Intravenous lipid emulsions: removal mechanisms as compared to chylomicrons

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Abstract We have compared the metabolism of chylomicrons and a labeled emulsion, similar to those used for parenteral nutrition. Both were labeled in their triglyceride moieties and by a core label. It is known that chylomicron triglycerides are cleared by two processes: removal of triglycerides from the particles through lipolysis and removal of whole or partly lipolyzed particles. It has been proposed that emulsion droplets are cleared by the same pathways. After intravenous injection to postprandial rats, triglycerides were cleared less rapidly from the emulsion than from the chylomicrons (half-lives of 6.4 and 4.0 min), whereas the core labels were cleared at the same rate (half-lives around 7.5 min). This suggests that there was less lipolysis of the emulsion droplets which was further supported by the finding that less label appeared in the plasma free fatty acids (FFA). In adipose tissue of fed rats given chylomicrons, the ratio between fatty acid and core label was above 6, showing that fatty acids had been taken up after lipoprotein lipase-mediated hydrolysis. In contrast, for rats given emulsion, that ratio was only 1.2 showing that nearly as much emulsion droplets as emulsion-derived fatty acids were present in the tissue. In the liver the ratio was 0.55 after chylomicrons but 0.93 after emulsion. In further support of more lipolysis, fatty acids were oxidized more rapidly from chylomicrons than from emulsion. These data suggest that a large fraction of the emulsion droplets was removed from plasma with little or no preceding lipolysis. A substantial proportion, more than 50%, of this uptake occurred in extrahepatic tissues.—**Hultin, M., C. Carneheim, K. Rosenqvist, and T. Olivecrona.** Intravenous lipid emulsions, removal mechanisms as compared to chylomicrons. *J. Lipid Res.* 1995. **36:** 2174–2184.

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Lipid emulsions have been used in parenteral nutrition for almost 30 years. Their clinical efficiency and safety are well established. The emulsion droplets have a size and lipid composition similar to chylomicrons (1, 2). In addition, the emulsions contain an excess of phospholipids which form liposome-like particles that are turned over more slowly than the triglyceride-rich droplets (1). On entering the plasma, the emulsions rapidly acquire apolipoproteins from circulating lipoproteins (3–5), among them apoC-II which is necessary for the activation of lipoprotein lipase (LPL) and apoE which is needed for recognition by lipoprotein receptors. It is generally assumed that triglyceride-rich emulsion droplets are metabolized in a manner similar to chylomicrons (1). This is based primarily on early studies that showed that emulsion triglycerides are cleared at rates similar to those for chylomicrons (6). Briefly, the postulated pathway is that the chylomicron/emulsion droplets bind transiently to sites at the vascular endothelium where some of the triglycerides are unloaded through the action of LPL (7). After several such cycles, the remaining triglyceride-poor droplets are recognized by remnant receptors in the liver, endocytosed, and degraded. This process has been visualized by Vilaró and Llobera (8), who used electron microscopy to study the metabolism of a commercial emulsion (Intralipid®) by the rat liver. They found emulsion droplets trapped in the spaces of Disse, and indications that the droplets entered hepatocytes where they were degraded. Large lipid droplets were present next to the mitochondria indicating that emulsion lipids, once inside the cell, were readily available for energy production.

Experimental emulsions are often prepared by extensive sonication of phospholipids and triglycerides followed by sequential ultracentrifugation. This generates emulsions with a homogenous droplet size and virtually without liposomes (9). Studies on the biological behavior of such emulsions have shown that the composition of surface lipids such as the type of fatty acids in the phospholipids (10), the amount of cholesterol (11, 12), and the presence of partial glycerides (13) has pronounced effects. This is to be expected as the metabolic steps depend on interactions with the surface layer of

Abbreviations: FFA, free fatty acids; LPL, lipoprotein lipase; apo, apolipoprotein; FCR, fractional catabolic rate; TG, triglyceride; RES, reticulo-endothelial system.

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the droplets, acquisition of apolipoproteins, recognition by LPL at endothelial binding-lipolysis sites and by catabolic receptors. However, these emulsions differ in many respects from those used for parenteral nutrition. In a recent study, we studied the effects of triglyceride structure on the metabolic fate of the fatty acids (14). On the one hand, we used synthetic triglycerides with medium chain fatty acids in the 1- and 3-positions and labeled oleic acid in the 2-position. We speculated that LPL would cleave off the 1 and 3 fatty acids and that the resulting monoglyceride would be taken up by the adjacent tissue. On the other hand, we used a mixture of medium chain triglycerides and oleic acid-labeled long chain triglycerides. In such a mixture LPL hydrolyzes mainly the medium chain triglycerides leaving a remnant droplet rich in the long chain component (15). This should divert most of the label to the liver with the remnant droplet. The expected differences in lipolysis were confirmed in vitro but surprisingly small differences were found in tissue distribution or oxidation of the labeled oleic acid when the emulsions were injected into rats. This raised the possibility that for these emulsions, droplet catabolism was more important than lipolysis.

To further explore the mechanisms by which lipid emulsions similar to the ones used for intravenous nutrition are metabolized, we have now prepared such an emulsion containing labeled triglycerides and labeled cholesteryl oleate. These labels makes it possible to follow both lipolysis and droplet catabolism. We have studied the plasma clearance and tissue distribution of the two labels, as well as oxidation of the fatty acids, and have compared this to the metabolism of lymph chylomicrons in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 190–210 g (Moellegaard Breeding Center, Ejby, Denmark) were used in the experiments. The rats were housed with free access to standard pellet diet and water in a 12-h light cycle (7 AM–7 PM). Experiments were carried out with both fed and fasted rats. The fed rats were used because this is the state when chylomicrons are normally metabolized. These rats were taken from their cages in the morning. We have observed that under these conditions, the thoracic duct lymph has a milky appearance, demonstrating ongoing chylomicron transport. At the end of each experiment, the stomach was inspected to make sure that it contained food. Fasted rats were used because in this state the interference from endogenous triglyceride-rich lipoproteins should be at a minimum. For these experiments, food was withdrawn 18 h before chylomicrons or emulsion was injected. All animal experiments were performed between 9 AM and 1 PM.

