Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells

Mark Bamberger, Jane M. Glick, and George H. Rothblat

Department of Physiology/Biochemistry, Medical College of Pennsylvania, Philadelphia, PA 19129

Abstract The objective of this study was to determine whether high density lipoproteins (HDL) that have been treated with hepatic lipase have an enhanced ability to deliver cholesterol to cells. Human HDL was incubated with rat hepatic lipase, reisolated, and subjected to compositional analysis. Approximately 28% of the HDL phosphatidylcholine was hydrolyzed by the hepatic lipase but no change was detected in the cholesterol or apoprotein content of the HDL compared to HDL incubated with heat-inactivated hepatic lipase. Cultured rat hepatoma cells exposed to hepatic lipase-modified HDL showed an increased uptake of HDL free cholesterol relative to cells exposed to control HDL. This increased delivery of HDL free cholesterol was demonstrated by both isotopic and mass determinations and it contributed to a 1.6-fold increase in total cellular cholesterol content relative to cells treated with control HDL. The free cholesterol delivered by the HDL is functionally available to the cell as evidenced by the conversion of radiolabeled free cholesterol to cholesteryl ester. The stimulation of free cholesterol delivery was dose-dependent up to a level of 100 μg of HDL free cholesterol/ml of extracellular medium, and was directly related to the extent of phosphatidylcholine hydrolysis. The enhanced cellular accumulation of HDL free cholesterol observed with hepatic lipase appears to be due to the phospholipase activity of this enzyme, since similar results were obtained with HDL that had been modified by snake venom phospholipase A_2. Experiments utilizing doubly labeled HDL (^{14}C-labeled free cholesterol, ^{125}I-labeled apoHDL) demonstrated that the mechanism does not involve an enhanced uptake and degradation of the entire lipoprotein particle. These results indicate that HDL exposed to hepatic lipase preferentially delivers cholesterol to cells and supports the hypothesis that hepatic lipase may modulate the delivery of cholesterol to the liver.—**Bamberger, M., J. M. Glick, and G. H. Rothblat.** Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells. *J. Lipid Res.* 1983. **24:** 869–876.

Supplementary key words phospholipase • reverse cholesterol transport • surface transfer of cholesterol • apoprotein catabolism

Hepatic lipase plays a role in lipoprotein metabolism that has yet to be determined. Located on the surface of cells, this enzyme has both phospholipase and triacylglycerol hydrolase activities (1). It has been demonstrated in vivo that blocking the activity of hepatic lipase results in an increase in the levels of plasma high density lipoprotein (HDL) phospholipid (2, 3), along with possible alterations in very low density lipoprotein and low density lipoprotein metabolism (2–4). A negative correlation has also been found between postheparin hepatic lipase activity and HDL_2 cholesterol and phospholipid levels (5). This putative link between hepatic lipase and HDL metabolism is further supported by studies in vitro that suggest that the main activity of hepatic lipase is against HDL_2 phospholipid (6, 7).

Jansen and Hulsmann (8) have proposed that hepatic lipase functions in the delivery of cholesterol to the liver or other lipase-containing tissues. In their model, cholesterol is taken up from peripheral cells by a phospholipid-rich HDL and subsequently delivered to the liver via a mechanism involving the depletion of phospholipid from HDL by hepatic lipase. This loss of phospholipid could result in the formation of a chemical gradient, with cholesterol partitioning from the lipoprotein surface to the cell membrane. This HDL cholesterol, delivered through the action of hepatic lipase, could then serve as a precursor for bile acids in liver or for steroidogenesis in adrenals and ovaries where enzymes with the characteristics of hepatic lipase have also been found (9). Using a cell culture system, we have attempted to test this model.

