

Effects of particle size and number on the plasma clearance of chylomicrons and remnants

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Abstract Lymph chylomicrons of different sizes are known to be cleared at different rates, but the underlying mechanism for this effect has not been resolved. To investigate the differences in clearance rates between small and large particles, chylomicron-like lipid emulsions labeled with radioactive triolein and cholesteryl oleate were injected into conscious rats. The clearance from plasma of small emulsion particles was significantly slower than large when equal lipid masses of small and large particles were injected. Similar results were obtained in clearance studies with lymph chylomicrons. When equal numbers of either small or large emulsion particles were injected into rats, the clearance of the triolein label from large particles was significantly slower than small particles, but no significant difference was found in the clearance of the remnants (traced by the cholesteryl oleate label) derived from small and large particles. However, when increased numbers of either small or large particles were injected, the clearances of emulsion triolein and remnants were significantly decreased. Larger particles were found to be lipolyzed significantly less than small. Simultaneous injections showed competition for removal of large and small particles, suggesting competition for a common, saturable removal process. Our findings provide evidence that particle number and size are determinants of the rates of plasma clearance of the triglyceride-rich lipoproteins and the results are consistent with a saturable process. Our data also show that particle number is more important than size and higher numbers of particles markedly affect the clearance of triglyceride-rich lipoproteins. However particle uptake by the liver is not sensitive to remnant size.—**Martins I. J., B-C. Mortimer, J. Miller, and T. G. Redgrave.** Effects of particle size and number on the plasma clearance of chylomicrons and remnants. *J. Lipid Res.* 1996. **37:** 2696–2705.

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Chylomicrons (CM) are the triacylglycerol-rich lipoproteins formed in the intestine during lipid absorption. Chylomicrons are heterogeneous in size and range in diameter from 75 to 600 nm (1). Chylomicron size depends on the flux of triacylglycerol through the absorptive cells of the intestine, and during fat absorption the particles become larger in diameter (2, 3). Fat ab-

sorbed from the intestine is transported in larger-sized particles when lymph triacylglycerol concentration is more than 3.0 mg/ml, while at lower rates of absorption smaller-sized particles predominate (3, 4).

Quarfordt and Goodman (5) separated CM from the lymph of fat-fed rats by centrifugation in sucrose density gradients. After intravenous injection, large CM were cleared from plasma faster than small CM, with less uptake in liver of the cholesterol label from small CM. Similar results were obtained with large and small CM subfractionated from rat chyle by Chajek-Shaul et al. (6), who obtained remnants by lipolysis in vitro of lymph CM collected from fasted rats, and after the remnants were fractionated by size, small remnants were cleared slower after injection in intact rats ($t_{1/2}$ 60–90 min) compared with larger remnants ($t_{1/2}$ 5–10 min). More recently it was found that small remnants prepared in hepatectomized rats were taken up by perfused rat livers slower than larger remnants (7).

A regulatory effect of particle size on CM clearance has potential physiological implications. During periods of fasting the intestine makes small CM, and an intestinal fat load is transported chiefly by increased particle size, with only minor increases in the numbers of particles secreted into the intestinal lymph (3). Therefore, the faster clearance of larger CM produced after a fat load could be important in limiting the extent of postprandial lipemia.

The present study was designed to compare the clearance of small particles with clearance of larger particles. To assess the physiological role of size in CM clearance, emulsions designed to model the lipid composition of chylomicrons were injected intravenously into rats. Tri-

Abbreviations: CM, chylomicron; VLDL, very low density lipoprotein; LDL, low density lipoprotein; EYPC, egg yolk phosphatidylcholine; apoE, apolipoprotein E; apoA-IV, apolipoprotein A-IV; TG, triglyceride; CE, cholesteryl ester; CO, cholesteryl oleate; TO, triolein; C, cholesterol; PL, phospholipid.

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olein (TO) and cholesteryl oleate (CO) labels were incorporated for assignment of the relative contributions to clearance of lipolysis and particle uptake. Chylomicrons and emulsions were analyzed for differences in their lipid and protein compositions. To assess the potential contribution of particle number on particle clearance, the clearance of small and large emulsion particles was compared by varying the dose of particles injected into rats.

METHODS

Preparation of emulsions

Emulsions of the required compositions were prepared by sonication and purified by ultracentrifugation as previously described (8). Triolein, cholesteryl oleate, cholesterol and egg lecithin (all from Nu-Chek Prep, Elysian, MN), each more than 99% pure, were dispensed from stock solutions into vials followed by radioactive [^{14}C]triolein and [^3H]cholesteryl oleate (Amersham, International Plc, Buckinghamshire, England). For large emulsions, emulsions were prepared from a lipid mixture of TO (70 mg), CO (3 mg), FC (2 mg), and PL (25 mg). Small emulsions were prepared from lipid mixtures containing TO (45 mg), CO (3 mg), FC (2 mg), and PL (25 mg). The ultracentrifugation conditions for purification of small particles were different from large particles. After sonication the crude emulsion was placed at the bottom of two centrifuge tubes, and then 2.5 ml of NaCl solutions of densities 1.065, 1.02, and 1.006 g/ml were sequentially layered above. The tubes were then centrifuged in a SW 41 rotor of a Beckman L8-70M ultracentrifuge for 20 min at 25,000 rpm and 20°C. The large coarsely emulsified particles were removed from the top of the gradient and replaced with 1.006 g/ml solution. This was followed by a second centrifugation at 40,000 rpm for 60 min. The emulsion particles (small) that floated to the surface were removed and used for the clearance studies.

