Effect of phosphatidylcholine molecular species on the uptake of HDL triglycerides and cholesteryl esters by the liver

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Abstract It has previously been shown that the hydrolysis of high density lipoprotein (HDL) phospholipids by hepatic lipase promotes the hepatic uptake of triglyceride and cholesteryl ester from HDL. Since the hydrolysis of HDL phospholipids promotes HDL cholesteryl ester uptake, then it is possible that the hepatic metabolism of HDL could be altered by changing the molecular species composition of HDL phosphatidylcholine (PC) (the major phospholipid in HDL). To test this possibility the uptake of [^{3}H]triolein and [^{14}C]cholesteryl oleate by the isolated perfused rat liver was determined in a nonrecirculating perfusion system following a bolus injection of reconstituted HDL (rHDL) prepared with rat serum HDL apolipoproteins, [^{3}H]triolein, [^{14}C]cholesteryl oleate, unesterified cholesterol, and one of five molecular species of phosphatidylcholine (16:0-18:2, 16:1-16:1, 18:0-18:2, 18:1-16:0, or 20:1-20:1), 18:1-16:0 phosphatidylcholine diether, or the total phosphatidylcholine fraction isolated from rat serum HDL. The apolipoprotein profiles and lipid compositions of all the rHDL were similar. The greatest uptake of [^{3}H]triolein was obtained with the rHDL prepared with 16:0-18:2 phosphatidylcholine. The amount of [^{3}H]triolein taken up by the liver when the rHDL were prepared with 16:1-16:1, 18:0-18:2, 18:1-16:0, 20:1-20:1, 18:1-16:0 phosphatidylcholine diether, or rat serum HDL phosphatidylcholine was 56.7%, 51.7, 39.9%, 27.6%, 31.8%, and 84.7%, respectively, of the amount taken up from the rHDL prepared with 16:0-18:2 phosphatidylcholine. Likewise, the greatest amount of [^{14}C]cholesteryl oleate was taken up by the liver when the rHDL was prepared with 16:0-18:2 phosphatidylcholine and the variation in [^{14}C]cholesteryl oleate uptake from the various rHDL displayed a pattern of dependence on the phosphatidylcholine molecular species of rHDL similar to triolein uptake. The variation in the rate of rHDL phosphatidylcholine hydrolysis by hepatic lipase in vitro as a function of the PC molecular species composition of the rHDL also resembled the variation in the hepatic uptake of [^{14}C]cholesteryl oleate and [^{3}H]triolein. Partial substitution of rHDL phosphatidylcholine with the minor phospholipids isolated from rat serum HDL affected neither the hepatic uptake of [^{3}H]triolein or [^{14}C]cholesteryl oleate nor phosphatidylcholine hydrolysis by hepatic lipase in vitro when 16:0-18:2 phosphatidylcholine or rat serum HDL phosphatidylcholine was used to prepare the rHDL. On the other hand, when rHDL was prepared with 20:1-20:1 phosphatidylcholine, the inclusion of the minor phospholipids increased [^{3}H]triolein and [^{14}C]cholesteryl oleate uptake and phosphatidylcholine hydrolysis approximately twofold. These results indicate that both the phosphatidylcholine molecular species composition and phospholipid composition of HDL can affect the rate of phosphatidylcholine hydrolysis by hepatic lipase and thereby also affect the hepatic metabolism of HDL.—**Kadowaki, H., G. M. Patton, and S. J. Robins.** Effect of phosphatidylcholine molecular species on the uptake of HDL triglycerides and cholesteryl esters by the liver. *J. Lipid Res.* 1993. **34:** 180-189.

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A number of studies have demonstrated that the uptake of both unesterified and esterified cholesterol from HDL by the liver depends on the hydrolysis of HDL phospholipids by hepatic lipase (1, 2). In vitro, hepatic lipase has been shown to hydrolyze a wide range of glycerides, including phosphatidylcholine (PC), at different rates depending on the fatty acid composition of the substrate (3-6). Since the hydrolysis of HDL phospholipids promotes the uptake of unesterified cholesterol (7-9) and appears to be required for the uptake of HDL cholesteryl ester (CE) by the liver (2), it is possible that the rate of HDL metabolism by the liver could be altered by changing the molecular species composition of HDL PC, the major phospholipid in HDL.

It has been shown that in the live rat the clearance from the blood of reconstituted HDL (rHDL) unesterified cholesterol and PC both vary depending on the PC molecular species composition of rHDL (10). However, in that study the PC molecular species composition of rHDL had no effect on the clearance of rHDL CE. In this study

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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we have reexamined the effect of the PC molecular species composition of rHDL on the uptake of CE from rHDL using a model which, unlike studies in the live rat, ensures that the rHDL PC composition is not changed by exchange with other lipoprotein phospholipids. Specifically, the uptake of [^{3}H]triolein and [^{14}C]cholesteryl oleate by the isolated perfused rat liver was determined in a single pass perfusion system following a bolus injection of rHDL prepared with rat serum HDL apoproteins, [^{3}H]triolein, [^{14}C]cholesteryl oleate, unesterified cholesterol, and one of five molecular species of PC (16:0-18:2, 16:1-16:1, 18:0-18:2, 18:1-16:0, or 20:1-20:1), 18:1-16:0 PC diether, or the total PC fraction isolated from rat serum HDL. We also examined the effect of the minor phospholipids of rat serum HDL on the uptake of rHDL triolein and cholesteryl oleate by the liver and have compared these results to the rate of rHDL PC hydrolysis by hepatic lipase in vitro.

TABLE 1. PC molecular species composition of rat serum HDL

Molecular species	%
16:0-18:1	4.83 ± 0.43
16:0-18:2	26.33 ± 0.67
16:0-20:4	10.34 ± 0.29
16:0-22:6	4.81 ± 0.26
18:0-18:1	1.63 ± 0.11
18:0-18:2	18.23 ± 0.51
18:0-20:4	10.95 ± 0.28
18:0-22:6	2.76 ± 0.19
18:1-18:2	3.99 ± 0.07
18:1-20:4	1.93 ± 0.15
18:1-22:6	0.67 ± 0.09
18:2-18:2	1.99 ± 0.07
Others	11.20 ± 2.14

The data shown are mean ± SD of three determinations. The PC fraction isolated from rat serum HDL was hydrolyzed with phospholipase C, the resulting diglycerides were converted to bezoyl esters, and the molecular species were separated by HPLC as described in Methods.

