Metabolic heterogeneity of post-lipolysis rat mesenteric lymph small chylomicrons produced in vitro

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Abstract The study was undertaken to investigate the metabolic fate of post-lipolysis mesenteric lymph small chylomicrons produced in vitro. Small chylomicrons doubly labeled with [^{3}H]cholesterol (more than 70% in cholesteryl esters) and [^{14}C]palmitate-labeled triglycerides were collected from rat mesenteric lymph during periods of fasting. Lipolysis was performed in vitro with lipoprotein lipase purified from bovine milk. More than 98% of the chylomicron-triglycerides could be hydrolyzed to fatty acids. Post-lipolysis chylomicrons were separated by zonal ultracentrifugation, characterized, and tested for biological behavior in intact rats. Following lipolysis the lipoproteins lost nearly all their triglycerides, apoA-I, and apoC, and were relatively enriched with cholesteryl esters, unesterified cholesterol, phospholipids, and apoB. Three preparations were tested for biological behavior: pooled (total) post-lipolysis chylomicrons (diameter ~250 Å); particles at the ascending part of the zonal effluent (diameter ~300 Å), and at the descending part (diameter ~200 Å). After intravenous injection to intact rats, [^{3}H]cholesteryl ester decay was very rapid with pooled lipoproteins and the 300-Å preparation ($t_{1/2}$ = 5–10 min). The 200-Å preparation in contrast stayed in circulation much longer ($t_{1/2}$ = 60–90 min). The study thus demonstrated metabolic heterogeneity of post-lipolysis small chylomicrons and indicated that some may form an LDL-like subpopulation with a plasma lifetime slower than "remnants" but faster than LDL.—**Chajek-Shaul, T., S. Eisenberg, Y. Oschry, and T. Olivecrona.** Metabolic heterogeneity of post-lipolysis rat mesenteric lymph small chylomicrons produced in vitro. *J. Lipid Res.* 1983. **24:** 831–840.

Supplementary key words lipoprotein lipase • low density lipoproteins • remnants • apolipoprotein B • triglyceride hydrolysis • zonal ultracentrifugation

VLDL and chylomicrons are the main triglyceride-carrying lipoproteins in mammalian plasma. VLDL is formed in the liver and carries triglycerides of endogenous origin; chylomicrons (small and big) carry triglycerides absorbed from the intestine of either dietary or endogenous origin. Both lipoproteins transport triglyceride fatty acids to tissue cells after interactions with the lipoprotein lipase enzyme system. The metabolic fate of nontriglyceride constituents of these two lipoproteins has been the subject of extensive investigations during the last 10–15 years. Hydrolysis of triglycerides leaves behind a triglyceride-depleted particle (core-particle) that contains residual cholesteryl esters, all of the apoB of the parent lipoprotein, and proportionate amounts of the surface lipids, phospholipids, and unesterified cholesterol (1–7). Surplus surface constituents (lipids and soluble proteins) are transferred to the HDL density range (3, 8–13), and are possibly the major source of the HDL lipids and apoproteins (14, 15).

In the rat, post-lipolysis core particles of either VLDL or chylomicron origin are rapidly removed from the circulation by the liver (16–19), via a high-affinity receptor-mediated process (20–22). These particles have been designated remnants. A small fraction (5–15%) of the post-lipolysis VLDL, however, forms LDL (17, 19). In humans, in contrast, most or even all of the VLDL eventually is converted to LDL (3, 23). The fate of post-lipolysis chylomicrons is not clear.

The present investigation was undertaken to critically determine whether some chylomicrons may form LDL in the rat. Our premise was that, in analogy to VLDL (6), after complete or nearly complete hydrolysis of chylomicron-triglycerides, the resulting core particles may exhibit LDL-like properties. To this end, we have characterized post-lipolysis chylomicrons formed in the test tube, and have tested their biological behavior in the intact rat. The study indeed demonstrated that a subpopulation of post-lipolysis chylomicrons exhibits LDL-like properties.

METHODS

Preparation of lipoproteins

Small chylomicrons were collected from male rats through a canula inserted into the mesenteric lymph

duct (24). During the period of lymph collection, the rats were infused intraduodenally with 0.9% NaCl solution at a rate of 2–3 ml/hr. To obtain biosynthetically labeled chylomicrons, the intraduodenal infusion solution contained 10–15 μCi (0.15–0.2 μmol) of [^{14}C]palmitate ([1-^{14}C]plamitic acid, sp act 59 mCi/mmol, Radiochemical Centre, Amersham, England) complexed to serum bovine albumin and 50–200 μCi of [^{3}H]cholesterol dissolved in 0.2 ml of ethanol ([1,2-^{3}H(N)]cholesterol, 53 Ci/mmol, New England Nuclear, Boston, MA). With these radioactive precursors, doubly labeled ([^{14}C]palmitate-triglycerides and [^{3}H]cholesterol) small chylomicrons were obtained. The following procedure was used. Fed rats were cannulated during morning hours, and lymph was allowed to flow for 4–6 hr while the rats were given 0.9% NaCl only. That lymph sample was discarded. Lymph collection for experimental use was started after the 4–6 hr initial period, with intraduodenal infusion of radioactive precursors. Lymph was collected over a period of 12–24 hr into glass tubes immersed in ice-cold water; the triglyceride concentration was 20–30 mg/10 ml of lymph. Usually, 40–60 ml of lymph (containing 100–200 mg of triglycerides) was obtained for each preparation. A typical fatty acid composition of small chylomicron lipids (in mol %) was 30.1, 1.9, 9.5, 14.2, 31.9, and 11.9 for palmitic, palmitoleic, stearic, oleic, linolenic, and arachidonic acids, respectively; the percentages for triglycerides were 29.8, 4.1, 8.3, 16.3, 31.6, and 8.8 for the same fatty acids. Chylomicrons were isolated by preparative ultracentrifugation in an L5-50 Beckman ultracentrifuge using 40.3 rotors at 35,000 rpm for 16–18 hr. The d < 1.006 g/ml fraction containing the small chylomicrons was separated by the tube-slicing technique (25). The chemical composition, electron microscopy, and SDS-PAGE of apoproteins of small chylomicrons are shown in Table 1 and Figs. 5 and 6. The distribution of radioactive [^{14}C]palmitic acid among chylomicron lipids was 3.0 ± 1.0% in phospholipids, 81.0 ± 1.2% in triglycerides, 3.4 ± 1.6% in fatty acids, and 7.7 ± 1.8% in cholesteryl esters; of the [^{3}H]cholesterol, 23.8 ± 3.3% of the radioactivity was in unesterified cholesterol and 73.8% ± 2.9% was in cholesteryl esters (mean ± SD of four chylomicron preparations).

