Regulation of the metabolism of lipid emulsion model lipoproteins by a saturated acyl chain at the 2-position of triacylglycerol

Bok-Cheng Mortimer, Wilfred J. Simmonds, Cynthia A. Joll, Robert V. Stick, and Trevor G. Redgrave

Departments of Physiology and Organic Chemistry, The University of Western Australia, Nedlands, 6009, Western Australia

Abstract Lipid emulsion particles were prepared by sonicating four different lipid mixtures (triacylglycerol (TAG), 70%; phospholipid, 25%; cholesteryl oleate (CO), 3%; and free cholesterol, 2%), then purified by density gradient ultracentrifugation. For three test mixtures, the TAG contained 50, 75, or 100% 1,3-dioleyl-2-stearylglycerol (OSO) with the remainder being triolein (OOO); 100% triolein in the lipid mixture was used as the control. After intravenous injection of the lipid particles into unanesthetized rats, removal of radioactive TAG fatty acid and CO from plasma was measured for 30 min, then liver and spleen uptakes were measured. When emulsions contained 75% or 100% OSO as TAG, the plasma removal rates of CO were, respectively, 60% or 30% of the rate when the TAG was 100% triolein; smaller recoveries of CO were found in the liver. The clearances of TAG fatty acid did not differ significantly and the recoveries of TAG fatty acid in the organs were not affected by the type of emulsion injected. Remnant particles were derived from donor rats in which uptake was blocked by exclusion of liver and other viscera from the circulation before injection of 100% OOO and 100% OSO emulsions. When injected into recipient intact rats, the removal of remnants from plasma was slower for remnants derived 15 min after injection of 100% OSO emulsions than from 100% OOO emulsions, showing that the slower removal of emulsion CO was due to slower remnant uptake from the plasma with OSO emulsions. The lipid compositions of remnants harvested at 15 and 30 min after the injection of emulsions suggested that OOO and OSO were hydrolyzed to the same extent for the first 15 min and then there was a slow down in the hydrolysis of the OSO emulsion. The protein content showed that remnants derived from OSO carried less apolipoprotein E and less of an unidentified protein similar in mobility to albumin.—**Mortimer, B-C., W. J. Simmonds, C. A. Joll, R. V. Stick, and T. G. Redgrave.** Regulation of the metabolism of lipid emulsion model lipoproteins by a saturated acyl chain at the 2-position of triacylglycerol. *J. Lipid Res.* 1988. **29:** 713–720.

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During digestion and absorption of dietary TAG, the acyl chain at the 2-position of glycerol is conserved as 2-monoacylglycerol to become, after reacylation, the major fatty acid component at the 2-position of TAG in chylomicrons (CM) (1). When remnants are formed from chylomicrons prior to removal by the liver, hydrolysis of TAG by lipoprotein lipase is through 2-monoacylglycerol (2). Thus the nature of the fatty acid at the 2-position of glycerol could be an important modulating factor in any effect of TAG composition on the metabolism of chylomicrons.

In earlier experiments (3), chylomicrons were prepared in donor rats by feeding TAG of known structure and composition. After intravenous injection of chylomicrons into recipient rats, removal from plasma of TAG and cholesteryl ester radioactivities were measured for 30 min. The fractional clearances of both TAG and cholesteryl ester, monitored by radioactive tracers, were significantly slower for chylomicrons prepared from donor rats fed TAG with the structure 1,3-dioleyl-2-stearylglycerol (OSO) than from those fed 1,2-dioleyl-3-stearyl-*sn*-glycerol (OOS). This was also the case when model emulsions were injected. They were prepared by sonication of mixtures of phosphatidylcholine, free cholesterol, and cholesteryl oleate with TAG being OSO or triolein (OOO), respectively. Thus stearic acid at the 2-position of the TAG in chylomicrons or model emulsions slowed lipolysis and remnant removal.

The object of the present experiments was to examine in greater detail the effects of OSO on the clearance of artificial emulsions and, in particular, to determine the proportion of OSO in the TAG emulsion required for the effect.