MATERIALS AND METHODS

Cells in culture

The Fu5AH rat hepatoma cell line was derived from the Reuber H-35 rat hepatoma (10) and has been well-characterized regarding cholesterol and cholesteryl ester metabolism (11). Stock cultures were maintained in Eagle's minimal essential medium (MEM, Flow Labs) supplemented with Basal Modified Eagle's vitamins and 5% (v/v) bovine serum (Microbiological Associates). For

Abbreviations: HDL, high density lipoprotein; FC, free (unesterified) cholesterol; EC, esterified cholesterol; TC, total cholesterol; ACAT, acyl CoA:cholesterol acyltransferase; PBS, phosphate-buffered saline; MEM, Eagle's minimal essential medium.

each experiment, cells were plated in 35-mm plastic petri dishes (Costar). After 24 hr, the medium was removed, the cells were washed with serum-free medium, and then refed with MEM containing 2.5 mg/ml delipidized bovine serum protein (12). The cells were allowed to grow to confluency at which time they were used for experimentation.

Lipoproteins

Human HDL was isolated from blood supplied by the blood bank of the Medical College of Pennsylvania. Rat HDL was isolated from pooled rat plasma. Both rat and human HDL were isolated at density 1.063 to 1.21 g/ml by density ultracentrifugation techniques (13). The lipoproteins were washed by recentrifugation at density 1.21 g/ml. Unless indicated otherwise, all experiments utilized human HDL that had been applied to a column of heparin-Sepharose CL-6B (Pharmacia) equilibrated with 50 mM NaCl, 2mM sodium phosphate buffer, pH 7.4 (14). The nonretained fraction was collected and concentrated on a PM-10 ultrafiltration membrane (Amicon). This column effectively removed all lipoproteins containing apoprotein B; however, trace amounts of a protein migrating as apoprotein E could be detected even after repeated passage of the lipoprotein preparation through the column. The purity of the lipoprotein fractions was assessed by electrophoresis on precast agarose slides (Bio-Rad) at pH 8.6 (15).

Labeling of lipoprotein lipid and apoproteins

To label the free (unesterified, (FC)) cholesterol of HDL, either [7-^{3}H(N)]cholesterol (34.6 Ci/mmol) or [4-^{14}C]cholesterol (60 mCi/mmol, New England Nuclear) was repurified by thin-layer chromatography on silica gel G plates developed in diethyl ether immediately prior to use. HDL was radiolabeled with free cholesterol by exchange from Celite (16). Analysis of the HDL cholesterol by thin-layer chromatography revealed that more than 99.5% of the label was present as free cholesterol. HDL was iodinated with ^{125}I following the iodine monochloride method of McFarlane as modified by Bilheimer, Eisenberg, and Levy (17). Specific activities ranged from 1600 to 2000 cpm/μg. Greater than 97% of the label was associated with protein and less than 3% with lipid. In some experiments HDL was delipidated and reassembled with [^{14}C]-cholesterol and ^{125}I-labeled apoprotein A-I (18) following the method of Scanu and Edelstein (19). Following reassembly, HDL of density 1.063 to 1.21 g/ml was reisolated by ultracentrifugation.

Preparation of hepatic lipase

Hepatic lipase was isolated from rat livers and purified by heparin-Sepharose chromatography following the procedure of Kuusi et al. (20). The fractions were assayed for triacylglycerol hydrolase activity using the procedure of Nilsson-Ehle and Schotz (21) except that 1 M NaCl was present and serum was omitted. Under the conditions of this assay, a unit of hepatic lipase is defined as that amount of enzyme activity required to generate 1 μmol of free fatty acid from triolein per hr at 37°C. The most active fractions were pooled and concentrated by ultrafiltration using a YM-30 membrane (Amicon). Recovery of enzyme activity from the column was approximately 35% of that applied. The purified hepatic lipase was stored at −20°C with little loss in activity.

