Triglycerides in Fish Oil Affect the Blood Clearance of Lipid Emulsions Containing Long- and Medium-Chain Triglycerides in Mice

Kemin Qi, Toru Seo, Zaifang Jiang, Yvon A. Carpentier, and Richard J. Deckelbaum

Abstract

Lipid emulsions containing long-chain triglycerides (LCT) and medium chain triglycerides (MCT) are widely used in parenteral nutrition. Recently, fish oil (FO) triglyceride (TG)-derived emulsions are considered therapeutic because of their many beneficial biological modulatory actions. We investigated in mice whether adding 10% FO to an intravenous lipid emulsion with MCT and LCT (MCT:LCT:FO = 50:40:10% by wt) would affect particle blood clearance and tissue targeting in comparison to LCT (100% by wt) and MCT:LCT (50:50% by wt) emulsions. The 3 emulsions were labeled with [3H]cholesteryl oleoyl ether and administered by bolus injection (400 μg TG/mouse) to C57BL/6J mice. Contributions of LDL receptor (LDL-R) and LDL-R-related protein to emulsion catabolism were assessed using LDL-R-deficient mice and preinjection of lactoferrin, and the effects of lipoprotein lipase (LPL) were determined by preinjection of heparin and Triton WR 1339. Although fractional catabolic rates did not differ among the 3 emulsions, blood removal at each time point after injection was greater for MCT:LCT:FO particles due to their higher initial margination volume. Compared with MCT:LCT and LCT emulsions, patterns of tissue uptake of the MCT:LCT:FO emulsions were different, e.g. MCT:LCT:FO emulsion particle uptake was lower in heart, adipose tissue, and muscle, and higher in lung, and the removal of MCT:LCT:FO emulsion particles was less dependent on LDL-R, LDL-R-deficient and lactoferrin-sensitive pathways. These data suggest that the addition of a low percentage of FO to MCT:LCT emulsions substantially changes their particle clearance and tissue uptake mechanisms.


Introduction

Lipid emulsions have been widely used in parenteral nutrition as energy sources and for supplementation of essential fatty acids for over 40 y, at first exclusively relying on soy oil or safflower oil based long-chain triglycerides (LCT) unveil (n-6) fatty acids (1,2). Over the past 2 decades, medium-chain triglycerides (MCT) and more recently fish oil (FO) unveil (n-3) fatty acids have been introduced into therapeutic emulsions. A series of clinical trials found that mixed lipid emulsions containing MCT and LCT at 1:1 wt ratios (MCT:LCT, 50:50, wt:wt) have advantages over conventional LCT emulsions in improving some endpoints, such as decreasing adverse liver outcomes and improving immunosuppression and pulmonary disease outcomes (3,4). Recently, FO-containing emulsions have received increasing attention, because the major fatty acids in FO, eicosapentaenoate [20:5(n-3)] (EPA) and docosahexaenoate [22:6(n-3)] (DHA) have beneficial biological activities in retinal and brain development, immune function, endothelial function, blood clotting, and prevention of cardiac arrhythmias (4-6).

The metabolism of lipid emulsions has long been considered to be similar to that of chylomicrons with intravascular lipolysis by lipoprotein lipase (LPL) being followed by tissue uptake of remnant particles (7-9). However, recent studies have suggested that lipid emulsions are cleared from blood with less lipolysis than chylomicrons and that substantial amounts of emulsions can be cleared as almost intact whole particles by different tissues (10,11). The metabolism of lipid emulsions is affected by many factors, including triglyceride (TG) composition (12-15). For example, MCT:LCT emulsion TG are cleared faster from blood than pure LCT emulsion TG (12,13). Recently, we reported that pure FO emulsion particles are removed from blood faster and by different pathways as compared with LCT emulsions. Removal of LCT emulsions is modulated by LPL, apolipoprotein E (apoE), LDL receptor (LDL-R), and lactoferrin-sensitive pathways. In contrast, clearance of FO emulsions relies on LPL to a much lesser extent and is apparently independent of apoE, LDL-R, and lactoferrin-sensitive pathways (14).
The FO emulsions used in our previous study (14) were made exclusively from FO with a high percentage (~50%) of fatty acids as EPA and DHA and are limited in clinical use, because they are usually added as an “adjunct” during infusion of other lipid emulsions. In this study, we explored the blood clearance and tissue targeting of a MCT:LCT:FO emulsions, in which EPA and DHA accounted for only 5% of total fatty acids, and compared it with MCT:LCT and LCT emulsions in mice. Based upon our earlier study of pure FO emulsions (14), we hypothesized that addition of FO to widely used emulsions would affect emulsion clearance and tissue delivery.

