	13 ± 2 (4.2)	0.0 ± 0.0 (0.0)	7.3 ± 0.4 (13.2)
3	Chylomicrons	188 ± 8 (89.8)	5.4 ± 0.7 (88.5)	11.4 ± 0.3 (85.7)	4 ± 1 (12.9)	1.2 ± 0.2 (19.0)	1.6 ± 0.3 (14.3)
	VLDL	16 ± 2 (7.6)	0.5 ± 0.1 (8.2)	1.1 ±0.2 (8.3)	6 ± 2 (19.4)	0.9 ± 0.1 (14.3)	0.5 ± 0.2 (4.5)
	IDL	2 ± 1 (1.0)	0.1 ± 0.1 (1.6)	0.2 ± 0.1 (1.5)	6 ± 1 (19.4)	1.9 ± 0.2 (30.2)	1.2 ± 0.1 (10.7)
	LDL	2 ± 1 (1.0)	0.1 ± 0.0 (1.6)	0.1 ± 0.1 (0.7)	4 ± 0 (12.9)	1.1 ± 0.1 (17.5)	3.2 ± 0.4 (28.6)
	HDL_2	1 ± 0 (0.5)	0.0 ± 0.0 (0.0)	0.1 ± 0.0 (0.7)	4 ± 1 (12.9)	0.7 ± 0.2 (11.1)	1.8 ± 0.2 (12.5)
	HDL_3	1 ± 0 (0.5)	0.0 ± 0.0 (0.0)	0.1 ± 0.1 (0.7)	3 ± 0 (9.7)	0.5 ± 0.1 (7.9)	0.8 ± 0.1 (7.1)
	1.21 B[b]	0 ± 0 (0.0)	0.0 ± 0.0 (0.0)	0.3 ± 0.1 (2.1)	4 ± 1 (12.9)	0.0 ± 0.0 (0.0)	2.5 ± 0.3 (22.3)
4	Chylomicrons	269 ± 12 (91.2)	9.2 ± 0.7 (88.5)	18.3 ± 1.4 (87.6)	5 ± 1 (13.5)	2.0 ± 0.4 (18.5)	2.8 ± 0.5 (13.9)
	VLDL	20 ± 3 (6.8)	0.7 ± 0.2 (6.7)	1.6 ± 0.3 (7.7)	8 ± 2 (21.6)	1.6 ± 0.2 (14.8)	1.0 ± 0.3 (5.0)
	IDL	2 ± 1 (0.7)	0.2 ± 0.1 (1.9)	0.3 ± 0.2 (11.4)	7 ± 1 (18.9)	3.1 ± 0.1 (28.7)	2.1 ± 0.2 (10.4)
	LDL	2 ± 0 (0.7)	0.1 ± 0.1 (1.0)	0.1 ± 0.1 (0.4)	4 ± 1 (10.8)	1.8 ± 0.2 (16.7)	5.6 ± 0.1 (27.7)
	HDL_2	2 ± 1 (0.3)	0.0 ± 0.0 (0.0)	0.1 ± 0.0 (0.4)	5 ± 1 (13.5)	1.4 ± 0.3 (13.0)	2.5 ± 0.2 (12.4)
	HDL_3	2 ± 0 (0.3)	0.2 ± 0.1 (1.9)	0.0 ± 0.0 (0.0)	4 ± 0 (10.8)	0.9 ± 0.1 (8.3)	1.8 ± 0.4 (8.9)
	1.21 B[b]	0 ± 0 (0.0)	0.0 ± 0 (0.0)	0.5 ± 2 (2.3)	4 ± 1 (10.8)	0.0 ± 0.0 (0.0)	4.4 ± 0.5 (21.8)
7	d < 1.006 g/ml	405 ± 11 (97.6)	11.4 ± 1.2 (95.0)	22.9 ± 1.7 (94.6)	4 ± 1 (18.2)	1.8 ± 0.3 (15.5)	1.1 ± 0.2 (15.1)
	IDL	4 ± 2 (1.0)	0.2 ± 0.1 (1.7)	0.5 ± 0.2 (2.1)	5 ± 2 (2.7)	2.0 ± 0.2 (17.2)	2.6 ± 0.2 (12.1)
	LDL	3 ± 2 (0.7)	0.2 ± 0.1 (1.7)	0.1 ± 0.1 (0.4)	8 ± 1 (36.4)	4.3 ± 0.1 (37.1)	4.6 ± 0.1 (21.5)
	HDL	2 ± 1 (0.5)	0.1 ± 0.1 (0.8)	0.2 ± 0.1 (0.8)	3 ± 1 (13.6)	3.4 ± 0.3 (29.3)	5.5 ± 0.3 (25.7)
	1.21 B[b]	1 ± 1 (0.2)	0.1 ± 0.0 (0.8)	0.5 ± 0.1 (2.1)	2 ± 1 (9.1)	0.1 ± 0.1 (10.9)	7.6 ± 0.4 (35.5)

[a] Incubations were carried out with and without lipoprotein lipase for 60 min at 37°C, values are given as mean ± SEM of analyses done in triplicate, and numbers in parentheses indicate percentages of individual constituents among lipoproteins.
[b] 1.21 B is the 1.21 g/ml infranate. The percent lipolysis in experiments 1, 3, 4, and 7 in the presence of LPL was 69.0%, 85.2%, 87.5%, and 94.6%, respectively.

