Regulation of bile acid synthesis in cultured rat hepatocytes: stimulation by apoE-rich high density lipoproteins

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Abstract Cultured rat hepatocytes obtained by liver perfusion with collagenase in the presence of soybean trypsin inhibitor were used to examine the role of high density lipoproteins (HDL) in supplying cholesterol to the hepatocyte for bile acid synthesis. Within 6 hr of adding HDL (d 1.07–1.21 g/ml) obtained from rat serum there was a significant stimulation of bile acid synthesis and secretion that reached 2-fold after 24 hr. The stimulation by HDL occurred at normal plasma concentrations (i.e., 500 μg/ml) and showed further stimulation in a dose-dependent manner reaching a maximum stimulation of 2- to 2.5-fold. The stimulation of bile acid synthesis was dependent on the cholesteryl ester content of the HDL. Several lines of evidence show that the HDL is taken up by a receptor-mediated process dependent on apoE. These include: *1*) at the same concentration (500 μg/ml) apoE-poor HDL (not retained by heparin affinity chromatography of HDL isolated from the plasma of rats fasted for 72 hr stimulated bile acid synthesis by 48%, whereas apoE-rich HDL stimulated bile acid synthesis by 110%; *2*) reductive methylation totally blocked the stimulation of bile acid synthesis by HDL; *3*) HDL_C, which contained apoE as its major protein component, also maximally stimulated bile acid synthesis; and *4*) human HDL, which contained no detectable apoE, failed to stimulate bile acid synthesis. Additional studies showed that apoE-enriched HDL and HDL_C both inhibited cholesterol synthesis (determined by the incorporation of ^3H_2O) and caused a net accumulation of cholesteryl esters in hepatocytes. These data show for the first time a direct link between hepatic receptor-mediated uptake of HDL and bile acid synthesis. Moreover, in the rat this process involves receptor recognition of apoE and is likely to be a major pathway through which cholesterol homeostasis is determined. — **Mackinnon, A. M., C. A. Drevon, T. M. Sand, and R. A. Davis.** Regulation of bile acid synthesis in cultured rat hepatocytes: stimulation by apoE-rich high density lipoproteins. *J. Lipid Res.* 1987. **28:** 847–855.

Supplementary key words hepatic cholesterol metabolism • receptor-mediated uptake

The liver is integrally involved in the removal of lipoproteins from plasma and their subsequent degradation (1, 2). Unlike most other cells, hepatic parenchymal cells have the unique ability to catabolize cholesterol into bile acids. This is the major process through which cholesterol is removed from the body and cholesterol homeostasis is maintained. The relationship between hepatic lipoprotein uptake and the regulation of bile acid synthesis is poorly understood. Previous studies using cultured rat hepatocytes show that the availability of cholesterol in hepatocytes varied in parallel with rates of bile acid synthesis (3). These data established that the hepatocyte adapts to the accumulation of cholesterol by increasing the synthesis and secretion of bile acids.

There is now accumulating evidence of the ability of the hepatocyte to take up HDL proteins and lipids (4–8). Two different processes for the uptake of HDL have been documented using different experimental approaches and models. HDL that is depleted of apoE (i.e., contains A-I as the major apolipoprotein) is taken up by a process that results in the accumulation of cholesteryl esters (as non-degradable ethers) within cells, while a greater fraction of the apoA-I is released back into the medium (7, 8). This apparent disparity of apolipoprotein and cholesteryl ester uptake has been suggested as a mechanism to account for the conversion of HDL_2 to smaller HDL_3 particles in perfused rabbit livers (9). Characterization of apoE-enriched HDL shows a process more in line with what has been

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43); SDS/PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; apo, apolipoprotein.

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described for the high affinity receptor-mediated uptake of LDL (10) and HDL_C[4] (11) by human fibroblasts. In the perfused rat livers, HDL_C (5) and apoE-rich HDL (6) are taken up by a receptor-mediated process that leads to a marked accumulation of cholesteryl esters. Moreover, apoE-rich HDL shows a greater ability to deliver cholesteryl ester to the liver than does apoA-I HDL (6).

In previous studies, we have found that following surgical removal of the liver (i.e., functional hepatectomy) cholesteryl ester and apoE accumulate in the HDL fraction of serum (12). Moreover, inhibition of LCAT abolished the increase in serum cholesteryl ester concentrations that occurs with hepatectomy (13). These studies were interpreted as supporting the hypothesis of Glomset (14) that LCAT and HDL remove cholesterol from extrahepatic cells for delivery to the liver. Moreover, this apoE-rich, cholesteryl ester-rich HDL was hypothesized to be an important source of cholesterol for the synthesis of bile acids (12, 13).

In this study we examine the role of HDL and its apolipoprotein composition on the ability to regulate bile acid synthesis. The results support the concept that, in the rat, HDL delivers cholesterol to the hepatocyte via a process dependent on receptor-mediated uptake involving apoE. Moreover, this uptake of cholesteryl ester- and apoE-containing HDL causes a quantitatively significant 2-fold stimulation of bile acid synthesis and secretion.

MATERIALS AND METHODS

Materials

Tissue culture reagents, radioisotopes, and chemical supplies were obtained from sources as described (3, 15). Affi-gel heparin was obtained from Bio-Rad Laboratories (Richmond, CA). Mevinolin was kindly supplied by A. Alberts, Merck, Inc.

Preparation of hepatocytes

Hepatocytes from male rats weighing 150–259 g were prepared as described in detail (16), with one important modification: the inclusion of soybean trypsin inhibitor (Sigma type IIS, 20 mg/150 ml) in the collagenase perfusion buffer. Several preliminary studies performed in setting up the experiments to be described showed that, without the soybean trypsin inhibitor, HDL did not stimulate bile acid synthesis.