Preparation of chylomicrons

Two groups of rats were used to prepare small or large chylomicrons from intestinal lymph. Male Wistar rats weighing between 250–300 g were obtained from the Animal Resources Centre (Murdoch, Western Australia) and fed a pelleted diet containing approximately 5% fat. Rats were fasted overnight, then prepared surgically for collection of intestinal lymph through a plastic tube implanted in the principal mesenteric lymph duct. After surgery the rats were placed in individual cages to recover from anesthesia and kept hydrated by a steady

infusion of 0.15 mol/l NaCl at 2.0 ml/h through a tube placed in the stomach at the time of lymph cannulation. Drinking water was available to the rats during the course of the experiments.

The first group of donor rats received only tracer amounts of labels, to obtain small lymph chylomicrons representative of the fasting state. Lymph was collected after a bolus intragastric injection of 3 ml phosphate-buffered saline (pH 7.4) containing 57 μmol sodium taurocholate, 10 μCi of [^{14}C] palmitic acid, and 20 μCi of [^3H]cholesterol. Lymph was collected at 20°C for 6 h into vessels containing EDTA (1 mg/ml) and reduced glutathione (1.6 mmol/l), while rats received a steady intestinal infusion through the gastrostomy tube of 0.15 mol/l NaCl. After removal of cells from the lymph by centrifugation at 3000 rpm for 10 min, solid KBr was added (1.96 g/14 ml) to raise the density to 1.1 g/ml. The lymph samples were degassed briefly under reduced pressure, then 14-ml portions were placed under discontinuous density gradients (9) consisting of 6 ml solutions with densities 1.065, 1.040, 1.020, and 1.006 g/ml. The gradients were then centrifuged at 20°C in the SW 28 rotor of the Beckman L8-M ultracentrifuge for 15 h at 28,000 rpm. Chylomicrons aspirated from the top 0.5 cm of the tube were analyzed and injected into rats within 1 day. Oxidation was prevented by the addition of reduced glutathione (50 μg /ml) and storage under argon.

The second group of donor rats, used to obtain larger CM particles, were infused with Intralipid at a rate of 25 mg triglyceride/h. Labeled chylomicrons were prepared by intragastric infusion of 3 ml of phosphate-buffered saline (pH 7.4) containing 57 μmol sodium taurocholate, 10 μCi [^{14}C] palmitic acid, and 20 μCi [^3H] cholesterol at 2 h after the start of Intralipid infusion. Lymph was then collected for 4 h into vessels containing EDTA (1 mg/ml) and reduced glutathione (1.6 mmol/l). The lymph was ultracentrifuged as described above except the gradients were centrifuged for 75 min.

In competition experiments, small and large CM were mixed on the basis of equal protein content. For these studies, the cholesteryl ester component of large or small particles was labeled with [^3H]CE and [^{14}C]CE, respectively, by including either [^3H] cholesterol or [^{14}C]cholesterol in the intestinal infusate.

Analysis of CM apolipoproteins by 2-dimensional electrophoresis

CM preparations were concentrated using Amicon ultrafiltration units with 5000 Dalton cutoff YM-5 filters. The CM samples were delipidated by adding equal volumes of isopropanol and pentanol (10). The aqueous phase was collected for isoelectric focussing and protein

was determined. Samples in a volume of 70 μl containing 1 $\mu\text{g}/\mu\text{l}$ of protein were mixed with 10 μl of reducing buffer (1.2% Tris, 1.6% dithiothreitol) and 60 mg of urea, then incubated at 37°C for 1 h. After pre-focussing the gel for 2 h, samples were loaded into wells before focussing was continued at 250 V. After electrophoresis for 16 h the gel was incubated in 50 ml of buffer (0.125 M Tris-HCl, 2% SDS, 10% glycerol, 0.8% dithiothreitol, pH 6.8, for 10 min (11). For electrophoresis in the second dimension, strips of the gel were cut out and placed on a vertical slab sodium dodecyl sulfate polyacrylamide gel 1.5 mm in thickness as previously described (10). The gels were imaged using an Epson Scanner 4000, and the image was analyzed by a computerized Scan Analysis program (Biosoft, Milltown, NJ). The absorbances of the apolipoprotein E-3 and A-IV bands were measured with subtraction of background absorbance.

Injection studies

For clearance studies, male rats weighing 250–350 g were prepared surgically with arterial and venous canulas as described earlier (8). Labeled chylomicrons or emulsions were prepared and injected to measure plasma clearance exactly as described previously (3, 8).

Measurements of particle diameters

Large CM or emulsions were analyzed by laser-light scattering using a BI-90 particle sizer (Brookhaven Instruments Corp., Ronkonkoma, NY). Small CM or emulsion particle size was determined by electron microscopy. Chylomicron samples on formvar-coated grids were negatively stained with 2% phosphotungstic acid at room temperature and viewed on a Joel 2000 FX transmission electron microscope. The diameters of particles ($n = 30$) were measured with a graduated scale bar which was determined from the magnification of the electron micrographs.

