Features of cholesterol structure that regulate the clearance of chylomicron-like lipid emulsions

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Abstract Cholesterol is an absolute requirement for the clearance from plasma of the remnants of triglyceride-rich lipoproteins. Our laboratory previously established that cholesterol was essential for the hepatic uptake of remnant particles after intravenous injection of chylomicron-like lipid emulsions (1). The aim of the present study was to determine the structural features of the cholesterol molecule that regulate the metabolism of chylomicrons. Chylomicron-like lipid emulsions, which reflect the size and composition and mimic the physiology of lymph chylomicrons, were prepared with tracer amounts of labeled triolein ([^{14}C]TO) and cholesteryl oleate ([^{3}H]CO) to follow the hydrolysis of triglyceride and the uptake of chylomicron remnant particles by the liver. Sterols selected as cholesterol congeners with functional group variations were incorporated into the emulsions in place of cholesterol and injected intravenously in rats. Control emulsions contained either no cholesterol or approximately 1% (by weight) cholesterol. The effects of the different sterol structures on lipolysis and hepatic remnant uptake were compared with controls to determine the significance of various functional groups. Clearance of emulsion CO was impaired when cholesterol was absent or replaced by cholesteryl chloride, cholesteryl formate, or 3-keto-cholesterol. Clearance of emulsions containing epicholesterol, where the OH group at the 3-position is in the α configuration, was similar to control emulsions containing cholesterol. Congeners with an additional hydroxyl group, viz. 7α-hydroxycholesterol, 7β-hydroxycholesterol, or 25-hydroxycholesterol, reduced CO clearance. Androstenol, which lacks the side chain at the C17-position, also retarded CO clearance from plasma. In contrast, emulsions incorporating congeners with side chain variations such as campesterol, β-sitosterol, stigmasterol, or saturated congeners of cholesterol such as cholestanol, coprostanol and its epimer, epicoprostanol, all were cleared similarly to emulsions containing cholesterol. In conclusion, for physiological clearance of a chylomicron-like emulsion, the presence of a hydroxyl (-OH) group at the 3-position and an alkyl side chain at the C17-position of cholesterol are essential, while the structure of the side chain and the saturation of the ring structure are not critical. The mechanism of the specificity of sterols on the metabolism of protein-free emulsions is unclear, but does not relate to changes in microfluidity of the surface lipids, nor to the amount or isoform of associated apolipoproteins.—**Mortimer, B-C., P. Tso, C. T. Phan, D. J. Beveridge, J. Wen, and T. G. Redgrave.** Features of cholesterol structure that regulate the clearance of chylomicron-like lipid emulsions. *J. Lipid Res.* 1995. **36:** 2038–2053.

Supplementary key words cholesterol congeners • chylomicron remnants • cholesteryl oleate • triolein

Dietary fat, mainly in the form of triacylglycerols, is transported into the bloodstream from the small intestine as triacylglycerol-rich lipoprotein particles known as chylomicrons (CM). CM are metabolized in the plasma, with a significant portion of the triacylglycerol lipolyzed by lipoprotein lipase and removed by peripheral tissues (2). The hydrolysis of particle triacylglycerol is incomplete, with about 10–30% of the triacylglycerol remaining (3, 4). Residual triacylglycerol and the cholesteryl esters remain with the particle and these particles are known as chylomicron remnants (5). Remnants then acquire apolipoprotein (apo) E, the ligand for binding to the low density lipoprotein receptor (6) and possibly other receptors (7) in the liver and peripheral tissues. The plasma clearance of labeled triacylglycerol in CM-like emulsions, therefore, reflects the sum of two processes, lipolysis and particle uptake by the liver, while clearance of labeled cholesteryl ester represents the uptake of chylomicron remnants by the liver. The difference calculated between the triglyceride clearance and the cholesteryl ester clearance is known as the "lipolysis index" which only measures lipolysis.

The study of CM metabolism has been impeded by the difficulty of manipulating the composition of a single component of the native CM particle. With a number of parameters changing simultaneously during

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Abbreviations: CM, chylomicrons; CO, cholesteryl oleate; TO, triolein; TG, triacylglycerol; VLDL, very low density lipoproteins; ACAT, acyl-CoA:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; DPH-PC, 1-palmitoyl-2-{[(2-[4-(6-phenyl-*trans*-1,3,5-hexatrienyl)phenyl]ethyl]carbonyl}-3-*sn*-phosphatidylcholine; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5,-triene; TLC, thin-layer chromatography; FCR, fractional clearance rate.

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CM metabolism, it is difficult to pinpoint regulatory roles of individual components. We have established previously that artificial emulsion particles with lipid composition and size comparable to lymph CM are metabolized in a fashion similar to the native CM (8). This has afforded a unique opportunity to establish the roles of the individual emulsion components on the metabolism of artificial chylomicron particles.

Using artificial emulsion particles, we demonstrated a slower plasma clearance of labeled cholesteryl oleate (CO) in emulsions made with triacylglycerol containing a saturated fatty acyl (stearate) at the *sn*-2 position of glycerol and oleate at positions-1 and -3 of the molecule (8). By utilizing the same model system, we also demonstrated that the amount of cholesterol in the emulsion particles had profound effects on the plasma clearance of CM-like particles. Increased amounts of cholesterol in the triacylglycerol–phospholipid emulsions changed the metabolism of these particles by promoting their rapid uptake by the liver without significant hydrolysis (9). In contrast, the absence of cholesterol had no effect on lipolysis but markedly impeded the hepatic uptake of remnant particles (1). These studies indicate that the presence of unesterified cholesterol plays a crucially important role in the metabolism of artificial CM.

To further understand how cholesterol affects lipolysis of triacylglycerol and uptake of remnant particles by the liver, we have now studied the contribution of various structural features of cholesterol on these parameters. CM-like lipid emulsions that reflect the lipid composition of lymph chylomicrons were prepared with either no cholesterol, or with approximately 1% cholesterol or selected congeners. Tracer amounts of radiolabeled TO and CO were incorporated to follow the hydrolysis of triacylglycerol and the uptake of remnant particles by the liver. The emulsions were injected into rats for the comparison of lipolysis and hepatic remnant uptake. Cholesterol congeners selected for the study had positional variations in *1)* the hydroxyl group at the 3-position; *2)* an additional hydroxyl group located else-

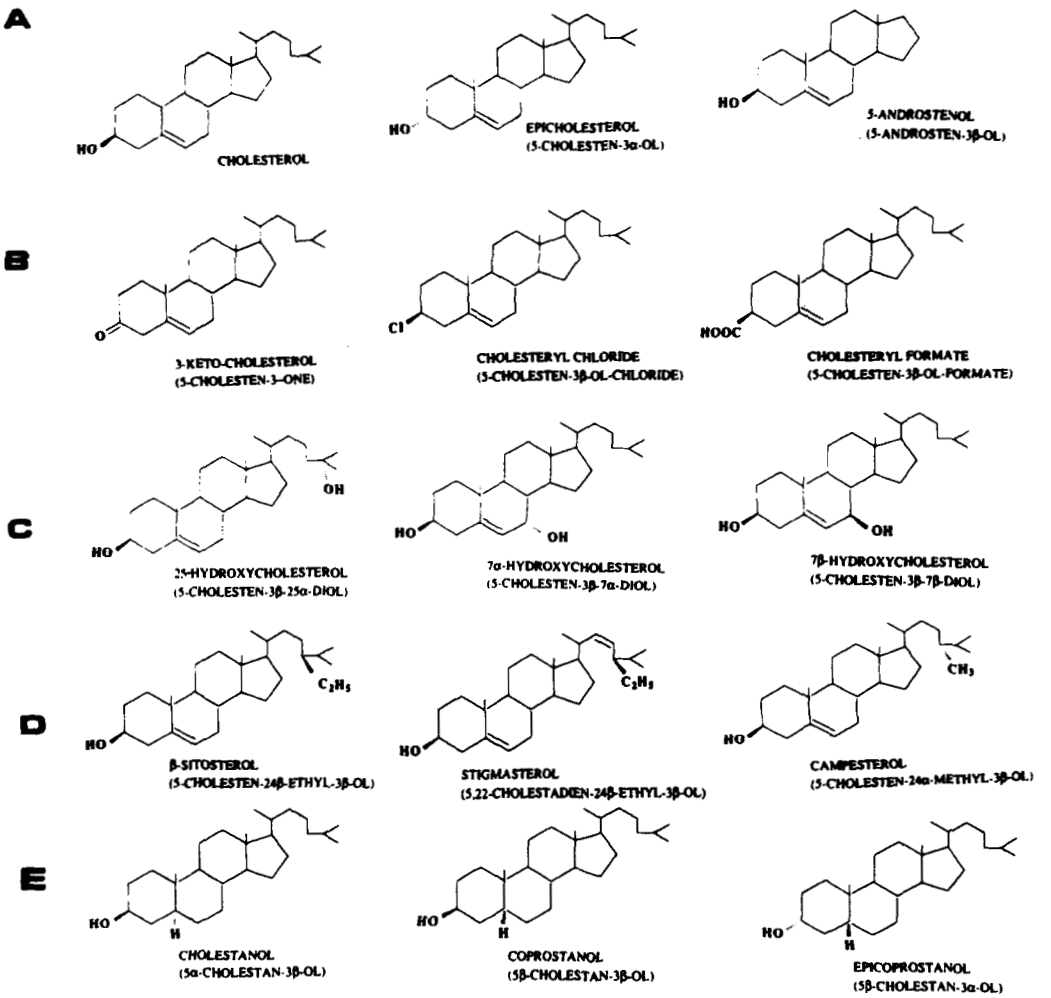

Fig. 1. Structures of (A) cholesterol, epicholesterol and androstenol, (B) congeners with variations in 3-functional groups, (C) di-hydroxycholesterols, (D) cholesterol congeners with C17 branched side-chain variations, and (E) saturated congeners.

where in the sterol; *3)* the side chain at the 17-position; and *4)* the double bond in the 5–6-position of the sterol ring.