Materials and Methods

Materials. [1α, 2α (n) – 3H] cholesteryl oleoyl ether ([3H]CEO) (TRK 888) was purchased from Amersham Pharmacia Biotech. Avertin (2,2,2-tribromoethanol) (T-4840-2) was purchased from Aldrich Chemical. Bovine lactoferrin (L-9507) and Triton WR 1339 (lysolecithin) (T-0307) were purchased from Sigma-Aldrich.

Lipid emulsions. Lipid emulsions (200 g TG/L) with different TG compositions were kindly provided by B. Braun for production of intravenous emulsions, as previously described in detail (16). Each emulsion contained 20 g TG emulsified by 1.2 g egg yolk lecithin and 2.5 g of glycerol in 100 mL solution. The lipid emulsions used in this study included: LCT (100% soy oil), MCT:LCT (50:50%, coconut oil:soy oil, by wt), and MCT:LCT:FO (50:40:10%, coconut oil:soy oil:FO, by wt) with different fatty acid compositions (Table 1). The 3 emulsions were similar in particle size and homogeneity, with mean diameters ranging from 285 to 300 nm. LCT, MCT:LCT, and MCT:LCT:FO emulsions contained 1.10 ± 0.04%, 1.33 ± 0.18%, and 1.40 ± 0.20%, respectively, of total fatty acids as nonesterified fatty acids, concentrations too low to greatly affect emulsion metabolism.

Emulsions were labeled with [0.2 mCi/100 mg TG] by sonication to trace emulsion particle catabolism using previously described methods (14,17). After sonication, the incorporation of [3H]CEt into the 3 emulsions and specific radioactivities were similar and emulsion properties were not changed during the radiolabeling procedures (14,17). Note that there is no exchange of emulsion neutral lipids (including added cholesteryl esters) with other lipoproteins in the absence of cholesteryl ester transfer protein (18) and that rodents have no cholesteryl ester transfer protein.

Animals. Wild-type (WT) C57 BL/6j mice (7- to 8-wk-old females) were purchased from Jackson Laboratory. Stock homozygous LDL-R deficient (LDL-R −/−) C57BL/6j mice (19) were kindly provided by Dr. J. Breslow (The Rockefeller University, New York, NY). Sibling mating was used to breed mice homozygous for the null mutation. For all experiments, we used 10–12-wk-old female mice weighing 18–22 g. All the mice were housed at Columbia University animal facilities in a 12-h light/12-h dark cycle, with free access to a standard pellet rodent semi-purified diet (No. 5001, laboratory rodent diet, W.F. Fisher & Co) and water. We measured plasma TG and total cholesterol using enzymatic kits (Boehringer Mannheim) according to the manufacturer’s procedures. Plasma cholesterol concentrations in homozygous LDL-R −/− mice (4.29 ± 0.45 mmol/L, n = 4) were higher than that of WT mice (1.82 ± 0.28 mmol/L, n = 4). In contrast, plasma TG levels did not differ between LDL-R −/− mice (0.97 ± 0.11 mmol/L, n = 4) and WT mice (0.72 ± 0.16 mmol/L, n = 4).

