

Metabolism of high density lipoprotein lipids by the rat liver: evidence for participation of hepatic lipase in the uptake of cholesteryl ester

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Abstract In order to determine the role of hepatic lipase in the hepatic uptake and metabolism of high density lipoprotein (HDL) triglycerides, cholesteryl esters, and phospholipids, isolated rat livers were perfused with a reconstituted HDL (rHDL) radiolabeled with [³H]triolein and [¹⁴C]cholesteryl oleate or palmitoyl-[¹⁴C]linoleoyl phosphatidylcholine. A bolus of radiolabeled rHDL was injected into the portal vein and livers were perfused for 5 min using a nonrecirculating perfusion system. Recovery of rHDL triolein in the liver as intact triolein was used to determine the amount of unmetabolized rHDL remaining in the liver. After correcting for the amount of unmetabolized rHDL remaining in the liver, about 30% of the rHDL triolein was hydrolyzed of which 19% was recovered in the liver and 11% in the perfusate. Moreover, about 7% of the rHDL phosphatidylcholine was hydrolyzed to lysophosphatidylcholine, all of which was recovered in the perfusate. Although there was no hydrolysis of rHDL cholesteryl oleate, about 30% of the cholesteryl oleate was taken up by the liver. Preperfusion of the liver with heparin to deplete the liver of hepatic lipase resulted in about a 70% reduction in rHDL triolein hydrolysis and about a 75% reduction in rHDL cholesteryl oleate uptake. Although hepatic lipase hydrolyzes both triglycerides and phosphatidylcholines, elimination of the triolein from rHDL had no effect on the uptake of rHDL cholesteryl oleate, but replacement of the rHDL phosphatidylcholine with a nonhydrolyzable phosphatidylcholine diether resulted in an 87% reduction in cholesteryl oleate uptake. ■ These results indicate that hepatic lipase is necessary for the hepatic uptake of both HDL triglycerides and cholesteryl esters and that the uptake of cholesteryl esters is not dependent on the hydrolysis of HDL triglycerides but is dependent on the hydrolysis of HDL phospholipids. — Kadowaki, H., G. M. Patton, and S. J. Robins. Metabolism of high density lipoprotein lipids by the rat liver: evidence for participation of hepatic lipase in the uptake of cholesteryl ester. *J. Lipid Res.* 1992. 33: 1689-1698.

Supplementary key words HDL • liver perfusion • triglycerides • phospholipids

HDL has a putative role in the removal of cholesterol from tissue stores and in the delivery of cholesterol to the liver for excretion from the body (1). In this connection, high affinity saturable HDL binding sites have been

identified in peripheral tissues (2), in the liver (3-6), and in a variety of cultured cells (7-10) including hepatocytes (11-13); and these binding sites have been implicated in the transfer of cholesterol from peripheral tissues to HDL and from HDL to liver (1). However, HDL, unlike low density lipoproteins or triglyceride (TG)-rich remnant lipoproteins, is not taken up by the liver as an intact particle. This conclusion is based largely on the observation that the various components of HDL turn over at different rates. For instance, in vivo studies have shown that in the rat both HDL phosphatidylcholines (PC) (14) and cholesteryl esters (CE) (15-20) are cleared from the blood much faster than HDL apolipoproteins. Selective uptake of the various HDL components has also been demonstrated with cultured hepatocytes (21, 22) and rat hepatoma cells (23, 24).

Except for unesterified cholesterol (1), the mechanism(s) by which the components of HDL are taken up by the liver is not clearly defined, but it has been suggested that hepatic lipase plays a major role in the initial processing of HDL lipids by the liver. Evidence for the involvement of hepatic lipase in the metabolism of HDL is as follows. *a*) Hepatic lipase catalyzes the hydrolysis of HDL glycerophospholipids and TG (25-28); *b*) HDL phospholipids and TG appear to be the preferred substrates for hepatic lipase (25); *c*) in patients with hepatic lipase deficiency, the concentration of plasma HDL phospholipids and unesterified cholesterol is increased (29, 30); and *d*) in rats injected with antibody to hepatic lipase, plasma HDL phospholipids and unesterified cholesterol

Abbreviations: HDL, high density lipoprotein(s); rHDL, reconstituted high density lipoprotein(s); PC, phosphatidylcholine(s); FFA, free fatty acid(s); CE, cholesteryl ester(s); TG, triglyceride(s); HPLC, high performance liquid chromatography.

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are increased (31, 32). Although, *in vitro*, hepatic lipase does not catalyze the hydrolysis of CE, transfer of esterified and unesterified cholesterol from HDL to isolated cells, including cultured hepatocytes, is enhanced by pre-treating HDL with hepatic lipase or other phospholipases (24, 33, 34).

The present studies were performed to determine the extent to which the uptake of HDL CE is dependent on the hydrolysis of a major surface lipid, PC, and an associated core lipid, TG, and the extent to which the uptake of HDL lipids is dependent on the activity of hepatic lipase. For these studies, we used a reconstituted HDL (rHDL) prepared with cholesteryl oleate, triolein, palmitoyl-linoleoyl (16:0-18:2) PC, unesterified cholesterol, and rat HDL apolipoproteins, and measured the metabolism of rHDL CE, TG, and PC by the isolated perfused rat liver.