Chemical analysis

The lipids extracted from chylomicrons with chloroform-methanol 2:1 (v/v) were separated by thin-layer chromatography on 0.2-mm layers of silica gel in the solvent system petroleum ether (40–60°C)-diethyl ether-formic acid 90:10:1 (by volume). The triacylglycerol, cholesteryl ester, and free cholesterol bands were scraped from the plate for assay of triacylglycerol by the chromotropic acid method (12), and free and esterified cholesterol by the *o*-phthaldialdehyde procedure (13). Protein assay was by the procedure of Lowry et al. (14), using crystalline bovine serum albumin as a standard, with extraction of turbidity due to lipids with chloroform. Phospholipid was measured directly on chylomicron samples (15). For measurement of CM lipid

radioactivity, lipid bands separated by thin-layer chromatography were scraped into vials and counted in 15 ml of scintillant.

Separation of phospholipids from chylomicron samples

Chylomicron samples were extracted in chloroform-methanol 2:1 and then applied to silica gel 60 TLC plates. Plates were run in a rectangular glass tank and a mobile phase ethyl acetate-n-propanol-chloroform-methanol-0.25% aqueous KCl 25:25:25:13:9 (v/v) was used for separation of phospholipids (16). The bands were scraped from the plate and phospholipids were extracted with chloroform-methanol-water 80:35:5. Aliquots of solvent were dried and then phosphorus was determined.

Fatty acid analysis

Triglyceride or phospholipid silica scrapings were placed into sealed glass tubes. Then 2 ml of 4% sulphuric acid/methanol mixture was added to the tubes and mixed under a stream of nitrogen. The tubes were heated at 100°C for 30 min and then cooled on ice. Four ml of distilled water was added and the tubes were mixed, then 2 ml of hexane was added. The tubes were vortexed thoroughly and centrifuged at 1500 rpm for 5 min. The hexane layer was removed and dried under a stream of nitrogen. The extracted fatty acids were placed at -20°C until analyzed by gas-liquid chromatography by using a Hewlett-Packard model 5980A gas chromatograph equipped with a 3393A computing integrator (Rockville, MD). The stationary phase was BPX70 coated on a (25 m \times 0.22 mm column 0.25 μm film thickness; SGE, Australia) with a temperature programmed from 150° to 245°C at 5°C/min with nitrogen as a carrier gas and a split ratio of 30:1. Peaks were identified by comparison with a known standard mixture. Individual fatty acids were calculated as a relative percentage, omitting peaks contributing <1% of total mass.

Calculation of particle numbers

The lipid compositions of emulsions were used to calculate the diameter of an average particle as described by Miller and Small (17). The calculated mass of phospholipid in an average particle was then divided into the total mass of phospholipid to derive the number of particles in each sample.

Statistical analysis

Statistical comparisons were made on the data of the plasma clearance (calculated from area under the curves) by two-way analysis of variance using the Systat Software (SYSTAT, Inc., Evanston, IL) or on the lipid

TABLE 1. Composition and size of lymph chylomicrons and emulsions

	n	Composition					Diameter nm
		TG	FC	CE/CO %	PL	Protein	
Small CM	3	61.8 ± 2.82 ^c	2.0 ± 0.63	6.2 ± 2.29	23.6 ± 1.97 ^c	6.4 ± 0.71 ^c	43 ± 9 ^{a,c}
Small EM	3	60.4 ± 1.65 ^c	1.8 ± 0.15	4.5 ± 0.84	33.3 ± 0.98 ^c		47 ± 4 ^{a,c}
Large CM	3	81.6 ± 1.49	0.5 ± 0.07	1.9 ± 0.39	13.9 ± 0.76	2.1 ± 0.43	140 ± 10 ^b
Large EM	3	81.8 ± 1.39	1.3 ± 0.28	2.9 ± 0.29	14.0 ± 1.04		143 ± 2 ^b

Isolation by centrifugation and lipid analysis of CM and emulsions were performed as described under Methods. The results are mean ± SEM; (n) is the number in each group.

^aParticle size determined by electron microscopy.

^bParticle size determined by laser light-scattering.

^cSignificantly different from large particles, $P < 0.01$.

radioactivity data of the plasma clearance by repeated measures analysis of variance using Statistical Package for Social Science software (SPSS Inc, Chicago, IL). The *t*-test for independent means was used to compare differences between means.

RESULTS

Composition and size of CM and emulsions

Table 1 shows the composition and size of small and large CM and emulsions. The small CM obtained from the lymph of fasted rats had average diameters of 43 nm which were similar to the diameters of small emulsions. Also, large CM from lymph absorbing fat and large emulsions were similar in diameter, 140 and 143 nm respectively. The large CM and emulsions contained significantly more TG and less of other constituents compared with small particles. The patterns of incorporation of radioactive labels were similar in large and small CM. Approximately 80% of the ³H label was found in the cholesteryl ester fraction and between 80–95% of the ¹⁴C label was in the TG fraction with both large and small CM.

CM triglyceride and phospholipid fatty acid composition

Figure 1 compares the phospholipid classes of small and large chylomicrons. It is clear that the phospholipids were similarly distributed with PC contributing about 80% in each case. The fatty acids of the chylomicron PC and PE are compared in Fig. 2A. Large CM contained less 16:0 and 18:0 and more 18:2 and 18:1 than small CM, in both PC and PE. The amounts of 20:4 were similar in large and small CM. The triglyceride fatty acids as illustrated in Fig. 2B, show large CM contained less 16:0, 16:1, 18:0, and 20:4 but more 18:2 and 18:3 than small CM. The amounts of 18:1 in CM

triglycerides were similar. Analysis of apolipoproteins by SDS-PAGE revealed only minor quantitative differences between small and large CM (data not shown).

