High Density Lipoproteins, But Not Other Lipoproteins, Provide a Vehicle for Sterol Transport to Bile

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Abstract

Unesterified cholesterol (UC) that is taken up by the liver from lipoproteins is rapidly mixed by exchange with liver UC. Thus, it is not possible to quantitate the transport of UC from different lipoproteins into bile using radiolabeled UC. However, plant sterols do not exchange with UC and are secreted in bile with the same kinetics as UC. To compare the contribution to bile of sterols from different lipoproteins, we perfused isolated rat livers with VLDL, LDL, and HDL that were obtained from patients with hereditary phytosterolemia and were rich in plant sterols. After 30-min recirculating perfusions, hepatic concentrations of plant sterols were not different after different lipoproteins were perfused. However, biliary plant sterol secretion was markedly different: with the perfusion of either VLDL or LDL there was no increase in plant sterols in bile, but with perfusion of HDL, the secretion of plant sterols was increased two- to threefold ($P = 0.0005$). The increase in biliary plant sterols was detected 5–10 min after HDL was added to perfusates and was similarly large for each of three individual plant sterols that was tracked. Results show that when sterol transport from lipoproteins into bile can be determined, only HDL provides a vehicle for UC elimination in bile that is consistent with its putative function in reverse cholesterol transport. (*J. Clin. Invest.* 1997. 99:380–384.) Key words: cholesterol • plant sterols • phytosterolemia • sitosterolemia • cholesterol exchange

Introduction

A major excretory route for cholesterol from the body is through the bile as unesterified cholesterol (UC).[1] Biliary UC originates predominantly from a preformed UC pool in the liver (1, 2) that may be replenished by cholesterol delivered to the liver by a variety of plasma lipoproteins. Studies performed with radiolabeled UC in humans (3) as well as in laboratory animals (4, 5) have indicated that plasma HDL rather than VLDL/LDL preferentially provides UC to the liver for secretion into bile. However, the interpretation of these studies is complicated by UC exchange, a process that results in the redistribution of radiolabeled UC by mixing with nonradiolabeled UC but does not represent UC mass transport. It has been demonstrated that UC on the surface of plasma lipoproteins rapidly equilibrates by exchange with UC in a variety of animal tissues (for review see reference 6) and, most particularly, with UC in the liver (7). Exchange might be especially relevant as a confounding variable when the uptake by the liver of radiolabeled UC from different lipoproteins is compared since (*a*) lipoproteins of different size with a different surface area might be expected to exchange UC at different rates and (*b*) the radiolabeled UC from lipoproteins that is exchanged with UC in the liver is mixed with and diluted by a much larger pool of nonradiolabeled UC that is already in the liver.

To avoid UC exchange in studies of sterol transport from lipid particles into bile, we have used an unesterified plant sterol, sitostanol, as an analogue of UC. We have reported previously (8) that sitostanol does not exchange with UC and, when added to a perfused rat liver in liposomes, is secreted in bile with the same kinetics and in direct proportion to UC. Since plant sterols are ordinarily present in the liver in extremely low amounts (for reviews see references 9 and 10), plant sterols that are taken up by the liver from a lipid particle will not be diluted by exchange with endogenous stores of these sterols. We have thus undertaken these studies with native human lipoproteins that have a relatively high content of plant sterols with the purpose of comparing the transport into bile of plant sterols from different lipoproteins.

Methods

Plasma lipoproteins that contain plant sterols were isolated from five patients with a known diagnosis of phytosterolemia (sitosterolemia). Four of the five patients were currently being treated to reduce the levels of plant sterols in their blood. Three patients from a single family (identified as KC, TC, and KCN) have been treated with ileal bypass surgery. One of the two other patients who are unrelated (identified as TK and MAPA) regularly takes a bile acid-binding resin. VLDL, LDL, and HDL were separated by flotation from EDTA plasma by sequential ultracentrifugation at 10°C at densities of 1.006, 1.06, and 1.21 g/ml, respectively. Lipoproteins were dialyzed for 24 h to remove KBr before use.

Each of the three lipoprotein fractions that were isolated from each plasma sample was used to perfuse an isolated rat liver with a bile duct cannula, as described previously (11). Donor livers were

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1. *Abbreviation used in this paper:* UC, unesterified cholesterol.