MATERIALS AND METHODS

Materials

[4-¹⁴C]cholesteryl oleate (50 mCi/mmol), [9, 10-³H(N)]triolein (20 Ci/mmol), and 1-palmitoyl-2-[1-¹⁴C]linoleoyl-*sn*-glycerophosphocholine (40 mCi/mmol) were obtained from DuPont New England Nuclear (Boston, MA); bovine serum albumin (fraction V) was from Sigma (St. Louis, MO), sodium taurocholic acid (A grade) was from Calbiochem (La Jolla, CA); cholesteryl oleate, cholesterol, and triolein were from Nu-Chek-Prep (Elysian, MN); L- α -1-palmitoyl-2-linoleoyl-PC was from Avanti Polar Lipids (Birmingham, AL); (1-oleyl-2-palmityl) DL-phosphatidylcholine diether was from Serdary Research Laboratories (Port Huron, MI); stigmaterol (5, 22-cholestadien-24b-ethyl-3 β -ol) and stigmaterol acetate were from Steraloids (Wilton, NH); heparin (porcine intestine) was from Elkins-Sinn (Cherry Hill, NJ); and ultra pure urea was from ICN Biomedicals (Costa Mesa, CA). Analytical and HPLC grade solvents were obtained from Fisher (Medford, MA).

Animals

Male Sprague-Dawley rats (Taconic Animal Farm, Germantown, NY) weighing 250-300 g were maintained on standard Purina rat chow *ad libitum*.

Preparation of rHDL

Fresh rHDL was prepared for each experiment as described by Pittman et al. (35) and as used previously by our laboratory (36). Rat serum HDL was isolated by equilibrium centrifugation at d 1.08-1.19 g/ml and delipidated with ethanol-diethyl ether as described by Rudel et al. (37). Radioactive lipids were purified by high performance liquid chromatography (HPLC) prior to use (38).

Each rHDL preparation contained 3 μ Ci of [³H]triolein and either 1.25 μ Ci of [¹⁴C]cholesteryl oleate or 2.5 μ Ci of 16:0-[¹⁴C]18:2 PC. Unesterified cholesterol, 0.82 μ mol; cholesteryl oleate, 2.24 μ mol; triolein, 0.34 μ mol; and 16:0-18:2 PC or oleyl-palmityl (18:1-16:0) PC diether, 2.30 μ mol were dissolved in chloroform, dried under nitrogen, and lyophilized. Lipids were suspended in 3.5 ml of 10 mM Tris buffer (pH 8.0) containing 100 mM potassium chloride and 3.9 mM sodium azide, and then sonicated under a stream of nitrogen gas at 40 watts output with a tapered microtip (1/8 inch diameter) using a Branson model 250 sonifier (Danbury, CT). After sonicating for 15 min, 2.5 mg of rat HDL apolipoproteins dissolved in 1.5 ml of 4 M urea in the Tris-potassium chloride-sodium azide buffer was added dropwise over 5 min. Sonication was continued for an additional 8 min. The sonicated sample was centrifuged at 150,000 *g* for 30 min at 20°C and rHDL was dialyzed overnight at 4°C against saline. Protein concentration was determined by the method of Bensadoun and Weinstein (39). Quantitation of lipid classes was by the method of Patton, Fasulo, and Robins (38, 40). The dialyzed rHDL contained 1.98 \pm 0.14 mg (mean \pm SD, *n* = 6) of apolipoproteins, 1.30 \pm 0.08 mg of cholesteryl oleate, 0.28 \pm 0.002 mg of unesterified cholesterol, 0.18 \pm 0.02 mg of triolein, and 1.30 \pm 0.14 mg of 16:0-18:2 PC or 1.41 mg of 18:1-16:0 PC diether (*n* = 1) in 5 ml. rHDL were either diluted with saline or concentrated with an Amicon YM5 membrane as required. Apolipoprotein profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were the same as previously reported by our laboratory (36).

Liver perfusion

Rats were anesthetized with sodium pentobarbital and livers, with the bile duct cannulated, were perfused *in situ* with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 25 mM glucose at a flow rate of 2.8 ml/min per g liver as previously described (41). Sodium taurocholate was infused directly into the portal vein cannula at 40 μ mol/h. Initially the liver was perfused with Krebs-Ringer bicarbonate buffer in a nonrecirculating system for 15 min and then for 5 min with the same buffer containing 3% bovine serum albumin (fatty acid-poor). At this point, a 1-ml bolus of radiolabeled rHDL (50-1000 μ g protein) was injected into the portal vein cannula and the liver was continuously perfused for another 5 min during which the perfusate was collected in 1-min fractions. After 5 min, the liver was removed, washed in cold saline, and blotted to remove excess saline. The liver was weighed and a 20% homogenate was prepared with ice-cold saline. Lipids were extracted from an aliquot of the homogenate by the method of Folch, Lees, and Sloane Stanley (42). In some experiments the livers were perfused with heparin before the administration of the rHDL. In these experiments the liver was perfused in sequence with Krebs-Ringer bicar-

bonate buffer (pH 7.4) containing 25 mM glucose in a nonrecirculating system for 5 min; with the same buffer containing 40 IU/ml of heparin for 5 min; with the same buffer without heparin for 5 min; and finally with the same buffer containing 3% albumin for 5 min in order to wash out the heparin prior to the injection of rHDL.