Bile acid and lipid synthesis studies

Four hr after plating the hepatocytes in Dulbecco's Modified Eagle's medium, the medium was changed to serum-free medium with the indicated amount of lipoproteins (described in legends). Bile acid synthesis was determined by electron capture detection of hexafluoroisopropanol (carboxyl groups) and trifluoroacetic anhydride (hydroxyl groups) derivatized bile acids as described in detail (3). The incorporation of ^3H_2O by hepatocytes into lipids was determined following extraction and separation by TLC as described (15). The cellular concentration of cholesterol and cholesteryl esters was determined by GLC as described (15). Protein was determined by the method of Lowry et al. (17) using bovine serum albumin as the standard.

Isolation of serum lipoproteins

Blood was obtained from overnight-fasted rats weighing 400–475 g. In some experiments (see legends), rats were fasted for 72 hr in order to increase the serum concentration of apoE (18). The blood was centrifuged at 2000 rpm for 30 min at 4°C to obtain serum. A solution containing EDTA and NaN_3 was added to bring the concentration up to 0.02%. In some experiments (see legends) serum was then incubated overnight at either 4° or 37°C. Lipoprotein fractions were obtained by ultracentrifugation using KBr as described (19). HDL was isolated as the fraction d 1.07–1.23 g/ml. To help minimize the loss of apoE that occurs with ultracentrifugation, the HDL fraction was obtained without "washing" (i.e., further ultracentrifugation). Lipoprotein fractions were dialyzed against 0.15 M NaCl at 4°C. The apoprotein content of the lipoprotein fractions was determined by SDS/PAGE as described (20). The content of cholesterol and cholesteryl ester was determined by GLC following extraction as described (15). ApoE-rich HDL was obtained using heparin affinity chromatography as described by Weisgraber et al. (21). Canine HDL_C was a gift from Dr. David Hui, Gladstone Research Foundation laboratories.

Unless otherwise stated, values of HDL concentrations are reported in μg of protein/ml.

Statistical analysis

All values are reported as the mean ± standard deviation. Analysis of differences was determined by Student's t test using double tailed P values. P values < 0.05 were considered to be significant.

RESULTS

Preliminary experiments showed that hepatocytes obtained using soybean trypsin inhibitor in the collagenase perfusion buffer were sensitive to HDL stimulation of bile

[4]It should be noted that HDL_C is not an HDL particle by either size or density of flotation in the ultracentrifuge. It is a cholesteryl ester-rich lipoprotein particle having apoE as its major component.

acid synthesis, whereas cells prepared without this proteolytic inhibitor were unresponsive (data not shown). We, therefore, used cells prepared in the presence of soybean trypsin inhibitor to examine the effect of HDL on bile acid synthesis. As shown in **Fig. 1,** HDL added at normal plasma concentrations (500 μg/ml) stimulated the secretion of bile acids throughout the 24-hr experiment. The stimulation by HDL was observed within 6 hr (50% increase, $P < 0.05$), reaching a twofold ($P < 0.01$) stimulation after 24 hr. Since the concentration of intracellular bile acids was not significantly changed throughout the 24-hr experiment (data not shown), the accumulation of bile acids in the culture medium reflects de novo synthesis.

The degree of stimulation of bile acid synthesis by HDL increased with increasing amounts of HDL (**Fig. 2**). At 500 μg/ml of HDL (the normal plasma concentration in rats) bile acid secretion was increased almost twofold (during the 24-hr incubation). Increasing the HDL concentration to 2 times the normal plasma concentration (1000 μg/ml) caused a further 50% stimulation so that the secretion rate was 2.5 times that of control cells. HDL in concentrations above 1000 μg/ml was toxic and the cells lysed and began coming off the culture dish (data not shown).

The influence of the cholesterol composition of HDL on bile acid synthesis was examined by incubating serum overnight at 37°C to stimulate the LCAT reaction. Control serum was incubated at 4°C. As expected, incubation at 37°C for 18 hr significantly decreased the HDL free cholesterol content by 58%, and resulted in an 18% increase in the cholesteryl ester content (**Table 1**). Moreover, this cholesteryl ester-enriched HDL stimulated bile acid synthesis to a greater extent than did HDL held at 4°C (**Fig. 3**). At both normal plasma concentrations and at twice this concentration, HDL incubated at 37°C stimulated bile acid synthesis greater than did HDL incubated at 4°C. Although this difference was not great enough to have reached statistical significance ($0.5 < P < 0.1$), in three different experiments using separate HDL and hepatocyte preparations the 37°C-incubated HDL always stimulated bile acid synthesis greater than did the 4°C-incubated HDL.

HDL was subfractionated into apoE-rich and apoE-poor particles using heparin affinity chromatography (6). Elution of HDL obtained from control rats yielded unretained HDL that contained small but detectable quantities of apoE (by densitometry apoE was 2% of the total protein, lane 4, **Fig. 4**). The retained fraction contained apoE-rich HDL (by densitometry apoE was 11% of the total protein, lane 3, Fig. 4). Separation of the HDL fraction obtained from rats that were fasted for 72 hr, a condition known to stimulated apoE synthesis by liver cells resulting in a 62% increase in apoE in serum HDL (18), yielded an apoE-depleted HDL (i.e., no apoE was detected by SDS/PAGE in the unretained fraction following heparin affinity chromatography, Fig. 4). Since this unretained fraction contained little cholesteryl ester (< 10 μg/ml protein) it is likely to be composed of free apoA-IV and albumin. It was not used for the incubation studies. The