Animal procedures

The animals were anesthetized with Hypnorm Vet® (AB Leo, Helsingborg, Sweden, 0.1 ml) and Stesolid Novum (diazepam, Pharmacia, Stockholm, Sweden, 0.1 ml, 10 mg/ml). Hypnorm Vet® is a combination of the narcotic analgesic fentanyl citrate (0.315 mg/ml) and the tranquilizer fluanisone (10 mg/ml). All experimental procedures were approved by the local Animal Ethics Committee. The blood volume of the rats was calculated as 5.5% of body weight (16).

In all experiments that involved blood samples, chylomicrons or emulsion (4 mg triglycerides in 0.20 ml) were injected into an exposed jugular vein of anesthetized rats. Blood samples (0.20 ml) were taken from the other jugular vein. Less than 10 sec after the blood withdrawal had begun, the samples were put into 2 ml Doles mixture (isopropanol–heptane–1 M H_2SO_4, 40:10:1, v:v:v (17)) in tubes. The exact amount of blood in each sample was determined by weighing the tube before and after addition of the sample. After the last blood sample, the rats were killed by cervical dislocation and tissue samples were rapidly excised, rinsed in cold saline, weighed, and frozen. In some experiments aimed to determine oxidation of labeled fatty acids (Table 2), the injections were made into a lateral tail vein on unanesthetized rats. Before this injection, the veins were vasodilated by immersing the tail in warm water. After the injection, the rats were put back into their cage. At the end of the experiment the rats were killed and immediately put into saponification mixture (see below).

Chylomicrons

For this, rats weighing 250–300 g were maintained on standard chow and tap water. Under anesthesia, the thoracic duct was cannulated (18) with PE-50 tubing (Clay Adams, Becton Dickinson and Co., Parsippany, NJ) and a similar tubing was inserted in the stomach. After the operation, the rat was placed in a restraining cage and infused intragastrically with a solution of 0.85% NaCl, 5% glucose and 0.05% KCl at a rate of 2 ml/h. The rat was allowed to drink the same solution ad libitum. The following day the rats were given an intragastric bolus of 2 ml 20% Intralipid® (Pharmacia Hospital Care, Stockholm, Sweden) to which 50 μCi [1-^{14}C]oleic acid (Amersham) and 50 μCi [11,12-^{3}H]retinol (Amersham) had been added. Lymph was collected at room temperature for 5 h in sterile tubes containing EDTA (0.1%, pH 7.4) and gentamicin (0.01%, Garamycina®, Schering Corporation USA, Kenilworth, NJ). The lymph flow was

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2-4 ml per h. The lymph was layered under 0.9% NaCl containing EDTA (0.1%, pH 7.4) and centrifuged for 35 min at 35,000 rpm (120,000 *g*) in a Beckman SW 50 rotor at 15°C. The top layer, containing the chylomicrons, was resuspended and centrifuged again under the same conditions. The final preparation was diluted in 0.9% saline. More than 93% of the [^{14}C]oleic acid label moved with triglycerides and more than 92% of [^{3}H]retinol label moved as retinyl esters on thin-layer chromatography on Silica G plates using heptane-diethyl ether-acetic acid 80:20:1 (v/v/v) as moving phase. The chylomicrons were stored at 8°C and used within 48 h.

Emulsion

The starting materials for preparation of the labeled experimental emulsion were chosen to mimic those used in commercially available emulsions. The triglycerides were from soy bean oil and the phospholipids from egg yolk. To 20 g triglycerides was added 12.5 mCi tri-[9,10-^{3}H]oleoylglycerol (prepared by Lennart Krabish, Dept. of Medical Biochemistry, University of Lund, Sweden) and 1 mCi cholesteryl-[1-^{14}C]oleate (NEN-638, NEN-DuPont, Stockholm, Sweden). To stabilize the emulsion, 1.2 g of egg yolk phospholipids was added and the tonicity of the emulsion was adjusted by addition of 2.25 g glycerol. The pH of the final emulsion was adjusted to 8.1 by addition of sodium hydroxide. The lipid phase was dispersed in the amount of water needed to make exactly 100 ml of the labeled experimental emulsion. The emulsion was prepared by high pressure homogenization in a Ranni Mini-lab 8.30H (PAV Rannies AS, Albertslund, Denmark) and then dispensed into 3-ml glass vials before heat sterilization.

Determination of droplet sizes

The sizes of the emulsion and chylomicron droplets were determined by Photon Correlation Spectroscopy (PCS) using a Malvern 4700 multiangle system with Malvern PCS for Windows software (version 1.1, Malvern Instruments Limited, Worchestershire, U.K.). Measurements were carried out at 90° scattering angle in highly diluted samples (1:12,500). The extensive dilution was necessary to avoid secondary scattering. The size of the droplets in each sample was determined as the intensity based zeta-average diameter.