To investigate the role of apolipoproteins in the plasma clearance of emulsions, selected emulsions were incubated with purified human apoE3, rat apoE, or rat plasma and then examined for the associated apolipoproteins. Moreover, membrane fluidity of selected emulsions was compared by measuring the anisotropy of two fluorescent probes, 1-[4-(trimethylamino)phenyl]-6-phenyl-hexa-1,3,5-triene (TMA-DPH) and 1-palmitoyl-2-{[(2-[4-(6-phenyl-*trans*-1,3,5-hexatrienyl)phenyl]ethyl]carbonyl}-3-*sn*-phosphatidylcholine (DPH-PC), which were incorporated into the emulsions.

MATERIALS AND METHODS

Materials

Egg yolk phosphatidycholine was purchased from Lipid Products, Surrey, UK. Cholesterol, cholesteryl oleate, and triolein were from Nu-Chek Prep, Elysian, MN. Radiochemicals including [7(n)-^{3}H]cholesterol, glycerol tri-[1-^{14}C]oleate, and glycerol tri-[9,10(n)-^{3}H]oleate were purchased from Amersham, Sydney, NSW, Australia. [^{3}H]cholesteryl oleate was synthesized as described previously (10). Cholesterol congeners were obtained from Steraloids (Wilton, NH) and used without further purification. As determined by thin-layer chromatography (TLC), the purity was > 99% for all sterols used in the emulsions. DPH-PC and TMA-DPH were purchased from Molecular Probes Inc. (Junction City, OR).

Preparation of chylomicron-like emulsions

Emulsions with the required composition and size were prepared by sonication and isolated after ultracentrifugation through density gradients. Details of these procedures have been described previously (8). Briefly, 70 mg triolein, 3 mg cholesteryl oleate, 2 mg cholesterol (or sterol congeners), and 25 mg egg yolk phosphatidylcholine were dispensed from stock solutions into a vial. Radiolabeled lipids or a fluorescent probe were then added. Fluorescent probes were dissolved in ethanol and added to the lipid at a probe/lipid ratio of 1/200. Solvents were evaporated under a stream of N_2 before overnight vacuum desiccation to eliminate residual solvent traces. The dried lipid mixture was sonicated in 8.5 ml of 10 mM HEPES (pH 7.4) in 0.15 M NaCl solution and then subjected to density gradient ultracentrifugation to isolate the appropriate emulsion fraction.

Injection studies

Non-fasted male albino Wistar rats weighing 280 ± 30 g were obtained from the Animal Resources Centre (Murdoch, Western Australia) and 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. The tip was advanced to lie in the superior vena cava. Clotting was prevented by treating the tubing with Aquasil™ (Pierce, Rockford, IL) prior to the surgical procedure. Heparin was not used as it may alter the circulating level of plasma lipoprotein lipase. After surgery, the animals were allowed to re-

TABLE 1. Lipid compositions and particle sizes of injected chylomicron-like lipid emulsions

Emulsion Sterol	n	Composition (% by weight) TO	CO	PL	Cholesterol or Congeners	Average Diameter	Median Size
		%				*nm*	*nm*
Cholesterol	4	82.3 ± 0.6	4.0 ± 0.4	12.4 ± 0.2	1.4 ± 0.1	144 ± 4	140 ± 2
No cholesterol	3	83.5 ± 0.9	3.4 ± 0.8	13.0 ± 0.2	-	134 ± 3	130 ± 2
Epicholesterol	1	84.5	3.9	9.7	1.9	135	132
3-Keto cholesterol	3	84.7 ± 0.7	3.8 ± 0.1	11.1 ± 0.8	0.4 ± 0.1	144 ± 2	134 ± 3
Cholesteryl chloride	1	82.4	6.1	11.0	0.5	142	139
Cholesteryl formate	1	86.1	2.6	9.7	1.4	136	131
7α-OH-cholesterol	3	85.3 ± 1.4	3.5 ± 0.8	10.9 ± 1.1	0.2 ± 0.0	143 ± 1	139 ± 2
7β-OH-cholesterol	2	86.3 ± 1.8	3.5 ± 0.5	10.0 ± 1.2	0.2 ± 0.1	148 ± 2	145
25-OH-cholesterol	3	85.9 ± 1.4	3.2 ± 0.6	10.7 ± 1.3	0.2 ± 0.1	160 ± 4	151 ± 4
Androstenol	1	85.9	2.4	10.2	1.5	134	129
β-Sitosterol	2	84.3 ± 1.4	3.6 ± 0.3	10.9 ± 1.8	1.2 ± 0.1	136	132
Campesterol	1	86.5	3.0	9.6	0.9	134	130
Cholestanol	1	85.8	3.3	10.5	0.4	142	138
Coprostanol	1	84.7	3.3	11.5	0.5	143	137

TO, triolein; PL, phospholipid; CO, cholesteryl oleate.

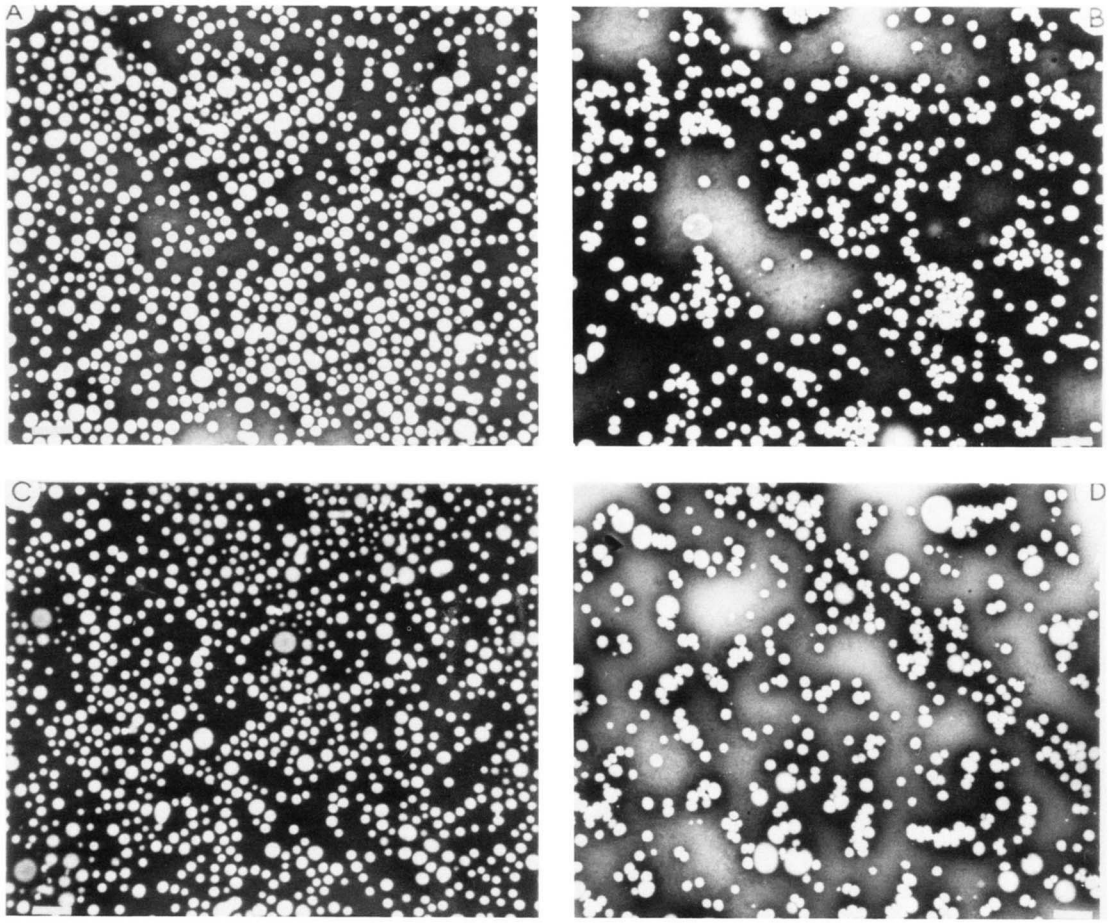

Fig. 2. Electron micrographs of (A) a control emulsion containing cholesterol labeled with TMA-DPH, (B) an emulsion with no cholesterol labeled with DPH-PC, (C) an emulsion incorporating 7α-OH-cholesterol labeled TMA-DPH, and (D) an emulsion incorporating cholestanol labeled with [^{3}H]CO and [^{14}C]TO. The bar represents 500 nm. The size distribution of these four emulsions as determined by a laser particle sizer (Brookhaven BI-90) had a 90 percentile of 204 ± 10 nm. The mean polydispersity is 0.07 ± 0.005 with an average median diameter of 138 ± 2.7 nm.

cover from anesthesia in individual restraint cages and were maintained at 30°C for 2-4 h before the study commenced. The emulsions were injected into the venous cannula as a bolus of approximately 3 mg lipid in a volume of 0.5 ml. Blood samples of 0.4 ml were then taken at 3, 5, 8, 12, 20, 25, and 30 min. Each withdrawal was replaced with an equal volume of 0.15 M NaCl. After the final blood sample a lethal dose of Nembutal was injected. The liver and spleen were excised for extraction of radioactive lipid from either 1 gram of minced liver or minced whole spleen. Lipids were extracted from the liver and spleen samples in 30 ml of chloroform–methanol 2:1 (v/v), aliquots were taken, the solvent was evaporated, and subsequently radioactivity was measured by liquid scintillation spectrometry. Radioactivity in plasma was measured, without extraction, in 150-μl samples using Emulsifier-safe™ (Packard). Plasma clearance kinetics were computed as fractional clearance rates from the mono-exponential curves fitted by least squares procedures to samples taken during the first 12 min after injection.