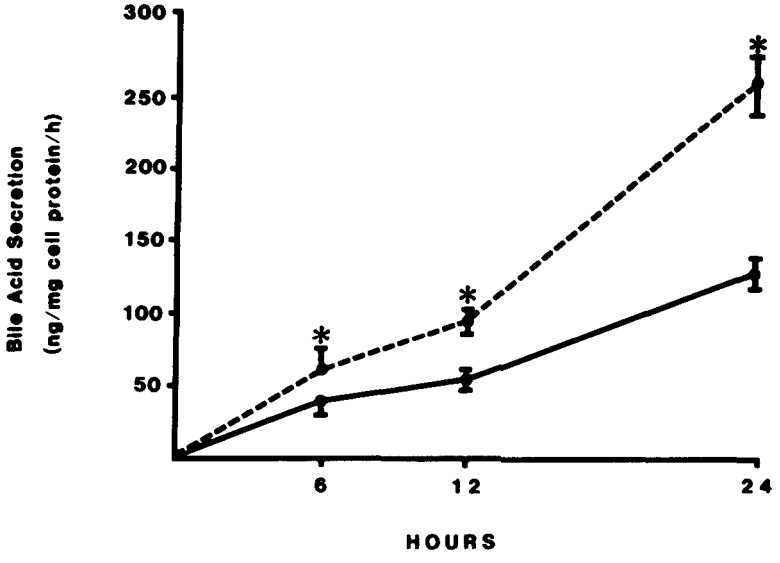

Fig. 1. Effect of HDL on bile acid secretion by cultured rat hepatocytes. Cultured rat hepatocytes, prepared as described in the Methods section, were incubated in serum-free culture medium (solid lines) or serum-free culture medium containing HDL (500 μg/ml, containing 42 μg/ml cholesteryl ester) (dashed lines) obtained from the serum of rats fasted for 72 hr. At the time indicated, medium was harvested and the concentration of bile acids was determined by GLC as described (3). Each point represents the mean ± SD of three individual plates of cells. Asterisks show significant differences caused by the HDL.

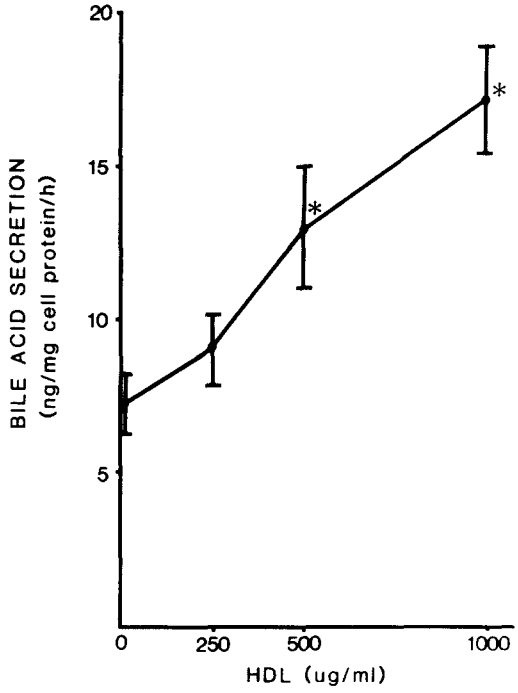

Fig. 2. Effect of increasing concentrations of HDL on bile acid secretion by cultured hepatocytes. Hepatocytes were incubated as in Fig. 1 with HDL at the indicated concentration. After 24 hr, the medium was harvested and the concentration of bile acids was determined. Each point represents the mean ± SD of three individual plates of cells. Asterisks show significant increases (from cells not incubated with HDL) in bile acid synthesis.

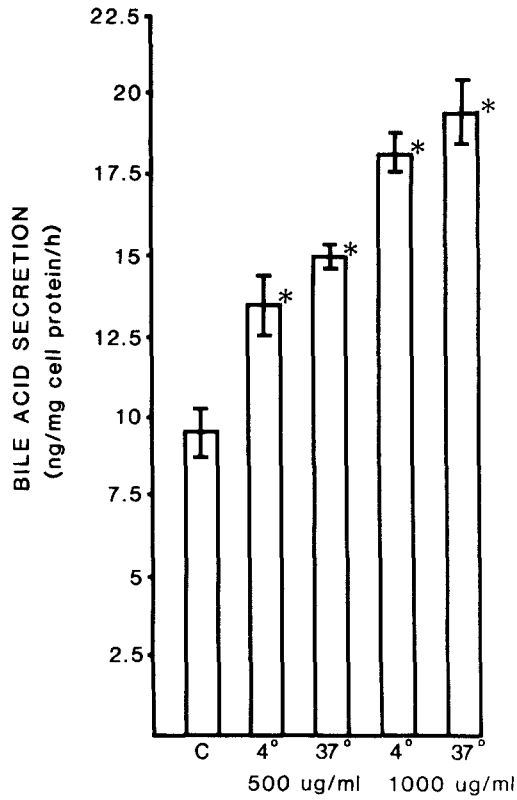

Fig. 3. Effect of LCAT-modified HDL on bile acid synthesis. HDL obtained as described in Table 1 was incubated with cultured hepatocytes for 24 hr, after which the secretion of bile acids was determined as in Fig. 1. The HDL, obtained from the serum of rats fasted for 72 hr, was incubated at 4°C for 18 hr; it contained significantly less cholesteryl ester than did HDL isolated from serum incubated at 37°C for 18 hr. Each point represents the mean ± SD of three individual plates of cells.

retained fraction contained apoE-rich HDL (by densitometry apoE was 8% of the total protein, lane 1, Fig. 4).