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TABLE 4. Lipid concentrations after incubation of lymph chylomicrons with and without lipoprotein lipase[a]

Experiment	Lipoprotein Fraction	Triglyceride	Unesterified Cholesterol	Esterified Cholesterol	Phospholipid
			mg/dl		
Without lipase					
8	d < 1.006 g/ml	411 ± 5 (98.2)	5.4 ± 0.2 (94.8)	8.2 ± 0.3 (95.5)	25.9 ± 1.0 (94.2)
9	d < 1.006 g/ml	392 ± 10 (97.4)	5.1 ± 0.2 (95.2)	8.1 ± 0.2 (94.8)	24.4 ± 0.8 (94.9)
With lipase					
8	d < 1.006 g/ml	30 ± 1 (34.9)	1.0 ± 0.1 (20.4)	1.6 ± 0.1 (19.8)	4.5 ± 0.5 (17.8)
	IDL	15 ± 1 (17.4)	0.6 ± 0.1 (12.2)	1.1 ± 0.2 (13.6)	2.1 ± 0.3 (8.3)
	LDL	14 ± 2 (16.3)	1.4 ± 0.2 (28.6)	2.3 ± 0.2 (28.4)	7.6 ± 0.4 (30.0)
	HDL_{2b}	11 ± 0 (12.8)	0.5 ± 0.2 (10.2)	0.7 ± 0.1 (8.6)	4.6 ± 0.2 (18.2)
	HDL_{2a+3}	14 ± 1 (16.3)	1.3 ± 0.3 (26.5)	2.3 ± 0.2 (28.4)	5.7 ± 0.8 (22.5)
	1.21 B	2 ± 1 (2.3)	0.1 ± 0.1 (2.1)	0.1 ± 0.1 (1.2)	0.8 ± 0.3 (3.2)
9	d < 1.006 g/ml	16 ± 1 (29.1)	0.9 ± 0.1 (19.7)	1.4 ± 0.1 (17.7)	3.5 ± 2.5 (14.9)
	IDL	9 ± 2 (16.4)	0.6 ± 0.2 (12.8)	0.9 ± 0.3 (11.4)	1.9 ± 0.2 (8.1)
	LDL	11 ± 0 (20.0)	1.5 ± 0.1 (31.9)	2.5 ± 0.2 (31.6)	7.8 ± 0.4 (33.2)
	HDL_{2b}	12 ± 2 (21.8)	0.4 ± 0.1 (8.5)	0.6 ± 0.1 (7.6)	4.7 ± 0.6 (20.0)
	HDL_{2a+b}	6 ± 1 (10.9)	1.3 ± 0.2 (27.7)	0.3 ± 0.3 (29.1)	5.0 ± 0.8 (21.3)
	1.21 B	1 ± 0 (1.8)	0.0 ± 0.0 (0.0)	0.2 ± 0.1 (2.6)	0.6 ± 0.1 (2.5)

[a] Incubations were carried out with and without lipase for 60 min at 37°C; values are given as mean ± SEM, and numbers in parentheses indicate percentages.

suggested the fragmentation of lipolyzed chylomicrons into smaller particles (**Fig. 5**). None of these alterations were observed in the absence of lipase.

The VLDL, IDL, LDL, HDL, and density >1.21 g/ml fractions were examined ultrastructurally. Some important differences between the enzyme-treated and control fractions could be noted, and these seemed to correlate well with our biochemical finding that the apoproteins and lipids from the LPL-digested chylomicrons were present in the d 1.019–1.063, 1.063–1.21, and >1.21 g/ml fractions (Tables 3, 4). In particular, the chylomicron and VLDL fractions were found to lose most of their larger (500–4,000 Å) particles during enzyme incubation (Fig. 5), large surface remnants were noted in the d 1.006–1.019 g/ml (IDL) fraction (**Fig. 6a**), and many small particles appeared in the d 1.006–1.019 g/ml (IDL) (Fig. 6b), 1.019–1.063 g/ml (LDL), (**Fig. 7a, b**), and 1.063–1.21 g/ml (HDL) (**Figs. 8 and 9**) fractions. The exact distribution and appearance of these larger particles varied somewhat, depending upon the degree of triglyceride lipolysis in a given preparation.

The LDL fraction from lipolyzed chylomicrons contained large irregularly shaped particles sometimes more than 1,000 Å in diameter in addition to smaller particles 150–400 Å in diameter (Figs. 7a, b). Occasional profiles in this fraction were suggestive of large chylomicron remnants.

Following lipolysis, particles in the d 1.063–1.21 g/ml (HDL) fraction ranged from 100–400 Å in diameter. When the HDL fraction from lipolyzed chylomicrons was subdivided into HDL_{2b} (d 1.063–1.10 g/ml) and HDL_3 (d 1.10–1.21 g/ml) fractions, a gradient in particle size from larger at the lower density to smaller at the higher density was noted, but the overall size of the particles in these fractions was seen to be larger than would be expected of normally occurring plasma lipoprotein particles (70–100 Å in diameter) in these same density ranges (44) (Figs. 8, 9).

The density >1.21 g/ml fractions contained primarily albumin and, following lipolysis, fatty acids attached to albumin. Negatively stained fractions of this density from control preparations lacking enzyme and from enzyme-treated chylomicrons were morphologically indistinguishable and contained no particulate components when Miles human fatty acid-free albumin (following filtration through a 0.2-micron filter) was used as the fatty acid acceptor. The bovine albumin fraction V from Armour was found to contain a particulate component and was therefore unsatisfactory for ultrastructural visualization of the fractions.

Fatty acid analysis

The fatty acid analysis of human lymph chylomicron triglyceride utilized in incubations 8 and 9 is given in **Table 2**, and the mean polyunsaturated:saturated fat ratio was 0.51. The percentage of cholesterol in the unesterified form in these chylomicrons was 40.0%.