Animal procedures. The animal experimental procedures were conducted as previously described (14,17). Briefly, each mouse was administered exactly 50 μL of emulsions (diluted with 0.9% NaCl) containing 400 μg of TG by a bolus injection via femoral vein. To explore the pathways of emulsion catabolism, we used bolus injections of lactoferrin (2 mg/mouse) (inhibits apoE mediated uptake pathways), heparin (10 U/mouse) (releases LPL from the endothelial wall into circulation), or Triton WR 1339 (10 mg/mouse) (blocks LPL activity) via femoral vein at 2, 1, and 15 min before emulsion injection, respectively, with a volume of 50 μL diluted with 0.9% NaCl. An equal volume of 0.9% NaCl was injected into mice as control (8,20–23). Retro-orbital blood was drawn at 0.5, 2, 5, 10, 15, and 25 min in capillary tubes at a volume of 20 μL following emulsion injection. At 25 min, mice were killed and the organs and tissues (heart, lung, liver, kidney, spleen, retroperitoneal fat, femoral bone, muscle, femoral shaft bone, and brain) were dissected out after vascular bed perfusion (14,17) and then were weighed and homogenized. Lipids in tissues were extracted with chloroform-methanol (2:1, v/v) as described by Van Bennekum et al. (17). The radioactivity in lipids of blood and tissues was measured by liquid scintillation spectrometry (Beckman LS 1800 Liquid Scintillation Counter). All the experiments were conducted from 0800 to 1200. The animal procedures were approved by the Institutional Animal Care and Use Committee at Columbia University.

Calculations. Based on the disintegrations per minute (dpm) of 20 μL sample blood, the total dpm in the whole blood at each time point was calculated using 4.9% of body wt as the whole blood volume for each mouse (24,25). Radioactivity in blood at different time points after injection was expressed as the percentage of injected dose remaining in the whole blood. Apparent distribution volumes of TG-rich particles (TGRP) have been reported to be greater than blood volumes due to margination (or sequestration or anchoring of TGRP to vessel/capillary walls without cellular or tissue internalization) during binding to endothelial LPL (26). Margination volume was determined as:

\[
\text{Margination volume (mL)} = \frac{\text{apparent distribution volume}}{\text{blood volume}}
\]

The apparent distribution volume (mL) = total dpm of lipid emulsion injected / blood radioactivity (dpm/mL), extrapolated to time zero (27).

Fractional catabolic rates (FCR) were calculated by the SAAM30/CONSAM program on the basis of first order linear kinetics during the first 10 min after emulsion injection. For experiments following preinjection of heparin and Triton WR 1339, FCR was determined according to the first order linear kinetics during the first 5 min, since after this time point linearity was lost. The equation \( N = N_0e^{-kt} \) was used to calculate the FCR (12,28), where \( N \) represents the radioactivity blood decay in function of time (t) and \( k \) is a rate constant for decay of N representing FCR in h\(^{-1}\). Tissue uptake was expressed as percentage of recovered injected dose/or organ except for adipose tissue and bone uptake, which were expressed as percentage of recovered injected dose/g tissue. The recovered injected dose was the sum of radioactivity from all organs and tissues assayed, including blood at the end of experiments.

Chromatography analysis of emulsions catabolism. To assess the in vivo lipolysis of lipid emulsions, column gel chromatography was used to

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>LCT</th>
<th>MCT:LCT</th>
<th>MCT:LCT:FO</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:0</td>
<td>31.4</td>
<td>31.2</td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>17.6</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>5.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>5.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>12</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>27.2</td>
<td>22.4</td>
<td></td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>2.9</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>0.7</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>22:0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td></td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>
monitor the changes in particle size and remnants produced during the process of emulsion catabolism in vascular compartment. Because radioactivity in individual diluted column fractions was considerably lower than in whole blood samples, higher levels of injected doses were required for these experiments. [3H]CET labeled emulsions containing 2 mg of TG were injected to mice and blood samples at each time point were drawn as described in Animal Procedures. The plasma separated from 60 μL blood at each time point was loaded onto Sepharose CL-2B Column (1.5 × 50 cm) with noninjected labeled emulsions as controls. Then fractions were collected at fixed time intervals (2 min) and the radioactivity in each elution fraction was measured to determine the amount of produced remnants. The changes in particle size of emulsions after injection into the blood were assessed by the amount of larger, medium and smaller remnants produced in each elution fraction.