These different HDL preparations were added to the medium of cultured hepatocytes to examine the role of apolipoproteins in the stimulation of bile acid synthesis. At a concentration of 500 μg/ml, apoE-rich HDL maximally stimulated bile acid synthesis by 2.1-fold (**Fig. 5**). The apoE-rich HDL obtained from overnight-fasted rats exhibited the same degree of stimulation (2-fold) as the apoE-rich HDL obtained from rats fasted for 72 hr (Fig. 5). In contrast, at the same concentration (i.e., 500 μg/ml) apoE-poor HDL stimulated bile acid synthesis by only 48%. This experiment was performed a total of three different times. In all experiments, apoE-rich HDL stimulated bile acid synthesis by 80–120%, whereas the stimulation by apoE-poor HDL was less (22–48%). Since the cholesteryl ester concentrations of the apoE-rich HDL (142 μg/ml medium) were 2-fold greater than the cholesteryl ester concentrations of apoE-poor HDL (62 μg/ml medium), the role of apoE was ambiguous. To further examine the role of apoE, HDL was isolated from fresh human plasma. The density of the fraction used was 1.09–1.21 g/ml and it contained no apoE and apoA-I, and apoA-II constituted 80% of the protein as determined by SDS/PAGE. In three separate experiments, this HDL at a concentration of 500 μg protein/ml medium, containing 94 μg of cholesteryl ester/ml did not significantly increase bile acid synthesis control: 7.3 ± 0.5 ng/mg of cell protein/hr; + HDL: 6.7 ± 0.5 ng/mg of cell protein/hr. With the proviso that human apoE-free HDL interacts normally with cultured rat hepatocytes, these data suggest

TABLE 1. Effect of incubation on HDL cholesteryl ester concentrations

Incubation Temperature	Cholesterol Concentration	
	Free	Ester
	μg/mg protein	
4°C	37 ± 4	292 ± 9
37°C	16 ± 2*	346 ± 12*

Serum was incubated at either 4°C or 37°C for 18 hr, after which HDL was isolated by ultracentrifugation. Cholesterol concentrations were determined by GLC (15), while protein concentrations were determined by the method of Lowry et al. (17). Each point represents the mean ± SD of three individual determinations. Asterisks show significant differences between the 4°C and 37°C values, $P < 0.05$.

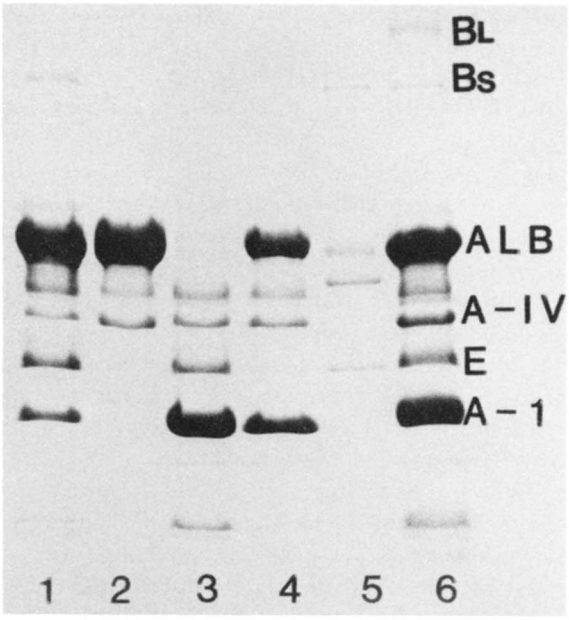

Fig. 4. SDS/PAGE analysis of apolipoproteins. HDL, obtained from rat serum via ultracentrifugation, was separated by heparin affinity chromatography. Individual fractions were then subjected to SDS/PAGE using a 2–20% linear gradient of polyacrylamide, as described (20). The Coomassie blue-stained bands corresponding in molecular weight to large molecular weight apoB (B_L), small molecular weight apoB (B_S), albumin (Alb), apoA-IV (A-IV), apoE (E), and apoA-I (A-I) are identified on the right. Lane 1, heparin affinity-retained fraction of HDL isolated from 72-hr-fasted rats; lane 2, HDL not retained by the heparin affinity column from 72-hr-fasted rats; lane 3, heparin affinity column-retained fraction of HDL isolated from overnight-fasted rats; lane 4, HDL obtained from overnight-fasted rats in the unretained fraction of the heparin affinity column; lane 5, molecular weight standards; lane 6, a total lipoprotein d < 1.21 g/ml fraction of rat serum.

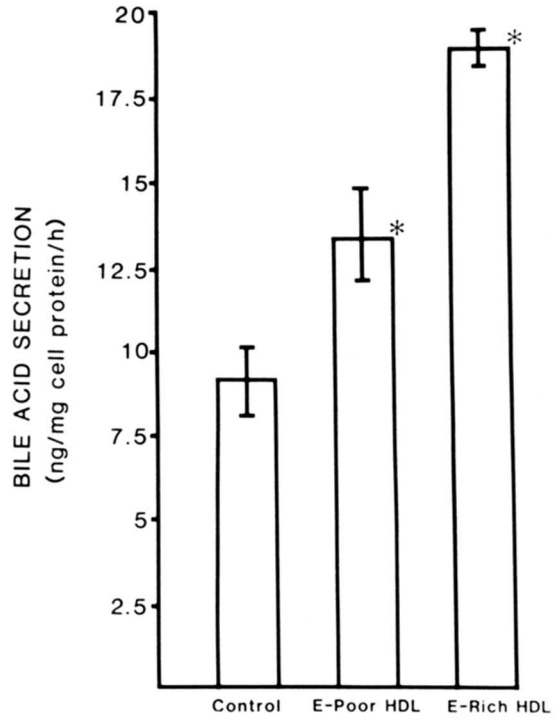

Fig. 5. Effect of different HDL fractions on bile acid synthesis. HDL fractionated by heparin affinity chromatography (see Fig. 4) was added to the serum-free medium of cultured rat hepatocytes. Bile acid synthesis was determined as in Fig. 1. The "E-rich HDL" was that obtained from the serum of rats fasted for 72 hr. Each point represents the mean ± SD of three individual plates of cells.

that apoA-I and apoA-II are not sufficient for mediating the HDL stimulation of bile acid synthesis.