Abbreviations: C, cholesterol; PL, phospholipid; TAG, triacylglycerol; CM, chylomicrons; OOO, trioleylglycerol; OSO, 1,3-dioleyl-2-stearylglycerol; OOS, 1,2-dioleyl-3-stearylglycerol; CO, cholesteryl oleate; TLC, thin-layer chromatography; DCC, 1,3-dicyclohexylcarbodiimide; DMAP, 4-N,N-dimethylaminopyridine; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Preparation of emulsions

Mixtures of pure lipids were emulsified by sonication in dilute NaCl solutions. The TAG, 1,3-dioleyl-2-stearylglycerol was synthesized as described below. ^{3}H-Labeled cholesteryl oleate was prepared by reacting cholesterol and [^{3}H]oleic acid in the presence of DMAP and DCC (4). Final purity was 99% by thin-layer chromatography (TLC). Triolein, CO, and cholesterol (Nu-Chek-Prep, Elysian, MN), dioleyl phosphatidylcholine (Avanti Polar Lipids, Birmington, AL), and [^{14}C]triolein (Amersham, Sydney) were each greater than 99% pure as judged by TLC. Lipids were dispensed from stock solutions into vials. Solvents were evaporated under a stream of N_2 before overnight vacuum desiccation to eliminate residual solvent traces. Then 100 mg of a lipid mixture was sonicated in 8.5 ml of 10 mM HEPES (pH 7.4) in 0.15 M NaCl solution using a Vibra Cell Disrupter (Danbury, CT) at a temperature of approximately 55°C, which is above the crystalline-to-isotropic liquid melting transition of CO. The temperature was monitored by a thermocouple in the vessel during sonication using a 1-cm probe with continuous output of 90–110 W. The crude emulsions were brought to density 1.10 g/ml by addition of solid KBr, divided into two, and then injected under duplicate preformed density gradients of 2.5 ml of NaCl solutions with densities 1.065, 1.020, and 1.006 g/ml (5). The preparations were then centrifuged in the SW41 rotor of a Beckman L8-70M ultracentrifuge at 20°C for 22 min at 14,000 rpm. The rotor was stopped and the coarse material that floated to the top of the 1.006 g/ml density solution was removed by aspiration and replaced with fresh 1.006 g/ml NaCl solution before a second period of centrifugation at 20°C for 20 min at 24,000 rpm. The emulsion droplets now floating to the top of the gradient were aspirated and used for analysis and subsequent metabolic studies. Contaminating titanium fragments from the sonicator and nonemulsified polar lipids remained in more dense fractions of the density gradient.

Synthesis of OSO

cis-1,3-*O*-Benzylideneglycerol was first prepared by the reaction of glycerol with benzaldehyde in the presence of concentrated sulfuric acid in benzene (6). Esterification with stearic acid was in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-N,N-dimethylaminopyridine (DMAP) in carbon tetrachloride (4). This was followed by hydrogenation with palladium catalyst in ethyl acetate to yield 2-stearylglycerol. 1,3-Dioleyl-2-stearylglycerol was prepared by esterifying the diol with oleic acid in the presence of DCC and DMAP. The purity of this compound was established by both ^{13}C NMR and differential scanning calorimetry (DSC). The ^{13}C NMR spectrum showed distinct signals for CH_2O and OCH and for C = O as previously reported (7). The thermal behavior of the compound was studied using a Du Pont model 1090 Thermal Analyzer. Samples were loaded in aluminum sealed pans and the heating and cooling rates were maintained at 5°C/min. The heating and cooling curves were comparable with those of Kodali et al. (8).

Preparation of remnants

Rats were anesthetized with ether plus Nembutal (30–40 mg/kg, intraperitoneally). The abdomen was opened. The liver and other viscera were excluded from the circulation by ligation of the superior and inferior mesenteric arteries and the portal vein, with ligatures at the rectosigmoid and esophagogastric junctions to exclude portosystemic anastomoses. About 1 ml of emulsion containing approximately 6–10 mg of total lipid was injected into the tail vein of each rat. Fifteen or 30 min later the animal was exsanguinated by heart puncture. The blood was mixed with EDTA, 4 μmol per ml, as anticoagulant and, unless for immediate injection into recipient rats, also with DTNB, 2 μmol per ml, to inhibit LCAT activity. After separation from blood cells by centrifugation, the plasma was immediately used for injection studies, or else 0.14 g/ml KBr was added to increase the density to 1.10 g/ml. Then 4-ml aliquots were layered under preformed step-density gradients made up with NaCl solutions as above. After centrifugation in the Beckman SW41 rotor at 20°C for 120 min at 40,000 rpm, the layer of remnants floating at the top of the gradient was aspirated for subsequent analysis.