EXPERIMENTAL PROCEDURES

Materials

[Cholesteryl-4-^{14}C]cholesteryl oleate (50 mCi/mmol) and [9,10-^{3}H(N)]triolein (20 Ci/mmol) were obtained from DuPont New England Nuclear (Boston, MA), bovine serum albumin (fatty acid-free and fraction V) from Sigma (St. Louis, MO), sodium taurocholic acid (A grade) from Calbiochem (La Jolla, CA), cholesteryl oleate, cholesterol, and triolein from Nu-Check-Prep (Elysian, MN), phosphatidylcholines from Avanti Polar Lipids (Birmingham, AL), (1-oleyl-2-palmityl)-DL-phosphatidylcholine diether (18:1-16:0 PC diether) from Serdary Research Laboratories (Port Huron, MI), stigmasterol (5,22-cholestadien-24b-ethyl-3β-ol) and stigmasterol acetate from Steraloids (Wilton, NH), heparin (porcine intestine) from Elkins-Sinn (Cherry Hill, NJ), DEAE Sephacel and heparin Sepharose from Pharmacia-LKB (Piscataway, NJ), and ultra pure urea 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 fed standard Purina rat chow ad libitum.

Preparation of rHDL

Isolation of HDL apolipoproteins from rat serum HDL (d 1.08-1.19 g/ml) and preparation of rHDL were as previously described (2) except that rHDL were prepared with one of five different PC molecular species (i.e., 16:1-16:1, 16:0-18:2, 18:0-18:2, 18:1-16:0, or 20:1-20:1), 18:1-16:0 PC diether, or the total PC fraction isolated from rat serum HDL. The PC molecular species composition of this rat serum HDL is shown in **Table 1.** rHDL were also prepared with 16:0-18:2 PC, 20:1-20:1 PC, or rat serum HDL PC, but PC was substituted in part (20%) with the minor phospholipids isolated from rat serum HDL. This "minor phospholipid" fraction consisted of sphingomyelin (62.7%), phosphatidylinositol (25.8%), phosphatidylethanolamine (7.4%), and other phospholipids (4.1%) (average of two determinations). Both the total PC fraction and the minor phospholipid fractions were isolated from a Folch extract of rat serum HDL by HPLC (11). Phospholipid fractions were quantitated by phosphorus analysis (12). Protein concentration was determined by the method of Bensadoun and Weinstein (13). Electron microscopy of negatively stained preparations of rHDL prepared with 16:0-18:2 PC, 20:1-20:1 PC, 18:1-16:0 PC diether, or rat serum HDL PC substituted in part with the minor phospholipids from rat serum HDL appeared identical. Particles were circular and over 90% of the particles measured 10-20 nm in diameter.

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 (14). Sodium taurocholate was infused directly into the portal vein cannula at 40 μmol/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. At this point, a 1-ml bolus of radiolabeled rHDL (100 μg apolipoproteins) was injected into the portal vein and the liver was continuously perfused for another 5 min. After 5 min the liver was removed, washed in cold saline, and blotted dry. 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 (15).

Lipid analysis

Known amounts of stigmasterol acetate, stigmasterol, trieicosenoin, and dimyristoyl PC were added to the lipid extract as internal standards for the quantitative analysis of cholesteryl oleate, unesterified cholesterol, triolein, and PC, respectively. The lipid extract was partitioned with saline (15) and the Folch lower-phase was separated into neutral lipids and individual phospholipid classes by HPLC and quantitated by the method of Patton, Fasulo, and Robins (16, 17). The neutral lipids were further separated into CE, triglycerides (TG), free fatty acids (FFA), and unesterified cholesterol by HPLC. Radioactivity was determined by liquid scintillation spectrometry. For the quantitation of the amount of [^{3}H]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) (2). 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. Statistical differences between groups were determined by ANOVA with one-way analysis of variance and comparisons were made using the Fisher procedure at a significance of 0.05.

Partial purification of hepatic lipase

Hepatic lipase was released from rat liver with heparin and purified as previously described by Jensen and Bensadoun (18). Briefly, the heparin-containing perfusate from four-five rat livers was applied to a heparin-Sepharose column equilibrated with 30% glycerol (v/v) and 10 mM phosphate buffer (pH 7.0). Hepatic lipase was eluted from the column with an increasing gradient of sodium chloride. The fractions containing hepatic lipase activity (measured by [^{3}H]oleate release from a [^{3}H]triolein–gum arabic emulsion) were pooled and dialyzed against saturated ammonium sulfate in 10 mM phosphate buffer (pH 6.5) for 16 h. The dialysate was centrifuged at 105,000 *g* for 60 min. The pellet was resuspended in 50 mM Tris buffer (pH 7.2) and dialyzed against the same Tris buffer for 3 h. The dialyzed hepatic lipase had a specific activity of 350 μmol FFA released/mg protein per h with the [^{3}H]triolein–gum arabic emulsion as substrate at pH 7.4.