Hepatocytes are known to exhibit at least two separate high affinity lipoprotein receptors: one that recognizes only apoE and one that recognizes both apoB and apoE (21, 22). Reductive methylation of lysine residues has been shown to block receptor-mediated uptake of lipoproteins both in vivo and in vitro (23). Moreover, reductive methylation has been shown by Hui et al. (21) to inhibit apoE binding to the hepatic B/E and E receptors. HDL was obtained from overnight-fasted rats (instead of 72-hr-fasted rats) in order to ensure that it contained both apoE-rich and apoE-poor HDL (see Fig. 4). This preparation of HDL (by densitometry, apoE was 6% of the total protein and it had a cholesteryl ester concentration of 42 μg/ml medium) stimulated bile acid synthesis by 80% (**Fig. 6**). In contrast, when this same HDL preparation was reductively methylated, it exhibited no stimulation of bile acid synthesis (Fig. 6). Additional studies showed that the methylation did not affect the cholesteryl ester content of the HDL (data not shown). These data show that reductive methylation blocks the ability of HDL to stimulate bile acid synthesis. To further examine the role of apoE-rich HDL, cells were incubated with canine HDL_C. Analysis of the HDL_C by SDS/PAGE showed that it contained only apoE and apoA-I in a ratio of 4:1 (data not shown). At a concentration of 200 μg of protein/ml (745 μg of cholesteryl ester), HDL_C stimulated bile acid synthesis by 2-fold (Fig. 6). Adding greater amounts of HDL_C did not further increase bile acid synthesis. It should be noted that HDL_C contained 5.2 times more cholesteryl ester than did apoE-rich HDL. Thus, the results obtained using HDL_C reflect increases in both the relative concentration of apoE as well as the amount of cholesteryl ester/unit protein.

The cellular concentration of free cholesterol was only affected by HDL_C (+12% increase) (**Fig. 7**). However, apoE-rich HDL (+31%) and HDL_C (+120%) both significantly increased the concentration of cholesteryl esters. Neither HDL nor reductively methylated HDL altered the cholesteryl ester content. The changes in cholesteryl ester concentrations were reflected by changes in cholesterol synthesis (**Fig. 8**). The incorporation of ^3H_2O into cholesterol was used to detect changes in cholesterol synthesis. This method avoids potential problems with substrate pool sizes that are associated with [^{14}C]acetate

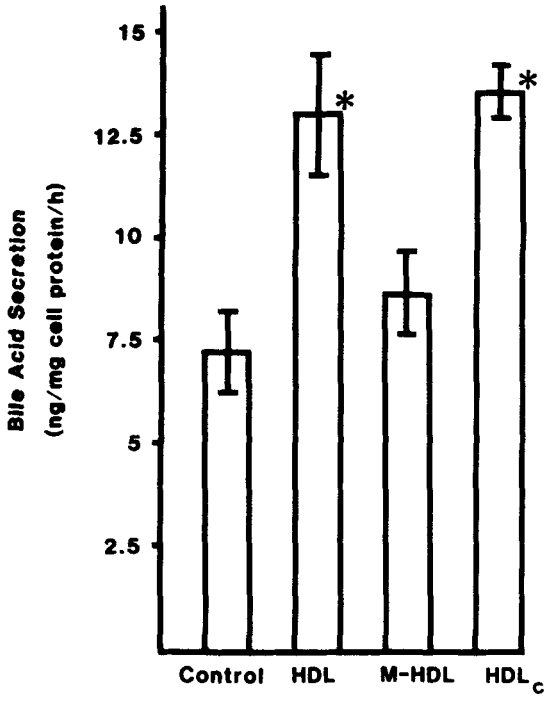

Fig. 6. Effect of HDL_C and reductively methylated HDL on bile acid synthesis. HDL (obtained from overnight-fasted rats) was reductively methylated using the procedure of Weisgraber, Innerarity, and Mahley (23). Each HDL preparation was added to the medium of cultured hepatocytes at a concentration of 500 μg/ml. The HDL_C preparation was added at a concentration of 200 μg/ml. After 24 hr, the concentration of bile acids was determined by GLC. Note that the same HDL preparation was used before and after reductive methylation. Points represent the mean ± SD of three individual plates of cells. Asterisks show significant differences from the values obtained without HDL.

(24). Both apoE-rich HDL and HDL_C significantly decreased cholesterol synthesis by 40% and 72%, respectively (Fig. 8). In contrast, neither HDL nor reductively methylated HDL significantly affected cholesterol synthesis.

DISCUSSION

Factors controlling the synthesis and secretion of bile acids are complex and not well defined. Conversion of cholesterol to bile acids is regulated, at least in part, by 7α-hydroxylase (25). Feedback inhibition of 7α-hydroxylase has been considered to be an important major regulatory mechanism (26). Data obtained from experiments using cultured hepatocytes (3, 27–29) and in vivo infusion studies (30) have challenged the view that bile acids act directly on the liver to regulate bile acid synthesis. Recent studies show that intracellular cholesterol availability (3) and microsomal free cholesterol concentrations in particular (30) are important determinants of bile acid synthetic rate. Although the mechanisms involved remain unknown, it appears that the activity of 7α-hydroxylase and rate of supply of cholesterol to the microsomes are sensitive to the amount of bile acids circulating through the gut.

Recently it was shown that the activity of 7α-hydroxylase is sensitive to agents that act directly on the microsomal membrane by a noncompetitive mechanism that may involve membrane lipid structure (31). Changes in membrane structure, perhaps mediated through lipid phase viscosity, may be the molecular mechanism through which the concentration of free cholesterol in the microsomal membrane affects the activity of 7α-hydroxylase (30).