Analyses

The blood samples (0.20 ml) were taken into 2 ml Doles mixture. After thorough mixing, 1.5 ml heptane and 1.2 ml distilled water were added for separation of polar and neutral lipids. One ml of the top-phase was taken to tubes containing 1 ml of 50 mM NaOH in 50% ethanol (19). After vortexing and centrifugation, the upper phase will contain neutral lipids, i.e., retinyl esters, cholesteryl esters, and triglycerides, and the lower phase will contain free fatty acids. An aliquot of the upper phase (0.7 ml) was transferred to a scintillation vial and 4 ml of scintillation liquid (OptiPhase HiSafe III, LKB-Pharmacia, Uppsala, Sweden) was added. The lower phase, containing free fatty acids, was washed twice with 3 ml heptane. Then, an aliquot (0.8 ml) was transferred to a scintillation vial and 4 ml of scintillation liquid was added.

Tissues were homogenized (Polytron, Kinematica, Basel, Switzerland) and extracted in 30 ml chloroform-methanol 2:1 per gram. Three-ml aliquots of the extract were taken and 1.2 ml of 2% KH_2PO_4 was added to break the system into a lower phase containing the lipids and an upper phase containing the water-soluble material (20). One ml of the chloroform phase was transferred to a scintillation vial. The chloroform was evaporated before scintillation liquid was added. The lipid separations were done in duplicate.

To estimate oxidation of labeled fatty acids, the carcass was digested in a mixture of 100 g KOH, 100 ml distilled water, and 300 ml 95% ethanol (21). The saponification was at room temperature for at least 20 h. At this time the only remnants that remained were some bone fragments which fell apart on stirring. Fifty percent ethanol in water (v/v) was added to a final volume of 1000 ml. After careful mixing, 2-ml aliquots were taken in triplicate. To extract the fatty acids, 2 ml heptane was added and the pH was adjusted by addition of concentrated HCl using thymol blue as indicator (pH < 1.2). After vortexing and a short centrifugation (10 min, 3000 *g*), 1 ml of the heptane phase was transferred to a scintillation vial and the radioactivity was determined. Oxidation of fatty acids was estimated as the difference between the amount administered and the amount recovered in the carcass by the saponification procedure plus what was extracted from tissue samples with chloroform-methanol.

Calculations

The SAAM II program (Resource Facility for Kinetic Analysis, University of Washington, Seattle, WA) was used to analyze the data (22). The values for radioactivity remaining in blood were fitted to the monoexponential decay function, i.e., $N = N_0 e^{-\lambda * t}$. The values reported are mean ± SEM for parameters derived from data for five individual rats in each group.

Statistical significance of differences fed versus fasted and emulsion versus chylomicrons was tested by ANOVA analysis and Scheffe's test. In some experiments Student's *t*-test was used to test differences between groups. The SPSS for Windows program was used for these calculations (version 6, SPSS Inc., Chicago, IL).

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In vitro lipolysis

To explore the availability of the labeled emulsion for hydrolysis by LPL, we compared it to Intralipid® 200 mg/ml (Pharmacia Hospital Care, Uppsala, Sweden) at 25°C. Final composition of the medium was triglycerides 5 mg/ml, Tris-HCl 20 mM, NaCl 0.1 M, heparin 100 μg/ml, BSA 60 mg/ml, heat-inactivated serum from fasted rats 50 μl/ml. The final volume was 8 ml and the pH was 8.5 (19). The reaction was started by addition of 130 mU bovine LPL (23). Samples were taken at 0, 15, 30, 45, and 60 min. One set of samples, each 1 ml, was extracted by 5 ml Doles mixture for later titration of the fatty acids (19). Another set, each 0.2 ml, was extracted in 2 ml Doles mixture and processed as described above for determination of radioactivity in released labeled fatty acids.

RESULTS

Emulsion and chylomicrons

To study the metabolism of chylomicrons and lipid emulsions, we prepared chylomicrons labeled with [11,12-^{3}H]retinol (core label) and [1-^{14}C]oleic acid (TG label) and a lipid emulsion, similar to commercially available lipid emulsions for parenteral nutrition, labeled with cholesteryl-[1-^{14}C]oleate (core label) and tri-[9,10-^{3}H]oleoylglycerol (TG label). To make the chylomicrons as similar as the possible to the emulsion, we gave lymph donor rats isotopes suspended in Intralipid®. As retinyl esters and cholesteryl esters are poor substrates for LPL and rats have no cholesteryl ester transfer activity in plasma (24), the labeled retinyl esters and cholesteryl esters will stay with the droplets until endocytosis and lysosomal degradation.

The diameter of the droplets in the labeled experimental emulsion was 0.40 μm as determined by photon correlation spectroscopy (Z-average, 90° scattering angle). The size of the droplets in a commercially available emulsion measured simultaneously as a reference was 0.31 μm (Intralipid® 20%). Due to the lower efficiency of the small scale process and equipment, the resulting droplet size was larger in the experimental emulsion compared to the commercial reference emulsion. Chylomicrons had a diameter of 0.21–0.22 μm (five different batches of chylomicron preparations). The estimated diameters were not different between whole lymph (after removal of lymphocytes) and isolated chylomicrons.

In vitro lipolysis

Figure 1 compares in vitro hydrolysis by LPL of the experimental emulsion and Intralipid®. Both the absolute rate and the time courses of hydrolysis were similar.