Distribution of lipid mass

Lymph chylomicrons utilized in this study had a mean (±SD) composition (weight percent) of 91.4 ± 1.1% triglyceride, 5.0 ± 0.8% phospholipid, 2.7 ± 0.9% cholesterol, and 0.9 ± 0.3% protein. The mean phospho-

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lipid:protein ratio in chylomicrons was 5.6. When lymph chylomicrons were incubated with albumin in the absence of LPL, 95% or more of the triglyceride, phospholipid, and cholesterol mass remained in the chylomicron and VLDL fractions (**Table 3**). When chylomicrons were incubated in the presence of LPL, a significant decrease in triglyceride occurred and there was a redistribution of the remaining triglyceride as well as the cholesterol and phospholipid components to IDL, LDL, HDL, and the 1.21 g/ml infranate. As lipolysis proceeded to completion, over 80% of the cholesterol and phospholipid was no longer found in the chylomicron or VLDL fraction, but rather in other lipoprotein density regions (see Table 3). Similar observations were made utilizing chylomicrons that were never brought below 30°C prior to incubation. Chylomicron unesterified cholesterol appeared to be distributed to similar density fractions as esterified cholesterol (see **Table 4**).

Distribution of apolipoprotein mass and radioactivity

ApoA-I, apoA-II, and apoC-II mass could be detected only in chylomicrons and VLDL in incubations without lipase (see **Table 5**). However when LPL was added to the incubation mixture, significant fractions of these apolipoproteins were transferred to LDL, HDL, and the 1.21 g/ml infranate. ApoA-II appeared to dissociate from chylomicrons during lipolysis more readily than apoA-I and apoC-II. In addition, apoA-I was as likely to transfer to HDL_{2b} as to HDL_{2a+3}, while a higher fraction of apoA-II was transferred to HDL_{2a+3}.

Purified apolipoproteins A-I, A-II, and C-III_2 utilized for radioiodinations and incubation studies as run on TMU PAGE are shown in **Fig. 10**. Transfer of apoprotein radioactivity was assessed utilizing TMU, 10% and 3.5% acrylamide SDS PAGE (Figs. 10–12). When radioiodinated apoA-I- and apoA-II-labeled chylomicrons were incubated in the absence of LPL, almost all of the apoA-I and apoA-II radioactivity remained in the d < 1.006 g/ml fraction (**Table 6**). When lipase was added to the incubation mixture, over 80% of apoA-I radioactivity and over 85% of apoA-II radioactivity were transferred to IDL, LDL, HDL, or the 1.21 g/ml infranate (Table 6). Similar differences between apoA-I and apoA-II redistribution with lipolysis were observed with radioactivity as previously observed with mass. A greater fraction of apoA-I radioactivity tended to remain with chylomicrons or to be transferred to HDL_{2b}, than was observed for apoA-II. A significant redistribution of apoC-III_2 radioactivity with lipolysis was seen in experiments performed with ^{125}I-labeled apoC-III_2-labeled chylomicrons. Without lipase, 94.6% of the apoC-III_2 radioactivity was found in the chylomicron and VLDL fraction; with LPL, 32.7% of the

TABLE 5. Distribution of lymph chylomicron apolipoproteins among lipoprotein fractions

Experiment	Lipoprotein Fraction	Without Lipase			With Lipase		
		ApoA-I	ApoA-II	ApoC-II	ApoA-I	Apo-A-II	ApoC-II
		mg/dl					
1	Chylomicrons	0.48 ± 0.05 (85.7)	—[a]	—	0.13 ± 0.02 (22.8)	—	—
	VLDL	0.08 ± 0.01 (14.3)	—	—	ND	—	—
	IDL + LDL	ND[b]	—	—	0.08 ± 0.01 (14.0)	—	—
	HDL	ND	—	—	0.36 ± 0.05 (63.2)	—	—
	1.21 B	ND	—	—	ND	—	—
8	1.006 T	0.58 ± 0.3 (100.0)	0.32 ± 0.01 (100.0)	2.14 ± (100.0)	0.10 ± 0.03 (16.4)	0.03 ± 0.01 (8.3)	0.43 ± 0.03 (21.1)
	IDL	ND	ND	ND	ND	ND	0.16 ± 0.01 (7.8)
	LDL	ND	ND	ND	0.04 ± 0.01 (6.6)	ND	0.10 ± 0.01 (4.9)
	HDL_{2b}	ND	ND	ND	0.24 ± 0.03 (39.3)	0.11 ± 0.02 (30.5)	0.42 ± 0.02 (20.6)
	HDL_{2a+b}	ND	ND	ND	0.23 ± 0.04 (37.7)	0.22 ± 0.03 (61.1)	0.93 ± 0.03 (45.6)
	1.21 B	ND	ND	ND	ND	ND	ND
9	1.006 T	0.64 ± 0.04 (100.0)	0.28 ± 0.02 (100.0)	1.93 ± (100.0)	0.07 ± 0.01 (11.9)	ND	0.30 ± 0.02 (15.0)
	IDL	ND	ND	ND	ND	ND	0.09 ± 0.01 (4.5)
	LDL	ND	ND	ND	ND	ND	0.06 ± 0.01 (3.0)
	HDL_{2b}	ND	ND	ND	0.23 ± 0.03 (39.0)	0.23 ± 0.02 (37.7)	0.37 ± 0.03 (18.5)
	HDL_{2a+3}	ND	ND	ND	0.25 ± 0.04 (42.4)	0.38 ± 0.02 (62.3)	1.06 ± 0.04 (53.0)
	1.21 B	ND	ND	ND	0.04 ± 0.01 (6.8)	ND	0.12 ± 0.01 (6.0)

[a] —, indicates interference of albumin with assay.
[b] ND, not detectable.

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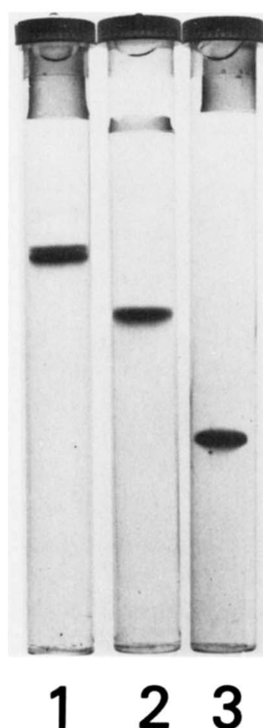

Fig. 10. Purified apoA-I (1), apoA-II (2), and apoC-III_2 (3) are shown as run on TMU PAGE.

radioactivity was found in LDL, 32.3% in HDL, and 21.5% in the 1.21 g/ml infranate (see **Table 7**).