Recently it has been shown that the liver is responsible for the removal from plasma of the majority of lipoprotein particles (1, 2). Furthermore, the majority of hepatic lipoprotein uptake is receptor-mediated (1, 2). While it has been hypothesized that hepatic receptor-mediated uptake of lipoproteins might be linked to the bile acid synthesis pathways, there is no direct evidence. The results of this study show for the first time a direct link between receptor-mediated HDL uptake and bile acid synthesis. It is likely that HDL stimulates bile acid synthesis by supplying cholesterol substrate to microsomal 7α-hydroxylase.

The finding of a finite stimulation of bile acid synthesis suggests that the cholesteryl ester contained within the HDL particle is involved in the stimulation of bile acid synthesis by HDL. The additional findings, that both the apoE-rich HDL and the HDL_C caused an accumulation of cholesteryl ester in the cells with a concomitant inhibition of cholesterol synthesis, show that the cholesteryl ester is taken up by the cells and this uptake exceeds the capacity of the hepatocyte to catabolize and/or secrete it.

Hepatic uptake of lipoproteins has been shown to be mediated through four different apolipoproteins: apoB and/or apoE (21, 22), apoA-I (8), and apoA-IV (32). Reductive methylation blocks uptake that is mediated by either apoB or apoE (21), but does not affect the uptake of cholesteryl esters mediated by apoA-I (8). Our results showing that reductive methylation of HDL blocked its ability to stimulate bile acid synthesis (Fig. 6) indicate that receptor-mediated uptake is required for HDL stimulation of bile acid synthesis and apoA-I is not involved. Our results support the findings of Dvorin et al. (32) showing that apoA-I does not participate in the uptake of HDL by cultured rat hepatocytes. Additional data that support the role of apoE (but not of apoA-I or apoA-IV) were obtained using lipoproteins containing different apolipoproteins. Since HDL_C, which contained only apoA-I and apoE, and no apoA-IV, maximally stimulates bile acid synthesis, these data suggest that apoA-IV is not essential for HDL stimulation of bile acid synthesis. To examine the apolipoprotein requirement further, rat HDL was enriched in apoE content by heparin affinity chromatography. Although apoE-rich HDL always stimulated bile acid synthesis to a greater degree than did apoE-poor (apoA-I-rich) HDL, the differ-

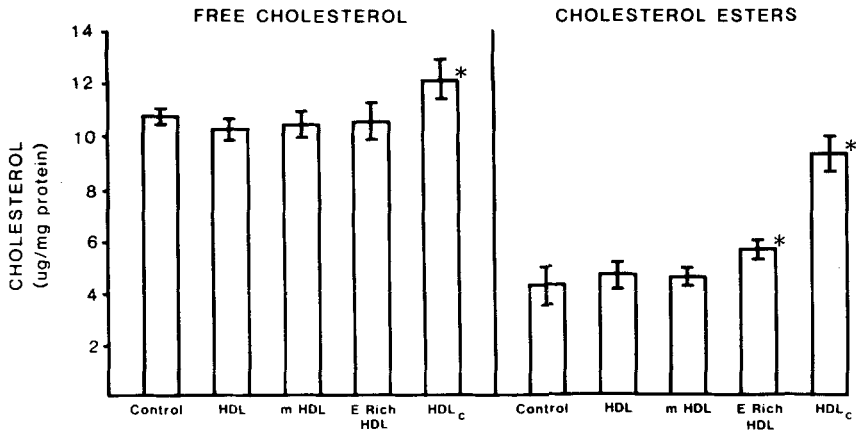

Fig. 7. Effect of HDL fractions on hepatocyte cholesterol concentration. Different HDL fractions, prepared as described in Figs. 4 and 6, were added to the medium of cultured hepatocytes at a concentration of 500 μg/ml. HDL_C was added at a concentration of 200 μg/ml. After incubating for 24 hr, the cells were harvested and the cholesterol concentrations were determined by GLC. Each point represents the mean ± SD of three individual plates of cells. Asterisks show significant differences from the values obtained in cells not incubated with HDL.

ences in cholesteryl ester content preclude definitive conclusions regarding apolipoprotein content. We were unable to obtain a rat serum HDL preparation that was completely free of apoE. However, using human serum as a source of apoE-free HDL, we found that this HDL was incapable of stimulating bile acid synthesis. In additional experiments we found that this HDL inhibited ACAT, decreased hepatocyte cholesterol concentrations, and stimulated cholesterol biosynthesis (C. A. Drevon and R. A. Davis, unpublished observations). The stimulation of hepatocyte HMG-CoA reductase by human HDL was first reported by Edwards (33). With the proviso that human apoE-free HDL behaves in a physiologic manner in the rat, we conclude that apoE, but not apoA-I, is involved in stimulating bile acid synthesis. Since apoE-rich HDL can bind both B/E and E receptors (23), it is not yet possible to conclude whether one or both of these receptors is (are) involved.

Recently, Ford et al. (34) showed that HDL_2 increased the secretion of bile acids by liver cells obtained from cholestyramine-treated rats (but not by cells from control rats). As in our earlier studies (3), Ford et al. (34) did not add soybean trypsin inhibitor to the collagenase perfusion buffer. Without this proteolytic inhibitor, Ford et al. were able to observe a stimulation of bile acid synthesis (in cells from cholestyramine-treated rats) and this might reflect a greater number of HDL receptors or proteolytically resistant receptors in the liver cells induced by the bile acid sequestrant. The expression of the B/E receptor is increased by the bile acid sequestrant cholestyramine, whereas the E receptor is not (35).