Five separate injection studies were performed.

(A) The clearances of emulsions with or without cholesterol were compared to confirm our previous observation that the absence of free cholesterol in emulsions delayed the clearance of remnant particles. The clearance of free cholesterol in emulsion particles was also followed by a tracer amount of labeled cholesterol.

(B) To assess the importance of the hydroxyl group at the 3β position of the cholesterol molecule, clearances were compared in rats injected with emulsions containing either cholesterol or congeners with the 3β-hydroxyl functional group replaced by a chloro group in cholesteryl chloride (5-cholesten-3β-chloro), or a keto group in 3-keto-cholesterol (5-cholesten-3-one), or a carbonyl group in cholesteryl formate (5-cholesten-3β-ol formate). The structures of these congeners are shown in **Fig. 1B.** The configuration of the 3-hydroxyl func-

tional group was assessed by comparing cholesterol (β-OH) with epicholesterol (α-OH), as shown in Fig. 1A.

(C) To study the effect of an additional hydroxyl group we examined the clearance of emulsions incorporating congeners with an extra hydroxyl (OH) group at different carbon positions, namely 7α-hydroxycholesterol (5-cholesten-3β-7α-diol), 7β-hydroxycholesterol (5-cholesten-3β-7β-diol), and 25-hydroxycholesterol (5-cholesten-3β-25α-diol). These structures are shown in Fig. 1C.

(D) Variations in the C17 alkyl side chain were also evaluated, using emulsions incorporating cholesterol analogues without the side chain, i.e., androstenol (5-androsten-3β-ol) as shown in Fig. 1A, or with an additional double bond at C22 (stigmasterol), or with an extra methyl (campesterol) or ethyl group (β-sitosterol) on the branched side chain, shown in Fig. 1D.

(E) The significance of the double bond at the C5–6 position in the sterol ring structure was examined in emulsions containing various saturated cholesterol congeners, cholestanol (5α-cholestan-3β-ol), coprostanol (5β-cholestan-3β-ol), and epicoprostanol (5β-cholestan-3α-ol), as shown in Fig. 1E.

Incubation of emulsions with rat plasma or with purified apoE

To obtain plasma with or without endogenous VLDL, rats were fasted for 16 h and blood was taken from the abdominal aorta into tubes containing EDTA (final concentration 1 mM). The plasma was centrifuged for 17 h at 40,000 rpm and 20°C in the Beckman SW41 rotor; the VLDL particles were then removed by aspiration of the top 10 mm. Five-ml portions of whole plasma or the VLDL-free plasma were incubated with various emulsions containing 3 mg of triolein at 37°C for 30 min. The emulsion particles were then separated from the mixture by ultracentrifugation, delipidated (10), and applied to 5–25% gradient SDS polyacrylamide gels for electrophoresis or to 5% polyacrylamide gels for isoelectrofocusing as reported previously (10). The apolipoprotein profiles from these gels showed no difference between incubations with whole plasma or VLDL-free plasma (results not shown). Hence, in subsequent studies, whole plasma from fasted rats was used for incubations with the emulsions.

Emulsions with and without cholesterol, and an emulsion containing 7β-hydroxycholesterol, were incubated with rat apoE (10) or human apoE3 (Calbiochem). Aliquots of 1-ml emulsion (in HEPES buffer, pH 7.4) were added to tubes containing the apolipoproteins (in 0.1 M ammonium carbonate buffer, pH 8.0/1 μl β-mercaptoethanol). The protein to lipid ratios were 15 μg human apoE3 or 50 μg rat apoE to 1 mg of emulsion. The mixtures were incubated at 37°C for 30 min then the emulsion particles were separated from the mixture by ultracentrifugation. The protein–emulsion mixtures were delipidated and applied to 5–25% gradient SDS polyacrylamide gels for electrophoresis (10). After staining with Coomassie blue the apolipoprotein bands were quantified with an UC 630 Maxcolor Densitometer, using the Photoshop program for Macintosh.

Immunoblots were prepared by electrophoretically transferring proteins from other non-stained SDS-PAGE gels to nitrocellulose filters and incubating with a polyclonal antiserum to rat apoE (10). ApoE bands were detected on these blots using the enhanced chemiluminescence (ECL) system (Amersham).

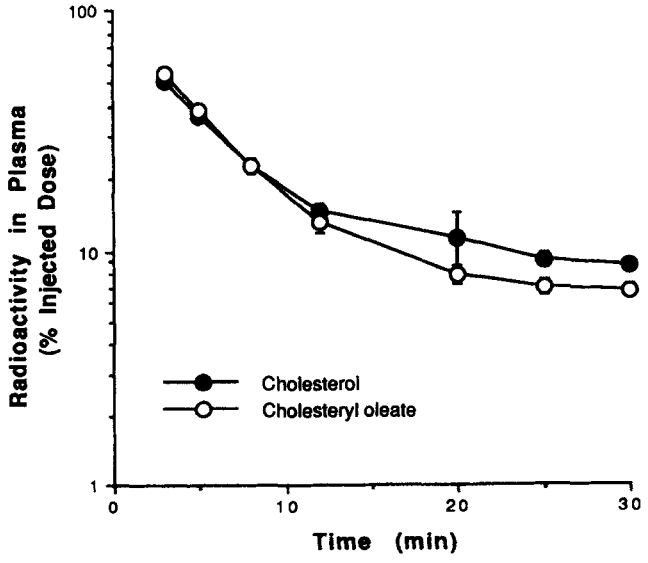

Fig. 3. Radioactivity in plasma after injection into conscious rats of a chylomicron-like lipid emulsion containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, and cholesterol. Plotted are the data for [^{3}H]cholesterol and [^{14}C]CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results are means ± SEM of six animals.

Fluorescence measurements

Steady-state fluorescence measurements were performed using an Hitachi F-3000 fluorescence spectrophotometer, equipped with a Xe 150W lamp, at a constant room temperature of 25°C. The spectrofluorometer was equipped with two polarizers (Hitachi Part No. 650-0155), one in each of the emission and excitation beams, and a thermostated cell holder with stirrer (Hitachi Part No. 6224-013). TMA-DPH and DPH-PC were both excited at 365 nm and emitted fluorescence was measured at 433 nm with a bandpass of 5 nm for both excitation and emission monochromators. Corrections for light scattering and intrinsic fluorescence were made by subtracting the signal from identical but unlabeled emulsions. The corrections (blank) were less than 4% for both TMA-DPH and DPH-PC in protein-free emulsions, which is within the

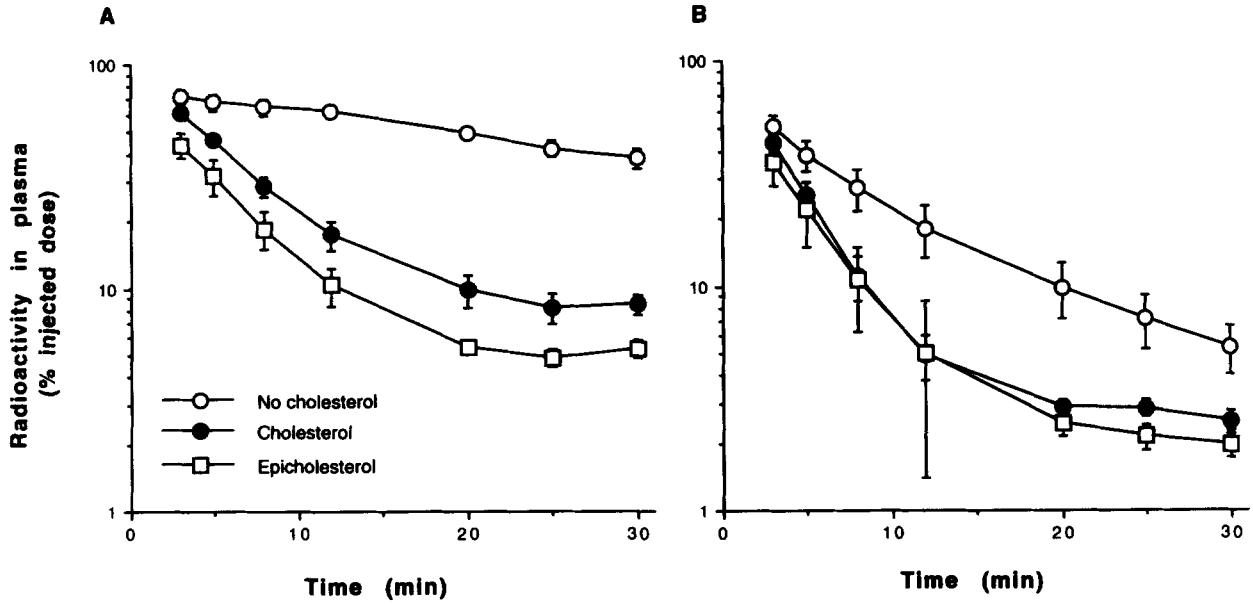

Fig. 4. Radioactivity in plasma of (A) cholesteryl oleate (CO) and (B) triolein (TO) labels after injection into conscious rats of chylomicron-like lipid emulsions containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, with and without cholesterol and with epicholesterol. Plotted are the data for TO and CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results are means ± SEM of 6–11 animals for each observation.

acceptable range for the calculation of anisotropy parameters (11). Fluorescence emission intensities parallel ($I_{parallel}$) and perpendicular ($I_{perpendicular}$) to the excitation plane of the probe were detected sequentially through a polarizer oriented parallel and then perpendicular to the direction of polarization of the excited beam. The measured fluorescence anisotropy (r_{obs}) was calculated by the following equation (12):

$$r_{obs} = (I_{parallel} - I_{perpendicular})/(I_{parallel} + 2\ I_{perpendicular})$$

The emulsion samples were turbid even in dilute solutions and the measured anisotropy was corrected from semilogarithmic plots of the observed values of r versus the concentration of the emulsion, h, according to Eisinger and Flores (13), from the relationship:

$$\ln r_{obs} = \ln r - K\ h$$

where K is the anisotropy attenuation coefficient and the intercept gives the true anisotropy of the fluorophores, r (13).