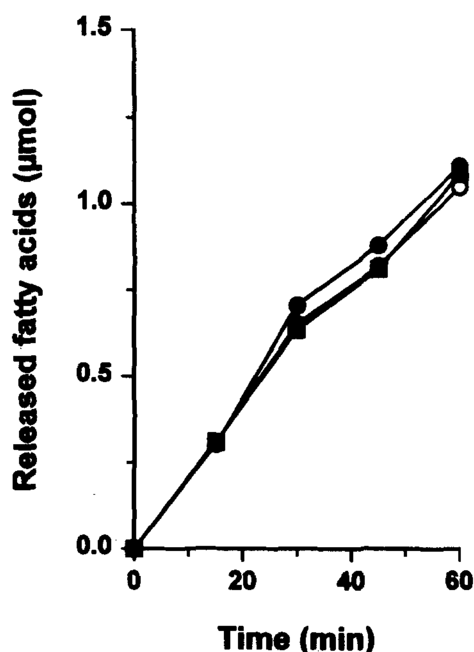

Fig. 1. In vitro lipolysis of the labeled emulsion compared to a commercial emulsion used for intravenous nutrition. The substrate concentration was 5 mg triglycerides per ml. The commercial emulsion used was Intralipid® 20%. Ten mU LPL was added per ml incubation mixture. Fatty acids were extracted after 0, 15, 30, 45, and 60 min and determined directly by titration (●, labeled emulsion; ■, commercial emulsion) or by calculation from radioactivity in released fatty acids (○).

We also compared hydrolysis of the labeled triolein (TG label) to that of bulk triglycerides in the experimental emulsion. For this we simultaneously monitored lipolysis by direct titration of the released fatty acids and by radioactivity in the released fatty acids. The release of fatty acids and of labeled fatty acids were similar, demonstrating that the tracer behaved similar to the bulk triglycerides (Fig. 1). There was no detectable release of ^{14}C-labeled oleic acid from the cholesteryl esters of the emulsion during in vitro lipolysis.

In other experiments we have observed that fatty acids are released at similar rates from chylomicrons and from Intralipid® (data not shown).

Turnover in blood

Four mg of chylomicrons or emulsion was injected to study the turnover in blood. This dose was chosen as the least amount that gave sufficient radioactivity for convenient measurement of blood and tissue radioactivity. Still, 4 mg TG is well below the level at which signs of saturation have been observed in previous studies (14, 25).

When chylomicrons or emulsion were injected, core label rapidly disappeared from the circulation following first order kinetics (**Fig. 2**). There were no major differences between the emulsion and the chylomicrons in

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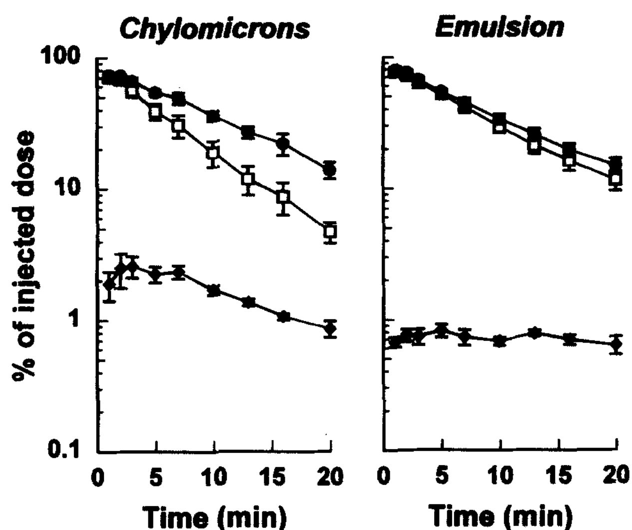

Fig. 2. Clearance from blood of the labeled lipid emulsion compared to chylomicrons. Fed anesthetized rats were given chylomicrons or emulsion (4 mg triglycerides in 0.2 ml). Blood samples were taken at the indicated times and immediately put into extraction mixture and later separated into a free fatty acid fraction, FFA (◆), and a neutral lipid fraction containing TG label (□) and core label (●). Values are means ± SEM, n = 5.

this respect. In contrast, there was a marked difference in disappearance rate for the TG label (Fig. 2). For chylomicrons, the TG label was cleared more rapidly than the core label. For instance, 13 min after injection only 11.9 ± 2.9% remained in blood compared to 26.9 ± 2.7% of the core label. For emulsion this difference was much less. Thirteen min after injection 21.1 ± 2.8% of the TG label and 25.6 ± 2.7% of the core label remained in blood. Label rapidly appeared in the plasma FFA after injection of chylomicrons (Fig. 2), demonstrating that some of the triglycerides had been lipolyzed. The FFA radioactivity reached a peak 3 min after injection and then decreased again. This time course suggests that the FFA were generated by hydrolysis of chylomicron triglycerides at endothelial sites. Much less label appeared in plasma FFA after injection of emulsion; the peak value was only 0.82% compared to 2.6% after chylomicrons. This suggests that emulsion triglycerides were lipolyzed more slowly than chylomicron triglycerides.

With time, the chylomicrons circulating in blood became more lipolyzed. **Figure 3** shows the lipolysis index: TG label divided by core label. This plot supports the view that emulsion triglycerides were less rapidly lipolyzed than chylomicron triglycerides. This was most marked in the fed rats where the lipolysis index remained above 0.75 for the emulsion droplets even after 20 min, whereas for chylomicrons the index dropped below 0.5 by 10 min.

The disappearance curves for individual rats were fitted by single exponential functions. The means of the derived fractional catabolic rates (FCRs) are given in **Table 1.** For core label the FCRs did not differ between emulsion and chylomicrons or with the nutritional state. The values were 0.092–0.095 min^{-1} corresponding to half-lives of 7.3–7.7 min. In fed rats, the FCR for TG label was 0.108 min^{-1} for emulsion compared to 0.172 min^{-1} for chylomicrons. This corresponds to half-lives of 6.4 and 4.0 min, respectively, and the difference was statistically significant. Also in fasted rats, the FCR was lower for emulsion than for chylomicron TG label, but the difference was not statistically significant. The relative rates of clearing were calculated as the FCR for TG label divided by the FCR for core label (Table 1). A relative rate of 1 would imply that core label was cleared from blood at the same rate as TG label. For chylomicrons the relative rates were 1.84 in fed and 1.65 in fasted rats whereas for emulsion the relative rates were only 1.15 and 1.26, respectively. The difference between emulsion and chylomicrons was significant in both nutritional states, confirming the conclusion that lipolysis occurred more slowly with the emulsion droplets.