**Statistics.** We tested clearance curves, changes in percentage of injected dose of lipid emulsions with time across emulsion types or different experimental variables (heparin, Triton WR 1339, LDL-R −/− and lactoferrin), by repeated-measures ANOVA. We used 1-way ANOVA to compare the differences among the 3 emulsions in FCR and tissue uptakes. In experiments with added modulators (heparin, Triton WR 1339, LDL-R −/− and lactoferrin), 2-way ANOVA was used to compare the effects of emulsion types and their interactions with specific experimental modulators on FCR and tissue uptakes. The Scheffe test was used for post-hoc analysis. When variances were unequal, we used Games-Howell analyses to compare the differences in blood clearance and tissue uptakes among the 3 emulsions and the effects of emulsion types and their interactions with experimental modulators. Statistical analyses were performed using SPSS Release 11.5 for Windows. All values were expressed as mean ± SEM.

### Results

**Differences in blood clearance and tissue uptake among the emulsions.** After injecting WT C57 BL/6J mice, the initial blood clearance of MCT:LCT:FO was faster than those of the other 2 emulsions. The radioactivity in the blood of mice injected with the MCT:LCT:FO emulsion was less than in those injected with MCT:LCT and LCT emulsions at different time points (P < 0.05) (Fig. 1). However, after 10 min, FCR did not differ among mice injected with MCT:LCT:FO (18.38 ± 3.23 pools/h), MCT:LCT (18.55 ± 3.77 pools/h) and LCT (16.95 ± 3.62 pools/h) (P = 0.096). The margination volume for the MCT:LCT:FO emulsion (1.018 ± 0.044 mL) was higher than that of MCT:LCT (0.625 ± 0.046 mL) and LCT emulsions (0.749 ± 0.041 mL) (P < 0.01). This initial “sticking,” or anchoring, of FO-containing emulsions to vessel walls could contribute to its early, rapid disappearance from blood without affecting the FCR of that remaining in blood.

**Heart uptake of the MCT:LCT:FO emulsion was less than those of MCT:LCT (41%) and LCT (53%) emulsions (P = 0.009) (Table 2).** Lung took up more MCT:LCT:FO emulsion than MCT:LCT (4.1-fold) and LCT (9.6-fold) emulsions (P < 0.001), consistent with our previous finding of higher lung uptake of pure FO emulsions (14). Adipose tissue and muscle took up less MCT:LCT:FO and MCT:LCT emulsions than LCT emulsion (P < 0.01) (Table 2).

**Effects of LPL.** Chromatography using Sepharose CL-2B columns showed that <5% of medium and smaller remnants from lipid emulsions were present in blood at each time point after injection of mice (at 5 min, 3.0, 2.1, and 1.9% for LCT, MCT:LCT and MCT:LCT:FO emulsions, respectively). They did not differ from noninjected emulsions, and the distribution of medium and small emulsion remnants was similar among the 3 types of emulsions (data not shown).

After injection of each type of emulsion, radioactivity remaining in the blood was significantly lower after preinjection of heparin and higher after preinjection of Triton WR 1339 compared with controls (P < 0.001) (Fig. 2A–C). Preinjection of heparin increased the FCR of all 3 emulsions to similar degrees (Fig. 2D), whereas preinjection of Triton WR 1339 decreased the FCR of all 3 emulsions but with substantially less effect on the MCT:LCT:FO emulsion (−33%) than on the MCT:LCT (−67%) and LCT emulsions (−85%) (P = 0.004) (Fig. 2D).

After preinjection of heparin or Triton WR 1339, most extrahepatic tissue uptakes were reduced compared with the controls (P < 0.05) after injection of all 3 emulsions, with similar effects of heparin and Triton WR 1339 on the 3 emulsions. For hepatic uptake, compared with controls and after preinjection of heparin, the uptake of LCT and MCT:LCT emulsions increased 91.9 and 81.2% for both, but only 44.8% for MCT:LCT:FO emulsions. Preinjection of Triton WR 1339 reduced the uptake of MCT:LCT:FO emulsions only 5.1% compared with 40.8 and 22.7% reductions in the uptake of LCT and MCT:LCT emulsions, respectively (P = 0.019) (Table 3).