Human plasma HDL$_3$ (d 1.125–1.21 g/ml) was prepared from fasting human plasma by preparative and zonal ultracentrifugation as described elsewhere (11).

Analytical methods

Lipoprotein-protein was determined by the method of Lowry et al. (26) using bovine serum albumin as a reference. Phospholipids were determined by the Bartlett procedure (27) and triglycerides in the AutoAnalyzer II following the Lipid Research Clinics' protocol. Unesterified cholesterol and total lipoprotein cho-

lesterol were determined by the cholesterol oxidase–cholesterol esterase method, using a commercial kit (Boehringer-Mannheim, Germany). Cholesteryl ester was calculated by difference. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of lipoprotein apoproteins in 15% acrylamide was performed according to established procedures (28), after delipidation with ethanol–diethyl ether and diethyl ether extraction of lipids. SDS-PAGE in 4% gels was performed according to Kane, Hardman, and Paulus (29). Triglycerides, di- and monoglycerides and fatty acids, and free and esterified cholesterol were separated by thin-layer chromatography of chloroform-extracted lipids using precoated silica gel cellulose nitrate plates (Schleicher and Schüll, Dassel, Germany) and a solvent system of petroleum ether–diethyl ether–acetic acid 80:20:1 (v/v/v) (10). Lipids were identified with the aid of purified reference compounds, and the corresponding plate regions were sliced and assessed for radioactivity content using a Packard model 2660 liquid scintillation spectrometer. Negatively staining electron microscopy of lipoproteins was performed as previously described (30) in a Phillips 300 electron microscope at an original instrument magnification of 90,000. For sizing of lipoprotein particles, the original negatives were enlarged by 2.5, and the size distribution of lipoproteins was measured with a final magnification of 225,000.

Separation of lipoproteins by centrifugation in a zonal system

Intact and post-lipolysis chylomicrons were separated by ultracentrifugation in a zonal system (31). The incubation mixture was applied in NaBr solution of d 1.30 g/ml at the periphery of a Ti-14 zonal rotor containing a 665-ml NaBr gradient of density range 1.0–1.30 g/ml with the rotor spinning at 3,000 rpm. This was followed by a 20-ml cushion of d 1.3 g/ml NaBr solution. The rotor was then accelerated to 42,000 rpm, lipoproteins were separated after 140 min centrifugation and collected in 25-ml fractions. With these conditions, human and rat LDL eluted at effluent volumes of 200–250 ml, while chylomicrons and VLDL eluted at the initial 50–75 ml. Radioactivity content was determined along the rotor effluent volume, and defined fractions were then identified, collected, and used for metabolic experiments.

Experimental conditions

Post-lipolysis chylomicrons were prepared in vitro, after incubation of doubly labeled ([^{14}C]palmitate-labeled lipids and [^{3}H]cholesterol) chylomicrons with lipoprotein lipase purified from bovine milk. The enzyme was prepared in Umea, Sweden (32) and was sent in dry ice to Jerusalem, Israel. A typical enzyme preparation contained 0.3 mg of enzyme protein/ml with activity

of 380 units/mg protein. The incubation mixture included chylomicrons (20–40 mg of triglycerides), human plasma HDL₃, bovine serum albumin (essentially fatty acid-free fraction V powder, Sigma Laboratories), and lipoprotein lipase. The amounts of HDL₃ and of albumin added were adjusted to that of chylomicron triglycerides, 1 mg HDL₃ protein/10 mg triglyceride, and 10 μmol albumin/10 mg triglyceride, respectively. Triglyceride hydrolysis was initiated with 25 μl of lipoprotein lipase and incubation at 37°C in a thermostated water bath was continued for 1–2 hr, when a complete clearing of the incubation system occurred. An aliquot of the incubation mixture was taken before the addition of lipoprotein lipase and at the end of the incubation to determine the distributions of labeled lipids and degrees of triglyceride hydrolysis. Post-lipolysis chylomicrons were separated by either preparative ultracentrifugation at density less than 1.04 g/ml, or by zonal ultracentrifugation. With this last method, the whole incubation system was applied to the zonal rotor immediately at the end of the incubation. After zonal centrifugation, the [³H]cholesterol peak was collected, pooled, dialyzed against 0.9% NaCl, 0.01% EDTA solution, and concentrated by vacuum ultrafiltration. (Concentration by an ultrafiltration technique using Amicon Diaflo membranes yielded preparations that were not stable metabolically). In some experiments, post-lipolysis lipoproteins in individual zonal fractions were investigated. In these experiments, dialyzed unconcentrated preparations were used, and the experiments were carried out within 24 hr of the initiation of the incubation.