The fluorescence anisotropy of membrane-associated probes reflects the re-orientational correlation time of phospholipid acyl chains in a membrane and provides a measure of membrane fluidity. The anisotropy parameter (r) varies directly with the rotational relaxation time of the probe and is related inversely to the lipid fluidity of the membrane (12, 14), which can be assessed by the order parameter, S, as shown in the following equation:

$$S = [(4/3\ r - 0.1)/r_o]^{1/2}$$

when r falls between 0.13 and 0.28 (14), and r_o represents the maximal hindered anisotropy of the probe, taken as 0.39 for TMA-DPH (15) and 0.40 for DPH-PC (16).

Chemical analysis

Lipid phosphorus was measured by a modified Bartlett procedure (17). Triacylglycerol, and free and esterified cholesterol were assayed from lipid extracts after separation by TLC. Triacylglycerol was quantified as glycerol by chromotropic acid (18), and free and esterified cholesterol by the *o*-phthaldialdehyde procedure (19) after saponification of the separated bands. Concentrations of cholesterol congeners were determined by densitometry of lipid extracts with standard solutions by TLC.

Electron microscopy

Emulsions on formvar-coated grids were negatively stained with 2% phosphotungstic acid at room temperature and viewed on a Jeol 2000 FX transmission electron microscope. Sizes of the emulsion particles were determined by laser light scattering using a B1-90 particle sizer (Brookhaven Instruments Corp., NY).

Statistical analysis

The significance of differences was tested by repeated measures analysis of variance or by one-way analysis of variance of the computed fractional clearance in rats with comparisons of individual means by Tukey's procedure. Two-tailed *P*-values less than 5% were considered to be significant.

RESULTS

Composition of emulsions

All the steroids were soluble in ethanol and could be incorporated into chylomicron-like emulsions as described in the Methods. **Table 1** shows the average diameter, median size, and composition of the various emulsions used for clearance studies. The average diameters of all emulsions varied in a narrow range of 134 to 160 nm. Electron micrographs of all the emulsions showed well-defined, round particles with distinct borders, indicating that the cholesterol congeners and the fluorescent and radioactive labels did not disrupt the emulsion structure. **Figure 2** shows the electron micrographs of four emulsion samples. The average median diameter of the four emulsions shown in Fig. 2 was 138 ± 2.7 nm, the mean polydispersity was 0.07 ± 0.005, and the average size distribution had a 90 percentile of 204 ± 10 nm.

An initial control experiment tested whether free cholesterol remained with the remnant particles after hydrolysis of chylomicron particles by lipoprotein lipase. Emulsions were double-labeled with tracer amounts of [^{3}H]cholesterol and [^{14}C]CO. **Figure 3** shows that the cholesterol and cholesteryl oleate were removed at the same rate throughout the whole clearance study. The organ uptakes of [^{3}H]cholesterol and [^{14}C]CO from these emulsion particles were also similar, with 60% of [^{3}H]cholesterol and 58% of [^{14}C]CO recovered in the liver.

Essentiality of cholesterol for clearance of CM-like emulsions from plasma

Figure 4 compares the clearance from rat plasma of emulsions incorporating no cholesterol, 1% cholesterol, or epicholesterol. Consistent with previous findings (1), emulsions containing approximately 1% (total lipid)

TABLE 2. Plasma clearance and organ uptake of emulsions incorporating cholesterol or cholesterol analogues injected into conscious rats

Emulsion Sterol	Removal from plasma		Lipolysis Index	Liver Uptake of Lipids	
	TO	CO		TO	CO
	FCR min^{-1}			% of injected radioactivities	
Cholesterol variations					
Cholesterol (11)	0.26 ± 0.02	0.14 ± 0.01	0.11 ± 0.01	9.0 ± 0.6	70.5 ± 3.1
No cholesterol (14)	0.19 ± 0.02	0.02 ± 0.01[a]	0.15 ± 0.02	9.0 ± 1.2	27.0 ± 4.3[a]
Epicholesterol (4)	0.25 ± 0.03	0.16 ± 0.01	0.08 ± 0.02	8.7 ± 1.3	52.3 ± 3.8[b]
Variations in 3-functional groups					
Cholesteryl formate (13)	0.22 ± 0.03	0.08 ± 0.01[a]	0.14 ± 0.03	9.5 ± 1.3	65.7 ± 2.1
3-Keto-cholesterol (11)	0.16 ± 0.02[b]	0.04 ± 0.05[a]	0.12 ± 0.02	9.7 ± 0.3	9.3 ± 0.4[a]
Cholesteryl chloride (5)	0.22 ± 0.22	0.03 ± 0.01[a]	0.18 ± 0.02[a]	7.3 ± 1.3	25.2 ± 1.6[a]
Di-hydroxyl sterols					
25-OH-cholesterol (5)	0.19 ± 0.03	0.01 ± 0.01[a]	0.18 ± 0.05[a]	9.1 ± 0.7	27.2 ± 5.3[a]
7α-OH-cholesterol (7)	0.16 ± 0.02[b]	0.01 ± 0.01[b]	0.15 ± 0.09	7.9 ± 0.5	30.1 ± 4.5[a]
7β-OH-cholesterol (6)	0.20 ± 0.02	0.03 ± 0.01[a]	0.16 ± 0.03	8.6 ± 0.5	26.2 ± 2.3[a]
Side-chain variations					
Androstenol (5)	0.14 ± 0.03[a]	0.01 ± 0.01[a]	0.13 ± 0.02	11.7 ± 1.4	25.0 ± 1.5[a]
β-Sitosterol (7)	0.26 ± 0.01	0.18 ± 0.01	0.08 ± 0.01	10.1 ± 0.5	65.5 ± 1.3
Stigmasterol (5)	0.18 ± 0.03[b]	0.14 ± 0.04	0.04 ± 0.01[b]	12.8 ± 1.1	66.0 ± 2.7
Campersterol (5)	0.28 ± 0.01	0.19 ± 0.02	0.09 ± 0.02	12.6 ± 2.2	67.0 ± 2.2
Saturated congeners					
Cholestanol (6)	0.24 ± 0.01	0.11 ± 0.01[b]	0.13 ± 0.02	11.8 ± 0.5	60.0 ± 2.1
Coprostanol (5)	0.29 ± 0.08	0.25 ± 0.02[a]	0.04 ± 0.02[a]	7.1 ± 0.7	71.3 ± 2.5
Epicoprostanol (6)	0.25 ± 0.02	0.14 ± 0.01	0.11 ± 0.02	7.2 ± 0.3	69.0 ± 2.4

The fractional clearance rates (FCR) were calculated from exponential curves fitted to the period 3–12 min after injection. Results are means ± SEM; (n) is the number of animals tested in each experimental group. Lipolysis index was calculated as the difference between the FCR of TO and CO. All liver uptakes were measured 30 min after injection. TO, triolein; CO, cholesteryl oleate.

[a] $P < 0.001$; [b] $P < 0.05$, significantly different from control emulsions containing cholesterol.

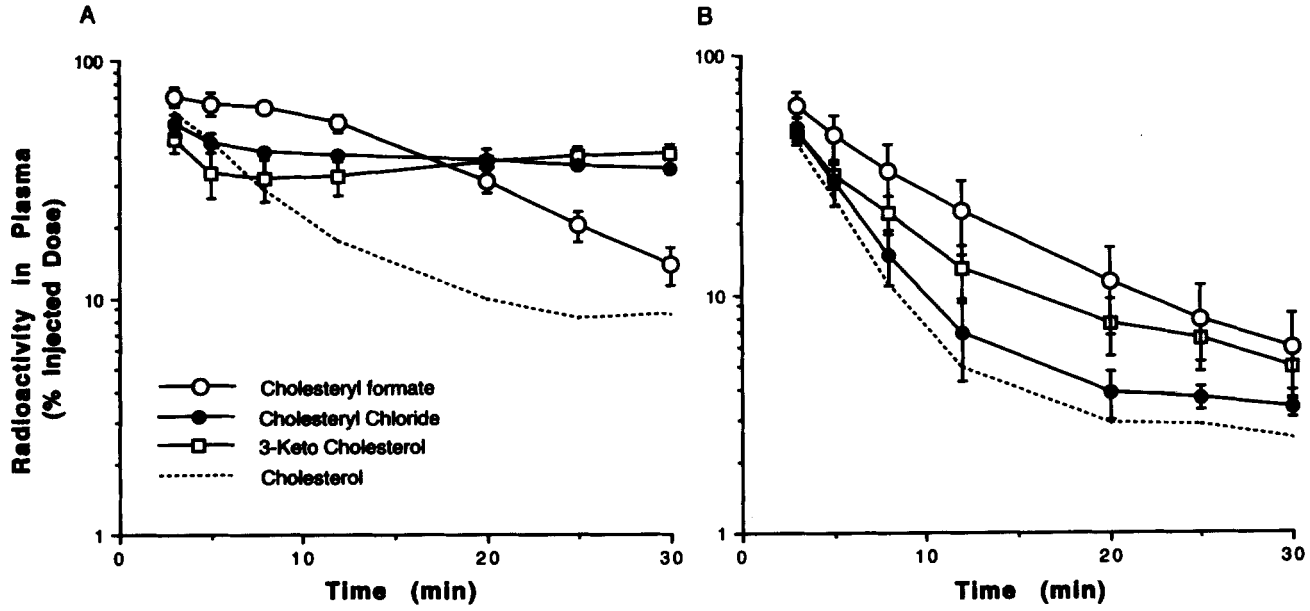

Fig. 5. Radioactivity in plasma of (A) cholesteryl oleate (CO) and (B) triolein (TO) labels after injection into conscious rats of chylomicron-like lipid emulsions containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, and cholesterol congeners with various functional groups at the 3β position of the sterol ring. Plotted are the data for TO and CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results are means ± SEM of 5–11 animals for each observation.