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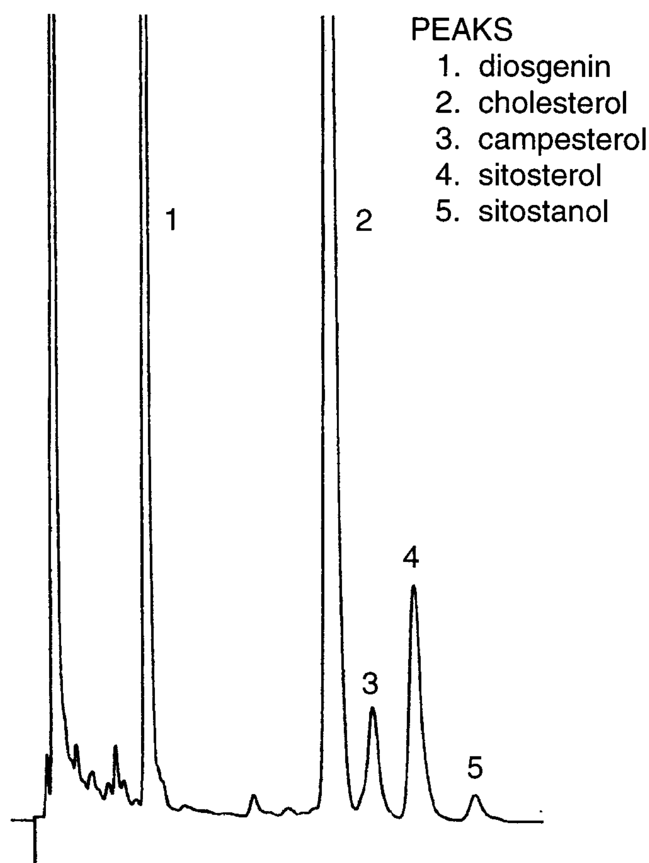

Figure 1. HPLC separation of sterols obtained from plasma LDL from a patient with hereditary phytosterolemia. Sterols were benzoylated to be detected at 230 nm. Conditions of chromatography are described in Methods. Diosgenin was added as an internal standard to quantitate individual sterol amounts by integration of peak areas.

taken from male Sprague-Dawley rats (Taconic Farms, Germantown, NY) that had been fed Purina chow. Briefly, rat livers were perfused through the portal vein with a recirculating Krebs-Ringer bicarbonate buffer (pH 7.4) that contained 3% bovine serum albumin and 25 mM glucose. Throughout perfusions, the bile salt, Na taurocholate (A grade; Sigma Chemical Co., St. Louis, MO), was infused directly into the portal vein at 40 μmol/h. Lipoproteins were added as bolus to the perfusate reservoir at the outset of perfusions that were continued for at least 30 min. All bile was collected during perfusions. At the conclusion of perfusions, livers were perfused free of media and taken for analysis.

Unesterified sterols were isolated by HPLC from lipid extracts of bile, perfusate lipoproteins, and liver, as described previously (12). Unesterified sterols were benzoylated (13) and separated on an Ultrasphere ODS column (5 μm, 4.6 mm × 25 cm) with a mobile phase of methanol at a flow of 0.5 ml/min. Detection was at 230 nm and quantitation was by integration of peak areas in conjunction with an internal standard of diosgenin (98% pure; Sigma Chemical Co.). Amounts of esterified sterols were separately determined in lipoproteins and liver by saponification of each sample and subtracting the amount before saponification (the unesterified sterols) from the amount after saponification (the total sterols). Bile acids, phospholipids, and the molecular species of phosphatidylcholines were determined as previously reported (14, 15).

Results

The HPLC system that was used permitted separation of cholesterol from three plant sterols that were found in all lipoprotein fractions from each patient (Fig. 1). These sterols were present in variable amounts in different patients. However, when LDL and HDL were perfused, sterols were added to perfusates in generally similar amounts and the percentages of unesterified sterols in each lipoprotein fraction were generally similar (Table I). In contrast, the amounts of VLDL obtained from patients were much less than LDL and HDL. Consequently, when VLDL was perfused, the amounts of all sterols added to perfusions were significantly less than when either LDL or HDL was used. The percentages of unesterified sterols were consistently higher in VLDL than in either LDL or HDL.