Plasma clearance of large and small CM

The removal of TG and CE radioactivity from the plasma after the injection of similar masses of labeled small and large CM is shown in Fig. 3. The clearances of TG and CE labels were both significantly slower ($P < 0.001$) in rats injected with small CM when compared with large CM. In these experiments, as equal lipid masses of small and large CM were injected into rats, the number of small CM were several fold greater than large CM. Less CE label (58.4 ± 5.7) was recovered in the livers from rats injected with small CM than with large CM (72.4 ± 1.6). The uptake by spleen was only

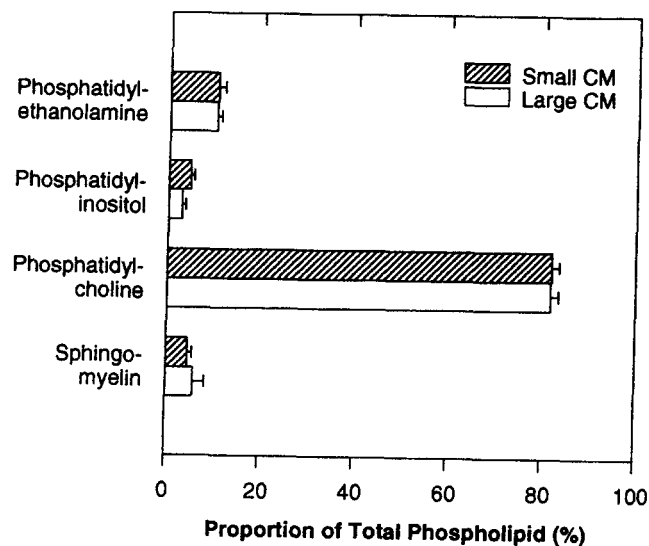


Fig. 1. The phospholipid classes of large and small chylomicrons (CM) are compared. The phospholipids were similarly distributed, with phosphatidylcholine contributing about 80% of total phospholipid in both small and large CM.

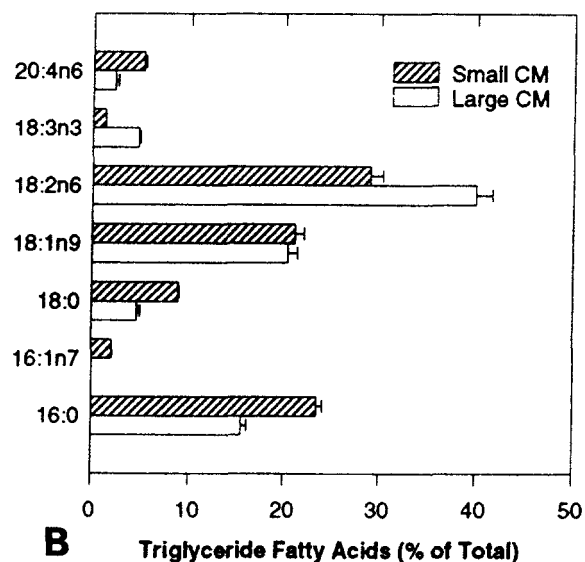
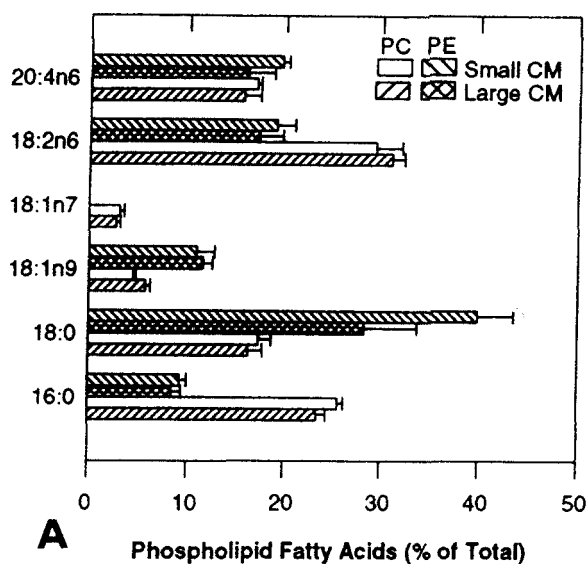


Fig. 2. A: The distribution of fatty acids in small and large chylomicrons (CM). Large CM contain less 16:0 and 18:0 and more 18:2 and 18:1 than small CM in both PC and PE, whereas the contents of 20:4 were similar in large and small CM phospholipids. B: The triglyceride fatty acids of small and large CM are compared. In large CM the contents of 16:0, 16:1, 18:0, and 20:4 were less, but 18:2 and 18:3 were more than in small CM. The contents of 18:1 in CM triglycerides were similar in both sized CM.

about 1% of the injected doses, but significantly more uptake was found with small CM than large CM. No differences were found with organ uptake of TG label with small or large CM.