**Effects of LDL-R and lactoferrin-sensitive receptors.** Compared with WT mice for each emulsion, blood clearance of LCT

### Table 2

Tissue uptakes of LCT, MCT:LCT, and MCT:LCT:FO emulsions in C57 BL/6J mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>LCT</th>
<th>MCT:LCT</th>
<th>MCT:LCT:FO</th>
</tr>
</thead>
</table>
| % injected dose recovered  
| Heart           | 13.35 ± 1.46a | 18.82 ± 1.43a | 7.89 ± 0.30b |
| Lung            | 1.81 ± 0.21c  | 3.74 ± 0.59b  | 19.16 ± 2.59a |
| Liver           | 48.54 ± 3.72  | 47.81 ± 4.80  | 44.51 ± 2.14 |
| Kidney          | 2.59 ± 0.32b  | 4.43 ± 0.38b  | 4.91 ± 0.29a |
| Spleen          | 7.10 ± 0.62   | 9.65 ± 0.54   | 8.87 ± 0.74  |
| Adipose         | 15.00 ± 3.36a | 7.45 ± 1.42a  | 8.16 ± 0.71a |
| Muscle          | 10.38 ± 1.35a | 8.73 ± 0.75a  | 5.82 ± 0.50b |
| Brain           | 0.01 ± 0.00a  | 0.02 ± 0.00a  | 0.06 ± 0.01a |
| Adrenal         | 0.05 ± 0.01   | 0.05 ± 0.01   | 0.06 ± 0.01  |

1 Values are means ± SEM, n = 16–20. Means in a row with superscripts without a common letter differ, P < 0.05.
2 Expressed per organ except for adipose tissue, which was expressed as per gram tissue.

![Figure 1](https://example.com/figure1.png)

Figure 1: Blood clearance of MCT:LCT:FO emulsions compared with MCT:LCT and LCT emulsions in C57BL/6J WT mice. Values are means ± SEM, n = 16–20. Means without a common letter differ, P < 0.05.
and MCT:LCT emulsions was slower in LDL-R −/− mice and after preinjection of lactoferrin in WT mice (P < 0.05), but no differences were found in blood clearance of MCT:LCT:FO emulsions in either group of mice. Compared with LCT and MCT:LCT emulsions, the absence of the LDL-R and lactoferrin preinjection had less effect on FCR of MCT:LCT:FO emulsions. In LDL-R −/− mice, decreases in FCR of LCT and MCT:LCT (24 and 21%) emulsions were more than that of MCT:LCT:FO emulsions (2%) (P = 0.005). In WT mice, lactoferrin decreased FCR of LCT and MCT:LCT (47 and 21%) to a greater extent compared with that of MCT:LCT:FO (7%) (P < 0.001) (Fig. 3).

The effects of lactoferrin and LDL-R on tissue uptake of the 3 emulsions differed (Table 4). Compared with controls for each emulsion, preinjection of lactoferrin in WT mice and absence of LDL-R in LDL-R −/− mice increased uptake by adipose tissue and decreased uptake by liver and heart both in LCT and MCT:LCT emulsions (P < 0.05). Only an increased uptake by adipose tissue was found for MCT:LCT:FO emulsions. Thus, analyses of the interaction of different emulsion types with the different modulating variables showed that lactoferrin, which blocks apoE mediated uptake pathways and the LDL-R, had much less influence on tissue uptakes of MCT:LCT:FO compared with LCT and MCT:LCT emulsions (P < 0.01).

### Discussion

TG composition of lipid emulsions affects blood clearance pathways and tissue targeting (12,13). Studies in humans and animals have reported that MCT- and FO-containing emulsions are cleared from blood faster than LCT emulsions (12–15). Of note, diets rich in FO are associated with lower postprandial lipemia (29,30). Our previous work showed that pure FO emulsions are cleared faster from blood and follow different removal pathways as compared with LCT emulsions (14,15).

In this study, we investigated the catabolism of an emulsion containing only 10 g/100 g FO that was associated with differences in blood clearance and tissue uptake mechanisms in comparison to LCT and MCT:LCT emulsions. Although the FCR of MCT:LCT:FO did not differ from those of MCT:LCT and LCT emulsions, in keeping with our recent study (31), the amount of MCT:LCT:FO emulsion particles remaining in blood was significantly less than for the other 2 emulsions. This may result in part from the higher margination volumes of MCT:LCT:FO emulsions, which are associated with more emulsion particles trapped in the capillary compartment (27) as compared with MCT:LCT and LCT emulsions. The lack of differences in FCR between MCT:LCT:FO and the other 2 emulsions was probably due to the relatively low amounts of DHA and EPA (5 g/100 g) in MCT:LCT:FO emulsions compared with higher concentrations.