At the conclusion of perfusions the amounts of plant sterols in the liver were determined and were not significantly different (with one exception), comparing livers that were perfused with VLDL, LDL, and HDL (Table II). The plant sterol content of the liver was far less than the content of cholesterol, averaging ∼ 3.0% of the total hepatic sterols.

The secretion in bile of plant sterols was markedly different with the perfusion of different lipoproteins (Fig. 2). With perfusion of either VLDL or LDL there was no increase in the secretion of plant sterols in bile. In contrast, with perfusion of HDL there was at least a threefold increase in the secretion into bile of each of the plant sterols administered. With HDL perfusion, an increase in all plant sterols was similarly detected in bile as early as 5–10 min after perfusions were begun and all plant sterols were similarly secreted in bile at a constant rate

Table I. Sterol Composition of Lipoproteins from Patients with Phytosterolemia That Were Added to Rat Liver Perfusions

Lipoprotein		Cholesterol	(unesterified)	Campesterol	(unesterified)	Sitosterol	(unesterified)	Sitostanol	(unesterified)
	n	*μmol*	%	*μmol*	%	*μmol*	%	*μmol*	%
VLDL	(3)	13.8±14.3	(50.9±6.5)	0.73±0.81	(52.4±3.8)	1.25±1.33	(45.4±8.2)	0.14±0.23	(38.7)
LDL	(4)	33.9±8.0	(26.2±2.2)	2.00±0.57	(32.8±6.8)	3.81±1.00	(31.6±6.9)	0.42±0.24	(32.1±3.9)
HDL	(5)	35.0±7.6	(17.5±4.7)	2.17±0.48	(23.5±6.4)	4.82±0.63	(21.4±7.5)	0.76±0.48	(15.9±12.5)
$P < 0.05$		a	b	a	a	a	a	a	

Amounts of sterols in lipoproteins added to isolated rat liver perfusions are shown as mean±SD. Lipoproteins were isolated from the plasma of five patients with phytosterolemia (see Methods). The percentages of lipoprotein-sterols that were unesterified are shown in parentheses. One perfusion with VLDL was performed with a mixture of VLDL from two patients to obtain an increased amount of plant sterols. In an additional case, VLDL was not perfused because of too little sample. One perfusion with LDL was unsuccessful. By ANOVA, $P < 0.05$ for (*a*) VLDL vs. LDL, VLDL vs. HDL; and (*b*) VLDL vs. LDL, VLDL vs. HDL, LDL vs. HDL.

Table II. Sterol Content of Livers That Were Perfused with Lipoproteins from Patients with Phytosterolemia

	Cholesterol	Campesterol	Sitosterol	Sitostanol	Plant sterols/ total sterols
	μmol	*μmol*	*μmol*	*μmol*	*μmol/μmol*
VLDL	58.9±2.4	0.72±0.14	0.66±0.07	0.08±0.08	0.024±0.002
LDL	65.4±6.3	0.86±0.11	1.05±0.41	0.06±0.05	0.030±0.008
HDL	63.1±12.2	0.85±0.13	0.93±0.17	0.05±0.05	0.029±0.006

Amounts of sterols in perfused livers are shown as mean±SD for the total liver. Lipoproteins were isolated from the plasma of five patients with phytosterolemia and perfused for 30 min (see Methods). By ANOVA, $P < 0.05$ for only sitostanol, comparing VLDL with LDL.