To test whether a simultaneous injection of small CM could delay the clearance of large CM, small and large CM were prepared as before except the palmitic acid

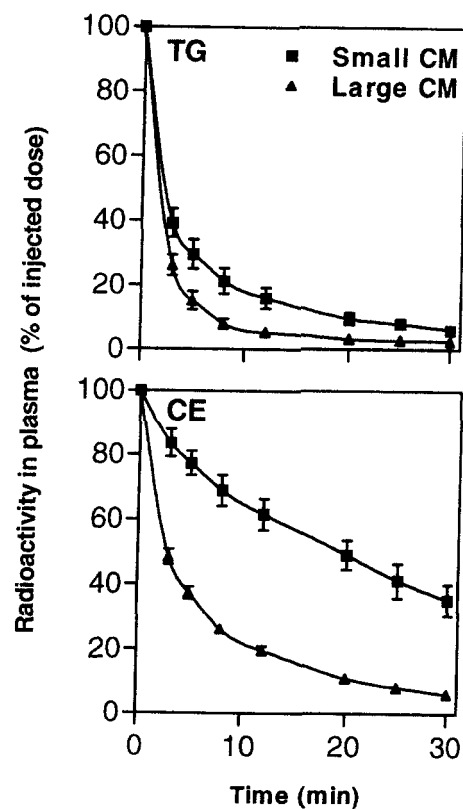


Fig. 3. The removal from plasma of triglyceride (TG) and cholesteryl ester (CE) labels after intravenous injections of similar triglyceride masses of small and large chylomicrons (CM) into rats. Both TG and CE labels were removed significantly more slowly from the plasma of rats injected with small chylomicrons compared with large chylomicrons.

label was omitted, and the donor lymph cannulated rats were given either [^3H]- or [^{14}C]-cholesterol to label the CM particles. The two preparations were mixed together on the basis of equal protein content for simultaneous injections of large and small CM. **Figure 4** shows that when the mixture was injected into rats the clearance of CE label from large CM (in the presence of an excess of small CM) was significantly slower when compared with CE label from large CM injected alone ($P < 0.001$).

Effect of dose and numbers of particles on plasma clearance of small and large emulsion particles

Figure 5 compares the clearance of lipid labels when small emulsion particles were injected at two doses. The figure clearly shows that when small emulsions were injected at a high dose (60×10^{13} particles) the clearances of TO and CO labels were significantly decreased ($P < 0.02$ and $P < 0.002$, respectively) when compared with emulsions injected at a low dose (1×10^{13} particles). Radioactivity was removed from plasma rapidly, with

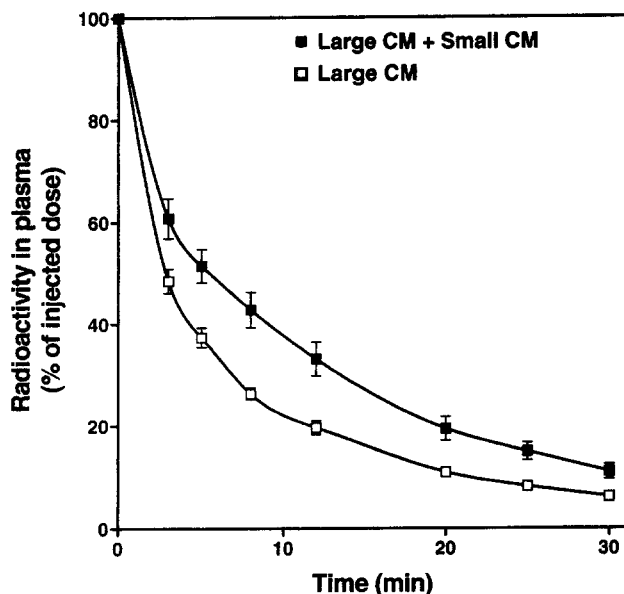


Fig. 4. The effect of a simultaneous injection of small chylomicrons (CM) on the clearance of large CM from the plasma of rats. The clearance of CE label from large CM (in the presence of an excess of small CM) was found to be significantly slower when compared with CE label from large CM injected alone ($P < 0.001$).

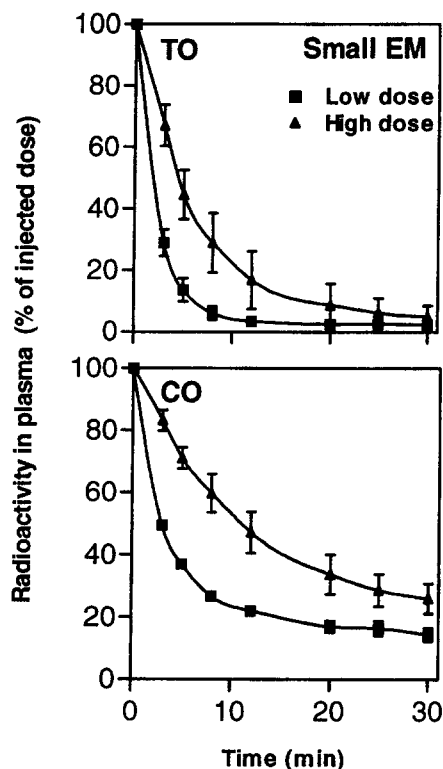


Fig. 5. The clearance of TO and CO labels from the plasma of rats after the injection of low or high doses of small emulsion (EM) particles. The clearances of the TO label (upper panel) and CO label (lower panel) from the plasma of rats were significantly decreased (by repeated measures of analysis of variance $P < 0.02$ for TO to 12 min and $P < 0.002$ for CO to 30 min).