Lipid analysis

Known amounts of stigmaterol acetate, stigmaterol, triecosenoin, and dimyristoyl PC were added to the lipid extract as internal standards for the quantitative analysis of cholesteryl oleate, unesterified cholesterol, triolein, and 16:0-18:2 PC, respectively. The lipid extract was partitioned with saline as described by Folch et al. (42). The Folch lower-phase was separated into neutral lipids and individual phospholipid classes by HPLC (38). The neutral lipids were further separated into CE, TG, free fatty acids (FFA), and unesterified cholesterol by HPLC, and quantitated by HPLC or gas-liquid chromatography as previously described (38, 40). Radioactivity was determined by liquid scintillation spectrometry. For the quantitation of the amount of [³H]triolein remaining in the liver, the TG fraction was separated into molecular species by HPLC on an Ultrasphere ODS column (4.6 mm × 25 cm, 5 μ) (Beckman, San Ramon, CA) with a mobile phase of methanol-2-propanol 75:25. The flow rate was 1 ml/min and the effluent was monitored at 205 nm. Pure triolein was used to determine the elution volume of the triolein peak. The peak that contained triolein was collected and its radioactivity was measured. For the determination of the triolein content in the liver, the peak containing triolein was separated into molecular species by HPLC on a Spherisorb S3 ODS2 column (2 mm × 25 cm, 3 μ) (Phase Separations, Norwalk, CT) with a mobile phase of acetonitrile-2-propanol 8:2. The flow rate was 0.3 ml/min and the effluent was monitored at 205 nm. For the determination of cholesteryl oleate, the CE fraction was separated into molecular species by HPLC on an Ultrasphere ODS column (4.6 mm × 25 cm, 5 μ) with a mobile phase of methanol-2-propanol 75:25. The flow rate was 1 ml/min and the effluent was monitored at 205 nm. Pure cholesteryl oleate was used to determine the elution volume of the cholesteryl oleate peak.

RESULTS

In order to determine to what extent the hepatic uptake of triolein and cholesteryl oleate from rHDL depends on the concentration of rHDL, increasing amounts of rHDL were injected as a 1-ml bolus into the portal vein cannula of perfused rat livers. In these single pass perfusions, the amount of [³H]triolein and [¹⁴C]cholesteryl oleate retained by the liver increased nearly linearly with the con-

centration of rHDL apolipoprotein in the bolus until about 300 μg of rHDL apolipoprotein (Fig. 1A and 1B). Above 300 μg of rHDL apolipoprotein there was almost no further increase in the amount of rHDL triolein and cholesteryl oleate retained by the liver. In order to ensure that the amount of rHDL lipids retained by the liver was well within the linear range, subsequent experiments were performed with a rHDL apolipoprotein concentration of 100 μg/ml.

As some of the rHDL [³H]triolein retained by the liver may not actually be metabolized, but merely bound or trapped (in sinusoids/space of Disse) by the liver, an attempt was made to differentiate between rHDL lipids that were merely bound or trapped and those that were actually metabolized by the liver. As triolein is present in liver TG in only trace amounts (Fig. 2), any [³H]triolein recovered in the liver at the conclusion of the perfusion was assumed to reside in rHDL that was retained by the liver but was not metabolized. Therefore, the amount of ³H re-

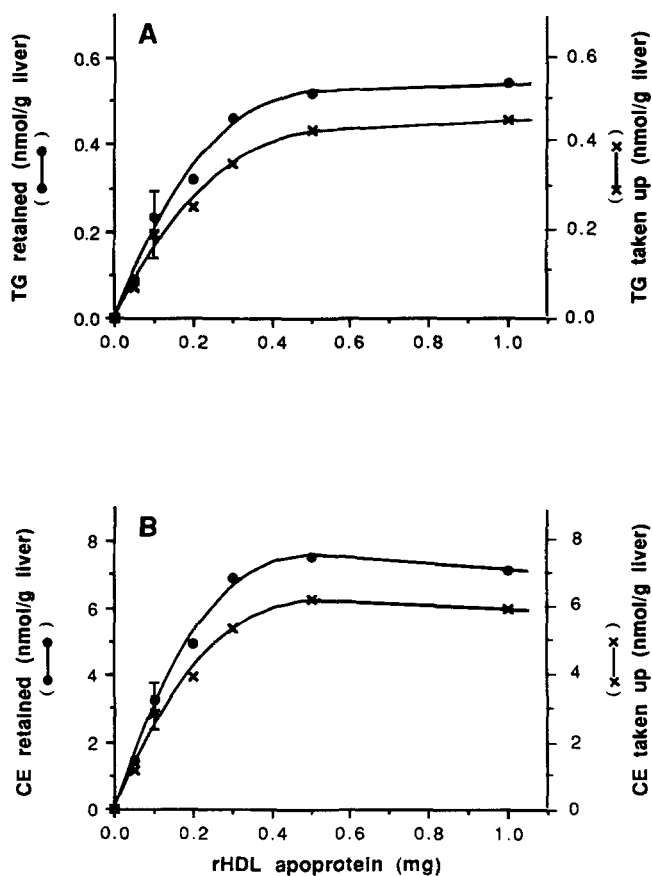


Fig. 1. Concentration-dependent uptake of [³H]triolein (A) and [¹⁴C]cholesteryl oleate (B) from rHDL. Rat livers were perfused for 5 min in a nonrecirculating perfusion system with various amounts of rHDL injected as a 1-ml bolus. The amount of [³H]triolein and [¹⁴C]cholesteryl oleate retained (●-●) and taken up (x-x) by the liver was determined as described in Methods. Values obtained with 100 μg apolipoproteins were mean ± standard deviation (SD) from five perfusions.