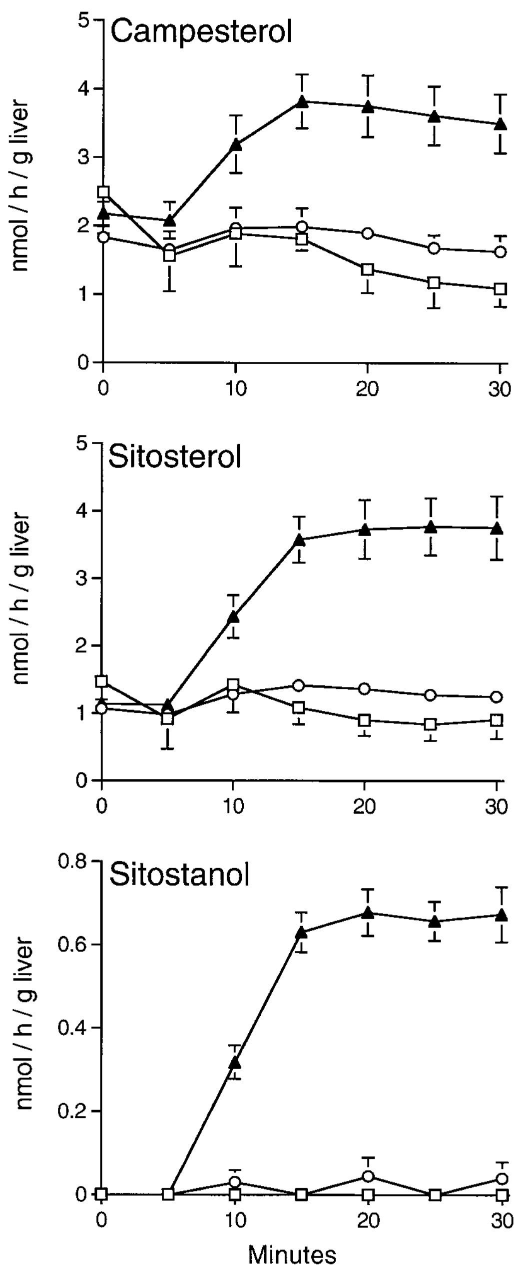

Figure 2. Biliary secretion of plant sterols by isolated rat livers perfused with VLDL (*open boxes*), LDL (*open circles*), or HDL (*filled triangles*), obtained from patients with phytosterolemias. Lipoproteins were added to recirculating perfusions at 0 min and perfusions were continued for 30 min. Results are shown as mean±SEM for the perfusions identified in Table I. From 10 to 30 min, biliary campesterol, sitosterol, and sitostanol secretion were significantly greater when HDL was perfused than when either VLDL or LDL was perfused (by ANOVA, $P < 0.05$).

after 15 min of perfusion. There were no significant differences in cholesterol secretion in bile with the perfusion of different lipoproteins (data not shown) and, consequently, the percentage change in the composition of biliary sterols paralleled the change in plant sterol mass. Thus, with perfusion of HDL from 0 to 30 min, plant sterols increased from 1.9±0.4 (SD) to 5.1±0.8% of the total bile sterols, while with LDL and VLDL perfusions the percentages of plant sterols remained stable, averaging (for VLDL and LDL perfusions combined) 2.4±0.8% of the total bile sterols at 0 min and 2.6±0.8% at 30 min. More prolonged liver perfusion (using lipoproteins from one patient) did not result in a change in this pattern of plant sterol secretion that for all three lipoprotein fractions remained constant from 30 to 120 min (data not shown).

Biliary UC secretion is known to be dependent on the coordinated secretion of phospholipids and bile acids. In perfusions with HDL, compared with perfusions with either VLDL or LDL, we found no significant differences in either biliary phospholipids or bile acid secretion. [Bile acid and phospholipid secretion were 3.6±0.4 (SD) and 0.63±0.12 μmol/h/g liver, respectively, when HDL was perfused and 3.1±0.6 and 0.48±0.18 μmol/h/g liver, respectively, when VLDL/LDL were perfused.] To ensure that the greater biliary plant sterol secretion with HDL compared with VLDL/LDL was not due to direct passage of intact HDL from the perfusate into bile, we measured the molecular species composition of biliary phosphatidylcholines before and after a perfusion with LDL and HDL. The pattern of phosphatidylcholines in bile was distinctly different than the pattern of phosphatidylcholines in either HDL or LDL and was not changed when either lipoprotein was perfused (data not shown).

Discussion

We undertook these studies to determine if there were differences in the transport of unesterified sterols from different kinds of lipoproteins into bile. To avoid the possibility of UC exchange between lipoproteins and the liver and between different UC pools within the liver, we have used plant sterols as a surrogate for UC. We have demonstrated previously that the

kinetics of secretion in bile of UC and the plant sterol, sitostanol, are the same (8). Although we found no difference in liver plant sterol amounts after isolated liver perfusion with human VLDL, LDL, and HDL that contained comparably high amounts of plant sterols, we demonstrated a clear difference in the secretion of plant sterols from these lipoproteins into bile. Only with perfusion of HDL did biliary plant sterol secretion substantially increase. Furthermore, with HDL, the increase in biliary plant sterol secretion was detected within 5–10 min after HDL was added to perfusions. This relatively rapid rate of plant sterol transport from HDL is consistent with the time of appearance in bile of radiolabeled UC after the intravenous injection of lipoproteins with radiolabeled UC in the intact rat (16) and may approximate the rate of transport of small water-soluble molecules from the blood into bile by a paracellular route (17).