### Table 3

<table>
<thead>
<tr>
<th>Emulsion (E)</th>
<th>Modulator</th>
<th>Control</th>
<th>Heparin</th>
<th>Triton</th>
<th>Control</th>
<th>Heparin</th>
<th>Triton</th>
<th>Control</th>
<th>Heparin</th>
<th>Triton</th>
<th>2-Way ANOVA</th>
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<tbody>
<tr>
<td>LCT</td>
<td>(M)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>12.50 ± 1.33a</td>
<td>2.04 ± 0.19b</td>
<td>0.19 ± 0.03a</td>
<td>10.97 ± 0.97a</td>
<td>0.65 ± 0.08a</td>
<td>0.09 ± 0.01a</td>
<td>3.56 ± 0.66a</td>
<td>0.58 ± 0.06a</td>
<td>0.11 ± 0.02a</td>
<td>0.04 ± 0.001 NS</td>
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</tr>
<tr>
<td>Lung</td>
<td>2.46 ± 0.21a</td>
<td>1.33 ± 0.13b</td>
<td>3.08 ± 0.29a</td>
<td>3.80 ± 0.31a</td>
<td>1.44 ± 0.10a</td>
<td>3.45 ± 0.37a</td>
<td>14.33 ± 0.85a</td>
<td>10.17 ± 1.95a</td>
<td>13.87 ± 0.16a</td>
<td>0.01 NS NS</td>
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<tr>
<td>Liver</td>
<td>39.15 ± 3.88a</td>
<td>75.12 ± 2.01b</td>
<td>23.19 ± 2.76a</td>
<td>45.32 ± 4.32a</td>
<td>82.12 ± 1.74a</td>
<td>35.01 ± 3.83a</td>
<td>48.04 ± 1.47a</td>
<td>69.55 ± 3.35a</td>
<td>45.59 ± 3.64a</td>
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<tr>
<td>Kidney</td>
<td>2.43 ± 0.17a</td>
<td>2.46 ± 0.20a</td>
<td>0.81 ± 0.08a</td>
<td>3.08 ± 0.39a</td>
<td>1.08 ± 0.06a</td>
<td>0.57 ± 0.07a</td>
<td>3.90 ± 0.22a</td>
<td>2.34 ± 0.16a</td>
<td>1.07 ± 0.20a</td>
<td>0.05 NS 0.053</td>
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</tr>
<tr>
<td>Spleen</td>
<td>5.50 ± 0.55a</td>
<td>3.74 ± 0.31a</td>
<td>9.17 ± 0.34a</td>
<td>6.38 ± 0.60a</td>
<td>4.28 ± 0.81a</td>
<td>6.78 ± 0.19a</td>
<td>7.09 ± 0.66a</td>
<td>6.04 ± 0.58a</td>
<td>6.42 ± 0.45a</td>
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<tr>
<td>Adipose</td>
<td>18.26 ± 3.20a</td>
<td>3.38 ± 0.72a</td>
<td>0.88 ± 0.09a</td>
<td>14.11 ± 3.23a</td>
<td>1.30 ± 0.16a</td>
<td>0.93 ± 0.12a</td>
<td>12.81 ± 2.18a</td>
<td>1.65 ± 0.28a</td>
<td>1.02 ± 0.05a</td>
<td>0.01 0.001</td>
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<tr>
<td>Muscle</td>
<td>12.06 ± 1.02a</td>
<td>8.34 ± 0.37a</td>
<td>5.89 ± 0.38a</td>
<td>11.33 ± 0.87a</td>
<td>5.14 ± 0.29a</td>
<td>4.82 ± 0.26a</td>
<td>5.16 ± 0.66a</td>
<td>4.11 ± 0.17a</td>
<td>1.68 ± 0.12a</td>
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<tr>
<td>Bone</td>
<td>3.22 ± 0.32a</td>
<td>1.79 ± 0.18a</td>
<td>1.33 ± 0.09a</td>
<td>2.53 ± 0.20a</td>
<td>2.78 ± 0.15a</td>
<td>2.08 ± 0.32a</td>
<td>3.86 ± 0.39a</td>
<td>3.99 ± 0.79a</td>
<td>0.80 ± 0.12a</td>
<td>NS 0.05</td>
<td></td>
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</tbody>
</table>