Injection studies

Emulsions were injected into the bloodstream of conscious rats for measurements of the plasma disappearance rates and the organ distributions of the injected radiolabels. Nonfasted male albino Wistar rats weighing 200–250 g were anesthetized with diethyl ether. A saline-filled Teflon cannula (0.76 mm o.d. × 0.33 mm i.d.; Small Parts, Inc., Miami, FL) was inserted through the left common carotid artery so that the tip was located in the aortic arch, and a venous cannula (0.8 mm o.d. × 0.5 mm i.d.; Dural, NSW, Australia) was inserted near the junction of the left jugular and subclavian veins and the tip was advanced to lie in the superior vena cava. Heparin was not used, but clotting was prevented by treatment of the tubing with Siliclad (Becton Dickinson & Co., Parsippany, NJ) before use. After surgery, the animals recovered from the anesthesia in individual restraint cages for 2–4 hr before the injection study commenced. The emulsions labeled with cholesteryl [^{3}H]oleate and [^{14}C]triacylglycerol were then injected, as a bolus of ~5 mg of lipid in a volume of ~0.5 ml, into the venous cannula. Blood samples of 0.35 ml were then taken (with saline injected to replace the blood volume) at 3, 5, 8, 12, 20, 25, and 30 min; at which time Nembutal was injected and the liver and spleen were excised for extraction of radio-

active lipid from minced whole spleen and from minced 1-g pieces of weighed liver. Lipids were extracted into 30 ml of chloroform–methanol 2:1. Aliquots were taken, the solvent was evaporated, and radioactivity was measured in Readysolv™ EP (Beckman) by liquid scintillation spectrometry (Beckman LS3800). Radioactivity in plasma was measured, without extraction, in 100-μl aliquots using Readysolv. Plasma clearance kinetics were computed from exponential curves fitted by least squares procedures to samples taken during the first 12 min after injection.

Chemical analysis

Lipid phosphorus was measured by a modified Bartlett (9) procedure. TAG and free and esterified cholesterol were assayed on lipid extracts after separation by thin-layer chromatography. TAG was quantified as glycerol by chromotropic acid (10); free and esterified cholesterol by the *o*-phthalaldehyde procedure (11) after saponification of lipids in the separated bands.

Total protein in remnant particles was measured by a fluorescamine assay according to Tajima, Yokoyama, and Yamamoto (12). For protein analysis, remnant suspensions were concentrated by ultrafiltration (Amicon, Danvers, MA) and applied to SDS-PAGE (13) without delipidation. Bands were stained by Coomassie Blue R-250 (Bio-Rad) and quantitated by laser densitometry (LKB, Bromma, Sweden).

HPLC of emulsion TAG was after conversion of the saponified fatty acids to the phenacyl esters (14). Detection was at 242 nm after separation with a 5 μ, 4.6 × 250 mm C-8 Ultrasphere column (Beckman Instruments, Australia), eluted with 80% acetonitrile in H_2O for 10 min, then increasing by 0.4%/min to 100% acetonitrile, in a Varian 8500 system.

RESULTS

Emulsions were prepared by sonicating four different lipid mixtures. The percentages by weight of lipid classes were the same in all four mixtures: TAG, 70; CO, 3; C, 2; PL, 25. For the control mixture TAG was 100% triolein. For the three test mixtures, OSO was 50, 75, and 100% by weight, the remainder being triolein. **Table 1** shows the composition of the emulsion particles prepared from these mixtures. In all cases the final emulsions contained larger proportions of core components (TAG and CO) than were present in the starting mixture, with a corresponding decrease in phospholipid, the principal surface component. There were no consistent differences between test and control emulsions in lipid class composition attributable to the presence of OSO. There was a tendency for OSO to accumulate from dilute mixtures during the preparation or purification of emulsions. Thus, a mixture in which 48% of TAG was estimated to be OSO by HPLC yielded emulsion particles in which 64% of TAG was OSO, whereas a mixture with 75% OSO yielded particles in which 82% of TAG was OSO. In the text, 50% and 75% OSO refer to the composition of TAG in the original mixtures.