When lymph chylomicrons were radiolabeled with ^{125}I, the distribution of apolipoprotein label was as follows: apoB 4.5 ± 0.3%, apoA-IV and apoH 19.2 ± 0.9%, apoE 1.4 ± 0.3%, apoA-I 15.2 ± 0.8%, apoA-II 8.1 ± 0.4%, apoC-II 7.4 ± 1.0%, apoC-III 42.3 ± 1.4%, and albumin 1.9 ± 0.4%. The distribution of apolipoprotein radioactivity among lipoprotein fractions with and without lipolysis for radiolabeled chylomicrons is shown in **Table 8**. The presence of apolipoproteins A-I, A-II, C-II, C-III, B, A-IV, and H was documented in isolated lymph chylomicrons prior to the addition of albumin both on SDS PAGE as well as in Ouchterlony plates (see **Figs. 11 and 12**). Without lipolysis over 94% of apoA-I, apoA-II, apoB, apoC-II, and apoC-III radioactivity remained in the chylomicron and VLDL density fractions. The addition of lipase resulted in the transfer of about 75% of apoA-I and apoA-II radioactivity to HDL and the 1.21 g/ml infranate. In contrast, apoB radioactivity was transferred mainly to IDL and LDL with lipolysis (see Table 8). It is of interest that apoB in lymph chylomicrons had a molecular weight slightly lower than most of the apoB in plasma VLDL, although plasma VLDL contained a

TABLE 6. Radiolabeled apoA-I and apoA-II lymph chylomicron radioactivity distribution among lipoprotein fractions[a]

Experiment	Lipoprotein Fraction	Without Lipoprotein Lipase		With Lipoprotein Lipase	
		ApoA-I	ApoA-II	Apo-A-I	ApoA-II
1	Chylomicrons	79.8 ± 2.4	85.3 ± 2.1	17.9 ± 0.9	6.0 ± 0.4
	VLDL	15.7 ± 1.9	12.7 ± 1.2	1.6 ± 0.7	0.3 ± 0.2
	IDL + LDL	1.2 ± 0.9	0.8 ± 0.4	16.7 ± 1.2	3.8 ± 0.8
	HDL	2.7 ± 0.4	0.9 ± 0.3	62.0 ± 2.7	84.8 ± 3.6
	1.21 B	0.6 ± 0.3	0.3 ± 0.2	1.8 ± 0.8	5.1 ± 1.6
3	Chylomicrons	76.7 ± 2.3	83.8 ± 1.7	8.7 ± 1.0	2.3 ± 0.8
	VLDL	11.8 ± 1.1	7.2 ± 1.0	7.7 ± 0.8	2.1 ± 0.6
	IDL	0.3 ± 0.2	0.3 ± 0.3	9.9 ± 1.1	4.7 ± 0.9
	LDL	2.1 ± 1.0	1.1 ± 0.9	10.6 ± 1.4	3.7 ± 1.4
	HDL_2	1.2 ± 0.5	2.5 ± 1.0	25.4 ± 1.5	32.2 ± 2.1
	HDL_3	2.9 ± 1.0	2.4 ± 0.3	13.6 ± 0.9	41.6 ± 1.2
	1.21 B	5.0 ± 0.9	2.7 ± 0.5	24.1 ± 1.7	13.4 ± 1.3
6	d < 1.006 g/ml	87.2 ± 2.2	92.4 ± 1.9	9.7 ± 1.7	3.2 ± 0.3
	IDL	0.4 ± 0.2	0.2 ± 0.1	4.9 ± 0.8	1.1 ± 0.7
	LDL	2.7 ± 0.5	1.7 ± 0.4	9.0 ± 0.7	2.2 ± 0.9
	HDL	3.7 ± 0.7	2.3 ± 0.6	49.8 ± 2.1	78.8 ± 3.1
	1.21 B	6.0 ± 1.1	3.4 ± 0.2	26.6 ± 1.3	14.7 ± 1.2
9	d < 1.006 g/ml	89.5 ± 1.8	93.1 ± 1.1	14.5 ± 2.1	3.1 ± 0.2
	IDL	0.2 ± 0.0	0.1 ± 0.1	1.4 ± 0.2	1.9 ± 0.4
	LDL	0.8 ± 0.1	0.2 ± 0.1	4.9 ± 0.6	3.0 ± 0.4
	HDL_{2b}	2.3 ± 0.4	2.4 ± 0.4	31.4 ± 1.0	32.4 ± 1.1
	HDL_{2a+3}	3.1 ± 0.8	2.0 ± 0.3	35.0 ± 2.1	55.1 ± 2.0
	1.21 B	4.1 ± 0.3	2.2 ± 0.4	12.8 ± 0.9	4.5 ± 0.2

[a] All values are given as means ± SEM. Incubations were carried out with and without lipoprotein lipase for 60 min at 37°C. 1.21 B is the 1.21 g/ml infranate. The percent lipolysis in experiments 1, 3, 6, and 9 in the presence of LPL was 69.0%, 85.2%, 94.0%, and 85.4%, respectively.