Modification of HDL

HDL was incubated with hepatic lipase for 4 hr at 37°C. Final concentrations in the incubation mixture were: 0.9 mM HDL phospholipid, 0.15 M NaCl, 2 mM sodium phosphate, 20 mM Tris-HCl, pH 7.4, 1% glycerol (v/v), 1% bovine serum albumin (w/v) (Sigma), and 100 units/ml of hepatic lipase. The typical incubation volume was 10 ml. Incubation of HDL with hepatic lipase longer than 4 hr resulted in no additional hydrolysis of phospholipid. HDL incubated with heat-inactivated hepatic lipase (20 min, 60°C) was used as a control. In some experiments, HDL was incubated with phospholipase A_2 from *Crotalus durissus* (Boehringer Mannheim) at 37°C under the following conditions: 0.9 mM HDL phospholipid, 0.15 M NaCl, 20 mM Tris-HCl, pH 7.4, 1% bovine serum albumin (w/v), 8 mM $CaCl_2$, and 1.0 μg/ml phospholipase A_2. The reaction was stopped after 15 min with EDTA at a final concentration of 16 mM. These conditions gave similar amounts of phospholipid hydrolysis as that found with hepatic lipase. For the control, EDTA was added before addition of the enzyme.

After modification, the HDL was reisolated by ultracentrifugation at d 1.21 g/ml. A second centrifugation at this density was found necessary to avoid contamination of the HDL with albumin. Modified and control HDL were extensively dialyzed against phosphate-buffered saline (PBS), 0.15 M, pH 7.4, before being sterilized by passage through a 0.2 μm filter (Gelman). The chemical composition of the HDL was determined after sterilization.

Cell incubation and processing of cells and media

HDL was incubated with hepatoma cells at concentrations ranging from 25 to 200 μg of HDL free cholesterol/ml in MEM buffered with 27 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid, pH 7.4, containing 2% (w/v) bovine serum albumin for various lengths of time at 37°C. To determine the uptake of HDL cholesterol by cells, the medium was removed

from the cells and the monolayer was washed three times with cold PBS. Cells were harvested with trypsin, the cell pellet was washed twice with PBS and disrupted by sonication (11). Aliquots were taken for the following determinations: *1*) protein, *2*) total cell-associated radioactivity, *3*) distribution of the label between cellular esterified (EC) and free cholesterol, and *4*) cellular cholesterol mass.

In experiments utilizing HDL doubly-labeled with ^{125}I and ^{14}C, an aliquot of the incubation medium was treated with trichloroacetic acid (TCA) and phosphotungstic acid to give a final concentration of 10% and 1%, respectively. The mixture was held on ice for 30 min after which it was spun at 1,500 *g* for 20 min. An aliquot was taken for determination of TCA-soluble radioactivity. Another aliquot was taken for determination of free iodide as described by Bierman, Stein, and Stein (22).

Analytical techniques

Protein was assayed by the sodium dodecyl sulfate-Lowry method (23). Cellular lipids were extracted by the procedure of Bligh and Dyer (24). Lipoprotein lipids were extracted and washed by the procedure of Folch, Lees, and Sloane Stanley (25). Esterified and free cholesterol were separated by thin-layer chromatography on silica gel IB2 (Baker) using petroleum ether–diethyl ether–acetic acid 70:30:1. Phospholipid species were separated by thin-layer chromatography on silica gel G plates developed in chloroform–methanol 95:5 followed by a second development in chloroform–methanol–acetic acid–water 50:30:8:4 (26). Phospholipid phosphorus was determined by the method of Sokoloff and Rothblat (27). Cholesterol mass was determined by gas–liquid chromatography using coprostanol (Applied Science) as an internal standard. ^{3}H and ^{14}C were quantitated by liquid scintillation techniques in a Beckman LS7500 counter using Scintiverse (Fisher). ^{125}I was quantitated in a Beckman G-300 counter. Apoproteins were analyzed by the procedure of Sparks and Marsh (28). Statistical significance was determined by the Student's independent *t*-test. Unless indicated, determinations from replicate plates were within 10% of the mean.