cholesterol hydrolyzed quickly, with more than 90% of particle TO disappearing from the plasma by 12 min after injection (Fig. 4B); followed by a rapid disappearance of particle remnants, with over 80% of labeled CO removed (Fig. 4A). In the absence of cholesterol, the removal of the emulsion triacylglycerol was little affected, with about 20% of the particle TO remaining in plasma by 12 min and with 80% being removed. The difference between the TG removal in emulsions with and without cholesterol was not significant (**Table 2**). In contrast, the absence of cholesterol in the emulsions markedly delayed CO clearance, with less than 30%

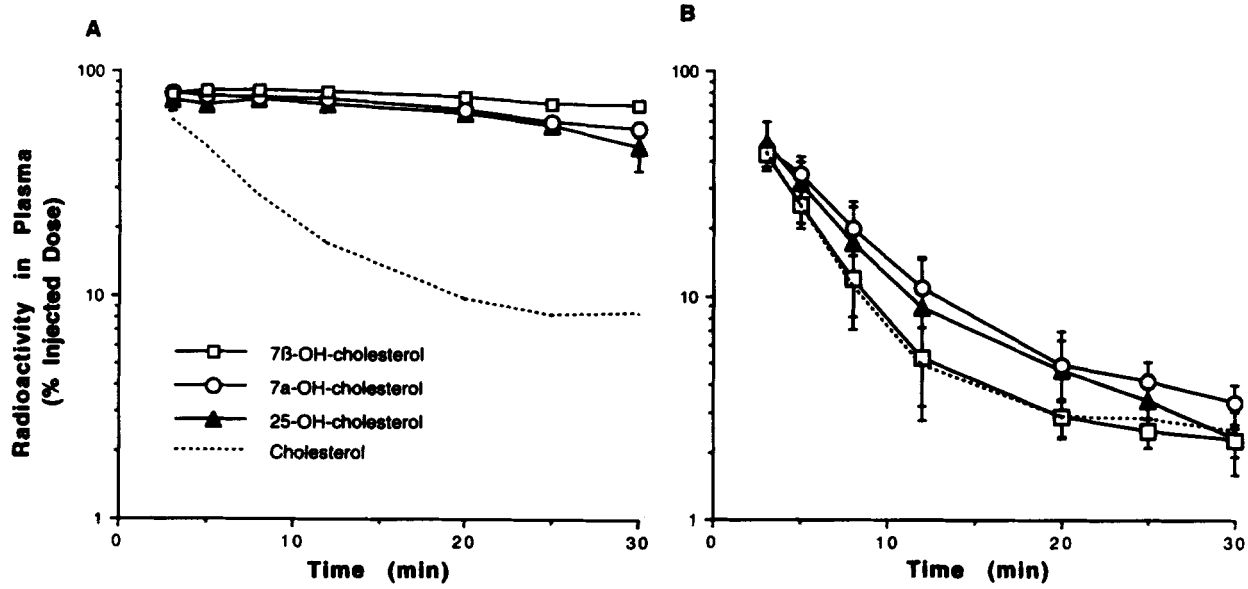

Fig. 6. Radioactivity in plasma of (A) cholesteryl oleate (CO) and (B) triolein (TO) labels after injection into conscious rats of chylomicron-like lipid emulsions containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, and cholesterol analogues with a second OH group. Plotted are the date for TO and CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results are means ± SEM of 4–7 animals for each observation.

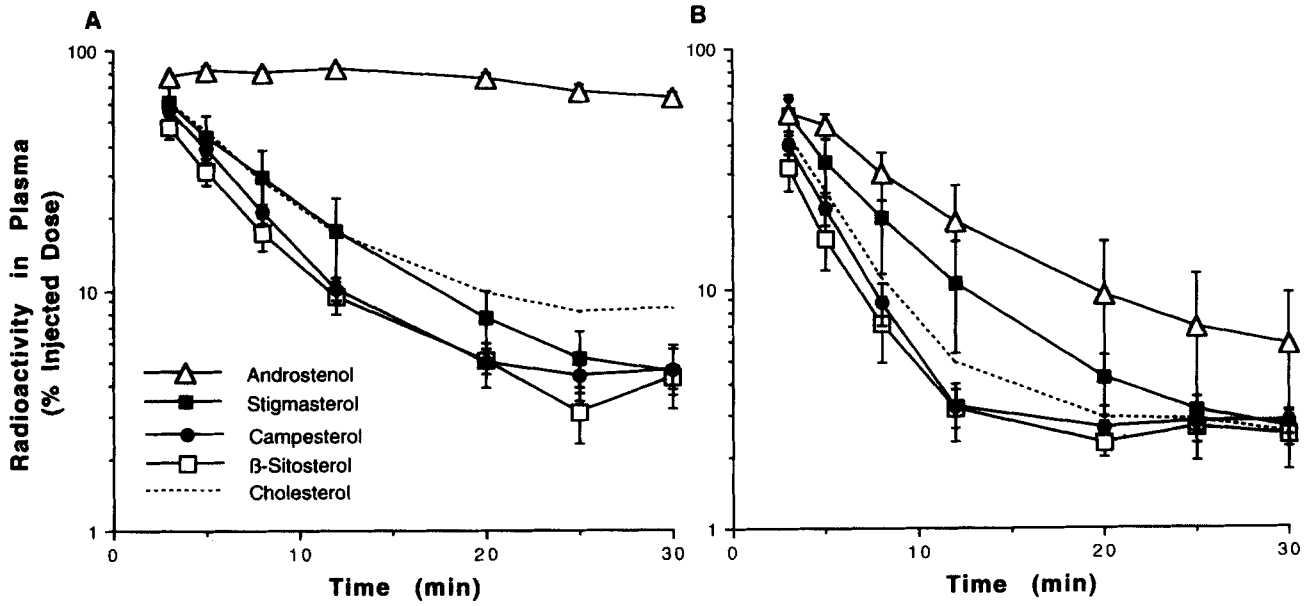

Fig. 7. Radioactivity in plasma of (A) cholesteryl oleate (CO) and (B) triolein (TO) labels after injection into conscious rats of chylomicron-like lipid emulsions containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, and cholesterol analogues with the side chain absent or modified. Plotted are the data for TO and CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results ± SEM are means of 5–7 animals for each observation.

removed from the plasma by 12 min after injection. Table 2 shows the fractional clearance rates (FCR, 3–12 min) and the hepatic uptake of the radiolabeled lipids at 30 min post-injection (expressed as a percentage of total radioactivity injected). Approximately 70% of labeled CO was found in the liver from emulsions containing cholesterol, significantly greater than the 27% recovered in the liver from emulsions with no cholesterol. Small quantities of radiolabels were taken up by the spleen in both cases (< 1%, results not shown). Emulsions incorporating epicholesterol, with the 3α-hydroxyl configuration, cleared from plasma at about the same rate as the control emulsion containing cholesterol, although liver uptake of labeled CO was slightly less than the control ($P < 0.05$, Table 2).

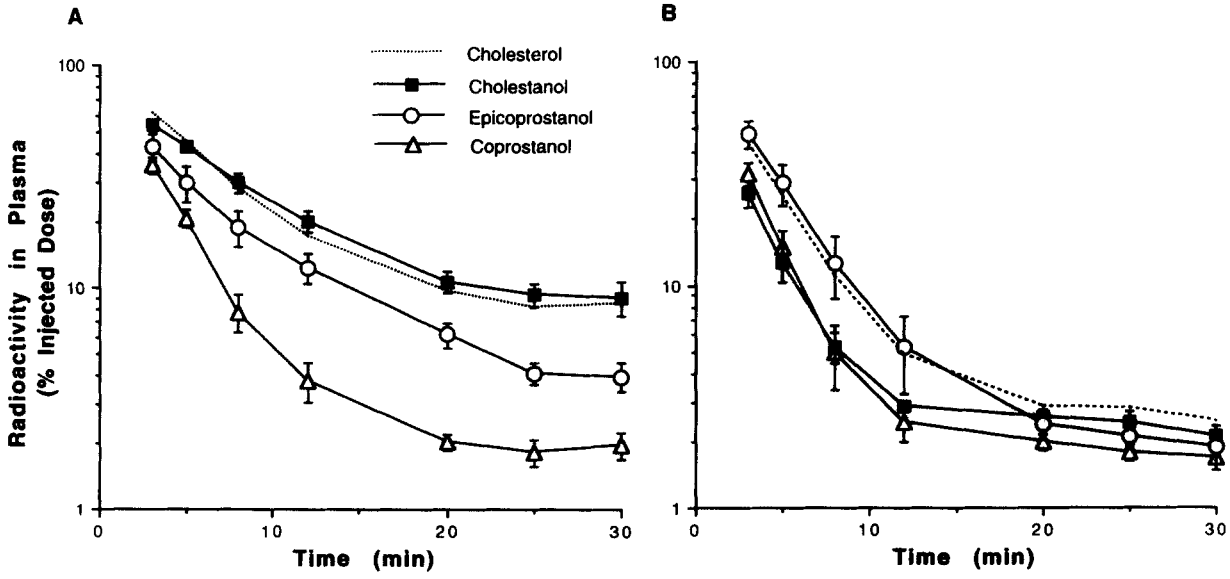

Fig. 8. Radioactivity in plasma of (A) cholesteryl oleate (CO) and (B) triolein (TO) labels after injection into conscious rats of chylomicron-like lipid emulsions containing triacylglycerol, cholesteryl oleate, egg yolk phosphatidylcholine, and cholesterol analogues with the ring-structure disrupted. Plotted are the data for TO and CO labels incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, 20, 25, and 30 min post injection. Results are means ± SEM of 5–7 animals for each observation.