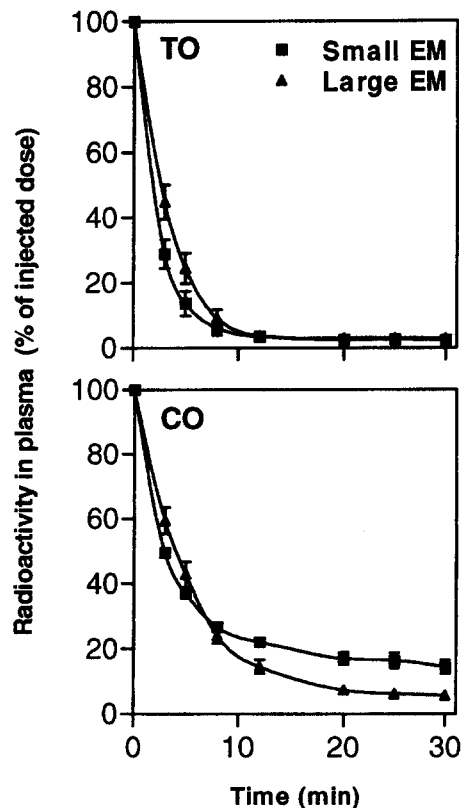


Fig. 6. The plasma clearance of equal numbers of either small or large emulsions (EM) injected intravenously into rats. The upper panel shows the clearance of large particles for the first two sampling points ($P < 0.01$ by analysis of variance) when rats were injected with either small or large emulsion. The clearance of the CO label (lower panel) from the plasma of rats was similar for small and large particles ($P > 0.1$ by repeated measures of analysis of variance).

faster disappearance of TO label (upper panel), predominantly a measure of lipolysis, than of CO (lower panel) which traces the clearance of triglyceride-depleted remnants. The numbers of particles injected into rats was calculated as described in Methods. A similar difference was found after injection of high and low doses of large particles (data not shown). **Figure 6** shows that when the doses were adjusted so that equal numbers were injected, the clearance of TO from large particles was slower than small particles for the first two sampling points ($P < 0.01$). By 8 min more than 90% of the injected doses had been cleared.

For easier comparison of clearance rates, the data were integrated by calculating the areas under the clearance curves. This integration is reciprocally related to clearance so the inverse was used for the comparisons, as shown in **Fig. 7**. Figure 7 summarizes the results of the plasma clearance (area under the curve)⁻¹ for a graded series of doses of large and small EM particles. The designations of 1, 2, and 3 refer to the dose of ei-