Hepatic lipase assays

The determination of hepatic lipase activity during the purification of the enzyme was performed with [^{3}H]triolein emulsified with gum arabic prepared by the methods of Ehnholm and Kuusi (19). The reaction mixtures contained 3.6 mM [^{3}H]triolein–gum arabic emulsion, 50 mM Tris buffer (pH 7.4), 1 M sodium chloride, 0.8 mM calcium chloride, 2% bovine serum albumin, and hepatic lipase preparation in a final volume of 250 μl. The reaction was started by the addition of hepatic lipase and the incubation temperature was 37°C. The reaction was stopped with methanol–chloroform–heptane and the amount of [^{3}H]oleate released was determined as described by Belfrage and Vaughan (20). The in vitro activity of hepatic lipase towards rHDL [^{3}H]triolein or rHDL PC was determined using the incubation conditions described above except that rHDL was used instead of the triolein–gum arabic emulsion. [^{3}H]triolein hydrolysis of rHDL was determined by the release of [^{3}H]oleate as described above. Hydrolysis of rHDL PC was determined by the disappearance of PC from the rHDL. The PC remaining in the rHDL was quantitated by the method of Patton, Fasulo, and Robins (16, 17). The enzymatic reaction was terminated with 4.8 ml of chloroform–methanol 2:1, dimyristoyl PC was added as an internal standard, and the chloroform–methanol extract was partitioned with saline (15). The PC fraction was isolated from the lower-phase by HPLC, hydrolyzed with phospholipase C (17), and the resulting diglycerides were converted to the benzoyl esters (21). The molecular species of PC were separated and quantitated by HPLC (17).

SDS-gel electrophoresis

rHDL apoprotein profiles were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to the procedure of Laemmli (22).

RESULTS

The apolipoprotein composition of rHDL prepared with 16:0–18:2 PC, 20:1–20:1 PC, 18:1–16:0 PC diether, and rat serum HDL PC together with the minor phospholipids (20%) was examined by SDS-gel electrophoresis. All of these rHDL appeared to have a similar apolipoprotein composition, but they differed from the rat serum HDL apolipoproteins from which they were prepared in that rHDL were relatively depleted in apoE and apoA-IV (**Fig. 1**). The lipid compositions of these rHDL as well as of the rHDL prepared with 16:1–16:1 PC, 18:0–18:2 PC, 18:1–16:0 PC, and rat serum HDL PC were the same, i.e., in no case was the lipid composition of any of the rHDL preparations outside the range of variation observed with 16:0–18:2 PC (the only rHDL for which multiple batches were prepared).

In order to determine to what extent the hepatic uptake of TG and CE from HDL is dependent on the PC molecular species composition of HDL, isolated rat livers were perfused in a nonrecirculating system with a 1-ml bolus of rHDL. Using this same perfusion system, we

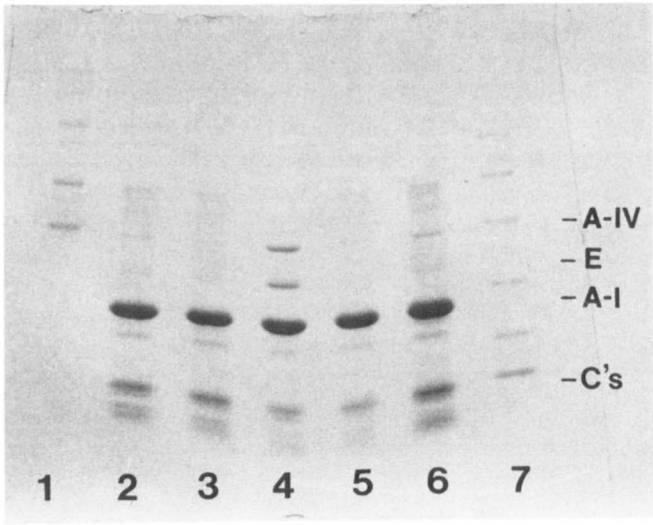

Fig. 1. Polyacrylamide gel electrophoresis of apolipoproteins isolated from rat serum HDL and rHDL. rHDL prepared with 16:0–18:2 PC, 20:1–20:1 PC, 18:1–16:0 PC diether or rat serum HDL PC partially substituted with the minor phospholipids from rat serum HDL were reisolated by equilibrium centrifugation (d 1.08–1.19 g/ml) and delipidated. Apolipoproteins were treated with sodium dodecyl sulfate and approximately 30 μg of apolipoproteins was applied to 3–20% gradient polyacrylamide gel with a 4% stacking gel. Protein was stained with Coomassie brilliant blue. Electrophoresis was performed for 16 h at 40 V. Bio-Rad molecular weight standards (lanes 1 and 7) are from top to bottom: *E. coli* β-galactosidase (116,000), rabbit muscle phosphorylase (97,400), bovine serum albumin (66,200), chicken ovalbumin (42,699), bovine carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and hen egg white lysozyme (14,400). Lane 2 contains apolipoproteins from rHDL prepared with rat serum HDL PC and the minor phospholipids from rat serum HDL; lane 3, apolipoproteins from rHDL prepared with 20:1–20:1 PC; lane 4, the original rat serum HDL apolipoproteins; lane 5, apolipoproteins from rHDL prepared with 18:1–16:0 PC diether; and lane 6, apolipoproteins from rHDL prepared with 16:0–18:2 PC.

have previously shown (2) that the hepatic uptake of triolein and cholesteryl oleate from rHDL was linear up to an rHDL apolipoprotein concentration of 300 μg. In order to ensure that the uptake of [^{3}H]triolein and [^{14}C]cholesteryl oleate was in the linear range, each rHDL preparation was adjusted to an apolipoprotein concentration of 100 μg/ml with saline. Thus, the 1-ml bolus of rHDL that was injected contained 100 μg apolipoproteins, 10.2 ± 2.1 μg (mean ± SD for 11 preparations) of [^{3}H]triolein, 62.6 ± 8.5 μg of [^{14}C]cholesteryl oleate, 13.4 ± 0.2 μg of unesterified cholesterol, and 64.0 ± 9.6 μg of PC.

We have also previously shown (2) that with the experimental protocol employed for this study, some of the [^{3}H]triolein retained by the liver was not actually metabolized and could therefore be recovered as unmetabolized, intact [^{3}H]triolein. This unmetabolized [^{3}H]triolein presumably resides in intact unmetabolized rHDL and does not reflect actual uptake. Therefore, the amount of ^{3}H in the liver remaining as [^{3}H]triolein was determined and that amount of ^{3}H was subtracted from the amount of ^{3}H retained by the liver to determine the amount actually taken up by the liver. For all of the different preparations of rHDL, the amount of [^{3}H]triolein (0.37 ± 0.12 nmol, mean ± SD for 26 determinations) retained but not metabolized by the liver was not significantly different from the rHDL prepared with 16:0–18:2 PC.