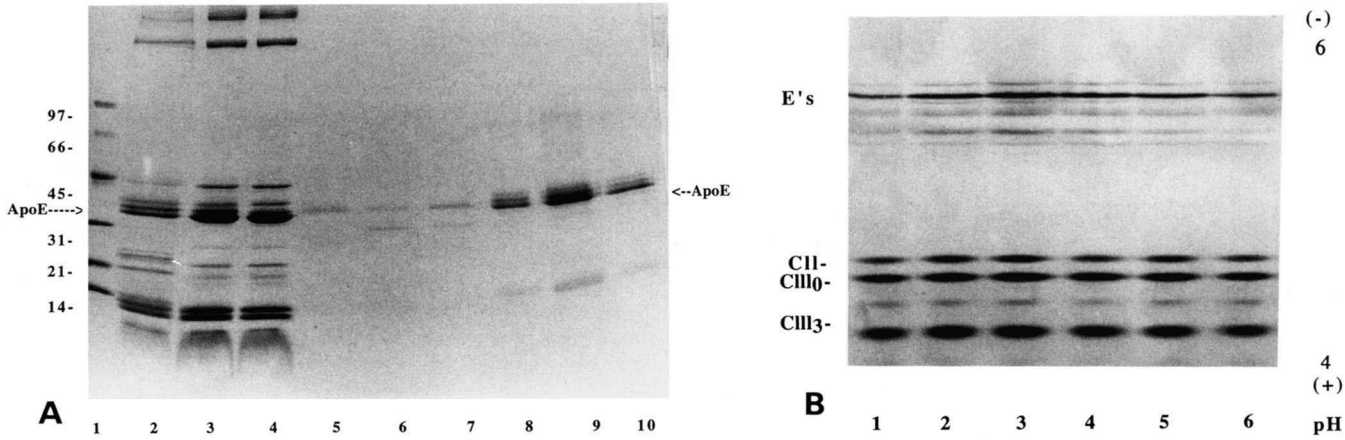

Fig. 9. (A) Five to 25% SDS gradient polyacrylamide gel electrophoretograms of apolipoproteins associated with various emulsions after in vitro incubation with rat plasma, with reconstituted rat apoE and with human apoE3 as described in the Methods. Lane 1 shows the molecular weight standards. Lanes 2–4 are, respectively, an emulsion with 1% cholesterol, with no cholesterol, and with 1% 7β-hydroxycholesterol after incubation with rat plasma. Lanes 5–7 are the same emulsions after incubation with human apoE3, and lanes 8 to 10 the same emulsions after incubation with rat apoE. (B) Isoelectric focusing of apoC isoforms after in vitro incubation with rat plasma as described in the Methods. Lanes 1 to 6 show, respectively, emulsions with 1% cholesterol, with no cholesterol, with 1% androstenol, stigmasterol, cholesteryl formate, and with 7α-hydroxycholesterol.

Importance of the 3-hydroxyl function of cholesterol on emulsion clearance

Figure 5 compares the plasma clearances of emulsion particles containing either 3-keto, 3-chloro, or 3-formyl in place of the 3β-hydroxyl group. The clearance of remnant particles in emulsions incorporating a keto or a chloro group, as determined by the plasma clearance of [^{3}H]CO, was greatly retarded when compared with control emulsions containing cholesterol. The fractional clearance rates (FCR) and the liver uptake of the radiolabeled lipids from these two emulsions were similar to the emulsion particles containing no free cholesterol (Table 2). In contrast, CO removal in emulsions containing cholesteryl formate was reduced to a much lesser extent, with normal liver uptake at 30 min. Emulsion TO removal was also slower in all three test emulsions when compared to that containing cholesterol. However, the lipolysis index calculated as the difference between the clearance rates of TO and CO (Table 2) showed that lipolysis was not affected.

Effect of an additional hydroxyl group

The presence of a second OH group in the sterol ring structure impeded CO clearance from the emulsions. **Figure 6** shows a retarded plasma clearance of the CO, accompanied by a marked reduction in liver uptake, in emulsions incorporating either the 7α, 7β, or the 25-hydroxycholesterol (Table 2). The rate of lipolysis was not affected (lipolysis index, Table 2), although the removal of TO was slower from emulsions containing 7α-hydroxycholesterol.

Side chain variations

Figure 7 shows the plasma clearance of emulsions containing the cholesterol analogues with the C17 side chain variations. Androstenol, which has the same ring structure as cholesterol but lacks the side chain, slowed TO removal from plasma, retarded plasma CO removal, and decreased liver uptake of CO. In contrast, β-sitosterol, with an additional ethyl group, and campesterol, with an extra methyl group on C24 of the side chain, did not affect the removal rates of emulsion TO or CO, or the hepatic uptake of the emulsion CO. With stigmasterol, TO removal was decreased and lipolysis was slower (lipolysis index, Table 2) while CO clearance was not affected.

Saturation of the sterol ring

Figure 8 shows the clearance of emulsions incorporating cholestanol, coprostanol, or epicoprostanol, selected as cholesterol congeners without the double bond at the C5–6 position in the sterol ring structure. Except for emulsions incorporating cholestanol, which had a slightly slower ($P < 0.05$) clearance rate, CO removal was similar to or faster than control emulsions containing cholesterol. TO removal was also similar to the control emulsion although lipolysis was reduced (lipolysis index, Table 2) with coprostanol. Liver uptakes of radioactive lipids 30 min after injection of the emulsions were all similar to control emulsions containing cholesterol.

Incubation studies

Emulsions were incubated with rat plasma, purified human apoE3, or purified rat apoE and re-isolated by ultracentrifugation as described in Methods. Neither

the SDS-polyacrylamide gradient gel (**Fig. 9A,** lanes 2, 3, 4) nor the isoelectric focusing gel (Fig. 9B) revealed any significant differences in the apolipoprotein profiles after incubation with rat plasma. The bands in Fig. 9A were measured by scanning densitometry. The relative amount of apoE obtained from the area and the intensity of apoE bands for a control emulsion (lane 2), an emulsion with no cholesterol (lane 3), and an emulsion containing 7α-OH cholesterol (lane 4) were 25.5, 25.5, and 21.3 (arbitrary units), respectively. Similarly, quantitative amounts of apoC in these lanes were 23.6, 23.3, and 19.6, respectively. The apoE/apoC ratio for all three samples was approximately 1:1. Immunoblotting of a similar gel with a rat apoE antibody confirmed the association of apoE with all three emulsions (results not shown). Likewise, the amount of human apoE3 that associated with the three emulsions did not appear to differ significantly (Fig. 9A, lanes 5, 6, 7). Similarly, after incubation, reconstituted rat apoE was also found to associate with the three emulsions (lanes 8, 9, 10 of Fig. 9A).

Fluorescence studies

The absorption and emission spectra of DPH-PC in a control emulsion containing 1% cholesterol are shown in **Fig. 10.** Emulsions containing TMA-DPH showed similar absorption and emission spectra (results not shown). The patterns of absorption and emission of the two fluorescence probes were consistent with earlier reports (15, 16). The top and middle panels of **Fig. 11** compare the intensity of fluorescence emission of TMA-DPH and DPH-PC in emulsions containing no cholesterol, 1% cholesterol, or 7α-OH-cholesterol, at a concentration of 1 mg TG/ml emulsion. When labeled with TMA-DPH, emulsions containing cholesterol consistently showed higher emission fluorescence than the emulsions containing either no cholesterol or 7α-OH-cholesterol. However, the steady-state fluorescence anisotropy (r), calculated from $I_{parallel}$ and $I_{perpendicular}$ as described in the Methods section, was not significantly different (**Table 3**) among the three emulsions studied, nor were there significant differences in the derived order parameters (S).

After incubation of emulsions containing TMA-DPH with plasma, re-isolation by ultracentrifugation and standardization to 1 mg TG/ml emulsion, the relative fluorescence intensities decreased to less than 10% of the original values, possibly due to transfer of the probe to plasma lipoproteins or to interference with the probe by associated apolipoproteins. In contrast, after incubation of emulsions containing DPH-PC with plasma, the fluorescent intensity (Fig. 11, bottom panel) decreased by about 50% compared with their original values (Fig. 11, middle panel). The anisotropy of DPH-PC in the emulsions containing cholesterol (Table 3) increased from 0.255 to 0.274 and the order parameter increased from 0.772 to 0.814. The increase was consistent in replicate experiments and indicates a decrease in fluidity in the environments of the probe after the association of apolipoproteins whereas the anisotropy and order