Extrapolation of the disappearance curves to zero time gives an apparent distribution volume (Table 1). In all cases this was the same for the core and TG labels. Studies with labeled red blood cells have shown that the blood volume in these rats is about 5.5% of body weight (16). Hence, a 200 g rat has about 11 ml blood. The calculated distribution volumes were larger than this. In fed rats the calculated volume was about 13 ml for both chylomicrons and emulsion. In fasted rats the apparent distribution volume for emulsion was significantly ($P <$ 0.05) larger, about 15 ml. The relation between the

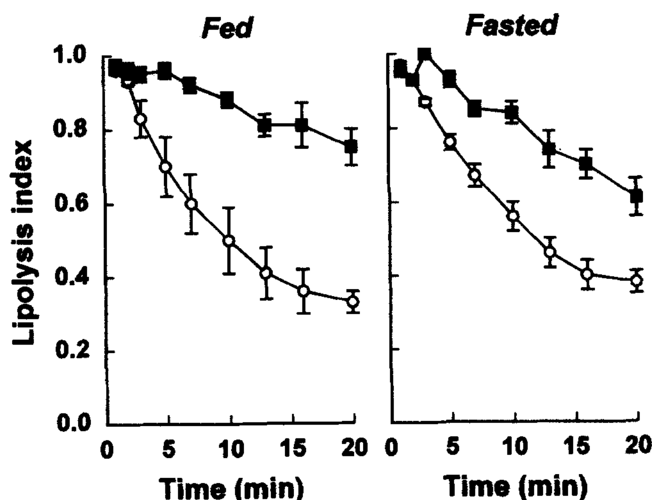

Fig. 3. Lipolysis index for chylomicrons/emulsion droplets remaining in blood. The lipolysis index is a measure of the extent to which triglycerides have been removed by lipolysis. It is the quotient of TG label over core label. The injected material has a quotient of 1, by definition. The figure shows the lipolysis index for emulsion (■) and chylomicrons (○) in fed rats (left panel) and fasted rats (right panel).

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TABLE 1. Kinetic parameters for clearance of chylomicrons and emulsion

	Apparent Distribution Volume		Rate Constant		Relative Rate λ(TG label)/λ(Core label)
	TG Label	Core Label	TG Label	Core Label	
	ml		λ, *min*⁻¹		
Chylomicrons					
Fed rats	12.8 ± 0.5	13.0 ± 0.4	0.172 ± 0.027	0.092 ± 0.008	1.84 ± 0.18
Fasted rats	12.8 ± 0.2	13.1 ± 0.2	0.147 ± 0.014	0.090 ± 0.009	1.65 ± 0.08
Emulsion					
Fed rats	12.8 ± 0.2	12.8 ± 0.3	0.108 ± 0.011	0.093 ± 0.008	1.15 ± 0.04[c]
Fasted rats	14.8 ± 0.8[a,b]	15.2 ± 1.0[a,b]	0.120 ± 0.008	0.095 ± 0.005	1.26 ± 0.05[c]

Fed and fasted rats were given chylomicrons or emulsion (4 mg triglycerides in 0.2 ml). The time-curves for disappearance of label from blood were fitted to the equation $N = N_0e^{-\lambda \cdot t}$ using the SAAM program. Only time points from 3 to 13 min were included. Data fitting was done for individual rats. Apparent distribution volume for the injected materials was calculated from the intercept of the fitted disappearance curves at time zero. Values are means ± SEM for the derived parameters (n = 5).

[a] $P < 0.05$, fed vs. fasted.

[b] $P < 0.05$, chylomicrons vs. emulsion.

[c] $P < 0.01$, chylomicrons vs. emulsion.

distribution volume for emulsion/chylomicrons and that for red blood cells gives a measure of the fraction seen in blood. This was 85% and 86% for chylomicrons and 86% and 74% for emulsion in fed and fasted rats, respectively.

In other experiments we followed the clearance of emulsion and chylomicrons for 40 min (data not shown). As we took fewer samples during the first 15 min the data are less precise, but rates for clearance of TG and core label as well as for apparent distribution volumes were entirely consistent with the data in Table 1. The longer time allowed us to follow the continued decay of core label after 20 min. The removal from blood continued to follow the same single exponential rate until less than 5% remained in the circulation.

Oxidation

Oxidation of labeled fatty acids was estimated as the difference between the injected dose and the radioactivity recovered with fatty acids after saponification of whole rats. Fatty acids were oxidized more rapidly from chylomicrons than from emulsion, particularly in the fed rats (**Table 2**). Twenty minutes after injection of chylomicrons, 37% and 50% of the fatty acid label had been lost in fed and fasted rats, respectively, compared to only 18% and 31% in the rats given emulsion. When comparing the effects of different factors in an ANOVA, emulsion versus chylomicrons and fed versus fasted were significant factors while anesthesia and different times were not significant.

Tissue distribution of radioactivity

Twenty min after injection of the labeled chylomicrons or emulsion, the tissue distribution of injected label was determined. At this time less than 20% of the core label remained in circulation, independent of vehicle. Thus, more than 80% of the droplets had left the circulating blood.