1 Values are means ± SEM, n = 9–12. Means in a row within an emulsion with superscripts without a common letter differ, P < 0.05.
2 Expressed per organ except for adipose tissue and bone, which were expressed as per gram tissue.
3 Nonsignificant, P > 0.05.
Many studies have shown that the metabolism of lipid emulsions is similar to that of chylomicrons with intravascular lipolysis by LPL as a first step followed by tissue uptake of remnant particles (7–9). As a multifunctional protein, LPL also functions as a “bridge” to mediate TGRP uptake by tissues independent of its hydrolytic activity (32–33). Hultin et al. (11) found that a phospholipid-stabilized emulsion, very similar to the LCT emulsion used in our studies, was removed from blood and taken up by tissues with less preceding lipolysis than chylomicrons, especially in the fed state. Consistently, in this study with fed mice, results from gel chromatography indicated that relatively little lipolysis of each of the 3 emulsions occurred before their blood removal. Results from electron microscopy and LPL-deficient mink indicated that intact emulsion and chylomicron particles were distributed to liver and peripheral tissues (36,37). Thus, tissue uptake of intact whole particles with little hydrolysis may be an important pathway for the clearance of lipid emulsions in vivo; although the role of LPL in this pathway remains unclear, it may substantially contribute as a “bridging” molecule for initial anchoring to tissues prior to cellular uptake.

To explore the role of LPL on metabolism of the different emulsions, we used heparin to release LPL from vascular endothelial walls and found that preinjection of heparin enhanced clearance of all 3 emulsions, especially during the first 2 min. Heparin injection increased hepatic uptake but decreased uptake by extra-hepatic tissues, suggesting that the released LPL from endothelial cells may target to liver for metabolism (38) with binding to, and trapping of, emulsion particles. The reduced extra-hepatic uptake of emulsions after heparin injection is consistent with our previous report that heparin inhibits uptake of TGRP and LDL particles by extra-hepatic cell types in vitro (39).

Triton WR 1339, a nonionic detergent that inhibits LPL's catalytic activity (40) and likely bridging activity as well, greatly impaired clearance of all emulsions, consistent with previous findings that Triton WR 1339 impaired TGRP blood clearance (21,41). In this study, we speculate that the influences of Triton WR 1339 on emulsion clearance may be likely ascribed to its actions on preventing LPL from binding to emulsions (42) and blocking the bridge function of LPL. This idea is supported by the relatively low levels of lipolysis (and hence LPL lipolytic activity) observed after injection of emulsions in the well-fed state (11). Still, compared with LCT and MCT:LCT, heparin and Triton WR 1339 preinjection had much less effect on the catabolism of MCT:LCT:FO emulsions. Thus, overall LPL-mediated removal mechanisms are less important for FO-containing

### Table 4. Effects of preinjection of lactoferrin and LDL-R deficiency on tissue uptake of lipid emulsions in C57 BL/6J mice