The biological life times of intact and post-lipolysis chylomicrons were determined after intravenous injection of the respective lipoproteins to male rats (about 250 g body weight). The following post-lipolysis chylomicron preparations were used: d < 1.04 g/ml isolated by preparative ultracentrifugation; pooled (fractions 3–9) zonal ultracentrifugation post-lipolysis lipoproteins; and lipoproteins at peak tubes of the zonal [³H]cholesterol elution profile (tubes 4 or 5) and at the far tail of the [³H]cholesterol (tubes 8 or 9). Rats were exsanguinated at time intervals after the injection, and plasma and ultracentrifugally separated lipoprotein radioactivity was determined. Radioactivity in liver was determined after perfusion of the liver through the portal vein with 30–50 ml of 0.9% NaCl solution when the liver appeared free of visible blood.

RESULTS

Small chylomicrons isolated from rat mesenteric lymph, were labeled biosynthetically with [³H]cholesterol and [¹⁴C]palmitate-labeled triglycerides. SDS-PAGE of small chylomicron apoproteins in 4% gels revealed the presence of B-48 only (**Fig. 1**). Hydrolysis of [¹⁴C]triglycerides in native chylomicrons and in chylomicrons activated by incubation with rat plasma after removal of VLDL (d > 1.006 g/ml plasma fraction) was followed in vitro. Incubation of the chylomicrons with a d > 1.006 g/ml fraction is known to increase the apoC-II content of chylomicrons (33). Preincubation of the chylomicrons with a d > 1.006 g/ml plasma fraction increased the rate of triglyceride hydrolysis (**Fig. 2**). Similar results were obtained when native chylomicrons were incubated with lipoprotein lipase in the presence of either human plasma HDL or soluble apoprotein fractions of VLDL origin (data not shown). In subsequent experiments, native chylomicrons (without prior incubation in plasma) were used and the lipolysis system was supplemented with human HDL₃.

Post-lipolysis small chylomicrons were isolated either by ultracentrifugation at density < 1.04 g/ml, or were separated on a rate zonal ultracentrifugation system. The elution profile in the zonal rotor of intact chylomicrons (incubated without LPL) and of chylomicrons with three different degrees of triglyceride hydrolysis (74%, 94%, and 99%) is shown in **Fig. 3**. In intact chylomicrons, more than 90% of the [³H]cholesterol eluted with the first 50–75 ml. With 74% and 94% triglyceride hydrolysis, increasing amounts of [³H]cholesterol eluted later in the rotor (100–150 ml), but a distinct peak of post-lipolysis particles was not observed. Only with nearly complete hydrolysis of triglycerides (>98%) did a distinct peak of radioactive ([³H]cholesterol) post-li-
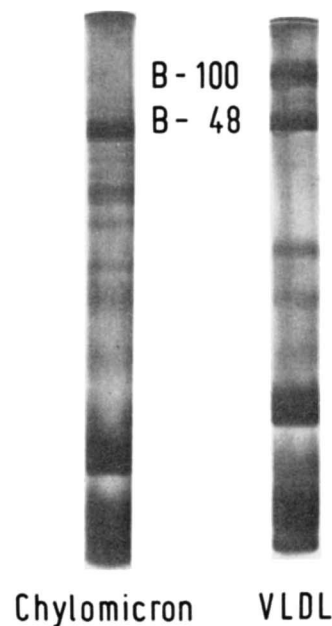

B - 100
B - 48

Chylomicron   VLDL

Fig. 1. SDS-PAGE of rat plasma VLDL and rat mesenteric lymph small chylomicrons in 4% acrylamide. Gels were loaded with 100 μg of protein.
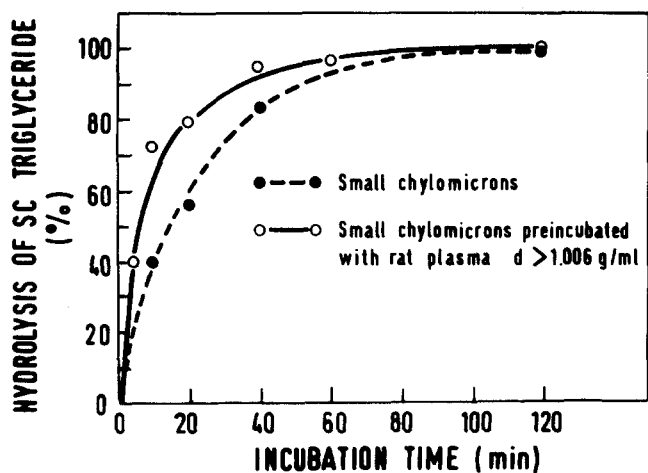
Fig. 2. In vitro hydrolysis of mesenteric lymph small chylomicrons (SC) by lipoprotein lipase purified from bovine milk. Incubation mixtures contained 1 mg of chylomicron triglycerides, 0.5 μl of lipoprotein lipase, 40 mg/ml of bovine serum albumin, and 0.1 M Tris buffer, pH 8.2. Percent hydrolysis of triglycerides was determined after lipid extraction and thin-layer chromatography of the extracted lipids. Two small chylomicron preparations were used: intact chylomicrons (● – – – ●) and chylomicrons preincubated for 60 min at room temperature with rat plasma of d > 1.006 g/ml (O——O). Data are mean of two experiments.