After injection of the emulsions described above into four groups of rats, the disappearance of radioactive lipid from plasma followed a pattern described previously for other artificial emulsions and for labeled chylomicrons (3), with almost complete removal of TAG during the first 12 min (**Fig. 1**). Most ^{14}C radioactivity in plasma was associated with TAG when analyzed by thin-layer chromatography. TAG fatty acid label was cleared much more rapidly than CO label for all four emulsions. However, when emulsion TAG was 75% or 100% OSO, the clearance rates of CO were 60% and 30%, respectively, of those for 100% triolein (OOO). These differences were statistically significant (**Table 2**). CO clearance was unaffected when 50% of TAG was OSO. The fractional clearance rates of TAG fatty acid label did not differ significantly with the type of emulsion injected.

Recoveries of CO in liver reflected the differences in plasma clearance (Table 2). The mean recoveries at 30 min for the four emulsions were: 100% OSO < 75% OSO < 50% OSO = 0% OSO. The differences between means were statistically significant ($P < 0.05$) by analysis of variance and the Newman-Keuls test (15). There were no differences

TABLE 1. Composition of emulsions containing 0%, 50%, 75%, or 100% OSO incorporated with triolein

Emulsion	(n)	Composition				Diameter	
		TAG	CO	C	PL		
		% by weight				*nm*	
100% OOO	(13)	81.2 ± 0.8	4.3 ± 0.4	1.5 ± 0.1	13.0 ± 0.9	155 ± 3.5[a]	160 ± 3.5[b]
50% OSO	(3)	81.1 ± 1.8	3.0 ± 0.4	1.3 ± 0.1	14.6 ± 2.0	193 ± 19.0	160 ± 2.0
75% OSO	(4)	81.6 ± 2.8	3.5 ± 0.1	1.3 ± 0.1	13.7 ± 2.7	177 ± 5.0	161 ± 0
100% OSO	(9)	79.9 ± 1.1	3.3 ± 0.2	1.6 ± 0.1	15.3 ± 1.1		158 ± 6.0

Results are means ± SEM, for (n) observations.
[a]Particle size determined by electron microscopy of osmium-stained particles applied directly to Formvar-coated grids. For each sample, 100 particles were measured.
[b]Particle size determined with a Brookhaven Instruments BI-90 laser light-scattering particle sizer.

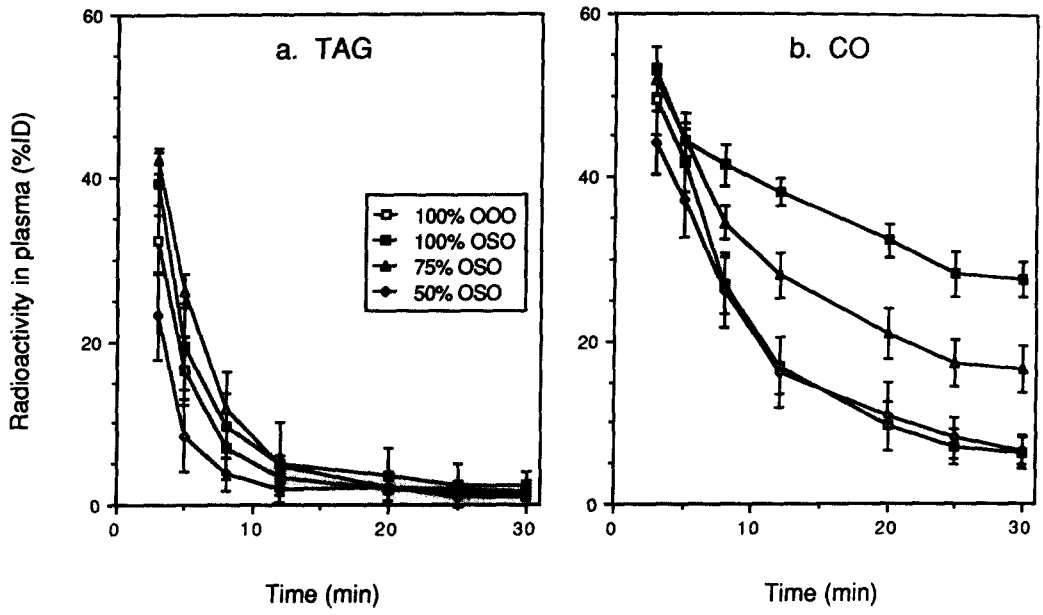

Fig. 1. a: Disappearance of triacylglycerol fatty acid radioactivity from injected chylomicron-like emulsions containing 100%, 75%, 50% OSO or 100% OOO as triacylglycerol. b: Disappearance of cholesteryl oleate radioactivity from injected chylomicron-like emulsions containing 100%, 75%, 50% OSO or 100% OOO as triacylglycerol. The results are means ± SEM for the groups described in Table 2.

in spleen recoveries of CO label, which were less than 2% of injected radioactivity, nor were there significant differences in recoveries of TAG fatty acid label in liver or spleen.