The mechanism of delivery and route of transit of cholesterol from the blood through the liver into bile has not been defined with any certainty. On the basis of much higher specific activities of radiolabeled UC in liver canalicular membranes than in the interior membranes of the hepatocyte soon after the administration of lipoproteins radiolabeled with UC, we have suggested (16) that lipoprotein UC might be transported into bile by lateral diffusion through the plasma membranes of the hepatocyte. This route of transport would not only be consistent with the rapid transport of sterols into bile, but also with the preferential delivery of sterols from HDL to bile. HDL, in contrast to VLDL/LDL, is not taken up by the liver by endocytosis as an intact particle but is dissociated at the surface of the hepatocyte. Many studies have demonstrated that the apoproteins and different lipid components of HDL are separately metabolized and/or transported upon first contact of HDL with the liver (18–21). This initial contact of HDL with the liver will result, in particular, in the hydrolysis of HDL-phosphatidylcholines, mediated by hepatic lipase on the sinusoidal surface (22, 23). Any reduction of HDL-phosphatidylcholines would be anticipated to result in a decrease in the solubilization of unesterified sterol on the HDL particle surface (24, 25) that, in turn, will promote an increased efflux of unesterified sterol from HDL to the liver (24–26). The absence of any increase in biliary plant sterol secretion with the perfusion of VLDL or LDL is further consistent with a mechanism of biliary sterol secretion that requires that a lipoprotein be catabolized at the cell surface of the hepatocyte to generate new unesterified sterol for direct delivery into bile by a plasma membrane route.

In these studies, the amounts of plant sterols that were added to perfusions and transported into bile relative to cholesterol were relatively small and the difference in plant sterol output in bile between HDL and VLDL/LDL perfusions did not result in any significant differences in the total amount of sterols that were secreted in bile. In a previous study (8), perfusing five- to sixfold greater amounts of a plant sterol in liposomes, we obtained a proportionately greater output of the plant sterol in bile than with HDL perfusion (∼ 40 nmol/h/g liver compared to ∼ 8 nmol/h/g liver when HDL was perfused). Furthermore, when administered in a larger amount in liposomes, plant sterols comprised a larger proportion of the sterols in bile (∼ 25–30% of the total sterols) (8).

Neither we nor any other group has determined the maximum capacity for biliary secretion of sterols that are taken up by the liver from the blood (or an analogous perfusate). That is, at this time, we do not know the capacity for transport into bile of sterols that are newly delivered to the liver compared with the sterols that are already in the liver. This might be accomplished by a formal balance study using reconstituted HDL particles to deliver different amounts of a plant sterol to the liver. Furthermore, using reconstituted HDL, it might be possible to define the specific characteristics of a transport vehicle that could optimally deliver sterols to the liver for biliary secretion.

High HDL-cholesterol seemingly protects against cardiovascular disease and this is generally attributed to the special role of HDL in delivering peripheral cholesterol to the liver for excretion from the body. Our finding that the transport of plant sterols into bile takes place only from HDL is entirely consistent with this putative role of HDL in reverse cholesterol transport (for review see reference 27). However, although there is a large literature demonstrating the capacity of HDL (or HDL-like particles) to remove cholesterol from peripheral sites (for reviews see references 27 and 28), there are relatively few studies that have focused on the delivery of HDL-cholesterol to the liver for biliary secretion. All of the studies that have been reported previously (5, 29–34) have determined the transport into bile of cholesterol from HDL particles and other lipoproteins that have been manipulated in a variety of ways to contain radiolabeled UC or both radiolabeled UC and radiolabeled cholesteryl esters. These studies (conducted in both humans and in animals) have uniformly found that the amount of radiolabeled cholesterol that is secreted in bile is extremely small when VLDL/LDL (29–32) as well as HDL (5, 32–34) are used as donor particles. As we have contended previously (35), these findings are consistent with extensive exchange and mixing of radiolabeled UC from donor lipoproteins with endogenous nonradiolabeled lipoproteins and a large amount of UC that is ordinarily present in the liver. This study may be the first demonstration of sterol transport from native lipoproteins showing that only sterols derived from HDL are directly secreted into bile.

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