polysis lipoprotein subpopulation became apparent (rotor effluent volume, 50–300 ml). A detailed analysis of radioactive lipids along the zonal rotor effluent, in a
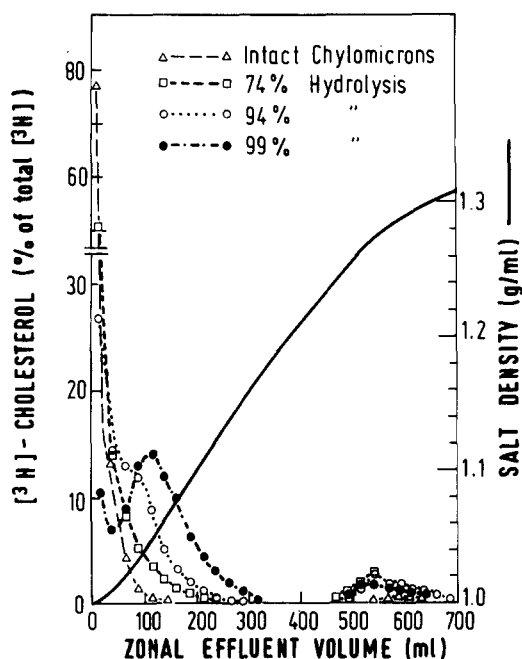
sample with 99% triglyceride hydrolysis (incubation carried out in the presence of human HDL₃), is presented in **Fig. 4.** The first lipoprotein fraction (50–300 ml), contained most of the [³H]cholesteryl ester, about half of the ³H-labeled unesterified cholesterol, and only trace amounts of [¹⁴C]palmitate-labeled lipids. ³H-Labeled unesterified cholesterol separated with the second lipoprotein fraction (450–600 ml), which corresponds to the elution profile of HDL. This fraction did not contain [³H]cholesteryl esters. [¹⁴C]Palmitic acid eluted with the albumin peak (550–650 ml). For further experiments, three different preparations were used: pooled post-lipolysis small chylomicrons (elution volume 75–250 ml, tubes 3–10); lipoproteins in tube four (75–100 ml) or tube five (100–125 ml) (peak [³H]cholesteryl ester content); and lipoproteins in tube 8 (175–200 ml) or tube 9 (200–225 ml). Chemical composition, SDS-PAGE of apoproteins, negatively stained electron micrographs, and size distribution of intact chylomicrons and pooled post-lipolysis products are shown in **Table 1** and **Figs. 5–7.** The composition of post-lipolysis chylomicrons was compatible with nearly complete loss of triglycerides and relative enrichment of the lipoproteins with all other constituents (Table 1). SDS-PAGE of intact chylomicrons revealed the presence of apoB, apoA-I, and apoC. ApoB remained conspicuous in post-lipol-

Fig. 3. Separation in a zonal ultracentrifugation system of intact and post-lipolysis mesenteric lymph chylomicrons. Conditions of lipolysis and zonal ultracentrifugation techniques are described in Methods. The figure contained preparations with zero (intact), 74%, 94%, and 99% triglyceride hydrolysis.
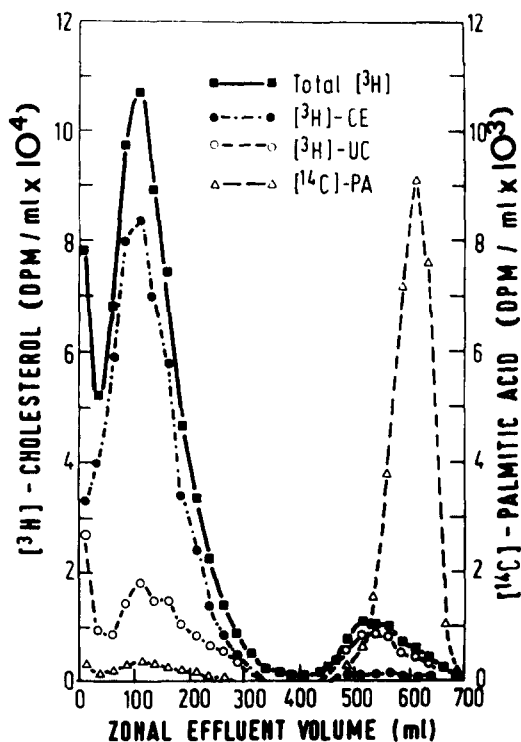

Fig. 4. Distribution along the zonal rotor effluent of post-lipolysis small chylomicron ³H-labeled cholesteryl esters (CE), ³H-labeled unesterified cholesterol (UC), and [¹⁴C]palmitate-labeled lipids (PA). Triglyceride hydrolysis in this experiment was 99%.

TABLE 1. Chemical composition of intact and post-lipolysis small chylomicrons

Lipoprotein	Protein	TG	PL	CE	UC
		mg / 100 mg lipoprotein			
Chylomicrons[a]	3.9 ± 0.7	80.8 ± 3.9	12.1 ± 2.6	2.4 ± 0.4	0.8 ± 0.3
Post-lipolysis chylomicrons[a]	25.3 ± 1.9	[b]	39.5 ± 4.7	27.5 ± 3.1	7.8 ± 0.5
Post-lipolysis chylomicrons, tubes 4 and 5[c]	22.0	[b]	33.7	33.1	11.2
Post-lipolysis chylomicrons, tubes 8 and 9[c]	29.7	[b]	41.9	19.3	9.1

[a] Data are mean ± SE of four preparations. Chylomicrons and post-lipolysis chylomicrons were double-washed in a fixed-angle rotor prior to analysis. After two washes, trace amounts of albumin were frequently found in intact chylomicrons. Albumin contaminated all post-lipolysis chylomicron preparations. By SDS-PAGE it is estimated that albumin contributed 30–50% of total post-lipolysis chylomicron protein.

[b] Too low to be determined accurately (less than 5% of mass).

[c] Post-lipolysis chylomicrons isolated by zonal ultracentrifugation in tubes 4 and 5 and tubes 8 and 9 of the rotor effluent. Data are means of two determinations.

ysis chylomicrons, whereas most of the apoA-I and the apoC disappeared (**Fig. 5**). Albumin frequently was detected in intact and post-lipolysis chylomicrons even after two washes. Both intact and post-lipolysis chylomicrons exhibited a marked heterogeneous size distribution (**Fig. 6** and **Fig. 7**). Mean diameter of intact chylomicrons was 477 Å (range 311–711 Å) and of post-lipolysis chylomicrons, 253 Å (range 133–356 Å).