Fig. 1 shows that the removal of TAG was rapid from all emulsions, whereas CO removal was obviously slower with the 75% and 100% OSO emulsions. This pattern suggested that OSO inhibited removal of remnants from the circulation but did not appear to retard lipolysis preceding remnant uptake. To test this interpretation, remnants were prepared in rats in which uptake was blocked by exclusion of liver and other viscera from the circulation before injection of emulsions in which TAG was either 100% OOO or 100% OSO. The remnants derived from the plasma of the donor rats were then injected into intact recipient rats. **Fig. 2** shows that the remnants were removed very rapidly when derived from 100% OOO emulsions, with more than

TABLE 2. Effect of 1,3-dioleyl-2-stearylglycerol on the metabolism of emulsions[a]

Emulsion	Removal from Plasma (Fractional Clearance Rate)		Organ Uptake of Lipid Radioactivity			
			TAG		CO	
	TAG	CO	Liver	Spleen	Liver	Spleen
	min^{-1}		*% of injected dose*			
100% OOO	0.253 ± 0.020 (12)	0.123 ± 0.016 (12)	9.6 ± 0.76 (7)	0.24 ± 0.02 (7)	59.7 ± 2.65 (7)	0.97 ± 0.08 (7)
50% OSO	0.265 ± 0.016 (5)	0.114 ± 0.020 (5)	9.2 ± 1.54 (5)	0.14 ± 0.02 (5)	63.0 ± 2.52 (5)	0.50 ± 0.05 (5)
75% OSO	0.245 ± 0.026 (6)	0.069 ± 0.011[b] (6)	11.0 ± 0.86 (6)	0.30 ± 0.04 (6)	50.2 ± 5.23[b] (6)	1.02 ± 0.11 (6)
100% OSO	0.221 ± 0.049 (6)	0.034 ± 0.005[b] (6)	11.4 ± 0.56 (6)	0.50 ± 0.20 (6)	39.5 ± 3.08[b] (6)	1.53 ± 0.26 (6)

[a]Radioactive emulsions labeled with [^{14}C]triolein and cholesteryl [^{3}H]oleate were injected into conscious rats. Blood was sampled from an implanted arterial cannula at intervals for 30 min. Clearance kinetics were calculated from the exponential curves fitted to the data for radioactivity remaining in the plasma during the first 12 min. After 30 min the rats were killed for removal of organs, extraction of lipids, and measurement of radioactivity. Results are means ± SEM; (n), number of observations. The lipid compositions of the emulsions are given in Table 1.

[b]$P < 0.05$, comparisons with OOO by analysis of variance and for organ uptake by the Newman-Keuls test (15). No other comparisons were statistically significant.

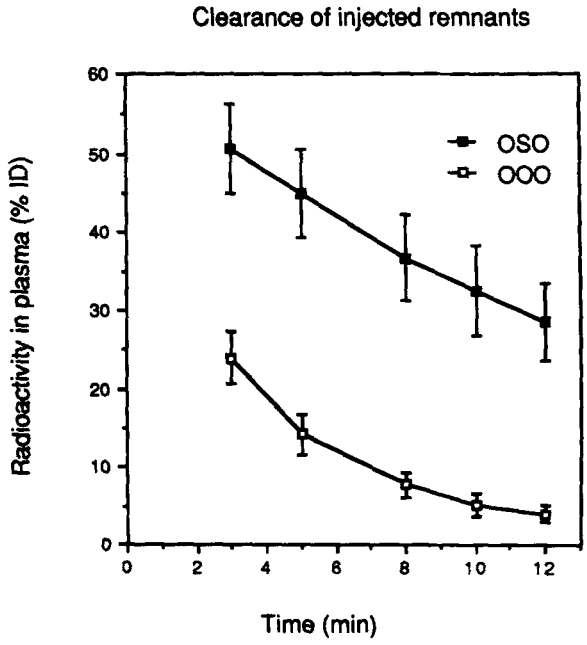

Fig. 2. Disappearance of cholesteryl oleate label from the plasma of intact rats injected with remnant particles prepared by injecting 100% OOO or 100% OSO emulsions into functionally hepatectomized donor rats, then allowing them to circulate for 15 min. The results are means ± SEM from 14 rats injected with OOO remnants and 10 rats injected with OSO remnants.