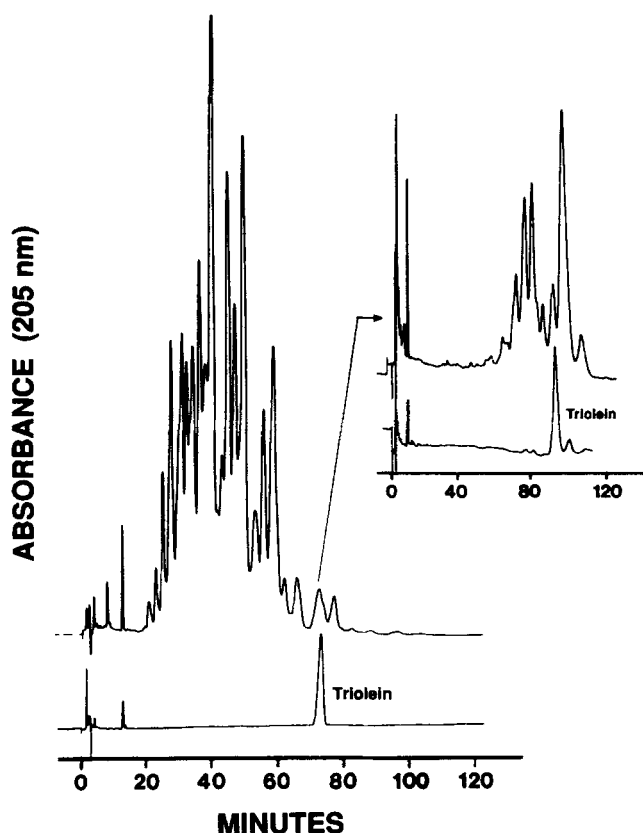


Fig. 2. Separation of TG molecular species by HPLC. Liver TG were separated into molecular species on an Ultrasphere ODS column (4.6 mm \times 25 cm, 5 μ) with methanol-2-propanol 75:25. The flow rate was 1 ml/min and effluent was monitored at 205 nm. The peak that contained triolein was collected and rechromatographed (inset) on a Spherisorb S3 ODS2 column (2 mm \times 25 cm, 3 μ) with acetonitrile-2-propanol 8:2. The flow rate was 0.3 ml/min and the effluent was monitored at 205 nm. The inset shows that triolein constituted a minor component of the initial "triolein" peak.

maining in the liver as triolein was determined (Fig. 2) and from that amount of ^3H and the specific activity of [^3H]triolein in the rHDL the nmoles of intact triolein in the liver were calculated. Then the amount of ^3H remaining as triolein was subtracted from the amount of ^3H retained by the liver to determine the nmoles of [^3H]triolein actually taken up by the liver. Furthermore, by assuming that the [^3H]triolein retained but not metabolized by the liver is a component of intact rHDL and that this retained but unmetabolized rHDL has the same composition as the rHDL originally injected, then it is also possible to calculate the nmoles of cholesteryl oleate and 16:0-18:2 PC that are retained but not metabolized and, therefore, the nmoles actually taken up by the liver. Using the correction procedure described above, it was possible to calculate the amount of rHDL [^3H]triolein and [^{14}C]cholesteryl oleate actually taken up by the liver (Fig. 1A and 1B, lower curves). At all concentrations of rHDL examined, approximately 80% ($81.3 \pm 3.6\%$, mean \pm SD for 11 determinations) of the rHDL [^3H]triolein and therefore [^{14}C]cholesteryl oleate retained by the liver was actually taken up.

When a 1-ml bolus of rHDL radiolabeled with [^3H]triolein (10.8 nmol) and [^{14}C]cholesteryl oleate (102.2 nmol) was injected into the portal vein, 23% of the ^3H and 34% of ^{14}C was retained by the liver (Table 1). Greater than 85% of the radiolabeled lipids that were not retained by the liver were recovered in the perfusate in the first minute and an additional 10% were recovered in the second minute. In the fifth minute of perfusion there was virtually no radioactivity in the perfusate. When the amount of ^3H remaining as triolein in the liver was determined, 19% of the ^3H retained by the liver was recovered in unmetabolized triolein. Therefore, approximately 81% of

TABLE 1. Recovery of ^3H (from triolein) and ^{14}C (from cholesteryl oleate) in the perfusate and liver

	^3H		^{14}C	
	nmol	(%)	nmol	(%)
Perfusate				
1 min	7.37 ± 1.52	(68.4)	56.42 ± 6.62	(55.2)
2 min	0.73 ± 0.31	(6.8)	6.30 ± 1.80	(6.2)
3 min	0.25 ± 0.30	(2.3)	2.49 ± 1.05	(2.4)
4 min	0.05 ± 0.03	(0.5)	1.51 ± 0.44	(1.5)
5 min	0.03 ± 0.02	(0.3)	1.19 ± 0.12	(1.2)
Total	8.30 ± 1.48	(77.0)	67.90 ± 7.55	(66.5)
Liver				
In unmetabolized rHDL ^a	0.46 ± 0.11	(4.3)	3.84 ± 0.90^b	(3.8)
Metabolized rHDL ^a	2.02 ± 0.48	(18.7)	30.44 ± 4.80^b	(29.8)
Total	2.48 ± 0.46	(23.0)	34.28 ± 5.43	(33.5)

Values are means \pm SD for five perfusions. Rat livers (weighing 10.7 ± 0.9 g) were perfused for 5 min in a nonrecirculating perfusion system as described in Methods. rHDL containing 10.8 ± 1.4 nmol ($n = 3$) of [^3H]triolein, 102.2 ± 12.1 nmol of [^{14}C]cholesteryl oleate, 81.7 ± 4.4 nmol of 16:0-18:2 PC, 31.1 ± 3.2 nmol of unesterified cholesterol, and 100 μg of apolipoproteins was injected as a bolus at zero min.