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TABLE 7. ^{125}I-Labeled apoC-III_2 lymph chylomicron radioactivity distribution among lipoprotein fractions[a]

Lipoprotein Fraction	^{125}I-Labeled ApoC-III_2 Percent of Total Radioactivity	
	Without Lipase[b]	With Lipase[b]
Chylomicrons	86.5 ± 1.7	0.8 ± 0.7
VLDL	8.1 ± 0.9	3.4 ± 1.2
IDL	0.0 ± 0	1.9 ± 1.2
LDL	0.0 ± 0	32.7 ± 1.4
HDL_2	2.6 ± 1.0	14.0 ± 2.1
HDL_3	0.0 ± 0	18.3 ± 1.8
1.21 B	3.4 ± 0.3	21.5 ± 2.0

[a] All values are given as means ± SEM. The data were derived from incubation 4. The percent lipolysis in the presence of lipase was 87.5%. 1.21 B is the 1.21 g/ml infranate.

[b] Incubated with and without lipoprotein lipase for 60 min at 37°C.

molecular weight form identical to chylomicron apoB (see Fig. 11). Of note was the fact that 86.9 ± 3.7% of the apoB radioactivity transferred to LDL was in the form of apoB-48, as assessed by 3.5% acrylamide SDS gels (44) in incubation 8. ApoC-II and apoC-III radioactivity was found mainly in LDL, HDL, and 1.21 g/ml infranate in incubations with LPL present as shown in Table 8.

DISCUSSION

The purpose of the present investigation was to define the products of chylomicron lipolysis following in vitro exposure to lipoprotein lipase utilizing human lymph chylomicrons. Our own electron microscopic data are similar to that reported for rat chylomicron morphologic changes by Blanchette-Mackie and Scow (47, 48). In both studies lipolysis resulted in finger-like projections extending from the chylomicron surface (Fig. 2) within 5 min after the addition of lipase, and the subsequent formation of particles with redundant surfaces, collapsed particles, particles with water spaces, and particle fragmentation into smaller particles (Figs. 2–5). None of these alterations were observed in the absence of lipase, or in chylomicrons prior to the addition of lipase and albumin (Fig. 1). The finger-like projections observed may represent fatty acid formation on the surface of chylomicrons prior to uptake by albumin molecules, as such configurations are mainly noted when limited amounts of albumin were used, as in incubation 1. Depletion of triglyceride core of chylomicrons after a significant amount of lipolysis has occurred appears to result in collapsed particles with redundant surface and water spaces, as well as fragmentation into smaller particles. The large particles with redundant surface were found mainly in VLDL, IDL, and LDL density regions, while smaller particles, produced by fragmentation or budding during lipolysis, were observed mainly in the LDL and HDL density regions (Figs. 6a–9). Similar lipoprotein morphologic alterations within IDL, LDL, and HDL have been observed following the injection of chylomicrons into rats (22, 23), with in vitro incubation of VLDL and lipoprotein lipase (49), and following 10-min heparin-induced lipolysis in human subjects (50).

Significant amounts of albumin must be added to incubations of triglyceride-rich lipoproteins with lipopro-

TABLE 8. ^{125}I-Labeled lymph chylomicron radioactivity distribution among lipoprotein fractions[a]

Lipoprotein Fraction	Distribution of Radioactivity				
	ApoA-I	ApoA-II	ApoB	ApoC-II	ApoC-III
			%		
Without lipase					
d < 1.006 g/ml	94.2 ± 4.1	95.6 ± 2.8	95.8 ± 2.5	96.5 ± 1.5	97.6 ± 4.7
IDL	0.0 ± 0.0	0.4 ± 0.2	3.3 ± 1.1	0.4 ± 0.2	0.3 ± 0.2
LDL	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
HDL	0.6 ± 0.2	0.6 ± 0.3	0.2 ± 0.1	1.7 ± 0.3	1.1 ± 0.4
1.21 B	5.1 ± 1.0	3.2 ± 0.7	0.3 ± 0.2	1.1 ± 0.4	0.6 ± 0.1
With lipase					
d < 1.006 g/ml	15.1 ± 2.1	13.3 ± 2.1	27.6 ± 1.9	6.1 ± 2.1	8.9 ± 1.3
IDL	4.7 ± 1.4	3.3 ± 0.6	17.3 ± 2.1	7.4 ± 1.7	4.3 ± 0.8
LDL	9.2 ± 1.1	6.4 ± 1.1	55.0 ± 4.1	31.9 ± 1.1	31.5 ± 2.3
HDL	41.8 ± 4.6	59.1 ± 4.1	4.1 ± 1.0	28.4 ± 2.2	29.4 ± 1.6
1.21 B	29.2 ± 2.7	17.6 ± 2.6	0.8 ± 0.6	26.2 ± 1.9	25.9 ± 2.1

[a] Values are given as mean ± SEM. Incubations were carried out with and without lipoprotein lipase for 60 min at 37°C. 1.21 B is the 1.21 g/ml infranate. The fraction d > 1.006 g/ml represents both chylomicrons and VLDL. ApoA-I and apoB radioactivity were determined on SDS PAGE, and apoA-II, apoC-II, and apoC-III radioactivity were determined on TMU PAGE. All analyses were performed in triplicate. The data were derived from incubation 7. Similar results were obtained in incubations 5 and 8 (data not shown). The percent lipolysis in the presence of lipase was 94.6%.