RESULTS

Effect of hepatic lipase on HDL composition

It has been previously reported (29) that in vitro incubation of rat hepatic lipase with rat HDL_2 resulted in a loss of HDL phosphatidylcholine without a change in total apoprotein, free cholesterol, or esterified cholesterol levels. The results shown in **Table 1** extend this observation to human HDL. While approximately 28% of HDL phosphatidylcholine was hydrolyzed by hepatic lipase, virtually no difference was detected in total apoprotein or cholesterol levels when compared to HDL incubated with heat-inactivated hepatic lipase. Also, no change was observed in the relative amounts of the individual apoproteins when examined by scanning densitometry of polyacrylamide gels (data not shown). Analysis of both total phospholipid and phosphatidylcholine revealed that, under the conditions used in this study, a fraction (approximately 22%) of the lysophosphatidylcholine generated by the action of hepatic lipase remained on the HDL particle after reisolation.

TABLE 1. Composition of lipoproteins

	Control	Hepatic Lipase-Modified
$\frac{\text{Apoprotein}}{\text{Free cholesterol}}$ (mg/mg)	15.3 ± 1.2	14.9 ± 1.3
$\frac{\text{Esterified cholesterol}}{\text{Free cholesterol}}$ (mol/mol)	3.5 ± 0.3	3.6 ± 0.3
$\frac{\text{Free cholesterol}}{\text{Phosphatidylcholine}}$ (mol/mol)	0.31 ± 0.01	0.43 ± 0.04
$\frac{\text{Free cholesterol}}{\text{Phospholipid}}$ (mol/mol)	0.22 ± 0.003	0.26 ± 0.01

Ratios reported are the mean ± standard errors for seven preparations of control and hepatic lipase-modified human HDL.

Response of rat hepatoma cells to hepatic lipase-modified HDL

The major objective of this study was to determine whether hepatic lipase-modified HDL has an enhanced ability to deliver cholesterol to cells. The Fu5AH rat hepatoma line has two advantages for such a study. First, it has a large capacity for storage of cholesterol as cholesteryl ester, and secondly, it has a highly regulated free cholesterol pool (11). Therefore, these cells will respond to an increased uptake of cholesterol by increasing their content of cellular cholesteryl ester. Such a response is shown in **Fig. 1B.** Cells incubated with hepatic lipase-modified HDL demonstrate increased levels of esterified cholesterol mass when compared to cells incubated with control HDL. In these experiments, HDL free cholesterol was radiolabeled to allow for quantitation of its incorporation and to examine its fate within the cell. Fig. 1A demonstrates the fraction of labeled free cholesterol incorporated that was recovered as cellular esterified cholesterol. This fraction, termed % esterification, has been shown previously to be a reflection of acyl-CoA:cholesterol acyltransferase (ACAT) activity (11). As is shown, the % esterification follows a similar pattern as cellular cholesterol mass.

To determine if this cellular response was dose-dependent, an experiment was performed using various

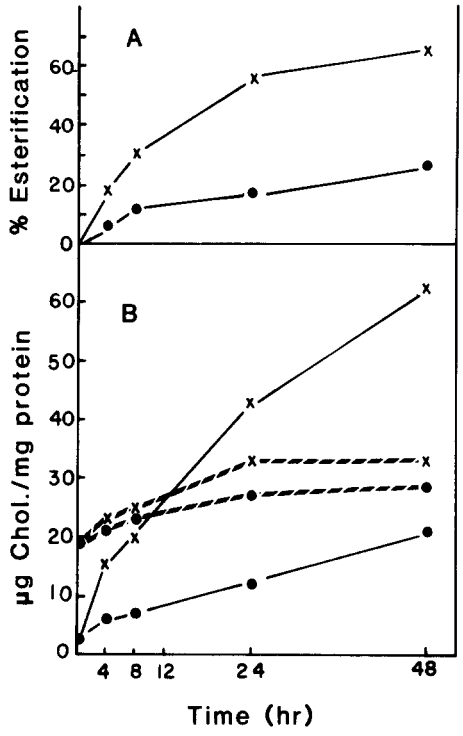

Fig. 1. Time course of the response of rat hepatoma cells to hepatic lipase-modified HDL (×) and control (●) HDL. Cells were incubated with non-heparin-Sepharose-treated HDL at 150 µg HDL-FC/ml for the times indicated after which cellular FC (– – –) and EC (——) mass (panel B) and % esterification (panel A) were determined. Percent esterification is that portion of radiolabeled HDL-FC incorporated by the cell and recovered as cellular cholesterol ester:

$$\frac{\text{cellular } {}^3\text{H-labeled EC}}{\text{total cellular } [{}^3\text{H}]\text{cholesterol}} \times 100.$$

concentrations of both control and hepatic lipase-modified HDL (**Fig. 2**). Both the cellular cholesteryl ester mass (Fig. 2B) and % esterification (Fig. 2A) were stimulated in those cells incubated with hepatic lipase-modified HDL, this stimulation occurring up to a dose of 100 µg of HDL free cholesterol/ml.

The results from several experiments comparing the incorporation of HDL free cholesterol and the cholesterol mass of cells incubated with either control or hepatic lipase-modified HDL are shown in **Table 2.** Modification of human HDL by hepatic lipase produced an 80% increase in the incorporation of HDL free cholesterol. Analysis of the distribution of the incorporated HDL free cholesterol between the cellular free and esterified cholesterol pools revealed that the major result of hepatic lipase modification was an increase in the esterification of incorporated HDL free cholesterol. The data given for cellular cholesterol mass follow a similar pattern. Comparison of both isotopic and mass data show that a large proportion of both cellular free and esterified cholesterol mass was derived from HDL free cholesterol.

The work described to this point has utilized rat hepatic lipase, human HDL, and cells of rat liver. Since it has been shown that rat and human HDL may be metabolized differently by cells in culture (30), it was of interest to determine whether similar results would be obtained from a homologous system. Hepatoma cells incubated with hepatic lipase-modified rat HDL accumulated 2 times more radiolabeled HDL free cholesterol per mg of protein after 24 hr than those cells incubated with control HDL (data not shown). These results are similar to those given for human HDL and demonstrate that the increase in cholesterol accumulation seen in cells exposed to hepatic lipase-modified human HDL is not unique to that system.

Effect of phospholipid hydrolysis

It has been proposed (8) that hepatic lipase plays a role in HDL metabolism via its ability to hydrolyze phospholipids, as opposed to triacylglycerols. If this is the case, then modification of HDL phospholipids with another phospholipase might yield results similar to those described for hepatic lipase-modified HDL. To test this hypothesis, hepatoma cells were incubated with HDL that had been modified by snake venom phospholipase A_2. As is shown in **Fig. 3,** cells incubated with phos-

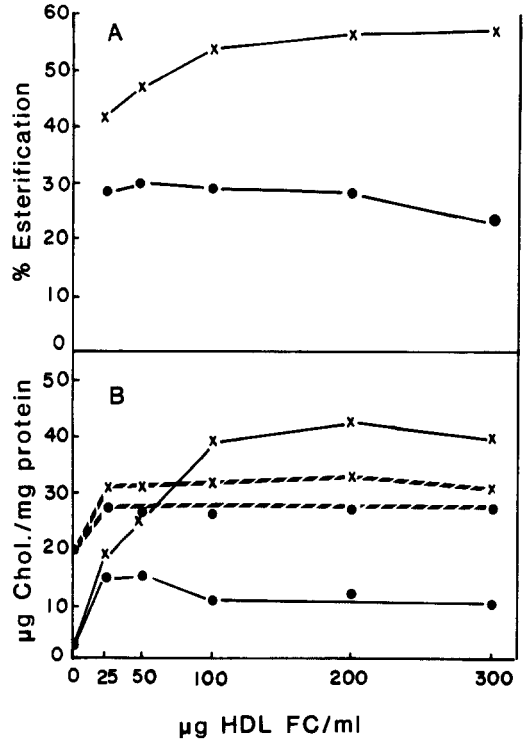

Fig. 2. Dose response of rat hepatoma cells to hepatic lipase-modified (×) and control (●) HDL. Cells were incubated for 24 hr after which cellular FC (– – –) and EC (——) mass, panel B, and % esterification, panel A, were determined. These experiments utilized HDL that had not been applied to a heparin-Sepharose column.