^aDetermined as described in Results.

^bValues were calculated from the amount of intact [^3H]triolein remaining in the liver as described in the text.

TABLE 2. Distribution of ³H (from triolein) in the perfusate and liver

	Perfusate		Liver	
	dpm		dpm	(%)
Unmetabolized triolein	402,219		25,180	
Metabolized triolein				
Triglycerides	0		60,085	(55.6)
Free fatty acids	82,382		3,997	(3.7)
Cholesteryl esters	0		5,329	(4.9)
Phosphatidylethanolamines	0		15,987	(14.8)
Phosphatidylcholines	0		18,652	(17.3)
Other phospholipids	0		3,997	(3.7)

Rat livers were perfused for 5 min in a nonrecirculating perfusion system and the radioactivity recovered in the lipids of the perfusate and liver was determined as described in Methods. rHDL contained 11.0 nmol (620,453 dpm) of [³H]triolein, 108.7 nmol of cholesteryl oleate, 83.5 nmol of 16:0-18:2 PC, 36.0 nmol of unesterified cholesterol, and 100 μg of apolipoproteins.

the triolein retained by the liver (or 18.7% of the injected rHDL triolein) was actually taken up by the liver (Table 1). When the aforementioned assumptions were used to calculate the amount of [¹⁴C]cholesteryl oleate remaining in unmetabolized rHDL and this amount (3.84 ± 0.90 nmol) was subtracted from the ¹⁴C retained by the liver, then 30.44 nmol (or 29.8%) of rHDL cholesteryl oleate was actually taken up by the liver (Table 1).

Of the ³H actually taken up by the liver, 56% was recovered in TG, 17% in PC, 15% in phosphatidylethanolamine, 5% in CE, and 4% each in FFA and other phospholipids (Table 2). Moreover, 17% of the ³H in the perfusate was recovered as FFA. When the ³H in the FFA fraction of the perfusate was added to the metabolized [³H]triolein in the liver, a total of 32% of rHDL [³H]triolein was actually hydrolyzed by the liver in a single pass. Thus, of the

32% of the rHDL triolein hydrolyzed by the liver, 57% was taken up by the liver and 43% was recovered in the perfusate. In contrast, all the ¹⁴C in both the perfusate and the liver was recovered as intact cholesteryl oleate.

The uptake of rHDL PC by the perfused rat liver was also determined using rHDL radiolabeled with 16:0-[¹⁴C]18:2 PC (84.1 nmol) and [³H]triolein (12.3 nmol) (Table 3.) The distribution of ³H in the perfusate and in the liver was similar to the distribution observed in the previous experiment (Table 1). Specifically, 20% of the ³H was retained by the liver of which 14% (or 2.9% of the original rHDL administered) was recovered in unmetabolized triolein. On the other hand, only 3.1% of the ¹⁴C in rHDL was recovered in the liver while 90% of the ¹⁴C was recovered in the perfusate as PC and 7% as lysoPC. All of the ¹⁴C recovered in the liver was in the PC fraction. Molecular species analysis of the PC showed that all of the ¹⁴C was recovered in the 16:0-18:2 PC peak both in the liver and in the perfusate. From the amount of unmetabolized [³H]triolein remaining in the liver (0.36 ± 0.08 nmol) and from the original rHDL triolein and 16:0-18:2 PC composition, it was calculated that the liver should contain 2.44 nmol of [¹⁴C]PC in unmetabolized rHDL. Therefore, virtually all of the [¹⁴C]PC found in the liver (2.67 ± 0.57 nmol) was in the unmetabolized rHDL and, consequently, virtually all of the rHDL PC which was hydrolyzed by the liver was recovered in the perfusate as lysoPC.

As hepatic lipase hydrolyzes both TG and phospholipids but not CE, it was not entirely clear whether CE uptake is actually dependent on hepatic lipase. In order to demonstrate that the uptake of rHDL TG depends on hepatic lipase and to determine whether hepatic lipase is also required for CE uptake, perfusions were performed in which the liver was depleted of hepatic lipase (by pre-

TABLE 3. Recovery of ³H (from triolein) and ¹⁴C (from PC) in the perfusate and liver

	³ H		¹⁴ C			
			PC		lysoPC	
	nmol	(%)	nmol	(%)	nmol	(%)
Perfusate						
1 min	9.07 ± 0.38	(73.7)	70.46 ± 2.12	(81.2)	2.86 ± 0.31	(3.3)
2 min	0.47 ± 0.05	(3.8)	4.63 ± 1.36	(5.3)	1.77 ± 0.47	(2.0)
3 min	0.19 ± 0.15	(1.5)	1.87 ± 1.16	(2.2)	0.83 ± 0.15	(1.0)
4 min	0.06 ± 0.03	(0.5)	0.73 ± 0.35	(0.8)	0.37 ± 0.12	(0.4)
5 min	0.04 ± 0.01	(0.3)	0.40 ± 0.01	(0.5)	0.20 ± 0.10	(0.2)
Total	9.83 ± 0.21	(79.9)	78.10 ± 0.46	(90.0)	6.03 ± 0.45	(6.9)
Liver						
In unmetabolized rHDL ^a	0.36 ± 0.08	(2.9)	2.44 ± 0.52 ^b	(2.8)		
Metabolized rHDL ^a	2.11 ± 0.16	(17.2)	0.21 ± 0.08 ^b	(0.2)		
Total	2.47 ± 0.20	(20.1)	2.67 ± 0.57	(3.1)	ND	