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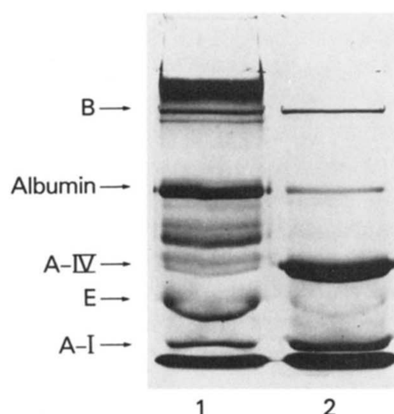

Fig. 11. Plasma VLDL (1) and lymph chylomicrons (2) are shown as run on 10% acrylamide, 1% bis SDS PAGE. Gel #2 documents the presence of only one form of apoB, as well as albumin, trace amounts of apoH, apoA-IV, trace amounts of E, apoA-I, and the C apolipoproteins.

tein lipase in order for lipolysis to occur. Albumin may represent a confusing component in these types of experiments. Deckelbaum, Olivecrona, and Fainaru (51) described the interaction of apoprotein found in several commercial preparations of albumin with the products of VLDL lipolysis, resulting in significant shifts in the proportion of VLDL-derived phospholipids and apoproteins found in HDL. The bovine albumin (Armour) utilized in incubations 1–7 was found not only to contain phospholipid, but to be particulate in solution when examined by electron microscopy. In addition, the use of this albumin appeared to interfere with our immunoassays of apolipoproteins in various density fractions. When human lipid-free and apolipoprotein-free albumin solution was used following filtration through a 0.2-micron filter, no particles were observed and no interference with the immunoassays was noted (incubations 8, 9). Use of either albumin preparation did not appear to affect lipid redistribution which occurred with lipolysis.

Because of a recent report that cooling chylomicrons isolated from monkeys fed saturated fat diets results in crystallization of triglyceride in the core of the particulate at 16–19°C (41), we carried out incubations 8 and 9 utilizing chylomicrons collected at 37°C, and isolated at 31°C. These particles were never brought down below 31°C prior to use in incubations. The chylomicrons were isolated from the thoracic duct lymph of patients who were on a regular diet (polyunsaturated fat:saturated fat (P/S) ratio of 0.44), and the mean triglyceride P/S ratio in the chylomicron triglyceride was 0.51. The results of incubations with these particles (Tables 4, 5) were similar to those obtained with particles isolated at 4°C (Tables 3, 5). Similar morphologic alterations with lipolysis were also noted. Temperature-dependent differences may not have been observed in the present study because the chylomicrons utilized were from patients on regular diets rather than saturated fat diets.

In vitro incubation studies with VLDL and lipoprotein lipase have documented the transfer of VLDL apolipoprotein (apoB, apoC-II, and apoC-III) radioactivity as well as lipid constituents (cholesterol and phospholipid) to IDL, LDL, HDL, and d > 1.21 g/ml density fractions (49, 52–54). The data indicated that with lipolysis VLDL-apoB radioactivity was transferred mainly to IDL and LDL, while C apolipoprotein radioactivity was found mainly in HDL and the 1.21 g/ml infranate. Of interest was the fact that these transfers occurred in the absence of LDL or HDL, suggesting that these latter particles did not need to be present to serve as "acceptors" for the products of lipolysis (49). In addition, these in vitro observations were consistent with human in vivo metabolic data (55–59).

Lymph chylomicrons differ from plasma VLDL in that they contain significant amounts of apoA-I and apoA-II (10, 13), and the apoB in chylomicrons appears to have a lower molecular weight than the major form of apoB found in plasma VLDL or LDL (44). Furthermore, lymph chylomicrons contain apoA-IV and apoH (B_2 glycoprotein-I), proteins which are generally not seen in significant amounts in normal plasma VLDL (13, 14). Both plasma VLDL and lymph chylomicrons contain apoC-I, apoC-II, and apoC-III. Previous in vivo studies in rats have documented the transfer of chylomicron phospholipid, cholesterol, and apo-A-I to HDL

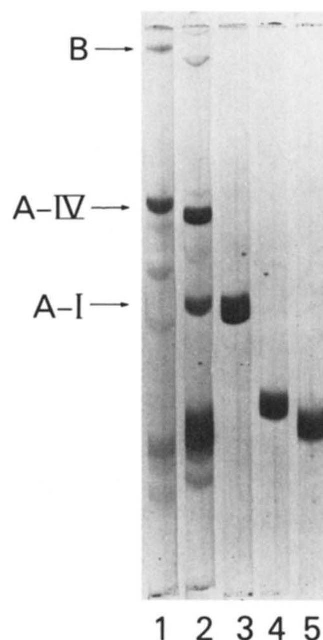

Fig. 12. Plasma VLDL (1), lymph chylomicrons (2), apoA-I (3), apoA-II (4), apoC-III_2 (5), are shown as run on 13% acrylamide, 0.5% diallytartardiamide, SDS PAGE.

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(22, 23). In vivo studies in man have also demonstrated that radiolabeled chylomicron apoA-I and apoA-II are rapidly transferred to HDL in plasma, and these apoproteins then have a similar kinetic behavior to HDL apoA-I and apoA-II (10).

In the present studies, human lymph chylomicrons were utilized for in vitro incubations with lipoprotein lipase. In the absence of lipase, almost all of chylomicron triglyceride, cholesterol, phospholipid, and apolipoproteins remained within the d < 1.006 g/ml density region. In the presence of LPL, the percent lipolysis of chylomicron triglyceride ranged between 69.0–94.6%. With almost complete lipolysis (94.6%), over 80% of the small amount of triglyceride remaining was found in IDL, LDL, HDL, or the 1.21 g/ml infranate, with the highest percentage being found in LDL. Similarly, a significant amount of chylomicron free and esterified cholesterol was transferred to IDL, LDL, and HDL, while most of the chylomicron phospholipid was transferred to LDL, HDL, and the 1.21 g/ml infranate.