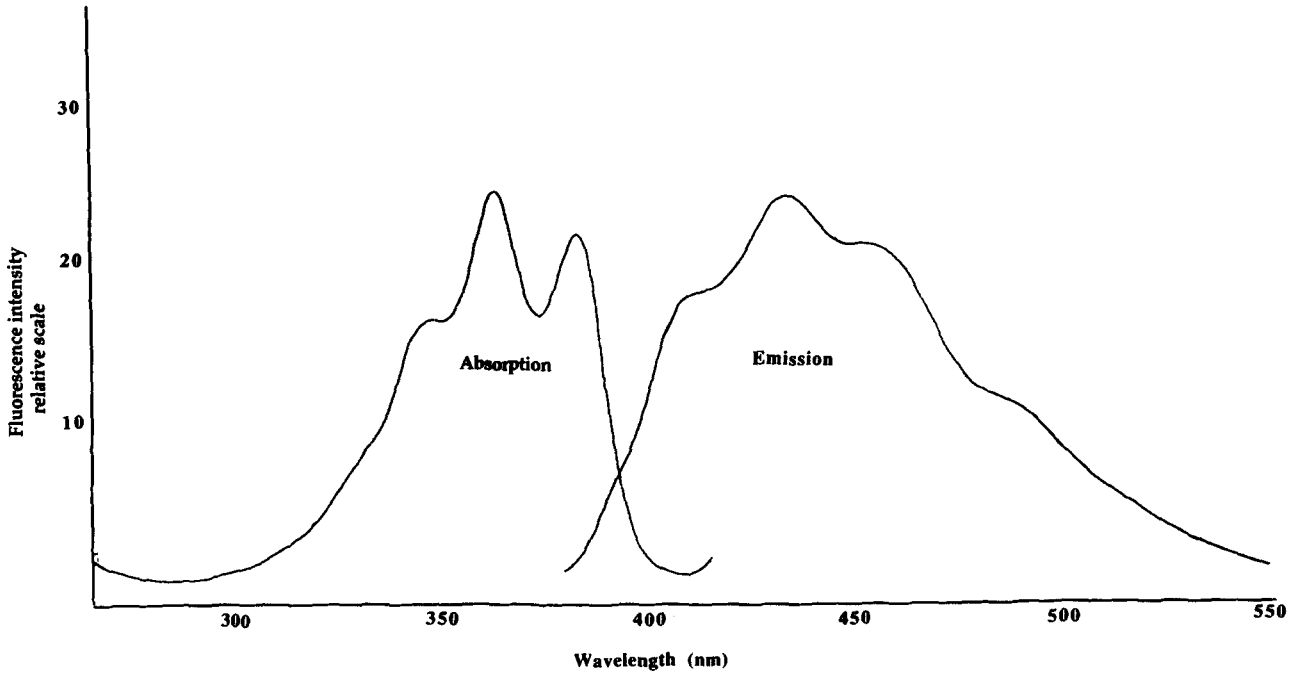

Fig. 10. Excitation and emission spectra of DPH-PC in chylomicron-like lipid emulsions (final concentration, 1 mg TG/ml emulsion) containing triacylglycerol, phosphatidylcholine, cholesteryl ester, and 1% free cholesterol. Similar spectra was observed for TMA-DPH in the same emulsion.

parameter of the emulsions containing no cholesterol or 7α-OH-cholesterol remained unchanged.

DISCUSSION

Lipid emulsions with a particle size and lipid composition similar to the triglyceride-rich lipoproteins are metabolized similarly to natural chylomicrons or VLDL when injected intravenously into the rat. As with chylomicrons, emulsion particles are metabolized in two steps. First, most of the triacylglycerol is removed through the lipolytic action of lipoprotein lipase in extrahepatic tissues, followed by the hepatic uptake of cholesteryl ester-enriched remnant particles by receptor-mediated endocytosis. Cholesterol is an obligatory component required in the process of liver uptake of these emulsion-remnants. Redgrave, Vassiliou, and Callow (1) showed that chylomicron-like emulsions excluding unesterified cholesterol were hydrolyzed normally but had a retarded removal of emulsion cholesteryl ester. In this study we have confirmed the previous results and strengthened the hypothesis that cholesterol is essential for the clearance of chylomicrons and their remnants from plasma. Cholesterol is also essential for the hepatic synthesis and secretion of VLDL, and transport of triacylglycerol in isolated perfused livers (22) and in intact animals (23).

Functional groups such as the C3-β-OH group, the C17 side chain and the C5-6 (Δ^5) double bond in the sterol ring of a cholesterol molecule are known to influence its biological properties. For example, intestinal absorption of some plant sterols varies with changes in the functional groups: the absorption rate ranged from 4.3% with sitosterol to 12.5% with campestanol, compared with 33% with cholesterol (24). The Δ^5 double bond is required (25), while the stereochemistry of the C-17 side chains (26) is not critical, for the down-regulation of hepatic HMG-CoA reductase by cholesterol congeners. The 3β-hydroxyl group and the C17 alkyl side chain are both necessary for sterols to act as the substrate for the enzyme acyl CoA:cholesterol acyltransferase (ACAT), but the absence of the double bond at C5-6 in ring B, as with cholestanol and the two epimers of coprostanol, does not affect esterification (27). McMullen, Lewis, and McElhaney (28) found that the C3-hydroxyl group, the rigid planar fused ring system, and the alkyl side chain at C17 were required for the cholesterol molecule to exert its characteristic effects in model bilayer membranes. Moreover, studies have also shown that the properties of cholesterol required for biological function could be met by a variety of other sterols. For example, in mycoplasma membranes (29), cholesterol can be replaced by β-sitosterol or ergosterol, both of which retain the planar sterol and the 3β-hydroxyl group but differ from cholesterol in the aliphatic side chain. Thus, certain features of the cholesterol

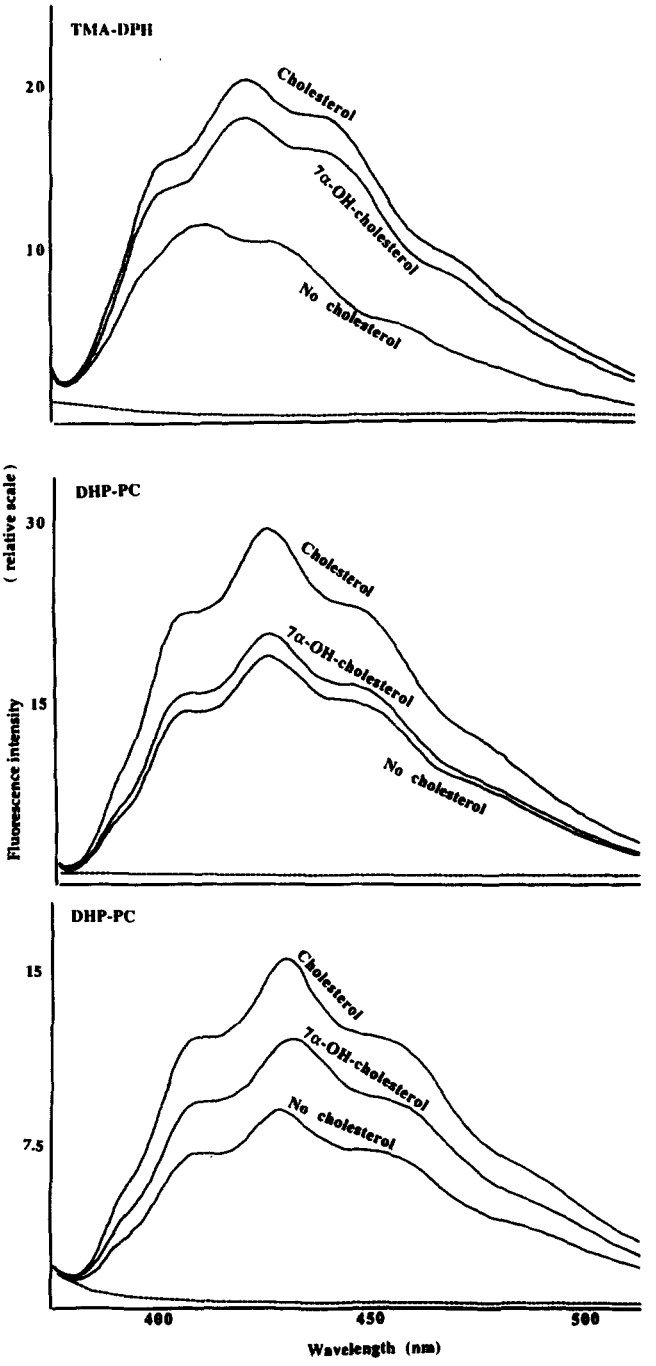

Fig. 11. (Top and middle panels) Comparison of fluorescence emission spectra of TMA-DPH and DPH-PC in emulsions containing 1% cholesterol, 7α-OH-cholesterol, or no cholesterol, standardized to 1 mg TG/ml emulsion. Each figure shows one of three experiments. (Bottom panel) The identical emulsions containing DPH-PC after incubation with rat plasma and re-isolation by ultracentrifugation as described in the Methods, standardized to 1 mg TG/ml emulsion. Each figure shows one of three experiments.

TABLE 3. Fluorescence anisotropy (r) and order parameter (S) of TMA-DPH and DPH-PC in chylomicron-like lipid emulsions

Emulsion type	Fluorescence Anisotropy (r)	r_o	Order Parameter (S)
TMA-DPH			
Cholesterol (n = 3)	0.265 ± 0.03	0.39	0.805 ± 0.04
No cholesterol (n = 3)	0.266 ± 0.01	0.39	0.808 ± 0.03
7α-OH-cholesterol (n = 2)	0.267	0.39	0.809
DPH-PC			
Cholesterol (n = 3)	0.255 ± 0.03	0.40	0.772 ± 0.06
No cholesterol (n = 3)	0.238 ± 0.02	0.40	0.736 ± 0.04
7α-OH-cholesterol (n = 3)	0.249 ± 0.03	0.40	0.761 ± 0.07
After incubation with rat plasma and re-isolation			
DPH-PC			
Cholesterol (n = 3)	0.274 ± 0.03	0.40	0.814 ± 0.05
No cholesterol (n = 3)	0.237 ± 0.02	0.40	0.737 ± 0.05
7α-OH-cholesterol (n = 3)	0.226 ± 0.05	0.40	0.707 ± 0.1

Emulsions labeled with TMA-DPH or DPH-PC in a probe:lipid ratio of 1:200 were prepared as described in the Methods. Emission intensity was measured at 433 nm and excited at 365 nm for both probes at 25°C. The fluorescence anisotropy (r) was derived from the measured anisotropy (r_{obs}) by correcting for sample turbidity (12). The values of r_o were from references 15 and 16. The order parameter (S) was calculated as defined in the Methods. Results are mean ± SEM for two to three determinations in each emulsion type. TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene; DPH-PC, 1-palmitoyl-2-{[(2-[4-(6-phenyl-*trans*-1,3,5-hexatrienyl)phenyl]ethyl]carbonyl}-3-*sn*-phosphatidylcholine.

molecule must be retained for the sterol to exhibit specific biological activities. In the present study we systematically explored variations of the cholesterol structure to determine the features that regulate the plasma clearance of chylomicron-like emulsions.