75% of CO label removed by 3 min. However, removal of remnants derived from 100% OSO emulsions was much slower with only about 50% removed by 3 min. The marked difference persisted to 12 min after injection of the remnants, when the experiment was terminated.

The lipid compositions of remnants harvested 30 min after injection of emulsion are shown in **Table 3.** Triacylglycerol clearance in vivo represents the sum of lipolysis and remnant removal. Furthermore, the plasma remnants are contaminated with partially degraded very low density lipoprotein remnants. For these reasons calculations are only approximate, and over-interpretations must be avoided. However, the results suggested that OSO was hydrolyzed somewhat more slowly than OOO. As a percentage of total lipid mass, TAG was higher in OSO remnants. The mass ratio TAG/CO fell from 24 in the original emulsion to 8.5 in OSO remnants, whereas it fell from 24 to 4.0 for OOO. Assuming that the amount of CO per particle remained constant during lipolysis (16), this indicated that 65% of OSO was hydrolyzed in 30 min, compared with 83% of OOO.

In the intact rat, plasma clearance rates of CO suggested that many of the remnant particles were taken up by the liver after residence in the plasma for less than 30 min. Also included in Table 3 are results from remnants obtained after only 15 min circulation. For the 15-min remnants the TAG/CO mass indices calculated as above showed little difference between lipolysis of OSO and OOO, for which values of 55% and 60% hydrolysis, respectively, were obtained. These findings were consistent with the plasma clearances of radioactive TAG fatty acid in the intact animals (Table 2).

When remnants are formed by the action of lipoprotein lipase, surface lipids, mainly phospholipid, transfer to the high density fraction of plasma lipoproteins (16, 17). This is consistent with the decreased PL/CO ratio of remnants in Table 3. Assuming once more that CO remains unchanged during conversion of emulsion particle to remnant, it can be calculated that both OOO and OSO emulsions lost 50% of their initial phospholipid in the first 15 min and that at 30 min, OOO emulsions had lost 70% of initial phospholipid compared with about 50% for OSO emulsions. Only very small amounts of partial glycerides were detected in purified remnants. At 15 min diacylglycerol made up less than 2% of total lipids. At 30 min diacylglycerol showed only as traces on TLC. No monoacylglycerol was detected

TABLE 3. Lipid compositions of emulsions and remnants

	Percentage by Weight of Total Lipids[a]									Ratios[b]			
	Emulsions				Remnants					Emulsions		Remnants	
	TAG	CO	C	PL	TAG	CO	C	PL	DG	TAG/CO	PL/CO	TAG/CO	PL/CO
15 min													
OOO	(1) 80.2	2.5	1.5	15.9	(1) 71.8	5.8	3.8	16.8	1.6	(1) 32.1	6.4	(1) 12.3	2.9
OSO	(2) 83.5	2.9	1.3	12.4	(2) 77.0	6.0	3.2	12.5	1.3	(2) 29.1	4.3	(2) 13.0	2.1
± Range	± 0.3	± 0.3	± 0.1	± 0.5	± 0.8	± 0.5	± 0.3	± 0.2	± 0.1	± 3.0	± 0.6	± 1.1	± 0.1
30 min													
OOO	(3) 81.0	3.4	1.3	14.2	(4) 56.0	16.2	7.3	20.5	trace	(3) 24.3	4.3	(4) 4.0	1.3
± SEM	± 1.5	± 0.4	± 0.4	± 1.6	± 6.0	± 2.7	± 0.7	± 3.9		± 2.3	± 0.9	± 1.1	± 0.0
OSO	(5) 78.8	3.3	1.4	16.5	(4) 66.0	8.3	4.5	21.1	0.0	(5) 24.3	5.0	(5) 8.5	2.6
± SEM	± 1.8	± 0.2	± 0.1	± 1.7	± 3.9	± 0.9	± 0.6	± 2.6		± 2.1	± 0.5	± 1.3	± 0.2

[a] Results relate only to the particular emulsion preparation used in these experiments, hence values differ slightly from those in Table 1.
[b] These ratios are calculated from the percentage by weight data to facilitate the comparisons discussed in the text.

on TLC. Overall, the emulsion particles became smaller, due to loss of both core TAG and surface phospholipid. The original OOO and OSO emulsions had particles of similar size with mean diameters about 160 nm (Table 1). Mean particle diameters of seven remnant preparations ranged between 34 and 47 nm by electron microscopy, and did not differ consistently between OOO and OSO remnants, nor between 15- and 30-min harvests.