When a 1-ml bolus of the various rHDL was injected into the portal vein of the perfused livers, the amount of [^{3}H]triolein taken up from rHDL by the liver varied depending upon the molecular species of PC used to prepare the rHDL (**Fig. 2**). The greatest amount of [^{3}H]triolein was taken up by the liver when the rHDL was prepared with 16:0–18:2 PC and was 18.7 ± 4.4% of the rHDL [^{3}H]triolein that was injected (2). The amount of [^{3}H]triolein taken up by the liver when the rHDL were prepared with 16:1–16:1, 18:0–18:2, 18:1–16:0, or 20:1–20:1 PC was 56.7%, 51.7%, 39.9%, and 27.6%, respectively, of the amount taken up from the rHDL prepared with 16:0–18:2 PC. When the rHDL was prepared with the total PC fraction isolated from rat serum HDL, the amount of [^{3}H]triolein taken up was comparable to that taken up from the rHDL prepared with 16:0–18:2 PC (i.e., 84.7% of the amount taken up from the rHDL prepared with 16:0–18:2 PC). When the rHDL was prepared with a nonhydrolyzable analogue of PC, i.e., 18:1–16:0 PC diether, the amount

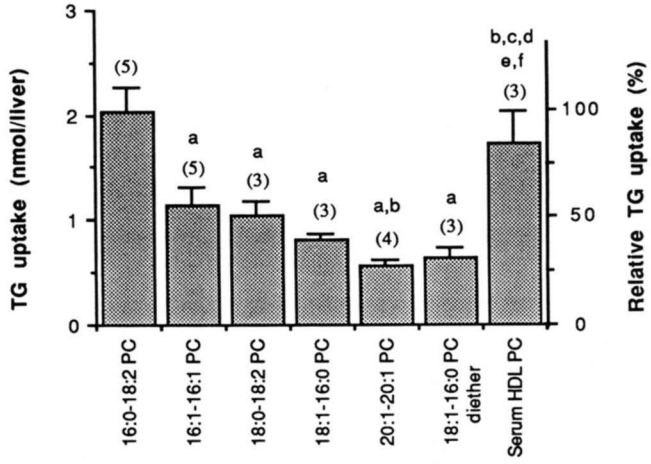

Fig. 2. Effect of rHDL PC composition on the uptake of [^{3}H]triolein by the perfused rat liver. A 1-ml bolus of rHDL (containing 11.5 ± 2.4 nmol (mean ± SD for 11 preparations) of [^{3}H]triolein, 96.2 ± 13.0 nmol of [^{14}C]cholesteryl oleate, 34.6 ± 0.5 nmol of unesterified cholesterol, 82.2 ± 10.4 nmol of PC, and 100 μg of HDL apolipoproteins) was injected into the portal vein of perfused rat livers (weighing 10.3 ± 1.0 g, mean ± SD for 26 livers), after which the livers were perfused for 5 min in a nonrecirculating perfusion system as described in Methods. Data are the mean values for the number of perfusions shown in parentheses. Error bars represent the SE. Relative TG uptake (right-side margin) expresses the [^{3}H]triolein uptake from the various rHDL as a % of the uptake from the rHDL prepared with 16:0–18:2 PC (2). a, Statistically different from 16:0–18:2 PC; b, from 16:1–16:1 PC; c, from 18:0–18:2 PC; d, from 18:1–16:0 PC; e, from 20:1–20:1 PC; f, from 18:1–16:0 PC diether.

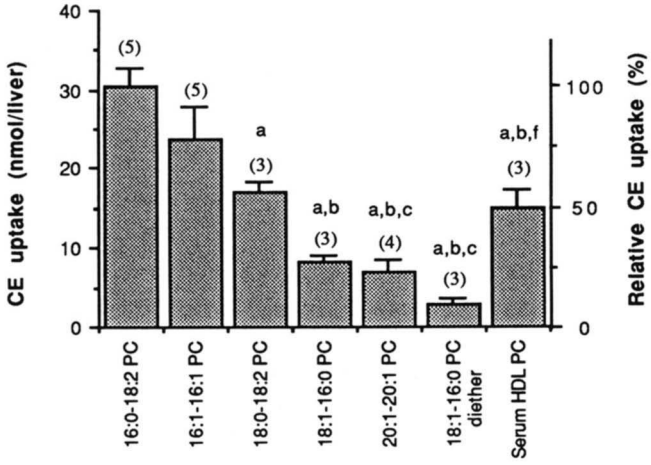

Fig. 3. Effect of rHDL PC composition on the uptake of [^{14}C]cholesteryl oleate by the perfused rat liver. rHDL compositions, liver weights, and perfusion protocol are the same as described in Fig. 2. Data are the mean values for the numbers of perfusions shown in parentheses. Error bars represent the SE. Relative CE uptake (right-side margin) expresses the [^{14}C]cholesteryl oleate uptake from the various rHDL as a % of the uptake from the rHDL prepared with 16:0–18:2 PC (2). a, Statistically different from 16:0–18:2 PC; b, from 16:1–16:1 PC; c, from 18:0–18:2 PC; d, from 18:1–16:0 PC; e, from 20:1–20:1 PC; f, from 18:1–16:0 PC diether.

of [^{3}H]triolein taken up was similar to the amount taken up from the rHDL prepared with 20:1–20:1 PC and was 31.8% of the amount taken up from the rHDL prepared with 16:0–18:2 PC.