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>LCT</th>
<th>MCT:LCT</th>
<th>MCT:LCT:FO</th>
<th>2-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulator</td>
<td>Control</td>
<td>Lactoferrin</td>
<td>LDL-R−/−</td>
<td>Control</td>
</tr>
<tr>
<td>Heart</td>
<td>11.83 ± 2.00b</td>
<td>3.61 ± 0.61b</td>
<td>4.99 ± 0.55b</td>
<td>13.24 ± 1.71b</td>
</tr>
<tr>
<td>Lung</td>
<td>2.20 ± 0.29a</td>
<td>2.18 ± 0.19a</td>
<td>1.05 ± 0.19a</td>
<td>4.43 ± 0.55a</td>
</tr>
<tr>
<td>Liver</td>
<td>52.06 ± 3.44a</td>
<td>34.69 ± 1.03a</td>
<td>43.48 ± 2.31b</td>
<td>54.77 ± 2.10a</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.60 ± 0.38b</td>
<td>2.62 ± 0.31a</td>
<td>3.13 ± 0.29a</td>
<td>3.15 ± 0.38a</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.63 ± 0.27a</td>
<td>5.91 ± 0.17a</td>
<td>4.33 ± 0.23a</td>
<td>8.27 ± 1.11a</td>
</tr>
<tr>
<td>Adipose</td>
<td>11.86 ± 2.43a</td>
<td>38.62 ± 2.51a</td>
<td>25.05 ± 2.69a</td>
<td>5.63 ± 1.98b</td>
</tr>
<tr>
<td>Muscle</td>
<td>7.16 ± 1.17a</td>
<td>5.89 ± 0.73a</td>
<td>7.94 ± 1.06a</td>
<td>6.44 ± 0.73a</td>
</tr>
<tr>
<td>Bone</td>
<td>2.70 ± 0.45a</td>
<td>2.64 ± 0.40a</td>
<td>2.19 ± 0.14a</td>
<td>2.73 ± 0.37a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8–10. Means in a row with an emulsion with superscripts without a common letter differ, P < 0.05.
2 Expressed per organ except for adipose tissue and bone, which were expressed as per gram tissue.
3 Nonsignificant, P > 0.05.
emulsions than for LCT and MCT:LCT emulsions. This might be one reason why LPL-rich tissues such as heart and adipose tissue take up substantially less FO-containing emulsions than MCT:LCT and LCT emulsions.

LDL-R, LDL-R related protein, and heparin sulfate proteoglycans are important pathways for TGRP remnant removal (7–9). Many effectors, such as lactoferrin, heparinase, and receptor-associated protein (RAP), have been used to block the function of LDL-R related protein and heparin sulfate proteoglycans (20–23,33,34,43). In our study, absence of LDL-R in LDL-R −/− mice or preinjection of lactoferron to WT mice impaired blood clearance and changed tissue targeting of LCT and MCT:LCT emulsions, but did not affect the blood clearance and tissue uptake of MCT:LCT:FO emulsions. This is consistent with our previous results that pure FO emulsion removal is much less dependent on LDL-R and apoE-mediated lactoferrin-sensitive pathways (14). Note the impaired clearance of LCT and MCT:LCT emulsions in LDL-R deficiency and after preinjection of lactoferron might have been offset, to some extent, by the increased uptake by adipose tissue. This compensating mechanism has been observed in other studies with increased adipose uptake of chylomicrons in scavenger receptor BI deficiency (44). Adipose tissue perhaps acts as a buffer system to clear emulsion particles when normal removal pathways are impaired.

When we compared contributions of LPL, lactoferron, and LDL-R pathways to catabolism of each emulsion, LPL contributed more to the catabolism of all 3 emulsions as compared with apoE and the LDL-R pathways (Figs. 2 and 3). Clearance of MCT:LCT:FO emulsions remained less dependent on LPL compared with the other 2 emulsions. Lesser effects of apoE and LDL-R pathways on the catabolism of emulsions could also be due to larger particle size compared with effects on smaller particles, as previously reported. (45).

Tissue uptakes of the 3 emulsions with different fatty acid compositions differ and the basis for this tissue-specificity remains to be elucidated. Reticulo-endothelial phagocytic systems may be another pathway for the catabolism of TGRP, and supplementation with FO emulsions increases total liver and lung macrophage number and phagocytosis (44,46,47). In this study, higher uptakes in lung with MCT:LCT:FO emulsions, as compared with LCT and MCT:LCT emulsions, possibly points to a role of resident macrophages in metabolism of FO-containing emulsions, and this idea needs further study.

In conclusion, our study suggests that the addition of (n-3) fatty acids to emulsions decreases their dependency on LPL, LDL-R, and apoE-mediated lactoferrin-sensitive removal pathways. Other “nonclassical” mechanisms may contribute more to clearance of MCT:LCT:FO emulsions, pathways that still need to be defined. In ongoing studies, our early data indicate a greater role of all surface proteoglycans in cellular uptake of (n-3) fatty acid-rich particles as compared with (n-6) fatty acid-rich particles (48). Moreover, inclusion of even small amounts of (n-3) fatty acids into TGRP is likely to modulate the pathways of particle blood clearance and tissue uptakes.

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Literature Cited