Values are means ± SD for three perfusions. Rat livers (weighing 9.2 ± 0.2 g) were perfused for 5 min in a nonrecirculating perfusion system as described in Methods. rHDL containing 12.3 nmol of [³H]triolein, 82.2 nmol of cholesteryl oleate, 84.1 nmol of 16:0-[¹⁴C]18:2 PC, 29.8 nmol of unesterified cholesterol, and 100 μg of apolipoproteins was injected as a bolus at zero min. ND, not detected.

^aDetermined as described in Results.

^bValues were calculated from the amount of intact [³H]triolein remaining in the liver as described in the text.

TABLE 4. Recovery of ^3H (from triolein) and ^{14}C (from cholesteryl oleate) in the perfusate and liver after preperfusion with heparin

	^3H		^{14}C	
	nmol	(%)	nmol	(%)
Perfusate				
1 min	6.47 ± 0.71	(72.1)	69.62 ± 3.31	(73.7)
2 min	0.59 ± 0.14	(6.6)	5.34 ± 1.90	(5.7)
3 min	0.20 ± 0.05	(2.2)	1.38 ± 0.11	(1.5)
4 min	0.12 ± 0.05	(1.3)	1.03 ± 0.20	(1.1)
5 min	0.06 ± 0.03	(0.7)	0.89 ± 0.16	(0.9)
Total	7.84 ± 0.04	(87.4)	78.26 ± 5.63	(82.8)
Liver				
In unmetabolized rHDL ^a	0.78 ± 0.14	(8.7)	8.78 ± 2.54 ^b	(9.3)
Metabolized rHDL ^a	0.35 ± 0.13	(3.9)	7.46 ± 3.01 ^b	(7.9)
Total	1.13 ± 0.06	(12.6)	16.23 ± 0.94	(17.2)

Values are means ± SD for three perfusions. Rat livers (weighing 10.7 ± 0.6 g) were perfused in a nonrecirculating perfusion system with 40 IU/ml heparin for 5 min prior to injection of rHDL as described in Methods. rHDL containing 9.0 nmol of [^3H]triolein, 94.5 nmol of [^{14}C]cholesteryl oleate, 78.2 nmol of 16:0-18:2 PC, 29.8 nmol of unesterified cholesterol, and 100 µg of apolipoproteins was injected as a bolus at zero min.

^aDetermined as described in Results.

^bValues were calculated from the amount of intact [^3H]triolein remaining in the liver as described in the text.

perfusion with heparin), and then [^3H]triolein and [^{14}C]cholesteryl oleate uptake from rHDL was examined. Heparin treatment decreased the total amount of ^3H retained by the liver from 23.0% (Table 1) to 12.6% (Table 4). Similar results were obtained for the amount of [^{14}C]cholesteryl oleate retained by the liver, i.e., heparin treatment decreased the total amount of ^{14}C retained by the liver from 33.5% (Table 1) to 17.2% (Table 4). However, after heparin treatment 69.0% of the ^3H retained by the liver was recovered as unmetabolized intact [^3H]triolein. Thus, the amount of ^3H actually taken up by the liver decreased from 18.7% (Table 1) to 3.9% (Table 4) and the amount of ^3H recovered in the perfusate as FFA decreased from 13.3% (Table 2) to 6.1% (data not shown). Therefore, the actual amount of triolein hydrolyzed by the heparin-treated livers decreased by 68.8%

compared to triolein hydrolysis by livers without heparin preperfusion. Similarly, heparin preperfusion decreased the amount of [^{14}C]cholesteryl oleate actually taken up by 73.5%.

As rHDL triolein was hydrolyzed and cholesteryl oleate was taken up by the liver to almost the same extent under all the conditions examined in this study, it appeared possible that TG hydrolysis and CE uptake were mechanistically linked. To test this possibility rHDL was prepared as described in the Methods but without any triolein. Elimination of triolein from the rHDL preparation had no effect on the amount of [^{14}C]cholesteryl oleate retained by the perfused liver (Table 5). If CE uptake is dependent on hepatic lipase but TG hydrolysis is not required for CE uptake, then it is likely that the hydrolysis of rHDL PC is necessary for the uptake of CE. To test this possibility,

TABLE 5. Effect of changes in the TG and PC components of rHDL on the uptake of ^{14}C (from cholesteryl oleate) by the liver

Elimination or Replacement of rHDL Components	n	Cholesteryl Oleate Uptake	
		Retained ^a	Taken up ^b
nmol/g liver			
None	5	3.24 ± 0.56	2.62 ± 0.47
Elimination of triolein	3	3.27 ± 1.27	— ^c
Replacement of PC with PC diether	3	0.59 ± 0.24	0.34 ± 0.11

Values are means ± SD; n, number of perfusions. Rat livers were perfused for 5 min in a nonrecirculating perfusion system as described in Methods. rHDL prepared with triolein was the same as in Table 1. rHDL prepared without triolein contained 117.7 nmol of [^{14}C]cholesteryl oleate, 84.0 nmol of PC, 38.6 nmol of unesterified cholesterol, and 100 µg of apolipoproteins. rHDL prepared with PC diether contained 11.0 nmol of [^3H]triolein, 95.6 nmol of [^{14}C]cholesteryl oleate, 83.5 nmol of oleyl-palmitoyl PC diether, 32.3 nmol of unesterified cholesterol, and 100 µg of apolipoproteins.