As was the case for the uptake of [^{3}H]triolein, the greatest amount of [^{14}C]cholesteryl oleate was taken up from rHDL by the liver when the rHDL was prepared with 16:0–18:2 PC and was 29.8 ± 4.7% of the rHDL [^{14}C]cholesteryl oleate injected (2). The amount of rHDL [^{14}C]cholesteryl oleate taken up by the liver also varied depending upon the molecular species of PC used to prepare the rHDL (**Fig. 3**). When rHDL was prepared with 16:1–16:1, 18:0–18:2, 18:1–16:0, or 20:1–20:1 PC, [^{14}C]cholesteryl oleate uptake displayed a similar pattern of dependence on the PC molecular species of rHDL as did [^{3}H]triolein uptake, i.e., the amount of [^{14}C]cholesteryl oleate taken up by the liver was 77.8%, 55.9%, 27.3%, and 22.5%, respectively, of the amount taken up from the rHDL prepared with 16:0–18:2 PC. However, when the rHDL was prepared with the total PC fraction isolated from rat serum HDL, the amount of [^{14}C]cholesteryl oleate taken up by the liver was proportionately less than the uptake of [^{3}H]triolein and was only 49.1% of the amount taken up from the rHDL prepared with 16:0–18:2 PC (compared to 84.7% for [^{3}H]triolein uptake). When rHDL was prepared with 18:1–16:0 PC diether, there was hardly any uptake of [^{14}C]cholesteryl oleate by the liver, i.e., only 9.4% of the amount taken up from the rHDL prepared with 16:0–18:2 PC (or 2.6% of the rHDL [^{14}C]cholesteryl oleate injected).

One possible explanation for the variation in the hepatic uptake of [^{3}H]triolein and [^{14}C]cholesteryl oleate from rHDL as a function of the PC molecular species composition of the rHDL is that CE uptake and to some extent TG uptake depend on PC hydrolysis and that the rate of PC hydrolysis by hepatic lipase varies depending on the molecular species of PC. Radiolabeled PC is required to measure accurately the rate of rHDL PC hydrolysis by the perfused liver, but most of the particular molecular species of PC used in this study are not commercially available. Therefore, the rate of hydrolysis of rHDL PC by hepatic lipase was examined in vitro using nonradiolabeled PC. We have previously shown that hepatic lipase was responsible for the hydrolysis of rHDL PC and triolein by the perfused rat liver (2). However, before attempting to determine the rate of rHDL PC hydrolysis in vitro, an experiment was performed to determine whether the variation in the rate of rHDL triolein uptake as a function of rHDL PC molecular species composition observed with the perfused livers would also be observed for the hydrolysis of rHDL triolein by partially purified hepatic lipase in vitro. First, an enzyme concentration curve was established to determine the amount of hepatic lipase preparation required for optimum hydrolysis of [^{3}H]triolein with rHDL prepared with 16:0–18:2 PC. The rate of rHDL [^{3}H]triolein hydrolysis was linear up to approximately 1 μg of the partially purified hepatic lipase preparation (data not shown). The rate of rHDL [^{3}H]triolein hydrolysis by 1.1 μg of the hepatic lipase preparation was then determined using rHDL prepared with 16:0–18:2, 16:1–16:1, 18:0–18:2, or 20:1–20:1 PC as the substrate. The hydrolysis of rHDL [^{3}H]triolein was linear for at least 60 min with all of the rHDL preparations (**Fig. 4**). Moreover, the relative rates of rHDL [^{3}H]triolein hydrolysis by hepatic lipase exhibited the same pattern of dependence on rHDL PC molecular species as did the uptake of rHDL [^{3}H]triolein by the perfused liver.

Because the hydrolysis of rHDL PC by the perfused liver is relatively low (7%) (2) and because the rate of PC hydrolysis in vitro is slow compared to TG hydrolysis (23), 2.2 μg of hepatic lipase preparation was used to determine the rate of rHDL PC hydrolysis. At that enzyme concentration, the hydrolysis of 16:0–18:2 PC was linear for up to 15 min (data not shown). Thus, a 15-min incubation was used to determine the relative rate of PC hydrolysis with rHDL prepared with 16:0–18:2, 16:1–16:1, 18:0–18:2, 18:1–16:0, 20:1–20:1, and rat serum HDL PC. The highest rate of PC hydrolysis was obtained with the rHDL prepared with 16:0–18:2 PC (12.6 ± 1.4 nmol/15 min, mean ± SE for three determinations) (**Fig. 5**). The rate of PC hydrolysis for rHDL prepared with 16:1–16:1, 18:0–18:2, 18:1–16:0, and 20:1–20:1 PC was 58.5%, 70.4%, 21.7%, and 12.3%, respectively, of the rate of hydrolysis of 16:0–18:2 PC. However, when rHDL was prepared with rat serum HDL PC, the rate of PC hydrolysis was only

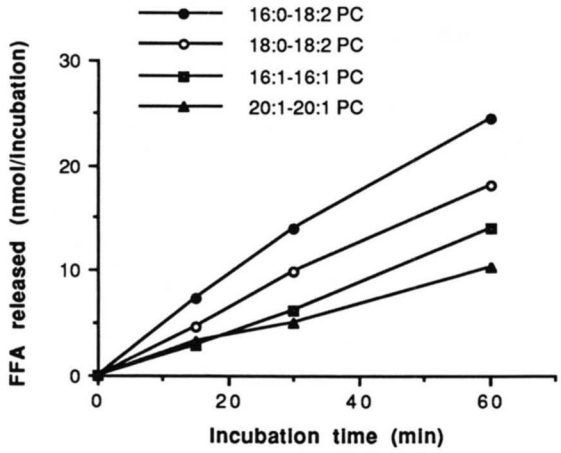

Fig. 4. Effect of rHDL PC molecular species on the hydrolysis of [^{3}H]triolein by hepatic lipase. The reaction mixtures contained 50 mM Tris buffer (pH 7.4), 1 M sodium chloride, 0.8 mM calcium chloride, 2% bovine serum albumin, rHDL prepared with various molecular species of PC, and 1.1 μg of hepatic lipase in a final volume of 250 μl. The rHDL contained 150.0 ± 14.2 μg (mean ± SD for four determinations) of rat serum HDL apolipoproteins, 21.3 ± 4.0 nmol of [^{3}H]triolein (0.05 μCi), 132.2 ± 20.1 nmol of cholesteryl oleate, 48.9 ± 5.0 nmol of unesterified cholesterol, and 138.5 ± 23.6 nmol of either 16:0-18:2, 16:1-16:1, 18:0-18:2, or 20:1-20:1 PC. The reaction was started by the addition of hepatic lipase and the mixture was incubated at 37°C. The reaction was stopped with methanol-chloroform-heptane at the indicated times and the amount of [^{3}H]oleate released was determined as described in Methods. The results are means of duplicate determinations.