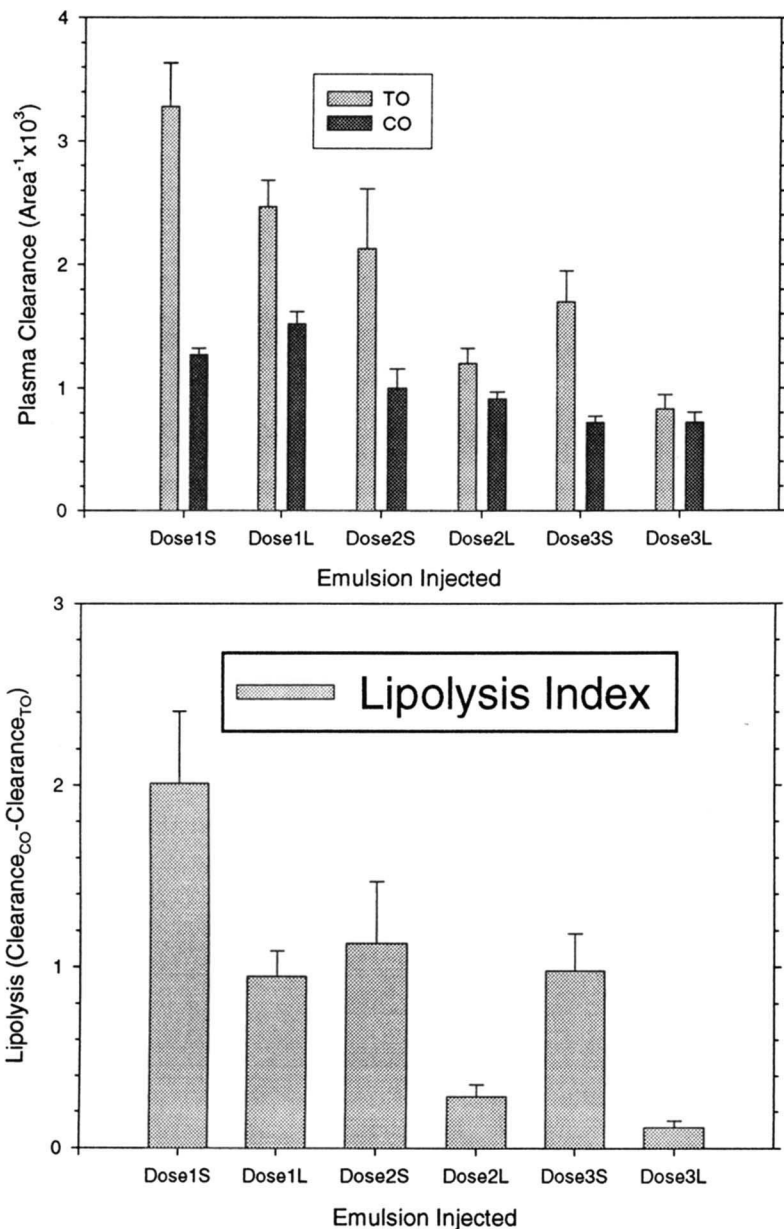


Fig. 7. The clearance of triolein (TO) and cholesteryl oleate (CO) labels from the plasma of rats after injection of different numbers of either small (S) or large (L) 1, 2, and 3 refers to the numbers of particles. The designations 1 and 2 correspond to 1×10^{13} and 10×10^{13} for both small and large particle numbers. S3 refers to 60×10^{13} particles and L3 to 30×10^{13} particles. The upper panel shows that with an increase in particle number (either small or large), the clearance (area under the curve)⁻¹ of the TO label was significantly slower with large particles when compared with similar doses of small particles. The lower panel shows the calculated lipolysis index (difference in areas under curves for TO and CO). Large particles (Dose 1L, 2L, and 3L) were lipolyzed significantly less than Small (Dose 1S, 2S, and 3S). The lower panel also shows clearly that lipolysis decreased for both small and large particles with increasing dose from 1–3.

ther small (S) or large (L) particles injected into rats. The designation of 1 and 2 corresponds with 1×10^{13} and 10×10^{13} particle numbers respectively. Dose3S refers to 60×10^{13} particles and Dose3L 30×10^{13} particles. In Fig. 7, the upper panel shows that when similar doses of large and small particles were injected, the clearances of TO labels were significantly faster ($P < 0.001$) for small particles, while clearances of CO were similar. By two-way analysis of variance, the effects of size and dose on TO clearance were both highly significant ($P < 0.002$ and $P < 0.001$, respectively), while the

effect of dose on CO clearance was highly significant ($P < 0.001$) but the effect of size on CO clearance was not significant ($P > 0.5$).

The lipolysis index (calculated by subtraction of the clearance of CO from clearance of TO) for large and small particles is shown in Fig. 7 (lower panel). The lipolysis index for large particles (Dose 1L, 2L, and 3L) was in each case markedly less ($P < 0.001$) than for small particles (Dose 1S, 2S, and 3S). The lower panel also shows that the lipolysis index decreased for small and large particles as the dose increased. By two-way

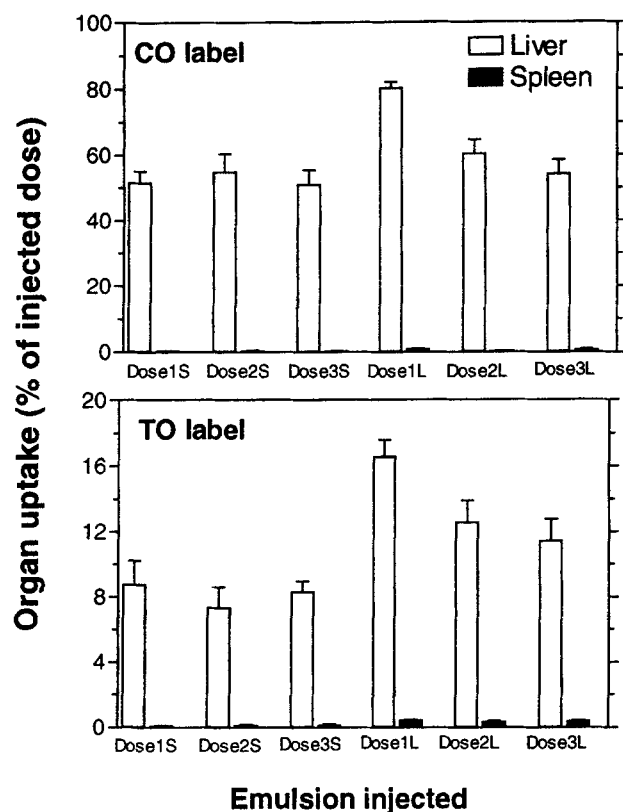


Fig. 8. The organ uptake of cholesteryl oleate (CO) and triolein (TO) labels in the spleen and liver after injections of rats with either small or large emulsion particles. The upper panel shows that the uptake of CO label in the liver with small emulsions was less when compared with large emulsion (Dose 1L). With higher doses of large emulsion (2L and 3L) the uptake by the liver of CO was also decreased. The lower panel shows that liver uptake of the TO label was less with small emulsions (Dose 1S, 2S, and 3S) when compared with Large (Dose 1L, 2L, and 3L). TO uptake was less with higher doses of large emulsions when compared to the lower dose (1L). Spleen uptake of both labels with either small or large emulsions was less than 1%.

analysis of variance for the lipolysis index data, the effects of size and dose were both highly significant ($P < 0.001$ and $P < 0.002$, respectively).

Figure 8 shows the organ uptakes of CO (upper panel) and TO (lower panel) label in the spleen and liver when rats were injected with small or large emulsion particles. In the upper panel the uptake of CO label in the liver with small emulsions was significantly less ($P < 0.002$) when compared with large emulsion (Dose 1L). With higher doses of large emulsion (2L and 3L) the uptake of CO label in the liver was also found to be significantly decreased ($P < 0.002$). A similar trend was found with the liver uptake of the TO label, uptake of TO label was significantly less ($P < 0.002$) with small emulsions when compared with large emul-

sion (Dose 1L). Also, uptake was significantly less with higher doses of large emulsions ($P < 0.04$) when compared to the lower dose. Spleen uptake of lipid radioactivities with small or large emulsions was less than 1%.