Characterization of post-lipolysis small chylomicrons present in tubes 4 and 5 (peak [³H]cholesterol radioactivity) and tubes 8 and 9 (tail of radioactivity) is also presented in Table 1 and Figs. 5–7. Both populations appeared quite homogeneous by electron microscopy,

but with very different diameters: mean diameter of post-lipolysis lipoproteins in tube 5 was 302 Å (range 222–400 Å) and in tube 9, 195 Å (range 133–267 Å) (Figs. 6 and 7). The smaller particles contained considerably less cholesteryl ester (19.3% of total mass) and
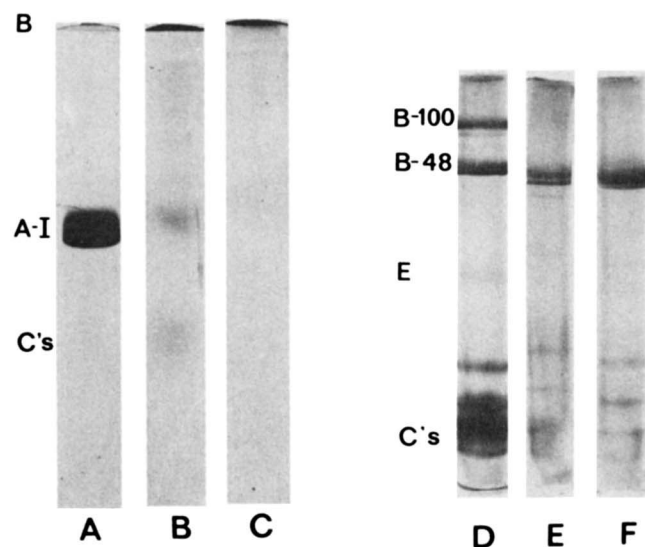

Fig. 5. SDS-PAGE of intact and post-lipolysis mesenteric lymph small chylomicrons. Gels A–C were run with 15% acrylamide, and gels D–E, with 4%. A, purified rat plasma apoA-I; B, intact small chylomicrons (triple washed); C, post-lipolysis small chylomicrons isolated at density of less than 1.04 g/ml; D, rat VLDL; E, post-lipolysis chylomicrons in zonal tubes 4 and 5; and F, post-lipolysis chylomicrons in zonal tubes 8 and 9. For each gel, 50 μg lipoprotein-protein was applied.
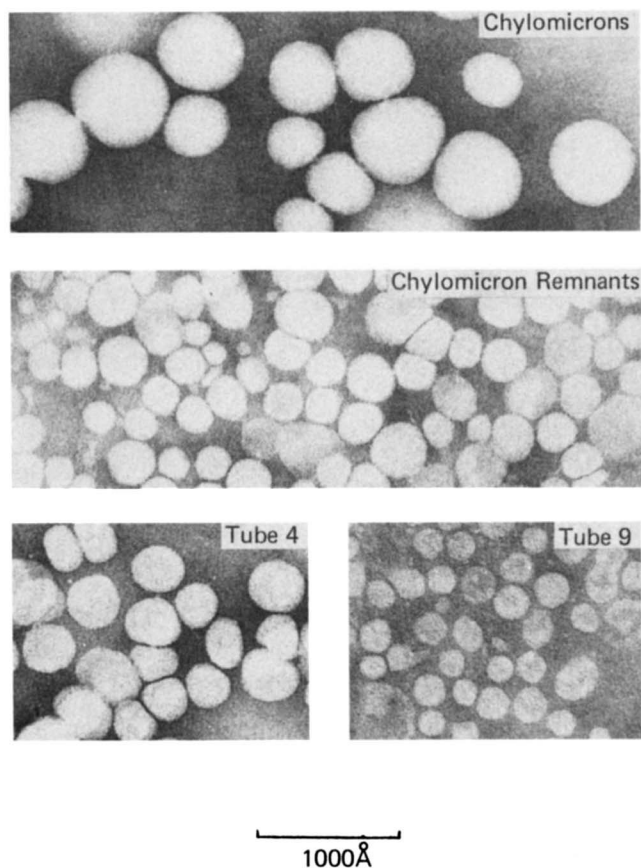