56.6% of the rate of 16:0-18:2 PC hydrolysis. Thus, the variation in the rate of rHDL PC hydrolysis as a function of the PC molecular species composition of the rHDL more closely resembled the variation in the hepatic uptake of rHDL [^{14}C]cholesteryl oleate than the variation in the hepatic uptake of rHDL [^{3}H]triolein.

Since rat serum HDL contains a total of about 20% various phospholipids other than PC, it is possible that these minor phospholipids could modulate the metabolism of rHDL prepared with PC alone. Therefore, we examined the uptake by the liver of [^{3}H]triolein and [^{14}C]cholesteryl oleate from rHDL prepared by substituting 20% of either 16:0-18:2 PC, 20:1-20:1 PC, or rat serum HDL PC with the minor phospholipids isolated from rat serum HDL. When the rHDL was prepared with 16:0-18:2 PC or rat serum HDL PC, the presence of the minor phospholipids had no effect on the uptake of [^{3}H]triolein or [^{14}C]cholesteryl oleate by the perfused liver (**Table 2**). However, when the rHDL was prepared with 20:1-20:1 PC and the minor phospholipids from rat serum HDL, the uptake of [^{3}H]triolein and [^{14}C]cholesteryl oleate by the liver increased more than 2-fold compared to rHDL prepared with 20:1-20:1 PC alone, and was comparable to the uptake obtained with rHDL prepared with rat serum HDL PC with or without inclusion of the minor phospholipids from rat serum HDL.

The hydrolysis of rHDL PC by hepatic lipase in vitro was also examined using as substrates the rHDL prepared with the minor phospholipid fraction of rat serum HDL together with 20:1-20:1 PC or rat serum HDL PC. At 15 min, there was no difference in the rate of PC hydrolysis between the rHDL prepared with rat serum PC alone and rat serum PC plus the minor phospholipids. On the other hand, there was a small (statistically insignificant) increase in the rate of PC hydrolysis between the rHDL prepared with 20:1-20:1 PC alone and the rHDL prepared with 20:1-20:1 PC plus the minor phospholipids (**Table 3**). Since the rate of PC hydrolysis was low and the variation in the rate of PC hydrolysis was relatively large, the hydrolysis of rHDL PC with and without the minor phospholipid fraction of rat serum HDL was examined after a 60-min incubation. At 60 min, the rate of 20:1-20:1 PC hydrolysis with the rHDL that included the minor phospholipid fraction was almost twice the rate obtained with the rHDL prepared with 20:1-20:1 PC alone, while there was no difference in the rate of rHDL PC hydrolysis between the rHDL prepared with rat serum HDL PC alone and the rHDL prepared with rat serum HDL PC plus the minor phospholipids.

DISCUSSION

In a previous study we demonstrated that the hydrolysis of rHDL PC by hepatic lipase was required for the uptake

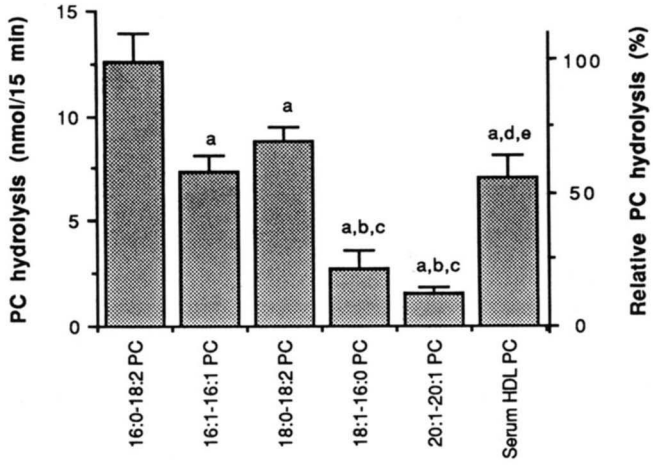

Fig. 5. Effect of rHDL PC molecular species on the hydrolysis of rHDL PC by hepatic lipase. The reaction conditions were the same as described for Fig. 4, except that 2.2 μg hepatic lipase was used. rHDL contained 118.1 ± 10.9 nmol (mean ± SD for 18 determinations) of PC. The incubation was for 15 min and the amounts of PC remaining were quantitated as described in Methods. The results are expressed as nmol of PC hydrolyzed during incubation. Data is shown as mean ± SE of triplicate determinations. a, Statistically different from 16:0-18:2 PC; b, from 16:1-16:1 PC; c, from 18:0-18:2 PC; d, from 18:1-16:0 PC; e, from 20:1-20:1 PC.

TABLE 2. Effect of minor phospholipids of rat serum HDL on the uptake of triolein and cholesteryl oleate from rHDL

PC Molecular Species	Minor HDL Phospholipids	Uptake	
		[^{3}H]Triolein	[^{14}C]Cholesteryl Oleate
		nmol/liver	
16:0-18:2 PC	–	2.03 ± 0.48 (5)[a]	30.44 ± 4.80 (5)[a]
	+	2.16 ± 0.17 (3)	32.92 ± 3.36 (3)
Serum HDL PC	–	1.72 ± 0.44 (3)	14.95 ± 3.46 (3)
	+	1.58 ± 0.04 (3)	13.24 ± 2.68 (3)
20:1-20:1 PC	–	0.56 ± 0.10 (4)	6.85 ± 2.93 (4)
	+	1.29 ± 0.18 (3)[b]	16.12 ± 3.14 (3)[b]

Values are expressed as means ± SD for three-five perfusions (shown in parentheses). Liver weights for the perfusions with rHDL prepared with PC alone were 10.7 ± 0.9 g (n = 12), and with rHDL prepared with PC and the minor phospholipids of rat serum HDL were 10.2 ± 0.7 g (n = 9). Livers were perfused for 5 min in a non-recirculating perfusion system as described in Methods. rHDL prepared with PC alone contained 10.3 ± 1.2 nmol (n = 6) of [^{3}H]triolein, 96.6 ± 11.0 nmol of [^{14}C]cholesteryl oleate. rHDL prepared with PC and the minor phospholipids contained 12.4 ± 0.8 nmol (n = 3) of [^{3}H]triolein, 99.4 ± 14.8 nmol of [^{14}C]cholesteryl oleate. The proportion of PC and minor phospholipids was 80% and 20%.