DISCUSSION

On the basis of the long standing finding of Quarfordt and Goodman (5) it has generally been accepted that large CM are cleared from plasma faster than small CM (5, 6). We also found that with either small lymph CM or emulsion particles the clearance was slower than large particles when similar lipid masses were injected. To determine an explanation for this finding we re-examined the effect of size on the clearance of either large or small emulsions particles at different doses.

Emulsions were mainly used in the present study as the amounts of lymph CM needed to complete the experiments at the higher doses were not practical. Appropriately formulated lipid emulsions have been found to mimic faithfully the physical and physiological properties of chylomicrons. Hultin et al. (18, 19) asserted on the basis of mathematical modeling that lipid emulsions differ in their metabolism from lymph chylomicrons. However for their comparisons, Hultin et al. (18) used inappropriately formulated emulsions that contained no unesterified cholesterol. When amounts of cholesterol similar to lymph chylomicrons are incorporated, extensive published data show that emulsions are metabolised similarly to chylomicrons (3, 8, 20–22). Conversely, similar emulsions from which cholesterol is omitted are substrates for lipoprotein lipase but do not mimic the metabolism of chylomicrons (21, 23).

We found when similar numbers of particles were injected that the effect of size was contrary to previous findings (5, 6). In fact, when similar numbers of particles were injected, the clearance of TO label from small particles was faster than from large particles (Fig. 7). The faster clearance from large particles occurred only when approximately equal masses of lipid were injected, which corresponded with a larger number of smaller particles. Our results showed that the TO clearance from both large and small particles was inversely proportional to the numbers injected. The lipolysis of large particles was markedly less when compared with small particles. It is possible that the faster clearance of TO from smaller particles is a reflection of the binding of the particles to proteoglycans during lipolysis by lipoprotein lipase. Clearly, the same dose of TO in smaller particles offers an increased number of sites for interaction with bound enzyme than distribution in a lesser

number of larger particles, and also provides an increased surface area.

In contrast with the findings of Quarfordt and Goodman (5) the clearance of CO label (tracing remnant clearance) was not significantly affected by particle size when similar numbers of particles were injected (Fig. 7). However, for both small and large particles, the clearance of the CO label was inversely proportional to the numbers of particles injected, indicating that clearance of remnants was saturable.

Our findings are therefore consistent with a receptor-saturable process that is independent of remnant size, but it is not clear whether the availability of apoE or the saturation of hepatic receptors is rate-limiting. ApoA-I was bound with higher affinity to smaller particles than larger particles but the binding of apoA-II, C-II, and C-III was not affected by particle size (24). ApoE was bound with higher affinity to larger particles at 37°C yet apoE bound to small particles was a better ligand for LDL receptors (25). The clearance of chylomicron remnants requires apoE as a ligand (26, 27) for binding to low density lipoprotein receptors (20, 21) and possibly to other receptors. Our results are not consistent with reports that smaller intravenous lipid emulsion particles were cleared more slowly in rats, but are consistent with evidence of lipolysis proportional to total surface area (28).

In liver perfusion studies, remnants from small CM were removed more slowly than remnants from large CM. The small CM used by Guldur and Mayes (7) contained apoB-100 whereas large CM did not, and it was suggested that the differences in apoB composition could account for the differences in hepatic uptake. However, the presence of apoB-100 could be due to contamination with plasma VLDL. In our present studies apoB-100 was consistently absent from the small CM fraction (3). When prepared by density gradient centrifugation as described in Methods, apoB-48 is always present, but no apoB-100 can be detected by polyacrylamide gel electrophoresis. Furthermore, emulsions do not contain any of the apoB apolipoproteins, yet with emulsions similar difference in clearance rates as for lymph CM were found to be associated with particle number rather than particle size. Also in perfused liver studies, Windler et al. (29) found that small remnants were removed more rapidly than large remnants. However, comparisons were on the basis of protein mass, and the relation of protein mass to particle number is unclear. Windler et al. (29) found that the smaller remnants were removed by the low density lipoprotein receptor, but asserted that the larger remnants were removed by a different mechanism. Windler et al. (29) did not perform competition experiments to test their hypothesis that large and small remnants were removed by differ-

ent mechanisms. In contrast, our in vivo clearance studies show competition between small and large particles (Fig. 4), suggesting common removal mechanisms for both sizes of particles. An additional complication with the experimental design of Windler et al. was labeling of the particles by iodination, which has been found to alter the metabolism of chylomicrons (30).

In conclusion, our studies show that particle number and size both regulate the metabolism of chylomicrons and remnants. However, our data show that particle number is more important than size when approximately equal numbers of particles are injected into rats. After injection of higher numbers of either small or large CM and CM-like emulsions, both lipolysis and remnant clearance were markedly decreased. Therefore, our findings provide evidence that particle number is an important factor affecting the clearance of particles. A liver-sieving mechanism has been proposed to restrict the permeation of particles into the space of Disse (31, 32). The sieve possibly accounts for more ready access of remnants than of unlipolyzed lipid particles, and may contribute to the faster clearance of TO from smaller particles (Fig 7, upper panel). However, our results for remnant uptake do not support a sieving effect for remnants within the range of sizes in our study, but are consistent with a saturable process that is independent of remnant size. ■

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