TABLE 2. Response of Fu5AH hepatoma cells to hepatic lipase-modified HDL

	Incorporation of HDL-FC[a]		Cellular Cholesterol Mass	
	Control	Hepatic Lipase-Modified	Control	Hepatic Lipase-Modified
Cellular total cholesterol	21.4 ± 2.0	38.5* ± 1.9	31.5 ± 2.4	50.1** ± 4.4
Cellular FC	15.9 ± 1.0	18.6 ± 1.2	23.2 ± 1.3	27.4 ± 1.5
Cellular EC	5.5 ± 1.3	19.9** ± 4.3	8.3 ± 1.6	22.7** ± 3.4
% Esterification[b]	22.8 ± 4.3	44.3** ± 3.4	25.1 ± 3.0	45.3** ± 2.9

Values are the mean ± standard error of nine experiments, expressed as μg per mg cell protein. Cells were incubated for 24 hr at 150 μg HDL-FC/ml.

[a] Based on radiolabeled cholesterol data.

[b] See legend to Fig. 1 for calculation of percent esterification.

*, $P < 0.02$; **, $P < 0.01$.

pholipase A_2-modified HDL demonstrated both an increased incorporation and increased esterification of HDL free cholesterol. These results are similar to those described for cells exposed to hepatic lipase-modified HDL and link the phospholipase activity of hepatic lipase to the stimulated uptake of HDL free cholesterol by cells. This relationship between phospholipid depletion and HDL cholesterol uptake receives additional support from the data in **Fig. 4.** Using values obtained from a series of experiments, the amount of HDL free cholesterol accumulated by cells, expressed as the % esterification, is directly related to the degree of hepatic lipase-mediated phospholipid hydrolysis.

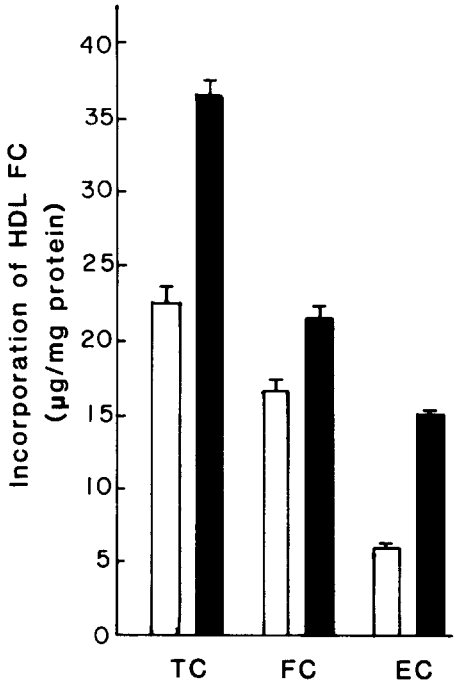

Fig. 3. Incorporation of HDL-FC from phospholipase A_2-modified HDL. Rat hepatoma cells were incubated at 150 μg HDL-FC/ml for 24 hr with phospholipase A_2 modified (■) or control (□) HDL, after which the distribution of the labeled HDL-FC among the cellular total cholesterol (TC), FC, and EC pools was determined. Incubation of HDL with phospholipase A_2 resulted in the hydrolysis of 30% of the HDL phosphatidylcholine. The data shown are representative of three experiments. Bars indicate the range of determinations from duplicate dishes.