Consistent with previous findings (1), the lipolysis index (Table 2) of most of the emulsions examined in this study were little affected by the absence of cholesterol or by substitution with congeners, although in some emulsions, TO removal was significantly slower than the control. As removal of emulsion-TO from the plasma reflects the sum of both lipolysis by lipoprotein lipase and the uptake of remnant particles, the latter process probably contributes to the slow total TO removal in some cases. However, in emulsions containing stigmasterol or coprostanol, lipolysis (Table 2) was significantly slower than the control ($P < 0.001$), whereas plasma removal of CO was unaffected in the emulsion containing stigmasterol and increased in the emulsion containing coprostanol. Liver uptakes of the radioactive labels were all similar to control emulsions containing cholesterol. The small recovery of radiolabels in the spleen (< 1%, results not shown) indicates that uptake by the endothelial reticular system was not involved in these processes. Similar amounts of various apoC isoforms (Fig. 9B) were associated with these emulsions after in vitro incubation with rat plasma, indicating that the slow lipolysis was not due to less apoC-II or more apoC-I and C-III. The slower rates of lipolysis in emulsions containing stigmasterol and coprostanol could probably be attributed to the differences in the sterol structure. As reported by Demel, Bruckdorfer, and Van Deenen (30), stigmasterol, with an additional double bond in the side chain, has a higher mean molecular area that renders it less effective for interactions in monolayers. In coprostanol, the *cis* structure between rings A and B also increases molecular area (30). Alternatively, the extra β-H atom at the juncture of rings A and B may sterically hinder the interaction of the 3β-OH group with other neighboring molecules. The slower lipolysis of these two emulsions did not affect the removal of emulsion-CO from plasma.

In keeping with delayed removal of remnants from plasma, emulsions showing slower CO clearance, namely those with no cholesterol, 3-keto cholesterol, cholesteryl chloride, androstenol, or any of the three diols, also showed diminished liver uptake. The amount of emulsion-CO recovered in the liver ranged from 9% to 30% in these emulsions (Table 2). The mechanism of this defective liver uptake is unclear but is probably not due to changes in association with apoE or apoCs, as the amounts of various apolipoproteins associated with selected emulsions after incubation with rat plasma were all similar (Figs. 9A and B). Although essential in the process of remnant clearance, apoE alone is not sufficient to mediate endocytosis and the rate of remnant

clearance is certainly not proportional to the amount of apoE present. Havel (20) reported that the presence of apoE on the remnant surface did not necessarily lead to their uptake by the liver, suggesting the involvement of other factors. Moreover, apoE alone does not bind to the receptor, as some lipids are required to preserve the apolipoprotein conformation necessary for binding to the receptor (21). Furthermore, normal $VLDL_1$, which contains approximately the same total mass of apoE as a specific class of VLDL from hypertriglyceridemic subjects (HTG-VLDL), failed to bind to the receptor (31). Thus, only apoE with the appropriate conformation is receptor-active.

The conformation of apoE can also be affected by the order/disorder of the lipid constituents of the particle to which it bound (32). By using TMA-DPH and DPH-PC as probes, the steady-state fluorescence anisotropy (r) and the derived order parameter (S), which measures the membrane fluidity (11, 12, 15), did not reveal any difference in the protein-free emulsions with or without cholesterol, nor with 7α-hydroxycholesterol (Table 3). However, the component emission fluorescence intensities ($I_{parallel}$ and $I_{perpendicular}$) of TMA-DPH, and hence the total fluorescence intensity (which is the sum of $I_{parallel} + 2\ I_{perpendicular}$) were all lower in emulsions with no cholesterol or with 7α-hydroxycholesterol (Fig. 11, top panel), suggesting a change in the microenvironments of the probes in these emulsions. A more detailed study of time-resolved anisotropy decay is needed to assess those changes.

A recent publication by Chang and Borensztajn (33) reported that treatment of chylomicrons with hepatic lipase did not alter the amount of apoE on the particle, but unmasked certain domains of apoE, making it receptor-active. The structural features of apoE that binds to the LDL receptor were investigated in depth by Dyer et al. (34). A synthetic peptide that contained a linear tandem repeat of apoE residues 141–155 inhibited LDL receptor binding, while a monomer of the same 141–155 residues did not. Using circular dichroism, the conformations of these two peptides were shown to be different, Dyer et al. (34) therefore proposed that the dimer apoE peptide bound the LDL receptor by making contact with more than one of the negatively charged repeat sequences. In our study, substitutions of the cholesterol structure may have hindered the contact of the particular part of apoE with the charged repeats of the LDL receptors. We propose that changes in the structure of surface coat sterol affect the conformation of the associated apoE, leading to defective binding of cell surface receptors, and hence endocytosis.

After incubation with plasma and the inclusion of proteins, the fluorescence emission of TMA-DPH in emulsions decreased by 90%. TMA-DPH orients closely to the lipid–water interface (14). Interactions of emulsions with enzymes, proteins, or apolipoproteins probably perturbs or replaces the fluorophore at the interface. In the DPH-PC molecule, DPH, the fluorescent moiety, is esterified at the *sn*-2 position of glycerol phosphatidylcholine (14). Emulsions labeled with DPH-PC showed high intensity of the emission fluorescence, with a slight variation in each preparation, probably proportional to the amount of phospholipids incorporated into the particular emulsion. The fluorescence emission of DPH-PC after incubation with plasma remained high and the anisotropy (r) of the emulsion containing cholesterol increased slightly, in keeping with the increased surface microviscosity after protein-binding (35, 36). In contrast, the anisotropy parameter remained constant or decreased slightly for emulsions without cholesterol or with 7α-hydroxycholesterol, probably implicating a difference in the surface structure or in the association with proteins.

The clearance studies indicate an absolute requirement for a hydroxyl group at the C3 position of the cholesterol molecule for effective emulsion CO removal from plasma. The configuration of the hydroxyl group with respect to the plane of the ring structure was not critical as emulsions containing epicholesterol showed behavior similar to emulsions containing cholesterol (Fig. 4). Emulsions incorporating cholesterol analogues with the 3-OH replaced by chloro or keto groups all showed impaired CO clearance. When substituted by a carbonyl (formate) group, clearance of emulsion CO was also significantly reduced, albeit to a lesser extent, and the liver uptake was not affected. This is possibly due to hydrogen bonding of the carbonyl group, highlighting the importance of the polar headgroup presented by the hydroxyl group. Although a direct hydrogen bonding between the sterol and the neighboring phospholipid molecules has not been demonstrated (37), the presence of the hydroxyl group in cholesterol is probably important for the interaction with plasma proteins and apolipoproteins.

Removal from plasma of emulsion-CO was unaffected by the orientation of the hydroxyl group in the 3α or 3β configurations, as emulsions incorporating epicholesterol were equal to cholesterol in facilitating CO removal from plasma. In facilitating remnant removal, the interfacial interactions between cholesterol and other molecules probably play a major role. Using force area measurements, Demel et al. (30) studied the interfacial properties of various sterols and found similar cross-sectional areas of the 3α or 3β sterols at the interface, whereas replacement of the hydroxyl group with a keto group increased the molecular area and decreased van der Waals interactions. It is possible that the addition of a second hydroxyl group, as in the 7α-, 7β-, or 25-OH-

cholesterol, probably changes the orientation of the sterol molecule or decreases the interactions between the sterol and other surface constituents resulting in markedly retarded remnant clearance. Recently, using lifetime fluorescence of DPH-PC in phospholipid bilayers, Li and Das (38) showed that 25-hydroxycholesterol was destructive to the bilayer organization.

Emulsion clearance was little affected by the double bond in cholesterol. Cholestanol, coprostanol, and epicoprostanol were all as effective as cholesterol in clearing chylomicron-like emulsions. In fact, coprostanol facilitated CO clearance better than cholesterol, although lipolysis of the emulsion-TO was slower as discussed above. Remnant (CO) clearance was greatly retarded with emulsions in which cholesterol was substituted for by androstenol, a C19 sterol without the side chain. This indicates that the presence of an alkyl side chain is critical. However, increases in the length of the side chain, as with campesterol and β-sitosterol, did not interfere with the function of the sterol in chylomicron catabolism. Our results, showing the absolute requirement of a planar sterol structure and side chain of cholesterol, support the view that hydrophobic interactions of phospholipids with cholesterol play important roles in lipid monolayers (37). Demel and Kruyff (37) also showed that the interaction forces between sterol molecules in monolayers were little affected by double bonds or modifications in the side chain.

In the present study we show for the first time that specific features of the cholesterol molecule, in particular the 3-hydroxyl group and the presence of the C17 side chain, are crucial structural features involved in facilitating clearance of chylomicron-like emulsions from plasma, whereas the C5-6 double bond in the sterol ring and the length of the side chain are not critical. To elucidate the mechanism for this requirement for specific features in the cholesterol structure, further investigation is necessary. Changes in the conformation of apoE are possible, which could in turn influence the binding of the emulsion remnants to membrane proteoglycans or to hepatic receptors (39).

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