[a] Data from ref. 2.

[b] $P < 0.01$, compared to rHDL prepared with 20:1-20:1 PC alone.

of triolein and cholesteryl oleate from rHDL by the perfused rat liver (2). In this study we demonstrate that the PC molecular species composition of rHDL has a profound effect on the uptake of triolein and cholesteryl oleate from rHDL by the perfused rat liver, and that the extent of cholesteryl oleate uptake by the liver shows the same dependence on rHDL PC composition as the rate of hydrolysis of rHDL PC by hepatic lipase in vitro.

It has previously been shown that there was no difference in the apolipoprotein composition of rHDL prepared with 16:0-18:2, 18:0-18:2, 16:1-16:1, or 20:1-20:1 PC (10). However, to verify that this was also the case in these experiments, the apolipoprotein composition of the rHDL prepared with the total phospholipid fraction isolated from rat serum HDL, 20:1-20:1 PC, 18:1-16:0 PC diether, and 16:0-18:2 PC was examined by SDS gel electrophoresis. The apolipoprotein compositions of all these rHDL were similar and they all had an apolipoprotein composition that differed from that of the rat serum HDL from which they were prepared in that the rHDL were relatively depleted in apoE and apoA-IV. All the rHDL had a lipid composition that was within the range of variation obtained with 16:0-18:2 PC.

The PC molecular species composition of rHDL had a similar effect on the uptake of both triolein and cholesteryl oleate by the perfused liver. However, the uptake of tri-

TABLE 3. rHDL PC hydrolysis by hepatic lipase

PC Molecular Species	Minor HDL Phospholipids	PC Hydrolysis	
		15 min	60 min
		nmol	
16:0-18:2 PC	–	12.56 ± 2.44	23.00 ± 1.97
	+	nd	nd
Serum HDL PC	–	7.11 ± 1.73	7.47 ± 0.89
	+	6.55 ± 1.79	8.07 ± 0.08
20:1-20:1 PC	–	1.47 ± 0.44	4.37 ± 0.66
	+	2.01 ± 0.17	7.69 ± 0.10[a]

The reaction conditions were the same as described for Fig. 4. The reaction was stopped at the indicated time with chloroform-methanol and the remaining PC was quantitated as described in Methods. The concentration of rHDL PC prepared without the minor phospholipids of rat serum HDL was 120.7 ± 3.7 nmol (n = 3) for 16:0-18:2 PC, 112.2 ± 1.5 nmol for rat serum HDL PC, and 111.6 ± 9.2 nmol for 20:1-20:1 PC. The concentration of rHDL PC prepared with the minor phospholipids was 86.6 ± 0.5 nmol for serum HDL PC and 98.7 ± 0.9 nmol for 20:1-20:1 PC. The results are expressed as the amount (means ± SD of triplicate determinations) of PC hydrolyzed in the incubation mixture; nd, not determined.

[a] $P < 0.02$, compared to rHDL prepared with 20:1-20:1 PC alone.

olein was not as sensitive to changes in the PC composition of the rHDL as was cholesteryl oleate uptake, i.e., triolein uptake varied about 4-fold while cholesteryl oleate uptake varied about 11-fold. It has been suggested that TG can account for 2–5 mol% of the surface lipid of HDL (24) which would account for 20–50% of the triolein incorporated into the rHDL. In model systems with mixed PC and TG monolayers at an air/water interface, the rate of TG hydrolysis by hepatic lipase was faster than PC hydrolysis (4, 6) and was relatively insensitive to PC composition. Thus, the triolein on the surface of rHDL should be rapidly hydrolyzed regardless of the PC composition of the rHDL. Clearly, the triolein uptake from the rHDL prepared with the PC diether (which is nearly a third of the maximum uptake observed with any rHDL) represents triolein hydrolysis that does not depend on PC hydrolysis. Based on these results, it is tempting to speculate that about a third of the triolein in rHDL is located on the surface of the rHDL.

The remaining triolein is presumably located in the core of the rHDL (with the cholesteryl oleate) and the metabolism of that triolein could depend on the hydrolysis of PC, i.e., it may be necessary to hydrolyze at least some PC in order for hepatic lipase to gain access to the core triolein. If this were the case, then the hydrolysis of PC and the hydrolysis and uptake of core triolein should exhibit the same dependence on phospholipid composition. Such a dependence was observed for rHDL prepared with all the PC examined except for the PC isolated from rat serum HDL, in which case the uptake of triolein was much greater than expected based on the rate of PC hydrolysis. We have no certain explanation for this discrepancy, but it is possible that the complex array of PC molecular species present in rat serum HDL PC affects the partitioning of triolein between the core and the surface such that a higher percentage of the triolein is located on the surface of that rHDL.