Apoprotein catabolism

To ascertain if the increase in incorporation of HDL free cholesterol observed after treatment with either phospholipase A_2 or hepatic lipase was accompanied by an increase in apoprotein degradation, experiments were performed utilizing doubly-labeled HDL (^{14}C-labeled free cholesterol, ^{125}I-apoprotein). **Table 3** gives the results of two such experiments. When exposed to either phospholipase A_2 or hepatic lipase-modified HDL, cells accumulated 52% more HDL free cholesterol than those incubated with control HDL, yet there

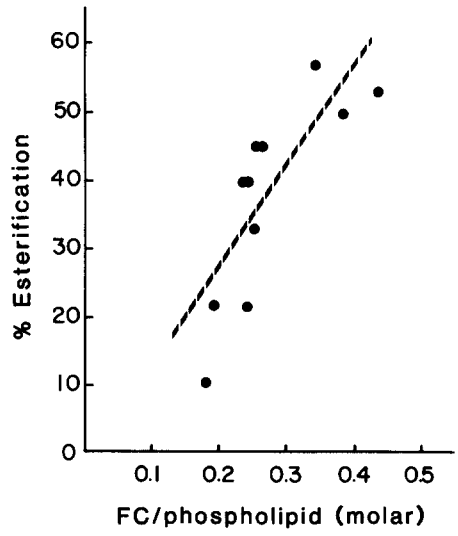

Fig. 4. Dependence of cellular % esterification upon HDL-FC to phospholipid molar ratio. Cells were incubated with hepatic lipase modified HDL or control HDL for 24 hr at 150 μg HDL-FC/ml. Percent esterification was found to be significantly correlated to the FC/total phospholiopid (●, $r = 0.80$, $P < 0.0025$). See legend to Fig. 1 for calculation of % esterification.

TABLE 3. Apoprotein catabolism of phospholipase A_2 and hepatic lipase-modified HDL by Fu5AH hepatoma cells

	Phospholipase A_2[a]		Hepatic Lipase	
	Control	Modified	Control	Modified
Apoprotein degraded	8.2 ± 0.4	7.4 ± 0.6	2.8 ± 0.3	2.8 ± 0.2
Cell-associated apoprotein	3.0 ± 0.1	3.0 ± 0.1	0.3 ± 0.01	0.3 ± 0.01
FC delivered via HDL degradation[b]	0.52 ± 0.01	0.51 ± 0.04	0.16 ± 0.04	0.16 ± 0.01
HDL-FC incorporated[c]	19.7 ± 0.2	29.8 ± 1.0	12.9 ± 1.0	19.9 ± 0.8

Values are the mean ± standard deviation expressed as μg/mg cell protein of triplicate dishes. Cells were incubated for 24 hr at 150 μg HDL-FC/ml.

[a] Experiments with phospholipase A_2 utilized reconstituted HDL, whereas those with hepatic lipase utilized radioiodinated HDL.

[b] This value is calculated from the amount of apoprotein degraded and the FC/apoprotein of the HDL.

[c] Based on radiolabeled cholesterol data.

was no increase in apoprotein degradation as judged by TCA-soluble ^{125}I in the culture medium. Also, there was no difference in cell-associated ^{125}I. Based on the ratio of HDL free cholesterol to apoprotein, it can be calculated that the degree of lipoprotein degradation, as determined by TCA-soluble ^{125}I plus the amount of cell-associated ^{125}I HDL seen in these experiments, accounts for only a small portion (<3.0%) of the free cholesterol that was incorporated by the cells. Thus, nearly all of the cholesterol uptake occurred in the absence of apoprotein degradation.

DISCUSSION

Although proposed by Glomset several years ago (31), a role for HDL in the process of "reverse cholesterol transport" has yet to be substantiated. While studies have shown that HDL promotes cholesterol efflux from cells in culture (32, 33), conflicting information exists concerning how the delivery of this cholesterol to the liver might be accomplished. Thus, while studies with perfused livers (34) and isolated hepatocytes (35) have shown that the liver can degrade HDL, other data indicate that it may not be the major site of HDL apoprotein catabolism. For example, Sigurdsson, Noel, and Havel (36) found that the catabolism of iodinated HDL by the perfused liver was approximately 7% of that obtained in intact rats. Van Tol and coworkers (37) reported that partial hepatectomy in the rat does not affect turnover rates of HDL apoproteins A and C in vivo, but decreases the removal rates of HDL phospholipid and cholesteryl ester (38). These observations suggest that HDL lipid may be taken up independently of apoprotein by the liver.