The emulsion particles were protein-free when injected, but substantial amounts of protein, mostly apolipoproteins E and C, became associated with remnants. Our preliminary data suggest a higher protein content for OOO remnants and there were also differences in class distribution of proteins separated by SDS-PAGE, (**Fig. 3**). **Table 4** summarizes these differences and shows the amounts of total protein and individual apolipoprotein molecules, calculated by assuming that CO per remnant particle was unchanged from the injected emulsions. The calculations were made on one representative sample of remnants pooled from several donor rats, but similar findings were observed with four other preparations. The band with electrophoretic mobility similar to plasma albumin was not identified and was reproducibly present only on OOO remnants. The calculations show that, compared with remnants from OOO, remnants from OSO contain similar numbers of apolipoprotein C molecules, about 30% less apolipoprotein E molecules, and substantially less of the unidentified protein with mobility similar to albumin.

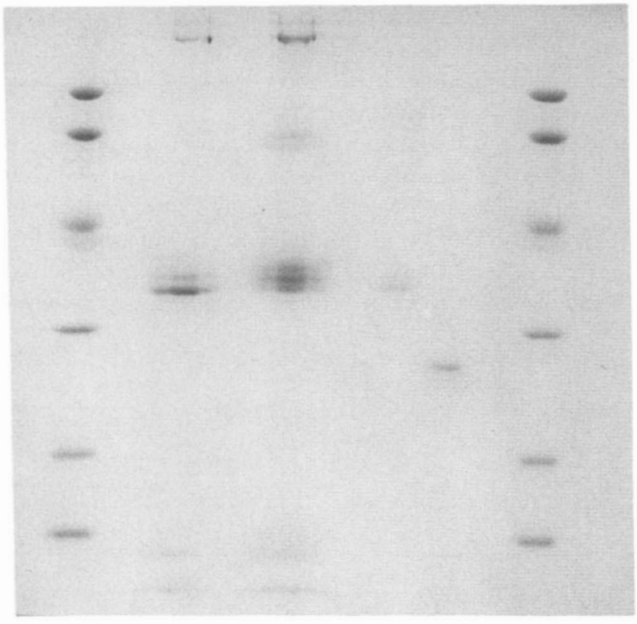

Fig. 3. SDS-polyacrylamide gel electrophoresis of proteins associated with the remnants harvested 15 min after injections of chylomicron-like emulsions containing 100% OSO or 100% OOO as TAG. Lipoproteins were dissolved without delipidation in 0.0625 M Tris–HCl buffer, pH 6.8, containing 2% SDS and 0.6% dithiothreitol. For each preparation 40 μg of protein was applied to 15% gels containing 0.1% SDS. From left to right: standard mixture, remnants from 100% OSO emulsions, remnants from 100% OOO emulsion, apolipoprotein E, apolipoprotein A-I, and the standard mixture containing phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme.

TABLE 4. Protein contents of OOO and OSO emulsion remnants[a]

	Injected Emulsion	
	OOO	OSO
Remnant protein		
Total mass (% by weight)	8.9	5.7
Mass per particle[b] ($g \times 10^{-18}$)	83.7	56.8
Percent distribution of total protein		
Apolipoprotein C	17.3	23.5
Apolipoprotein E	69.8	73.3
Other[c]	13.0	3.5
Number of molecules per particle[d]		
Apolipoprotein C	872	804
Apolipoprotein E	1006	717
Other[c]	101	18

[a] Remnants were recovered from the plasma of liver-excluded rats 15 min after the injection of 100% OOO or 100% OSO emulsions. The injected emulsions were 160 nm in diameter, giving a particle mass of 1.99×10^{-15} g at the calculated density of 0.93 g/ml. CO was 2.5% and 2.6% by weight, respectively.

[b] Remnant protein mass was measured by fluorescamine assay. To calculate mass per particle, protein mass was related to the measured mass of CO, which was calculated for the injected emulsion particles, and assumed to stay unchanged during remnant formation.

[c] By SDS polyacrylamide gel electrophoresis, this band had an apparent molecular mass of ~65,000.