The results showing that HDL stimulates bile acid synthesis supports the concept proposed by Glomset (14) that this lipoprotein is involved in transporting extrahepatic cholesterol to the liver. Several studies have shown the ability of HDL to remove cholesterol from several different cells in culture (36, 37). Cholesterol removal from peripheral cells is likely to involve the interaction of apoA-I HDL with specific sites on the plasma membrane (38). The equilibrium of the process through which cellular cholesterol is transferred into lipoproteins is driven by LCAT both in vitro (39) and in vivo (13). In the rat, which has little or no cholesteryl ester exchange activity (40), the HDL fraction is almost exclusively loaded with cholesteryl ester as a consequence of the LCAT reaction. Our results showing that cholesteryl ester-rich, apoE-rich HDL delivers cholesterol to rat hepatocytes for subsequent

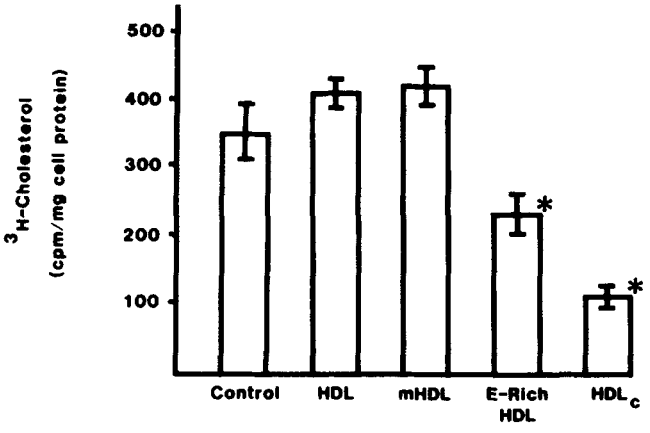

Fig. 8. Effect of HDL fraction on the incorporation of ^3H_2O into cholesterol. This experiment was performed as described in Fig. 7 except that the medium contained ^3H_2O (1 mCi/ml). After 24 hr, the cells were harvested and the formation of [^{3}H]cholesterol was determined as described (15). Each point represents the mean ± SD of three individual plates of cells. Asterisks show significant differences from the values obtained in cells not incubated with HDL.

stimulation of bile acid synthesis is consistent with this lipoprotein particle playing a major role (as outlined above) in the rat. However, in other species, e.g., humans, the plasma contains active cholesteryl ester exchange protein(s) (39–42). In these species cholesteryl ester is transferred to LDL, which may play an important role in delivering cholesteryl ester for hepatic bile acid synthesis and secretion. Because of these well-documented species specific differences, the physiologic implications of our results should be confined to the rat.

Our results show for the first time a direct link between receptor-mediated lipoprotein uptake and bile acid synthesis using cultured rat hepatocytes. These studies support previous studies (3) showing that the cultured rat hepatocyte responds to increased cholesterol availability by increasing the synthesis and secretion of bile acids. The increased cholesterol availability can be caused by increasing cholesterol synthesis (3), increasing cholesterol consumption (3), and increasing the uptake of cholesteryl ester-rich chylomicron remnants (3) and apoE-rich, cholesteryl ester-rich HDL (this study). Since cholesterol and cholesteryl ester concentrations of cultured rat hepatocytes are similar to those of fresh rat liver, the ability to stimulate bile acid synthesis by increasing HDL cholesteryl ester uptake cannot be ascribed to a cholesterol substrate deficiency caused by the culture conditions. We propose that bile acid synthesis and excretion pathways act via sensing the availability of cholesterol within the hepatocyte. By compensatory changes in bile acid synthesis, the hepatocyte maintains cholesterol homeostasis. It is likely that changes in both the availability of microsomal free cholesterol and the activity of 7α-hydroxylase both contribute to the adaptive response of the hepatocyte to plasma lipoproteins. The molecular mechanisms through which these changes occur remain to be elucidated.

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REFERENCES

1. Steinberg, D. 1983. Lipoproteins and atherosclerosis. A look back and a look ahead. *Arteriosclerosis.* **3:** 283–301.
2. Cooper, A. D. 1985. Role of the liver in the degradation of lipoproteins. *Gastroenterology.* **88:** 192–205.
3. Davis, R. A., P. M. Hyde, J. C. Kuan, M. M. McNeal, and J. A. Schexnayder. 1983. Bile acid secretion by cultured rat hepatocytes. *J. Biol. Chem.* **258:** 3661–3667.
4. Drevon, C. A., T. Berg, and K. R. Norum. 1977. Uptake and degradation of cholesterol ester-labelled rat plasma lipoproteins in purified rat hepatocytes and nonparenchymal liver cells. *Biochim. Biophys. Acta.* **487:** 122–136.
5. Sherrill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of the canine lipoproteins containing only the E apolipoprotein by a high affinity receptor. *J. Biol. Chem.* **255:** 1804–1807.
6. Quarfordt, S., J. Hanks, R. S. Jones, and F. Shelburne. 1980. The uptake of high density lipoprotein cholesteryl ester in the perfused rat liver. *J. Biol. Chem.* **255:** 2934–2937.
7. Stein, Y., O. Stein, and G. Halperin. 1982. Use of ^{3}H-cholesteryl linoleyl ether for the quantitation of plasma cholesteryl ester influx into the aortic wall in hypercholesterolemic rabbits. *Arteriosclerosis.* **2:** 281–289.
8. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* **260:** 744–750.
9. MacKinnon, M., J. Savage, R. Wishart, and P. Barter. 1986. Metabolism of high density lipoproteins by the perfused rabbit liver. *J. Biol. Chem.* **261:** 2548–2552.
10. Brown, M. S., P. T. Kovanen, and J. L. Goldstein. 1981. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212:** 628–635.
11. Bersot, T. P., R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1976. Interaction of swine lipoproteins with the low density lipoprotein receptor in human fibroblasts. *J. Biol. Chem.* **251:** 2395–2398.
12. Davis, R. A., A. D. Hartman, L. Dory, B. J. Ven Lenten, and P. S. Roheim. 1981. Metabolic fate of VLDL apolipoproteins B and E in hepatectomized rate. *Biochim. Biophys. Acta.* **665:** 154–164.
13. Davis, R. A., P. Helgerud, S. Dueland, and C. A. Drevon. 1982. Evidence that reverse cholesterol transport occurs in vivo and requires lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta.* **689:** 410–414.
14. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
15. Davis, R. A., M. M. McNeal, and R. L. Moses. 1982. Intrahepatic assembly of very low density lipoprotein. Competition by cholesterol esters for the hydrophobic core. *J. Biol. Chem.* **257:** 2634–2640.
16. Davis, R. A. 1986. Lipoprotein synthesis and secretion in cultured rat hepatocytes. *Methods Enzymol.* **129:** 272–282.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
18. Davis, R. A., J. R. Boogaerts, R. A. Borchardt, M. M. McNeal, and J. A. Schexnayder. 1985. Intrahepatic assembly of very low density lipoproteins. Varied synthetic response of individual apolipoproteins to fasting. *J. Biol. Chem.* **260:** 14137–14144.
19. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
20. Davis, R. A., G. M. Clinton, R. A. Borchardt, M. M. McNeal, T. Tan, and G. R. Lattier. 1984. Intrahepatic assembly of very low density lipoproteins. Phosphorylation of small molecular weight apolipoprotein B. *J. Biol. Chem.* **259:** 3383–3386.
21. Hui, D. Y., W. J. Brecht, E. A. Hall, G. Friedman, T. L. Innerarity, and R. W. Mahley. 1986. Isolation and characterization of the apolipoprotein E receptor from canine and human liver. *J. Biol. Chem.* **261:** 4256–4267.