For chylomicrons, about 25% of the core label and 12–15% of the TG label was in the liver in both nutritional states (**Table 3**). Hence, the chylomicrons had lost at least half of their triglycerides by lipolysis. In fasted rats given emulsion, radioactivity in the liver was similar to that for rats given chylomicrons, 26% of core label and 15% of TG label. In fed rats, however, only 12.7% of core label was in the liver. This was accompanied by 11.8% of TG label, suggesting that most of the label was in droplets that had undergone very little lipolysis. This

TABLE 2. Oxidation of TG label administered in chylomicrons or in emulsion

	Fed		Fasted	
	Emulsion	Chylomicrons	Emulsion	Chylomicrons
		% of injected dose		
20 min, anesthetized	18.0 ± 5.8	37.0 ± 7.3	31.2 ± 6.2	49.8 ± 5.1
40 min, anesthetized	24.6 ± 3.4	33.2 ± 3.0	46.2 ± 1.9	52.2 ± 2.1
40 min, unanesthetized	17.9 ± 4.9	41.8 ± 6.4	59.3 ± 3.6	60.6 ± 5.8

Rats were injected with labeled chylomicrons or with the labeled emulsion. After the indicated times the rats were killed and the amount of lipid soluble radioactivity was determined as described in Methods. Oxidation was calculated as the difference between injected label and label recovered as lipid. In an ANOVA, the nutritional state and whether the rats were given emulsion or chylomicrons were significant factors, but not the time after injection or the anesthetic state. Values shown are mean ± SEM, n = 5.

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TABLE 3. Distribution of radioactivity from chylomicrons or emulsion in some selected tissues

Label	Chylomicrons		Emulsion		Effect
	Fed	Fasted	Fed	Fasted	
		% of injected dose			
Triglyceride					
Blood	4.7 ± 0.8	6.6 ± 1.5	11.6 ± 2.0	7.9 ± 1.4	B
Liver, per organ	14.3 ± 1.7	12.5 ± 2.4	11.8 ± 2.9	14.9 ± 2.0	
Heart, per organ	0.60 ± 0.33	0.88 ± 0.14	0.21 ± 0.02	0.95 ± 0.05	A
Lung, per organ	0.41 ± 0.02	0.41 ± 0.07	0.30 ± 0.02	0.30 ± 0.03	B
Kidney, per organ	0.32 ± 0.02	0.34 ± 0.04	0.15 ± 0.01	0.18 ± 0.01	B
Spleen, per organ	1.08 ± 0.20	0.91 ± 0.09	3.36 ± 0.28	3.00 ± 0.24	B
Epididymal fat pad, per g	2.06 ± 0.25	0.51 ± 0.05	1.69 ± 0.17	0.75 ± 0.17	A
Diaphragm, per g	0.50 ± 0.05	1.69 ± 0.13	0.21 ± 0.01	0.87 ± 0.01	A B C
Core					
Blood	13.9 ± 2.0	17.5 ± 3.9	15.1 ± 1.9	12.5 ± 1.5	
Liver, per organ	26.1 ± 5.5	25.0 ± 5.5	12.7 ± 1.8	26.5 ± 2.0	A B C
Heart, per organ	0.32 ± 0.08	0.79 ± 0.05	0.36 ± 0.06	3.29 ± 0.31	A B C
Lung, per organ	0.30 ± 0.02	0.49 ± 0.10	0.40 ± 0.01	0.45 ± 0.04	
Kidney, per organ	0.34 ± 0.10	0.26 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	
Spleen, per organ	1.30 ± 0.21	1.51 ± 0.09	4.70 ± 0.33	4.85 ± 0.35	B
Epididymal fat pad, per g	0.32 ± 0.03	0.27 ± 0.06	1.40 ± 0.13	0.45 ± 0.04	A B C
Diaphragm, per g	0.36 ± 0.07	0.64 ± 0.03	0.38 ± 0.02	0.93 ± 0.05	A B C

Same experiment as Table 1. After the final blood sample had been taken at 20 min, the rats were killed and the tissues were dissected out, homogenized, and extracted as described in Methods. Statistical differences were tested using ANOVA. The results of this analysis are shown at the right end of the table. A: denotes a significant effect of nutrition; B: denotes a significant effect of chylomicrons versus emulsion; and C: denotes a significant two-way interaction. In all cases, $P < 0.05$ is considered a significant effect. The data are expressed as % of injected dose and are means ± SEM for groups of five rats.

is in agreement with the observations in blood, where the lipolysis index remained above 0.75 even at 20 min.

A major difference between emulsion and chylomicrons was the recovery in the spleen. For chylomicrons 1.3% (fed) and 1.5% (fasted) of the core label was found here. Hence, only a small fraction of the chylomicrons/chylomicron remnants had been removed by the spleen. For emulsion, three times as much of the core label, almost 5%, was in the spleen.

For chylomicrons there were major effects of nutritional state, as expected, in the distribution of TG fatty acids into adipose tissue and into the muscle tissues studied, heart and diaphragm. In the epididymal fat pad more than four times as much fatty acid label was found in fed compared to fasted rats. This increase occurred without a corresponding increase of core label, which was low, suggesting that the uptake was directed by increased LPL activity in combination with a metabolic pattern that enhanced esterification of fatty acids in the tissue.

There was also more uptake of emulsion-derived fatty acids into adipose tissue in fed than in fasted rats, but the nutritional effect was less than for chylomicrons. There was more core label in adipose tissue after emulsion than after chylomicrons. The ratio of TG label/core label was more than 6 in fed rats given chylomicrons, whereas it was less than 1.2 in fed rats given emulsion. This suggests that uptake of fatty acids from chylomicron triglyceride is largely dependent on LPL-mediated lipolysis, while the uptake of fatty acids from emulsions is largely dependent on capture of droplets.