[d] The contribution of each protein to total protein mass per particle was measured by laser densitometry of the stained protein bands, assuming that staining by Coomassie blue was proportional to mass, and similar for the different proteins. Each mass was then multiplied by Avogadro's number and divided by 10,000, 35,000, or 65,000 for apolipoprotein C, E, and "other," respectively.

DISCUSSION

Artificial emulsions provide a useful tool for the study of factors affecting the disposal of natural TAG-rich lipoproteins, provided that certain precautions are observed in their preparation (18). A lipid mixture is sonicated which provides an appropriate balance of core components (TAG and CO) and surface components (mostly PL) in the emulsified particles. Free cholesterol, C, partitions between the core and the surface components but is mostly in the surface for particles > 75 nm in diameter (19). The initial crude emulsion is purified by flotation through a step-density gradient to remove phospholipid-rich vesicles formed by sonication of excess phospholipid in the original mixture and to provide a harvest of fairly uniform particles of size and composition within the range for chylomicrons (20).

Like chylomicrons, artificial emulsions are metabolized in two stages with rapid early lipolysis of TAG by lipoprotein lipase and slower, but still rapid removal of remnants by the liver, as shown by clearance of labeled TAG fatty

acid and labeled CO, and recovery of labeled CO in the liver. Unlike chylomicrons, artificial emulsion particles are free of apolipoproteins when they enter the circulation. Nevertheless, rapid association with apolipoproteins must follow, as shown by quantitative agreement between clearance values for natural chylomicrons and those for emulsion particles of similar lipid composition. Thus, artificial emulsions provide a model for investigating the effects of the lipid composition of TAG-rich lipoproteins on the formation and disposal of remnants.

The present work confirms the effect of OSO in slowing the clearance of lipid particles from the circulation. In previous work (3), clearance of radioactive cholesteryl ester, as a marker for remnant cores, was slowed both for chylomicrons from rats fed a fatty meal of OSO and for artificial OSO emulsions. These findings have now been extended by showing that the effect of OSO was only seen when it formed > 64% of TAG in the emulsion particles and that the effect then increased progressively with increasing preponderance of OSO. The slower clearance of remnants was not explained by a slower production of remnant-sized particles by lipolysis. In the present experiments there were no significant differences in the fractional clearance rates of labeled TAG fatty acid although somewhat slower clearance of TAG was found in the earlier experiments. In the previous experiments, OSO was labeled both in the 2-position of glycerol and in stearic acid, compared with labeling with a tracer amount of radioactive triolein in the present experiments. Specific double-label experiments are planned to test for the possibility that lipolysis of OOO is faster than lipolysis of OSO in the same emulsion particle.

If lipolysis is indeed unimpaired, then the observed slower clearance of emulsion CO must be explained by slower remnant uptake by the liver. Direct support for this interpretation was provided by injecting intact recipient rats with remnants prepared in functionally hepatectomized rat donors. Fig. 2 shows clearly that the removal from plasma of remnants derived from OSO emulsions was significantly slower than removal of remnants derived from OOO emulsions.

One possible mechanism for slower hepatic uptake of remnants derived from OSO was that partial glycerides with stearic acid at the 2-position of glycerol, generated transiently by lipoprotein lipase, altered the affinity of the surface of the particle for apolipoproteins and, beyond a certain point, decreased the affinity for remnant receptors on the hepatocyte. So far, analysis of remnants from liver-excluded rats has not provided support for this hypothesis. No monoacylglycerol was detectable by TLC. There were no differences in the small amount of diacylglycerol present in 15-min remnants and only traces were present in 30-min remnants.

Differences were observed between the proteins associated with OSO remnants and OOO remnants in the contents of apolipoprotein E and of an unidentified protein, which has a molecular weight similar to albumin but which could be albumin, cholesteryl ester transfer protein (21), or LCAT (22). We are continuing with efforts to identify the proteins associated with remnants harvested at different times, in vivo, with liver uptake excluded.

Production and removal of remnants are overlapping processes in the intact animal. Studying the continuous transformation of a cohort of emulsion particles of uniform initial size and composition may provide a quantitative description of the interrelated changes involved. We recently described a kinetic model of chylomicron metabolism (23), and we are currently investigating emulsion clearance data with a similar multicompartmental model of plasma clearances and hepatic uptake of remnants (C. F. Ramberg, R. C. Boston, and T. G. Redgrave, unpublished results). A quantitative description of remnant production from different types of particles should increase the predictive power of the model; conversely, the model will assist in attributing the nature of observed metabolic changes.

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