Fig. 6. Negatively stained electron micrograph of intact small chylomicrons, post-lipolysis (remnants) chylomicrons of pool zonal effluent (50–250 ml), post-lipolysis chylomicrons present in tube 4 of the zonal effluent profile (75–100 ml), and in tube 9 (200–225 ml). Instrument magnification ×90,000.
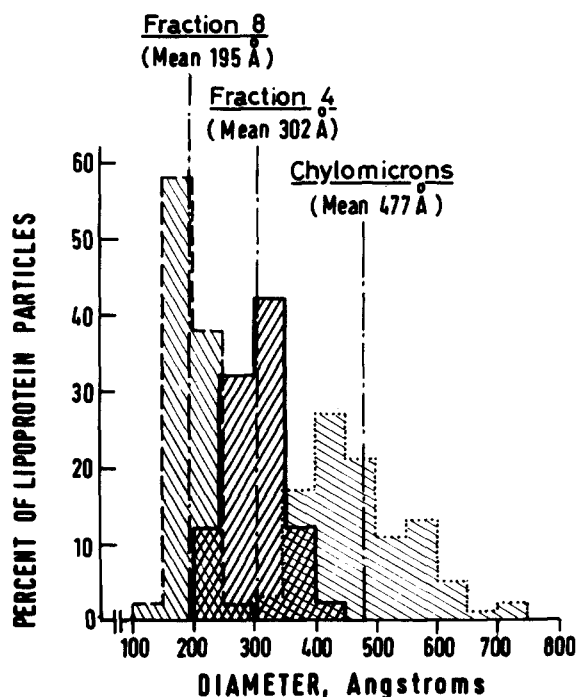
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Fig. 7. Size distribution of intact mesenteric lymph small chylomicrons and post-lipolysis chylomicrons present in tubes 4 and 9 of the zonal effluent profile (volumes of 75–100 ml and 200–225 ml, respectively). The size distribution pattern is based on diameter measurements of at least 200 particles.
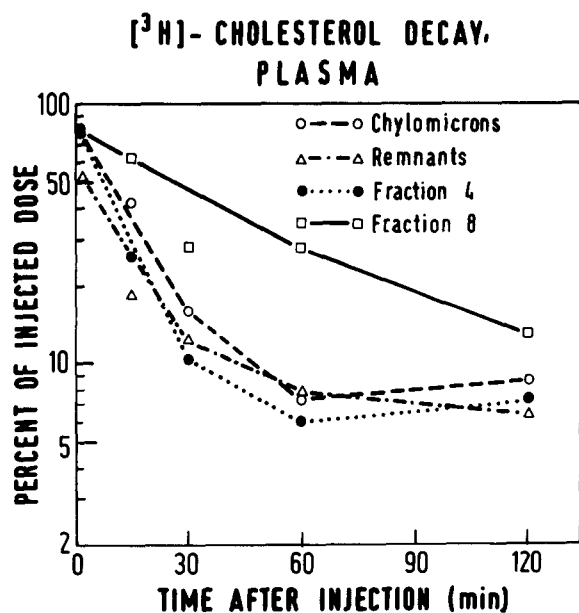
more surface constituents, particularly protein and phospholipids (Table 1). Apoprotein profiles on 10% SDS (not shown) or 4% SDS (Fig. 5) however, were almost identical with apoB-48 as the predominant apoprotein species. ApoE was not detected in either population.

Biological behavior of intact chylomicrons, of post-lipolysis chylomicrons, and of post-lipolysis lipoproteins in tubes 4 and 5 and 8 and 9 (five and four preparations, respectively) was investigated after intravenous injection to male rats. [³H]Cholesterol decay from whole plasma and from plasma lipoproteins of d < 1.04 g/ml is shown in **Fig. 8** and **Fig. 9**. Thirty min after the injection of either intact or post-lipolysis chylomicrons as well as lipoproteins in tubes 4 and 5, less than 10% of the injected radioactivity remained. The mean plasma half-life time of the [³H]cholesterol in these preparations was less than 10 min. Throughout the first 60 min of the study, [³H]cholesteryl ester constituted 78.3–91% of total [³H]cholesterol either in plasma or the plasma fraction of d < 1.04 g/ml (**Table 2**). Thus, the disappearance of total [³H]cholesterol during that period was also representative of the disappearance of ³H-labeled cholesteryl ester. Disappearance of [³H]cholesterol (and therefore ³H-labeled cholesteryl ester) from the plasma lipoprotein fraction of d < 1.04 g/ml was more rapid

Fig. 8. Decay of [³H]cholesterol from plasma of rats injected with [³H]cholesterol-labeled intact mesenteric lymph small chylomicrons and post-hydrolysis (remnants) chylomicrons of pooled zonal effluent (50–250 ml), post-lipolysis chylomicrons present in tube 4 of the zonal effluent profile (75–100 ml), and in tube 9 (200–225 ml). Data are mean of four to seven experiments.

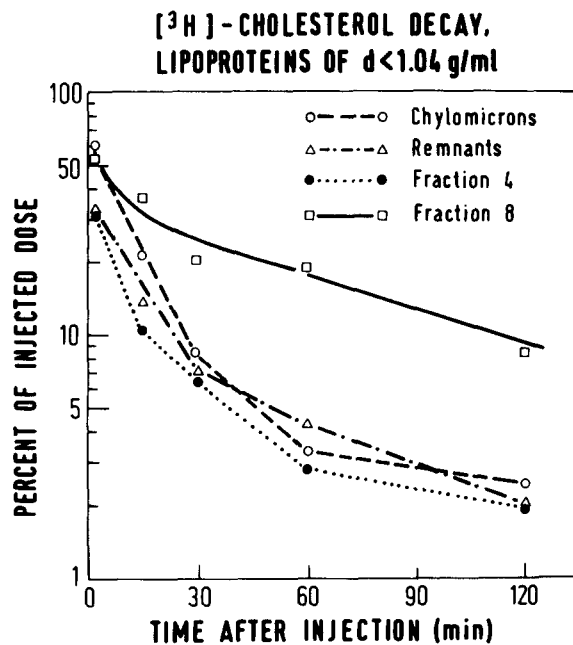**Fig. 9.** Decay of [³H]cholesterol from plasma lipoproteins of density < 1.04 g/ml of rats injected with [³H]cholesterol-labeled intact mesenteric lymph small chylomicrons and post-hydrolysis (remnants) chylomicrons of pooled zonal effluent (50–250 ml), post-lipolysis chylomicrons present in tube 4 of the zonal effluent profile (75–100 ml), and in tube 9 (200–225 ml). Data are mean of four to seven experiments.