Twice as much of the chylomicron core label was recovered in the hearts of fasted rats compared to fed rats. In rats given emulsion the effect of nutrition was even more dramatic, there was nearly 10 times as much core label in hearts of fasted rats, where it amounted to more than 3% of the administered dose. The amount of TG label recovered in the heart did not change as dramatically. For the group that showed the highest amount of core label in the heart, fasted rats given emulsion, the ratio TG label over core label was approximately 0.3, significantly less than the ratio in blood and in the liver, where the ratio was around 0.6. This suggests that the emulsion droplets bound to/taken up by the heart had been rather efficiently lipolyzed.

DISCUSSION

The main conclusion of this study is that emulsion catabolism cannot be fully accounted for by the mechanisms proposed for chylomicron metabolism; triglyceride unloading in extrahepatic tissues through the action of LPL followed by endocytosis of remnant droplets in the liver. In fed rats, more than 80% of the core label, i.e., the droplets, disappeared from the circulating blood within 20 min with little sign of triglyceride lipolysis. The lipolysis index remained above 0.7 showing that the droplets in blood had lost only a small proportion, less than 30%, of their triglycerides, whereas for chylomicrons the lipolysis index decreased below 0.4 showing that more than 60% of the

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triglycerides had been lost. A corresponding difference was evident in appearance of label in the plasma FFA. In rats given emulsion the peak radioactivity in plasma FFA corresponded to less than 0.9% of the injected dose, whereas in rats given chylomicrons the peak radioactivity in plasma FFA was three times higher. The same pattern was evident in several tissues. In adipose tissue of fed rats given chylomicrons the ratio between fatty acid and core label was above 6, showing that fatty acids had been taken up after LPL-mediated hydrolysis of the chylomicron triglycerides, but few chylomicrons or remnants remained in the tissue. In contrast, for rats given emulsion that ratio was only 1.2 showing that nearly as much emulsion droplets as emulsion-derived fatty acids were present in the adipose tissue. In the liver of fed rats given chylomicrons there was 14% of TG label and 26% of core label, giving a ratio of 0.55. Hence, the particles taken up by the liver had lost at least 45% of their triglycerides. In fact, that value must be higher as some labeled fatty acids must have arrived in the liver with plasma FFA. Rats given emulsion had a ratio in their livers of 0.93 indicating that the droplets taken up had undergone only little lipolysis. In this case the contribution of plasma FFA was less as the radioactivity in that fraction was much less than for the rats given chylomicrons. All in all, the data indicate that for emulsion the droplets left plasma with much less lipolysis than for chylomicrons.

There are several reports that clearance of triglycerides occurs more rapidly from large than small particles, both for chylomicrons (26) and for emulsion droplets (2). In our study, the emulsion droplets were considerably larger than the chylomicrons. Nonetheless, TG label was cleared less rapidly from the emulsion. Core label, i.e., the droplets, was cleared at the same rate for emulsion as for chylomicrons. Hence, the slow clearing of emulsion triglycerides cannot be explained by a difference in how the droplets as such were cleared, but was due solely to decreased lipolysis of the droplet triglycerides. The reason for the limited lipolysis is unclear. It is unlikely that it is due to an inadequate activation of LPL by apoC-II for several reasons. First, when incubated in vitro in rat plasma the emulsion was an excellent substrate for LPL. Second, in other experiments with a similar emulsion it was shown that addition of apoC-II in vitro causes immediate activation of LPL (27). Third, apoC-II transfers more rapidly than apoC-III and does not seem to be the determining factor for Intralipid TG removal, at least not in humans (28).

It is not clear what the mechanism is by which the emulsion droplets leave the circulating blood after only limited lipolysis. In the liver this could be by the remnant receptor pathway, but the blood plus the liver accounted for 40% or less of the core label after 20 min. Hence, most of the droplets must have located in extrahepatic tissues. Closer inspection of the data for chylomicron label shows that here, too, a large proportion of the particles must have located in extrahepatic tissues. After 20 min only about 40% of the core label was in the liver or blood, as for emulsion. In other experiments with chylomicrons we have extracted core label from all parts of the rat and have found more than 40% in extrahepatic tissues (M. Hultin and T. Olivecrona, unpublished results). This is in contrast to the view which states that, after extensive hydrolysis of triglycerides by LPL, the overwhelming majority of the remnants end up in the liver (7). In other studies, with somewhat differing design, we have recovered 25–60% of injected chylomicron core label in the liver 20 min after injection. The present data are in the lower end of this range, but not unique. To shed further light on the role of the liver we have reexamined the literature for reports on how much core label has been found there (**Table 4**). If we only consider experiments of 20 min or more, i.e., when most of the label has disappeared from plasma, the range is 20–88% of core label in the liver. Hence, it is a general finding that a large proportion, often about half of the chylomicrons/remnants, are recovered in extrahepatic tissues. The data suggest that the proportion varies widely with the conditions.

There are previous reports that core components from chylomicrons are taken up by extrahepatic tissues. Scow, Blanchette-Mackie, and Smith (29) and Zinder et al. (30) perfused chylomicrons through rat mammary glands and found that not only triglycerides but also cholesteryl esters and phospholipids were extracted by the gland. Fielding (31) perfused rat hearts with chylomicrons and found that appreciable amounts of cholesteryl esters were taken up. The molecular and cellular mechanisms involved in these processes have not been defined.

Hussain et al. (32, 33) injected canine chylomicrons into rats and found that the spleen took up 4.7–9.1% of the core label. They concluded that the reticulo-endothelial system (RES) participates in the clearing of chylomicrons. We found only 1.0–1.5% of the chylomicron core label in the spleen. An uptake of 1–2% has been found in several other studies (25, 34–37). Somewhat more of the core label from emulsion droplets was found in the spleen. This did not amount to more than 5% and there was less than 1% in the lung. Hence, the RES was not a major component in clearing of this emulsion.