^aAmount retained in the liver as described in Results.

^bAmount actually taken up by the liver calculated from unmetabolized [^3H]triolein as described in Results.

^cAn amount could not be calculated in the absence of [^3H]triolein.

rHDL was prepared in which PC was replaced by an unmetabolizable PC analogue, i.e., a PC diether. With this rHDL preparation, the amount of [^{14}C]cholesteryl oleate retained by the liver decreased by 81.8% and the actual uptake of [^{14}C]cholesteryl oleate decreased by 87.0% (Table 5).

DISCUSSION

Reconstituted HDL was used in these studies to separately radiolabel and separately follow specific lipid components of HDL during the metabolism of this lipoprotein by the isolated rat liver. The rHDL used in this study was relatively rich in apoA-I and poor in apoE and apoA-IV. In this respect and in the proportion of apolipoprotein, unesterified cholesterol, cholesteryl ester, triglyceride, and phospholipid, our rHDL was similar to the HDL₂ described by Oschry and Eisenberg (43).

In this study, we used the difference between the total amount of ^3H (from rHDL [^3H]triolein) recovered in the liver and the amount of ^3H recovered in triolein per se to calculate the amount of rHDL triolein actually taken up and metabolized by the liver. We then made the assumptions that the [^3H]triolein remaining in the liver was in intact rHDL and that that intact rHDL had the same composition as the rHDL that was injected, and therefore that a proportionate amount of cholesteryl oleate and 16:0-18:2 PC was also in that intact rHDL. Based on the amount of [^3H]triolein remaining in the liver, 4% of the rHDL originally injected (or about 20% of ^3H retained by the liver) was recovered in intact rHDL. We do not know to what extent the 4% of the injected rHDL remaining intact represents binding of rHDL to high affinity HDL binding sites, or rHDL that was taken up intact but which was not yet metabolized, or rHDL that was merely trapped by the liver. Taking this 4% correction into account, when rHDL was injected into the portal vein of the perfused rat livers, nearly 30% of the cholesteryl oleate was taken up by the liver and 30% of the triolein and only 7% of the 16:0-18:2 PC was hydrolyzed in a single pass. This pattern is characteristic of the hepatic metabolism of HDL in that the various components of HDL turn over at different rates. Numerous studies have shown that the major core component of HDL, CE, is taken up by the liver several times faster than apolipoproteins (15-20). In our study there was a four- to fivefold difference in the extent of metabolism of the major core component, cholesteryl oleate, compared to the major surface lipid, 16:0-18:2 PC.

Although 30% of the cholesteryl oleate was taken up by the liver, all of the ^{14}C in both the perfusate and the liver was recovered as cholesteryl oleate at 5 min, i.e., in 5 min there was no hydrolysis of cholesteryl oleate. This agrees with the results of Arbeen, Rifici, and Eder (19) who also

observed little or no cholesteryl ester hydrolysis at 5 min. The hydrolysis of HDL CE is thought to occur in the lysosomes and, therefore, no hydrolysis of cholesteryl oleate would be expected in 5 min as the transfer of CE from the cell membrane to lysosomes requires about 15 min (19). On the other hand, there was extensive hydrolysis of triolein but only 57% of the [^3H]oleate arising from the hydrolysis of rHDL [^3H]triolein was recovered in the liver where, with the exception of a trace amount of [^3H]FFA, [^3H]oleate was incorporated into phospholipids, CE, and TG other than triolein. The remaining 43% of the [^3H]oleate was recovered in the perfusate as FFA. All of the ^3H in the perfusate was either [^3H]triolein or [^3H]FFA (Table 2), indicating that in 5 min the newly synthesized [^3H]glycerides and [^3H]CE were not secreted into the perfusate by the liver.


All of the ^{14}C -labeled PC, with the exception of the 3.1% associated with the intact rHDL in the liver, was recovered in the perfusate either as lysoPC or 16:0-18:2 PC. This observation differs somewhat from what has been observed in similar experiments (44, 45). Scagnelli et al. (44) found that when reconstituted HDL prepared with 16:0- ^{14}C]18:2 PC was injected into rats in vivo, 18:2-lysoPC was the major product of rHDL PC metabolism, but the radiolabeled lysoPC was rapidly re-esterified by the liver yielding a variety of PC with [^{14}C]18:2 in the *sn*-2 position of PC. At 5 min they found that only 1.5% of the radiolabeled PC originally injected was recovered as lysoPC in the plasma. Likewise, Marques-Vidal et al. (45), using 16:0- ^{14}C]18:2 PC-labeled human HDL as the substrate in a recirculating rat liver perfusion system with 1% BSA, recovered only 1.1% of the original PC in the perfusate as lysoPC. Moreover, they found that almost all of the FFA derived from TG hydrolysis was incorporated into liver phospholipids and TG while less than 1% of the FFA derived from [^3H]TG hydrolysis was in the perfusate. A possible explanation for these disparate results can be the presence in our nonrecirculating perfusion system of 3% albumin (fatty acid-poor) which has unoccupied high affinity binding sites for both lysoPC and FFA (46). In the in vivo experiments of Scagnelli et al. (44) these sites would already be occupied and therefore the lysoPC generated from the hydrolysis of rHDL PC would be directed primarily to the cell membranes. Likewise, in a recirculating liver perfusion, large amounts of lysoPC derived from liver PC accumulate in the perfusate (47). Thus, in a recirculating perfusion system with only the 1% albumin used by Marques-Vidal et al. (45), the high affinity lysoPC binding sites on the albumin would soon be largely occupied by lysoPC derived from the liver. However, in our nonrecirculating perfusion system the unoccupied binding sites on albumin (3%) effectively compete for the lysoPC and FFA generated by the metabolism of rHDL lipids. The recovery in the perfusate of all the lysoPC and almost half of the FFA generated by