TABLE 2. Distribution of [^{3}H]cholesterol in density fractions after injection of intact and post-lipolysis chylomicrons to rats

| Lipoprotein | Time after Injection | [^{3}H]Cholesterol, % of Total Plasma [^{3}H] | | [^{3}H] in UC and CE, % of Total [^{3}H] | | | |
| | | | | Plasma | | d < 1.04 g/ml | |
		d < 1.04 g/ml	d > 1.04 g/ml	[^{3}H]UC	[^{3}H]CE	[^{3}H]UC	[^{3}H]CE
	min	%				%	
Chylomicrons	2	86.5 ± 3.0[a]	13.5 ± 2.8	21.7[b]	78.3	15.0	85.0
	60	46.2 ± 12.6	53.8 ± 12.6	21.0	79.0	14.3	85.7
Post-lipolysis chylomicrons	2	82.5 ± 10.5	17.5 ± 10.6	9.0	91.0	11.3	88.7
	60	53.0 ± 13.5	47.0 ± 13.6	14.1	85.9	13.3	86.7

[a] Mean ± SD of five experiments.
[b] Mean of two experiments.

than from total plasma or the lipoproteins of d > 1.04 g/ml. This is evident from data shown in Table 2 where the contribution of radioactivity in the d < 1.04 g/ml fraction to total plasma decreased from 86.5% to 46.2% for intact chylomicrons and from 82.5% to 53.0% for post-lipolysis chylomicrons. A markedly different biological behavior was found with lipoproteins in tubes 8 and 9 (Figs. 8 and 9). The $t_{1/2}$ of [^{3}H]cholesterol either in plasma or in lipoproteins of d < 1.04 g/ml was about 60 min.

Radioactive cholesterol content in liver was determined at the 2-min and 60-min intervals. In both instances, and for all four preparations, over 90% of the radioactivity that disappeared from the plasma was found in the rat liver.

DISCUSSION

Numerous studies have been published during the past 10 years on the intraplasma metabolism of VLDL and the metabolic fate of core and surface degradation products (34). Less is known about chylomicrons. The present investigation was initiated to study systematically the nature of products of interaction of chylomicrons with lipoprotein lipase and their metabolic fate in the rat. For that purpose, we have begun with rat mesenteric lymph chylomicrons collected during post-absorptive periods (small chylomicrons). These small chylomicrons are triglyceride- and phospholipid-rich lipoproteins that contain apoB-48 and relatively large amounts of apoA-I, similar to analogous preparations as reported by other investigators (33, 35, 36). To prepare post-lipolysis lipoproteins, small chylomicrons were incubated in vitro with lipoprotein lipase purified from bovine milk. More than 99% of the chylomicron's triglycerides could be hydrolyzed either in the presence or absence of plasma activators. Rate zonal ultracentrifugation was used to separate post-lipolysis small chylomicrons. It is only with this method that we were able to conclude that hydrolysis of at least 95% of triglyc-

erides is necessary before a distinct density profile of post-lipolysis particles is obtained. Our observations (Fig. 3) demonstrate marked differences of the elution profile of post-lipolysis chylomicrons with hydrolysis of triglycerides of 94% as compared to 98% or more. Hence, only preparations with degrees of triglyceride hydrolysis higher than 98% were used. Rate zonal ultracentrifugation also enabled us to sample individual fractions of the total post-lipolysis chylomicrons with distinct metabolic behavior (see below). This method therefore seems to be especially suitable for the study of post-lipolysis lipoproteins.

Post-lipolysis chylomicrons, isolated either by rate zonal ultracentrifugation or at density less than 1.04 g/ml, contain only trace amounts of triglycerides and are relatively enriched with cholesteryl esters, unesterified cholesterol, phospholipids, and apoproteins. The relative enrichment of different constituents in post-lipolysis chylomicrons was, however, different. The highest relative enrichment in post-lipolysis lipoproteins was observed for cholesteryl ester, 11–12-fold (from 2.4% to 27.5% of total lipoprotein mass). The relative enrichment of protein was at most 8-fold, and the apoprotein profile of the post-lipolysis particles was different from the intact lipoprotein: there was an almost complete disappearance of apoA-I and apoC, and in most preparations, post-lipolysis chylomicrons contained appreciable amounts of albumin. The relative enrichment of unesterified cholesterol was about 9-fold and of phospholipids only 3.5-fold. The different degrees of retention of constituents in the post-lipolysis particles is most probably due to removal of surface constituents from the lipolyzed chylomicrons, as demonstrated for VLDL (3, 8–11). Indeed, during lipolysis we observed transfer of ^{3}H-labeled unesterified cholesterol (but not [^{3}H-labeled cholesteryl ester, see Fig. 4) to HDL. As for phospholipids, substantial amounts of phosphoglycerides are hydrolyzed to lyso-compounds during incubation of either VLDL (10, 37) or chylomicrons (38) with bovine milk lipoprotein lipase and these lyso-compounds be-

come associated with the albumin. Following all these lipolysis-induced alterations of the small chylomicrons, the final post-lipolysis particles were apoB-rich and cholesteryl ester-rich lipoproteins with no (or only trace amounts) other proteins and a proportionate amount of surface lipids, mainly cholesterol and phospholipids.

The aim of the present investigation was to determine whether all post-lipolysis small chylomicron particles are remnants or some form of LDL-like lipoproteins. The distinction between the two is based on their biological behavior, even when other properties (composition, apoprotein profile, size, etc.) are similar. A remnant is a post-lipolysis particle that is rapidly removed (within minutes) from the circulation by the liver (1, 2, 20–22). LDL, in contrast, circulates for much longer periods of time (hours or days as compared to minutes for remnants). The behavior of intact chylomicrons was typical for a lipoprotein that forms predominantly remnants. After the injection, ^{3}H-labeled cholesteryl esters disappeared from the plasma with a very short half-life time, and the radioactivity was found in the liver. Very similar results were recently reported by Van't Hooft et al. (39) while following the fate of biosynthetically labeled small chylomicrons almost identical to those used here. Thirty min after the injection, Van't Hooft et al. (39) found about 10% of the injected radioactivity in plasma; we find 15%. Pooled post-lipolysis chylomicrons (cholesteryl ester- and apoB-rich) disappeared even faster, although their composition was similar to LDL. Next, we have explored the possibility that only a subpopulation of the post-lipolysis chylomicrons might exhibit LDL-like behavior. To this end, two zonal fractions were used, one at the peak (fraction 4 or 5) and the other at the far tail (fraction 8 or 9) of the zonal profile. Both must have contained predominantly cholesteryl ester-rich lipoproteins, as less than 1% of the original chylomicron triglycerides remained in the total lipolysis system. After injection into rats, lipoproteins at peak tubes exhibited typical remnant behavior; the biological behavior of lipoproteins at tubes 8 and 9 was distinctly different, as they were removed from the circulation with a rate one-tenth that of remnants. Yet, the half-life time of rat LDL labeled biosynthetically with [^{3}H]cholesterol and prepared similarly is considerably longer, 6–9 hr.[2]