22. Innerarity, T. L., D. Y. Hui, and R. W. Mahley. 1982. Hepatic apoprotein E receptors. *In* Lipoproteins and Coronary Atherosclerosis. G. Noseda, C. Fragiacomo, R. Fumagalli, and R. Padetti, editors. Elsevier Biomedical Press, Amsterdam. 173–181.
23. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053–9062.
24. Turley, S. D., J. M. Andersen, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
25. Myant, N. B. and K. A. Mitropoulis. 1977. Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18:** 135–153.
26. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.
27. Botham, K. M., M. E. Lawson, G. J. Beckett, I. W. Percy-Robb, and G. S. Boyd. 1980. The effect of portal blood bile salt concentrations on bile salt synthesis in rat liver. Studies with isolated hepatocytes. *Biochim. Biophys. Acta.* **666:** 238–245.
28. Davis, R. A., W. E. Highsmith, M. M. McNeal, J. A. Schexnayder, and J. C. Kuan. 1983. Bile acid synthesis by cultured hepatocytes. Inhibition by mevinolin, but not by bile acids. *J. Biol. Chem.* **258:** 4079–4082.
29. Kubaska, W. M., E. C. Gurley, P. B. Hylemon, P. S. Guzelian, and Z. R. Vlahcevic. 1985. Absence of negative feedback control of bile acid biosynthesis in cultured rat hepatocytes. *J. Biol. Chem.* **260:** 13459–13463.
30. Davis, R. A., C. A. Musso, and G. R. Lattier. 1985. Regulation of bile acid synthesis by microsomal free cholesterol. *In* Enterohepatic Circulation of Bile Acids and Sterol Metabolism. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press Ltd., Lancaster, England. 37–45.
31. Davis, R. A., T. S. Elliott, G. R. Lattier, R. B. Showalter, and F. Kern. 1986. Regulation of bile acid synthesis via direct effects on the microsomal membrane. *Biochemistry.* **25:** 1632–1636.
32. Dvorin, E., N. L. Gorder, D. M. Benson, and A. M. Gotto. 1986. Apolipoprotein A-IV. A determinant for binding and uptake of high density lipoproteins by rat hepatocytes. *J. Biol. Chem.* **261:** 15714–15718.
33. Edwards, P. A. 1975. Effect of plasma lipoproteins and lecithin dispersions on the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase of isolated rat hepatocytes. *Biochim. Biophys. Acta.* **409:** 39–50.
34. Ford, R. P., K. M. Botham, K. E. Suckling, and G. S. Boyd. 1985. The effect of a rat plasma high-density lipoprotein subfractions on the synthesis of bile salts by rat hepatocyte monolayers. *FEBS Lett.* **179:** 177–180.
35. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes. Metabolically distinct apo-E and apo-B, E receptors. *J. Biol. Chem.* **256:** 5646–5655.
36. Bates, S. R., and G. H. Rothblat. 1974. Regulation of cellular sterol flux and synthesis by human serum lipoproteins. *Biochim. Biophys. Acta.* **360:** 38–55.
37. Stein, O., J. Vanderhock, and Y. Stein. 1976. Cholesterol content and sterol synthesis in human skin fibroblasts and rat aortic smooth muscle cells exposed to lipoprotein-depleted serum and high density apolipoprotein/phospholipid mixtures. *Biochim. Biophys. Acta.* **431:** 347–358.
38. Oram, J. F., E. A. Brinton, and E. L. Bierman. 1983. Regulation of high density lipoprotein receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. *J. Clin. Invest.* **72:** 1611–1621.
39. Fielding, C. J., and P. E. Fielding. 1981. Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin: cholesterol acyltransferase. *Proc. Natl. Acad. Sci. USA.* **78:** 3911–3914.
40. Barter, P. J., and J. I. Lally. 1978. The activity of an esterified cholesterol transferring factor in human and rat serum. *Biochim. Biophys. Acta.* **531:** 233–236.
41. Pattnaik, N. M., A. Montex, L. B. Hughes, and D. B. Zilversmit. 1978. Cholesteryl ester exchange protein in human plasma. Isolation and characterization. *Biochim. Biophys. Acta.* **530:** 428–438.
42. Fielding, P. E., and C. J. Fielding. 1980. A cholesteryl ester transfer complex in human plasma. *Proc. Natl. Acad. Sci. USA.* **77:** 3327–3330.