The present emulsion was prepared to resemble the emulsions used for intravenous nutrition. The lipid composition was identical to that of a commercial emulsion but the mechanical method for preparing the emulsion differed from that used in industrial production, and the droplets were larger than in the commercial

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TABLE 4. Literature data on liver recoveries of core label

Reference	Time	% of Injected Dose		Experimental Conditions					
		Liver	Blood	Label	Dose	Anesthesia	Rat Strain	Sex	Rat Weight
Belcher et al., 1985 (40)	10	18		^{14}C-CE/C		pentobarbital	SPRD	M	290–320 g
Staprans et al., 1990 (41)	10	28		^{3}H-RE	10		SPRD	M	180–250 g
Redgrave et al., 1985 (9)	10	33	25	^{3}H-CE		unanesthetized	Holtzman	M	250–250 g
Staprans et al., 1990 (41)	10	42		^{3}H-RE	10		SPRD	M	60 g
Staprans et al., 1990 (41)	10	42		^{3}H-RE	10		SPRD	F	60 g
Staprans et al., 1990 (41)	10	46		^{3}H-RE	10		SPRD	F	180–250 g
Redgrave, 1977 (42)	10	46	44	^{3}H-CE			SPRD	M	209–233 g
Blomhoff et al., 1982 (43)	15	16	36	^{14}C-CE	5	anesthetized	Wistar		
Green et al., 1993 (44)	20	30		^{3}H-RE		unanesthetized	Wistar	M	308–479 g
Blomhoff et al., 1982 (43)	20	50	23	^{3}H-RE	0.5	anesthetized	Wistar	M	200–250
Quarfordt et al., 1967 (45)	20	70	21	^{14}C-CE	23	diethyl ether	SPRD	M	150–200 g
Redgrave et al., 1987 (35)	30	20	9	^{3}H-CE	5, 8		Holtzman	M	219 ± 35.8 g
Redgrave et al., 1988 (46)	30	23		^{3}H-CE	6	unanesthetized	Holtzman	M	-
Jäckle et al., 1993 (47)	30	37	41	^{125}I-CR		diethyl ether	SPRD	M	200–300 g
Blomhoff et al., 1982 (43)	30	45		^{3}H-RE	50	anesthetized	Wistar		
Mamo et al., 1993 (48)	30	50		^{3}H-CE		pentobarbitone	Wistar	M	220–250 g
Blomhoff et al., 1982 (43)	30	50	12	^{14}C-CE	5	anesthetized	Wistar		
Hussain et al., 1989 (33)	30	54	4.7	^{3}H-RE	33	methoxyflurane	SPRD	M	200–400 g
Ly et al., 1992 (49)	30	57	10	^{3}H-CE		unanesthetized	Wistar	M	200–250 g
Hussain et al., 1989 (33)	30	66	7.6	^{3}H-RE	200	methoxyflurane	SPRD	M	200–400 g
Blomhoff et al., 1982 (43)	30	74	24	^{3}H-RE	5	anesthetized	Wistar		
Hussain et al., 1989 (33)	30	88	0.2	^{3}H-RE	24	methoxyflurane	SPRD	M	200–400 g
Green et al., 1984 (50)	32	70	15	^{14}C-CE		diethyl ether	SPRD	M	100 g + 5 w
Green et al., 1984 (50)	44	70	15	^{14}C-CE		diethyl ether	SPRD	M	100 g + 5 w
Olivecrona et al., 1970 (51)	60	80	0.2	^{14}C-CE			SPRD	M	200 g

The amount of label recovered, in different studies, in rat livers after chylomicron injection. In all studies, except in those by Hussain et al. (33), rat lymph chylomicrons were studied. Abbreviations used: C, cholesterol, CE, cholesteryl esters, CR, chylomicron remnants, F, female, M, male, RE, retinyl esters, SPRD, Sprague-Dawley, w, weeks. Time is in min after injection; dose is in mg triglycerides; rat weight is in grams.

emulsion. Other investigators have prepared emulsions by extensive sonication of mixtures rich in phospholipids (9–12). This yields smaller droplets that are apparently metabolized similar to chylomicrons with extensive hydrolysis of the triglycerides (9). When such emulsions are prepared without any cholesterol, lipolysis proceeds at a normal rate but the droplets are cleared slowly as if they must first acquire a certain amount of cholesterol (11, 12). When 2-monostearin was included, the resulting droplets were cleared slowly (13, 38). This decrease in clearance could also be accomplished by use of triglycerides with oleic acid in the 1,3-position and stearic acid in the 2-position. 2-Monoolein did not decrease the emulsion clearance. When the emulsions were prepared with high amounts of free cholesterol (22%) and phospholipids (23%), the resulting droplets were lipolyzed slowly but the droplets were readily cleared and the TG label disappeared from plasma at the same rate as the core label (11). Emulsions prepared with different phospholipids differ much in metabolism (10, 39). A saturated fatty acid (palmitic acid) at the *sn*-2-position of phosphatidyl choline appears to decrease the catabolism of an injected emulsion. Taken together, these data show that for small emulsion droplets prepared by extensive sonication the metabolic pattern is highly dependent on composition.

Previous studies on emulsions of the type used for intravenous nutrition have generally been interpreted to show that metabolism proceeds as for chylomicrons. This has been based on similar first order kinetics for triglycerides and similar rate constants (6). This would be true also for our data if we only considered plasma triglycerides. The additional data on core label and on tissue distribution of the labeled lipids revealed, however, marked differences in metabolism. Future comparisons of different emulsions, and development of new emulsions, must take this into account and study not only the rate of clearance but also the pathways involved. ■

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