Post-lipolysis chylomicrons separated at tubes 8 and 9 of the zonal gradient are obviously different from the bulk of the preparation and cannot be defined as remnants. Although only 5–10% of the ^{3}H-labeled cholesteryl ester was present in this fraction, it constituted as much as 30–50% of the number of post-lipolysis particles. This is inferred from the size differences of lipoproteins at peak tubes (300 Å) and in tubes 8 and 9

(195 Å); 300-Å particles contain 4–6-fold more cholesteryl ester molecules than 195-Å particles. Also, the mean diameter of pooled post-lipolysis chylomicrons (253 Å) is only slightly more than the average diameter of lipoproteins in fractions 4 and 5 and fractions 8 and 9. Characterization of post-lipolysis chylomicrons in these fractions revealed only differences due to size: lipoproteins in tubes 8 and 9 contained less core cholesteryl ester and more surface lipids and proteins than those in tubes 4 and 5. We were not able to detect apoproteins other than B-48 and C in either population. However, we cannot rule out the possibility that larger post-lipolysis particles may acquire apoprotein E when injected into plasma whereas smaller particles do not. ApoE was shown recently to be responsible for rapid uptake of remnants (and other lipoproteins) by the rat liver (40–44).

Regardless of mechanism, we demonstrate in the present investigation marked biological variation within the spectrum of an otherwise seemingly homogeneous lipoprotein species, i.e., small chylomicrons. Structural and/or metabolic heterogeneity within rat lipoprotein families have been described in several studies. Fidge and Poulis (45) described in 1978 two subpopulations of apoB-rich LDL in rats, possibly of different origin. More recently, Elovson et al. (46) and Sparks and Marsh (47, 48) have shown metabolic heterogeneity of apolipoprotein B in total triglyceride-rich rat plasma lipoprotein fractions (VLDL, d < 1.006 g/ml). In those studies, the metabolic heterogeneity of the apoB moiety of the triglyceride-rich lipoproteins was ascribed to structural heterogeneity of the apoprotein, although Sparks and Marsh (47) conclude that other possible differences between the lipoproteins cannot be ruled out. Our studies extend the observations on the metabolic heterogeneity of rat plasma triglyceride-rich lipoproteins to a more discrete preparation, intestinal lymph small chylomicrons. The uniqueness of our observation is the ability to clearly demonstrate such heterogeneity while carrying out in vitro lipolysis of small chylomicrons (using purified lipoprotein lipase) followed by biological testing of the post-lipolysis particles. With this system, heterogeneity of the apoB moiety of the chylomicron is unlikely. Apparently, other chemical and/or physical properties of the chylomicrons seem to play major roles in determining their biological fate. More studies are obviously needed to elucidate the factors responsible for the heterogeneity of core constituents of the apoB-containing rat plasma lipoproteins, chylomicrons, VLDL, IDL, and LDL.

Several investigations have indicated that chylomicrons may contribute to LDL. Schaefer (49) have demonstrated that after the injection of radioiodinated chylomicrons to a human subject, as much as 27% of the apoB is found in LDL. Krishnaiah et al. (50) have more

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[2] Eisenberg, S. Unpublished observations.

recently demonstrated the presence of small molecular weight apoB in rat LDL. This "small B" is the only apoB species present in chylomicrons (29, 50) but may also be derived from the liver (51). Kane et al. (29), in contrast, reported complete absence of LDL in a human patient with normal capacity to form chylomicrons, and speculated that in this patient hepatic VLDL is not formed and that chylomicrons are not precursors of LDL. Our study clearly demonstrates that LDL-like particles can be formed by the action of lipoprotein lipase on mesenteric lymph small chylomicrons. Previous studies have shown that in vitro-produced post-lipolysis VLDL is in fact an LDL-like lipoprotein as judged by compositional and structural properties (6). Some of the post-lipolysis chylomicrons studied here also exhibit biological behavior similar, but not identical, to LDL. The plasma decay of ^{3}H-labeled cholesteryl ester associated with the LDL-like particles derived from small chylomicrons is faster than that reported for total rat LDL, studied with labeled apoB (52, 53). This behavior may reflect heterogeneity of the LDL system in the rat. Accordingly, it is possible that some LDL is indeed derived from chylomicrons, but the circulating life time of this intestinal-derived LDL may be shorter than that of hepatic LDL. However we cannot rule out the possibility that the faster decay from the circulation of LDL-like particles derived from small chylomicrons reflects the in vitro system used for their preparation. For example, interactions of LDL-like particles with tissue cells may be necessary for a final conversion of LDL-like particles to true LDL. Alternatively, some of the experimental procedures used for preparation of post-lipolysis small chylomicrons might have shortened the biological life times of the particles. Although these and other questions remain unanswered, the observations reported here clearly demonstrate metabolic heterogeneity of chylomicrons and indicate their potential contribution to the circulating LDL mass.

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