The objective of the present investigation was to examine the proposal that the delivery of HDL cholesterol to the liver is mediated by the enzyme hepatic lipase. Our studies indicate that modification of HDL by hepatic lipase results in an increased uptake of HDL cholesterol by rat hepatoma cells. This stimulation in cholesterol uptake can be demonstrated by the accumulation of both radiolabeled free cholesterol by cells and actual cellular cholesterol mass (Table 2). In either case, it is evident that ACAT activity is increased. Thus, the HDL cholesterol delivered to the cell is functionally available and does not represent surface bound lipoproteins or a one-for-one molecular exchange of free cholesterol between the lipoprotein and cell membrane. The increased accumulation of cholesterol seen with hepatic lipase-modified HDL could be the result of either an enhanced uptake and degradation of the entire lipoprotein particle or a selective removal of HDL cholesterol alone. Experiments utilizing radioiodinated HDL preclude the former possibility (Table 3). It appears, then, that there is a selective uptake of free cholesterol over protein by cells incubated with hepatic lipase-modified HDL. Such a process would be consistent with observations in both man (39) and monkey (40) that HDL free cholesterol is the major precursor of bile acids.

Since hepatic lipase has both a phospholipase and triacylglycerol hydrolase activity, it was possible that either one, or both, of these activities is required to obtain the observed stimulation in cholesterol uptake. A role for the phospholipase activity is supported by studies in which the net movement of free cholesterol between lipoproteins and erythrocyte membranes (41, 42) appeared to be related to the relative free cholesterol to phospholipid ratio between donor and acceptor. Also, previous work from this laboratory with the Fu5AH hepatoma cell line has shown a direct relationship between the uptake of HDL cholesterol and the HDL free cholesterol to phospholipid molar ratio (43). Removal of HDL phospholipids by the action of hepatic lipase would increase the free cholesterol to phospholipid

molar ratio. This, in turn, could result in a shift in the equilibrium of free cholesterol between the lipoprotein surface and the cell membrane, causing a net influx of free cholesterol to the lipase-containing tissue without corresponding apoprotein degradation. The finding that modification of HDL phospholipids by phospholipase A_2 produced similar results as that seen with hepatic lipase (Table 3, Fig. 4) support such a mechanism. Moreover, it can be demonstrated that the cellular response observed with hepatic lipase is directly related to the amount of phospholipid hydrolyzed (Fig. 4).

Our studies in vitro establish a role for hepatic lipase in the process of cholesterol delivery to cells. How such a process might occur in vivo is unclear. The hepatic lipase-mediated delivery of HDL free cholesterol to the cell could occur by either the formation of a complex between the HDL and the cell membrane (44) or by the diffusion of cholesterol through the aqueous phase (45). Our studies have investigated the delivery of HDL free cholesterol: whether other HDL lipid components are involved is not yet known. Van't Hooft, van Gent, and von Tol (38) have suggested that the lysophosphatidylcholine generated by the action of hepatic lipase promotes a fusion of HDL and hepatic membrane, resulting in a transfer of both free and esterified cholesterol plus phospholipid. In this respect, all of the modified HDL preparations used in the present study contained lysophosphatidylcholine. Whether lysophosphatidylcholine plays a role in the delivery of cholesterol, and whether it stimulates uptake of esterified cholesterol by hepatoma cells, is currently under investigation. Another mechanism is that of a "retro-endocytotic" process similar to that which has been described for LDL (46). This would involve the receptor-mediated endocytosis of hepatic lipase and HDL, followed by selective degradation of HDL lipid components with subsequent release of the 'modified' HDL from the cell surface (47). Although recognition may be apoprotein-mediated, such a mechanism might not result in apoprotein degradation, and therefore would also be consistent with the results described here.

The authors are grateful to Dr. Michael C. Phillips for valuable discussion and to Holly McCloskey for assistance in the isolation of the hepatic lipase. This study was supported by National Institutes of Health grants HL 07443, HL 22633, and HL 25938.

Manuscript received 28 September 1982 and in revised form 28 January 1983.

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