The transfer of chylomicron apolipoproteins to various lipoprotein density fractions with lipolysis was assessed in three different ways in our experiments: *1)* actual measurement of apolipoprotein concentration (for apoA-I, apoA-II, and apoC-II); *2)* use of purified radioiodinated apolipoproteins reassociated with lymph chylomicrons by incubation and reisolation (for apoA-I, apoA-II, and apoC-III_2); and *3)* use of radioiodinated chylomicrons (for apolipoproteins A-I, A-II, B, C-II, and C-III). Similar results were obtained by all three methods when apoA-I and apoA-II redistributions were compared (see Tables 5, 6, and 8). With lipolysis, chylomicron apoA-I and apoA-II mass and radioactivity were transferred mainly to HDL and the 1.21 g/ml infranate, similar to what has previously been observed in vivo in both man and the rat (10, 22, 23). The present studies, however, were carried out in the absence of HDL, and are consistent with the concept that acceptor particles need not be present for the transfer of chylomicron lipid and apolipoprotein to HDL. In addition, apoA-I was not as readily removed from chylomicrons and was more likely to be transferred to the HDL_{2b} density region than apoA-II was. If a similar phenomenon takes place in vivo, it may account for the higher apoA-I:apoA-II ratio observed in HDL_2 than in HDL_3 (60).

As previously observed in in vitro studies with VLDL and lipoprotein lipase (49, 52), chylomicron C-II mass and radioactivity, and chylomicron C-III radioactivity were transferred to LDL, HDL, and the 1.21 g/ml infranate, while apoB was transferred mainly to IDL and LDL. Most of lymph chylomicron apoB is comprised of apoB-48, while plasma LDL apoB in normal man is composed of a higher molecular weight apoB species (apoB-100) (44). Recently it has been shown in the rat that over 95% of chylomicron apoB-48 radioactivity is removed from the circulation prior to its conversion to LDL (61). These data are consistent with the concept that almost all of LDL apoB is derived from non-chylomicron sources. However, in our in vitro studies, chylomicron apoB-48 can be transferred to LDL, presumably because of the absence of liver uptake. It has been suggested that chylomicron remnants after in vivo lipolysis contain mainly apoB-48 and apoE, and that these particles are taken up by the liver via an apoE receptor (62–64). Indeed, in patients with type III hyperlipoproteinemia due to apoE deficiency, there is accumulation of apoB-48 and apoA-IV within IDL and LDL (65).

The data presented are consistent with the concept that chylomicron protein and lipid constituents following lipolysis in vitro can serve as precursors for these constituents within LDL and HDL, and that this transfer does not require the presence of "acceptor" lipoproteins.

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REFERENCES

1. Green, P. H. R., and R. M. Glickman. 1981. Intestinal lipoprotein metabolism. *J. Lipid Res.* **22:** 1153–1173.
2. Zilversmit, D. B. 1965. The composition and structure of lymph chylomicrons in dog, rat, and man. *J. Clin. Invest.* **44:** 1610–1625.
3. Alaupovic, P., R. H. Furman, W. H. Falor, M. L. Sullivan, S. L. Walraven, and A. C. Olson. 1968. Isolation and characterization of human chyle chylomicrons and lipoproteins. *Ann. NY Acad. Sci.* **149:** 791–807.
4. Glickman, R. M., and P. H. R. Green. 1977. The intestine as a source of apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA.* **74:** 2569–2573.
5. Imaizumi, K., R. J. Havel, M. Fainaru, and J. Vigne. 1978. Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats. *J. Lipid Res.* **19:** 1038–1046.
6. Schonfeld, G., E. Bell, and D. H. Alpers. 1978. Intestinal apoproteins during fat absorption. *J. Clin. Invest.* **61:** 1539–1550.
7. Wu, A. L., and H. G. Windmueller, 1978. Identification