the hydrolysis of [¹⁴C]PC and [³H]triolein, respectively, suggests that the metabolism of these components occurs extracellularly.

When livers were preperfused with heparin, there was a decrease of 23 nmol (from 30.4 nmol to 7.5 nmol) in the amount of rHDL cholesteryl oleate actually taken up by the liver compared to livers without heparin preperfusion. The difference in the amount of rHDL cholesteryl oleate taken up by the liver with and without heparin preperfusion indicates that approximately 75% (23/30.4 nmol) of the hepatic lipase was removed by the heparin preperfusion (40 IU/ml for 5 min). This is similar to the 80% reduction in hepatic lipase reported by Marques-Vidal et al. (45) who perfused rat livers with 20 IU/ml of heparin for 10–12 min. They also found that when the liver was depleted of hepatic lipase, both TG and phosphatidylethanolamine hydrolysis were greatly reduced (they used radiolabeled phosphatidylethanolamines instead of PC in their model HDL). Specifically, they found that at 5 min both [³H]triolein uptake and phosphatidylethanolamine hydrolysis were decreased by about 80% after heparin preperfusion compared to control livers. Similarly, we found that heparin treatment resulted in about a 70% decrease in triolein hydrolysis.

The substrate concentration curves in Fig. 1 show that the amount of both triolein and cholesteryl oleate retained by the liver increased linearly with rHDL concentrations up to 300 μ g of rHDL apolipoprotein. Arbeeney et al. (19) using apoE-deficient rat HDL as substrate and a similar nonrecirculating perfusion system reported that when up to 500 μ g of apolipoprotein was injected as a 0.4 ml bolus, a constant 15% of the HDL CE was retained by the liver at 5 min. The difference in the amount of CE retained by the liver in the two experiments is probably due to differences in the lipid composition of the substrates. The more relevant point, however, is that both we and they found that regardless of the amount of HDL injected (up to 300 μ g or 500 μ g of apolipoprotein, respectively), a constant percentage of the HDL CE was retained. Above that level (300 or 500 μ g) virtually no additional CE was retained by the liver. This peculiar concentration dependence has also been observed by van Berkel et al. (3) for the binding of HDL to the high affinity HDL binding sites of nonparenchymal hepatocytes and is distinct from the more traditional hyperbolic concentration dependence observed for HDL binding to liver parenchymal cells. If rHDL binds to the same nonparenchymal cell binding sites described by van Berkel et al. (3), then the similarity in the concentration dependence of HDL binding and the uptake of CE by the liver suggests that the binding of rHDL to the nonparenchymal hepatocytes is the rate-limiting step in hepatic CE uptake. This nonparenchymal cell binding of rHDL is consistent with the dependence of rHDL metabolism on hepatic lipase activity since hepatic lipase is located on the surface of

sinusoidal endothelial cells (48). It is almost certain that hepatic lipase is responsible for the metabolism of HDL by endothelial cells because PC is hydrolyzed by a phospholipase A1, because both the TG hydrolase and phospholipase A1 activities are extracellular (i.e., the products of rHDL PC and triolein hydrolysis were recovered all (PC) or in part (triolein) in the perfusate), and because the metabolism of rHDL decreased when the liver was depleted of hepatic lipase by heparin preperfusion.

Although the initial metabolism of HDL takes place on the surface of endothelial cells, most of the CE in HDL is taken up by parenchymal cells (49, 50). Thus, it is likely that after being metabolized by hepatic lipase, HDL is released from the endothelial cells and then binds to parenchymal cells where CE is taken up. Bamberger et al. (24) and Collet et al. (33, 34) have demonstrated that when HDL was exposed to hepatic lipase or a phospholipase A2 in vitro, HDL phospholipids are partially hydrolyzed and that these lipase-modified HDL are more rapidly metabolized by cultured hepatocytes than HDL that was not exposed to phospholipase. This study does not directly address the mechanism by which CE is taken up by the hepatocytes. However, based on our results it is unlikely that the mechanism of CE uptake involves the fusion of HDL with the hepatocyte membrane. Membrane fusion would almost certainly result in exchange of PC between the fused membranes, but in this study virtually all of the PC and lysoPC from rHDL was recovered in the perfusate indicating that no exchange occurred. Whatever the mechanism, phospholipid hydrolysis but not TG hydrolysis is required for CE uptake, since when rHDL was prepared with a nonhydrolyzable PC analogue (a PC diether) there was very little uptake of cholesteryl oleate. 

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