From the data presented in Figs. 3 and 5, and Tables 2 and 3 it is clear that PC hydrolysis was required for cholesteryl oleate uptake, and that the extent of cholesteryl oleate uptake from rHDL by the perfused liver was proportional to the rate of rHDL PC hydrolysis by hepatic lipase in vitro. In a previous study (2), in which we examined both the hydrolysis of 16:0-[^{14}C]18:2 PC and the uptake of [^{14}C]cholesteryl oleate by the liver in a single pass perfusion, 5 mol of cholesteryl oleate was taken up by the liver per mol of PC hydrolyzed. In the current study cholesteryl oleate uptake and PC hydrolysis vary in parallel. Thus, even though cholesteryl oleate and PC are present in approximately a 1:1 molar ratio and even though there is a wide variation (9-fold) in the rate of PC hydrolysis, this 5:1 ratio of cholesteryl oleate taken up to PC hydrolyzed was maintained for all the rHDL. Therefore, the variation in the extent of rHDL metabolism as a function of PC molecular species composition is probably due more to a difference in the number of rHDL particles metabolized rather than to a variation in the extent of metabolism of a given rHDL particle.

In a previous study (10) we found that when a small amount of rHDL prepared with either 16:1–16:1, 16:0–18:2, 18:0–18:2, or 20:1–20:1 PC and [^{14}C]cholesteryl oleate was injected into the live rat, there was no difference in the fractional clearance rate of rHDL [^{14}C]cholesteryl oleate among the various rHDL. There are a number of explanations for the apparent discrepancy between those experiments and the current study. First, since in vivo CE uptake is a relatively slow process with 0.34% being cleared per min and since clearance was measured by the disappearance of radioactivity from the plasma, the precision of the clearance curve at the early time points was fairly poor. This poor precision at early times could easily mask small differences in the rate of CE uptake. Second, since the rate of CE clearance is so slow and since the rHDL PC comprised only about 5% of serum phospholipids, there was ample opportunity for exchange of phospholipids between the serum lipoproteins and the rHDL. Thus, at the later time points in the clearance curve as rHDL phospholipids and serum HDL phospholipids equilibrate, the rates of CE clearance should become the same for all the rHDL.

Since the hydrolysis of HDL phospholipids is necessary for CE uptake, it is of some interest to examine the factors affecting the hydrolysis of HDL phospholipids by hepatic lipase. As 16:0–18:2 PC is hydrolyzed more rapidly than 18:0–18:2 PC and as 16:1–16:1 PC is hydrolyzed more rapidly than 20:1–20:1 PC, it seems that the more hydrophilic (shorter chain) molecular species of a homologous series is a better substrate than the more hydrophobic (longer chain) molecular species. The hydrophilic effect is limited, however, because 16:1–16:1 PC which is more hydrophilic than 16:0–18:2 PC is hydrolyzed less rapidly. Likewise, 18:1–16:0 PC, which is more hydrophilic than 18:0–18:2 PC and much more hydrophilic than 20:1–20:1 PC, is hydrolyzed much more slowly than 18:0–18:2 PC and is hydrolyzed at about the same rate as 20:1–20:1 PC. The fact that 18:1–16:0 PC is hydrolyzed so slowly supports the contention of Marques-Vidal et al. (25) that hepatic lipase prefers an unsaturated fatty acid at the *sn*-2 position of glycerol. That 16:0–18:2 PC and 18:0–18:2 PC were hydrolyzed more rapidly than 16:1–16:1 PC suggests that an *sn*-1 saturated, *sn*-2 unsaturated molecular species is the preferred substrate.

We observed an increase in 20:1–20:1 PC hydrolysis by hepatic lipase in vitro and in cholesteryl oleate (and triolein) uptake by the liver when the minor phospholipid fraction of rat serum HDL was substituted for part of the 20:1–20:1 PC, but not when the rHDL was prepared with either 16:0–18:2 PC or rat serum HDL PC. Thuren, Sisson, and Waite (26) also reported a 1.5- to 2.5-fold increase in HDL PC hydrolysis by hepatic lipase when

small amounts of phosphatidylethanolamine, phosphatidylserine, or phosphatidic acid were included in mixed rabbit liver PC/Triton X-100 micelles. That the hydrolysis of 20:1-20:1 PC by hepatic lipase increased when the minor phospholipids of serum HDL were included in the rHDL indicates that factors other than the specificity of the active site of hepatic lipase for the substrate (20:1-20:1 PC) are controlling the rate of rHDL PC hydrolysis. Miller et al. (3) using monoglyceride as substrate also observed that the catalytic site of hepatic lipase exhibits only limited acyl group specificity. This suggests that the variation in rHDL metabolism as a function of PC molecular species is due to differences in the surface properties of the various rHDL.

With the single pass perfused liver as a model, we (using rHDL prepared with rat serum HDL phospholipids) and Arbeeny, Rifici, and Eder (using modified human HDL) (27) both found that approximately 15% of the HDL CE was taken up by the liver in 5 min. From an analysis of the perfusate it appears that most of the metabolism of rHDL occurs in the first minute after the injection of a 1-ml bolus of rHDL (2). We have also reported that 300 μg of rHDL apolipoproteins/bolus injection is required to saturate CE uptake (2). That amount of rHDL also contains approximately 300 nmol of CE. Thus, with saturating levels of rHDL prepared with rat serum HDL phospholipids, about 45 nmol of cholesteryl oleate (300 nmol of CE $\times$ 15% uptake) can be taken up by the liver in a single pass. In the live rat the concentration of HDL is well above that required to saturate hepatic CE uptake, i.e., there is approximately 1 mg of HDL apolipoprotein and 1 μmol of HDL CE/ml of serum (28). Assuming that there are approximately 10 ml of serum in a 300-g rat, then from the fractional clearance rate (0.0034/min) of rHDL CE (10) approximately 34 nmol (1 μmol CE/ml $\times$ 10 ml $\times$ 0.0034) of HDL CE would be cleared from the serum per min. Thus, the amount of rHDL CE uptake observed with the perfused liver can account for most of the rHDL CE clearance observed in the live rat. This suggests that the amount of hepatic lipase on the endothelial cells is one of the limiting factors in HDL metabolism. Moreover, because hepatic lipase hydrolyzes rHDL PC at different rates depending on the PC molecular species and/or phospholipid composition of the rHDL, the PC molecular species and/or phospholipid composition of HDL could be another factor controlling the rate of HDL metabolism.

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