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of circulating apolipoproteins synthesized by rat small intestine in vitro. *J. Biol. Chem.* **253:** 2525–2528.
8. Havel, R. I., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
9. Kostner, G., and A. Holasek. 1972. Characterization and quantitation of the apolipoproteins from chyle chylomicrons. *Biochemistry.* **11:** 1217–1223.
10. Schaefer, E. J., L. L. Jenkins, and H. B. Brewer, Jr. 1978. Human chylomicron apolipoprotein metabolism. *Biochem. Biophys. Res. Commun.* **80:** 405–412.
11. LaRosa, J. C., R. I. Levy, P. N. Herbert, S. E. Lux, and D. S. Frederickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41:** 57–62.
12. Havel, R. J., C. J. Fielding, T. Olivecrona, V. G. Shore, P. E. Fielding, and T. Egelrud, 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoprotein lipase from different sources. *Biochemistry.* **12:** 1828–1833.
13. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the d < 1.006 g/ml lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
14. Polz, E., and G. M. Kostner. 1979. The binding of β_2-glycoprotein-I to human serum lipoproteins. *FEBS Lett.* **102:** 183–186.
15. Polz, E., and G. M. Kostner. 1979. Binding of β_2-glycoprotein-I to intralipid: determination of the dissociation constant. *Biochem. Biophys. Res. Commun.* **90:** 1305–1312.
16. Nakaya, Y., E. J. Schaefer, and H. B. Brewer, Jr. 1980. Activation of human post-heparin lipoprotein lipase by apolipoprotein H (β_2-glycoprotein I). *Biochem. Biophys. Res. Commun.* **95:** 1168–1172.
17. Higgins, J. M., and C. J. Fielding. 1975. Mechanism of formation of triglyceride-rich remnant particles from very low density lipoproteins and chylomicrons. *Biochemistry.* **14:** 2288–2293.
18. Scow, R. O., E. J. Blanchette-Mackie, and L. C. Smith, 1980. Transport of lipid across capillary endothelium. *Federation Proc.* **39:** 2610–2617.
19. Nestel, P. J., R. J. Havel, and A. Bezman. 1963. Metabolism of constituent lipids of dog chylomicrons. *J. Clin. Invest.* **42:** 1313–1321.
20. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
21. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–473.
22. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
23. Tall, A. R., P. H. R. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apoproteins in the rat. *J. Clin. Invest.* **64:** 977–989.
24. Bengtsson, G., and T. Olivecrona. 1977. Interaction of lipoprotein lipase with heparin-Sepharose. Evaluation of conditions for affinity binding. *Biochem. J.* **167:** 109–120.
25. Church, C. F., and H. N. Church. 1975. Food Values of Portions Commonly Used. Lippincott, Philadelphia, Pa.
26. Brewer, H. B., Jr., S. E. Lux, R. Ronan, and K. M. John. 1972. Amino acid sequence of human apoGln-II (apoA-II), an apolipoprotein isolated from the high density lipoprotein complex. *Proc. Natl. Acad. Sci. USA.* **69:** 1304–1308.
27. Shulman, R. S., P. N. Herbert, K. Wehrly, and D. S. Frederickson. 1975. The complete amino acid sequence of apoC-I (apoLP-Ser), an apolipoprotein from human very low density lipoproteins. *J. Biol. Chem.* **250:** 182–190.
28. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
29. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
30. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53.
31. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
32. Chalvardjian, A., and E. Rudnicki. 1970. Determination of lipid phosphorus in the nanomolar range. *Anal. Biochem.* **36:** 225–226.
33. Technicon Instruments. AutoAnalyzer Manual. 1965. Tarrytown, New York. 345.
34. Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
35. Wentz, P. W., R. E. Cross, and J. Sanory. 1976. An integrated approach to lipid profiling: enzymatic determination of cholesterol and triglyceride with a centrifugal analyzer. *Clin. Chem.* **22:** 88–194.
36. Dietschy, J. M., L. E. Weeks, and J. J. Delente. 1976. Enzymatic measurement of free and esterified cholesterol in plasma and other biological preparations using the oxygen electrode in a modified glucose analyzer. *Clin. Chim. Acta.* **73:** 407–412.
37. Rapport, M. M., and M. Alonzo. 1959. Photometric determination of long chain fatty acids in plasma and tissues. *J. Biol. Chem.* **217:** 195–198.
38. Curry, M. D., P. Alaupovic, and C. A. Suenram. 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassays. *Clin. Chem.* **22:** 315–322.
39. Goodnight, S. I., Jr., W. S. Harris, and W. E. Connor. 1981. The effect of dietary omega 3 fatty acids on platelet composition and function in man: a prospective controlled study. *Blood.* **58:** 880–885.
40. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
41. Parks, J. S., D. Atkinson, D. M. Small, and L. L. Rudel. 1981. Physical characterization of lymph chylomicra and very low density lipoproteins from nonhuman primates fed saturated dietary fat. *J. Biol. Chem.* **256:** 12992–12999.
42. Blum, C. B., R. I. Levy, S. Eisenberg, M. Hall, R. H. Goebel, and M. Berman. 1977. High density lipoprotein metabolism in man. *J. Clin. Invest.* **60:** 795–807.
43. Laemmli, U. K. 1970. Cleavage of structural proteins during the assemblage of the head of bacteriophage T_4. *Nature.* **227:** 680–685.

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44. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
45. Ouchterlony, O. 1968. Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor Science Publishers, Ann Arbor. 25–31.
46. Forte, T. M., and A. V. Nichols. 1972. Application of electron microscopy to the study of plasma lipoprotein structure. *Adv. Lipid Res.* **10:** 1–41.
47. Blanchette-Mackie, E. J., and R. O. Scow. 1973. Effect of lipoprotein lipase on the structure of chylomicrons. *J. Cell Biol.* **58:** 689–708.
48. Blanchette-Mackie, E. J., and R. O. Scow. 1976. Retention of lipolytic products in chylomicrons incubated with lipoprotein lipase; electron microscope study. *J. Lipid Res.* **17:** 57–67.
49. Glangeaud, M. C., S. Eisenberg, and T. Olivecrona. 1977. Very low density lipoprotein. Dissociation of apolipoprotein C during lipoprotein lipase induced lipolysis. *Biochim. Biophys. Acta.* **486:** 23–25.
50. Forte, T. M., R. M. Krauss, F. T. Lindgren, and A. V. Nichols. 1979. Changes in plasma lipoprotein distribution and formation of two unusual particles after heparin-induced lipolysis in hypertriglyceridemic subjects. *Proc. Natl. Acad. Sci. USA.* **76:** 5934–5938.
51. Deckelbaum, R. J., T. Olivecrona, and M. Fainaru. 1980. The role of different albumin preparations on production of human plasma lipoprotein-like particles in vitro. *J. Lipid Res.* **21:** 425–434.
52. Eisenberg, S., and T. Olivecrona. 1979. Very low density lipoprotein. Fate of phospholipids, cholesterol, and apolipoprotein C during lipolysis in vitro. *J. Lipid Res.* **20:** 614–623.
53. Eisenberg, S., and D. Rachmilewitz. 1975. Interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma. *J. Lipid Res.* **16:** 341–351.
54. Eisenberg, S., and D. Schurr. 1976. Phospholipid removal during degradation of rat plasma very low density lipoprotein in vitro. *J. Lipid Res.* **17:** 578–587.
55. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
56. Eisenberg, S., D. W. Bilheimer, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. II. Studies on the transfer of apolipoproteins between plasma lipoproteins. *Biochim. Biophys. Acta.* **280:** 94–104.
57. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. The metabolism of very low density lipoproteins in hyperlipidemia: studies of apolipoprotein B kinetics in man. *Eur. J. Clin. Invest.* **6:** 167–177.
58. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. *J. Clin. Invest.* **56:** 1481–1490.
59. Berman, M., M. Hall, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
60. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60:** 43–50.
61. Van't Hooft, F. M., D. A. Hardman, J. P. Kane, and R. J. Havel. 1982. Apolipoprotein B (B-48) of rat chylomicrons is not a precursor of the apolipoprotein of low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **79:** 179–182.
62. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652–658.
63. Sherill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of canine lipoproteins containing only the E apoprotein by a high affinity receptor. *J. Biol. Chem.* **255:** 1804–1807.
64. Windler, E., Y. S. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255:** 5475–5480.
65. Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1241.

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