The effect of plasma low density lipoprotein apheresis on the hepatic secretion of biliary lipids in humans

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Abstract

Background—The liver is a key organ in the metabolism of cholesterol in humans. It is the only organ by which substantial amounts of cholesterol are excreted from the body, either directly as free cholesterol into the bile or after conversion to bile acids. The major part of cholesterol synthesis in the body occurs in the liver. Cholesterol is also taken up by the liver from plasma lipoproteins. The relative contributions of newly synthesised cholesterol and plasma lipoprotein cholesterol to bile acid synthesis and biliary cholesterol secretion, respectively, are not known in detail.

Aims—To determine how a rapid lowering of plasma low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol influences the biliary secretion rates of cholesterol and bile acids in patients with cholesterol gallstones and complete biliary drainage. In this model with a completely interrupted enterohepatic circulation, the secretion of bile acids equals the new synthesis of bile acids in the liver.

Patients—Eight patients with common bile duct stones of cholesterol type undergoing conventional cholecystectomy and choledocholithotomy.

Methods—At operation a balloon occludable Foley catheter attached to a T tube was inserted into the bile duct with the balloon placed just past the distal limb of the T tube. The T tube was allowed to drain the bile externally. One week after the operation the Foley catheter balloon was inflated, creating complete biliary drainage. Twelve hours following the inflation plasma LDL apheresis was carried out for two hours. Bile was collected for 15 minute periods starting one hour before the apheresis and ending two hours after its termination. During the collection of bile, plasma lipids were analysed on several occasions.

Results—The plasma level of LDL cholesterol decreased by 26% from (mean (SEM)) 2.19 (0.29) to 1.63 (0.17) mmol/l during the LDL apheresis while high density lipoprotein (HDL) cholesterol in plasma was unaffected. During LDL apheresis apolipoprotein B containing lipoproteins bind to the column, causing a significant decrease of not only plasma LDL but also of VLDL cholesterol. The secretion rate of bile acids decreased significantly by 31% from 131 (38) to 90 (16) µmol/15 minutes (p=0.045). The output of phospholipids also decreased by 19%. The biliary secretion rate of cholesterol was not, however, affected by the plasma LDL apheresis.

Conclusions—The results suggest that, in patients with cholesterol gallstones and complete biliary drainage, lowering of plasma LDL and VLDL cholesterol reduces the biliary secretion rate—synthesis—of bile acids without affecting the biliary secretion rate of cholesterol.

Keywords: bile acids; biliary lipids; cholesterol; lipoproteins; plasma apheresis

The liver is a key organ in the metabolism of cholesterol in humans. It is the only organ by which substantial amounts of cholesterol are excreted from the body, either directly as free cholesterol into the bile or after conversion to bile acids.1 Human liver has, in comparison with other species, a relatively low capacity to convert cholesterol to bile acids—the rate determining enzyme being cholesterol 7a-hydroxylase2—and therefore human bile usually contains a relatively high amount of cholesterol.34 The liver efficiently clears cholesterol from plasma chylomicron remnants, which are enriched with dietary and intestinal cholesterol, and it also takes up a major part of plasma low density lipoprotein (LDL) cholesterol.1 The uptake and catabolism of LDL are highly dependent on the expression and activity of specific LDL receptors.5,6 The hepatocytes, like all other cells in the body, are also capable of endogenous synthesis of cholesterol from mevalonate, the rate limiting enzyme here being 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase.1

The mechanisms responsible for the regulation of all these processes are not known in detail. Neither has it been finally established to what extent bile acids and biliary cholesterol originate from newly synthesised cholesterol in the liver, from cholesterol taken up from plasma lipoproteins, or from stored cholesteryl esters. Current knowledge is based on investigations performed in vivo under steady state conditions. Therefore, we wanted to study how the biliary secretion rates of cholesterol and...
bile acids, and biliary lipid composition, may be affected by an acute lowering of plasma lipoprotein levels.

The present study was performed in cholesterol gallstone patients with complete biliary drainage, who were treated with selective apheresis of apolipoprotein B containing LDL and very low density lipoproteins (VLDL) (LDL apheresis), resulting in lowering of these lipoproteins. The results show that lowering of plasma apolipoprotein B containing lipoproteins reduced the secretion rates of bile acids and phospholipids but, surprisingly, did not significantly affect the biliary secretion rate of cholesterol.

Patients and Methods

Eight patients (two males and six females) volunteered for the study (Table 1). They were all healthy except for their cholesterol gallstone disease; none had evidence of diabetes mellitus, hyperlipoproteinaemia, or diseases affecting intestinal, thyroid, or kidney function. None was addicted to alcohol or narcotics. All patients underwent elective conventional open cholecystectomy with exploration of the common bile duct. All patients had common bile duct stones and stones in the gall bladder. Before the operation, the patients had normal or, in five cases, only slightly abnormal serum levels of aminotransferases and alkaline phosphatase (Table 1). Peroperative cholangioscopy was performed in a standardised manner to ensure that all stones had been removed from the common bile duct. Alongside the T tube, a balloon occludable Foley catheter (no. 8) was inserted with the balloon distal to the T tube in the common bile duct. The T tube was allowed to drain externally until the LDL apheresis was performed, six to eight days after the operation. This biliary drainage stimulates the liver to increase the synthesis of bile acids to its maximum. The balloon was inflated on the evening before the plasma LDL apheresis took place creating a complete bile fistula. Four of the patients experienced slight abdominal discomfort when the balloon was inflated but thereafter it was well tolerated. The patients all fasted overnight to eliminate the influence of dietary lipids. To confirm the completeness of the bile fistula and to exclude retained stones, a postoperative cholangiogram was performed on the morning before starting LDL apheresis. Plasma LDL cholesterol levels were measured before the balloon was inflated and then just before the LDL apheresis was started; no difference in levels was found. In all cases, the LDL apheresis was started after a minimum of 10 hours fasting. Bile from the T tube was collected in graduated tubes for 15 minute periods starting one hour before LDL apheresis, which was carried out for two hours, and continued until two hours after termination of the apheresis. Blood was sampled for analysis of lipoproteins immediately before, during, and after the apheresis.

Informed consent was obtained from all patients. The ethical aspects of the study were approved by the Ethical Committee of the Karolinska Institute.

PLASMA LDL APERESIS

The apheresis treatment was performed in the blood transfusion unit. Figure 1 shows the outline of the LDL continuous apheresis system. The apheresis was performed using an MA-0–1-system (Kanegafuchi Chemical Industrial Company Ltd, Osaka, Japan). The plasma separator consisted of polysulphone hollow fibres (Sulflux, Kanegafuchi) with an average pore diameter of 0.2 µm and an effective surface area of 0.5 m², in a 140 ml chamber. Two columns each containing 150 ml of cellulose beads covalently bound to dextran sulphate (Liposorber, Kanegafuchi) were used for specific adsorption of apolipoprotein B containing lipoproteins. After injection of a bolus...
dose of heparin (Kabi Vitrum, Stockholm, Sweden; 1000 IU), blood was drawn from an antecubital vein, continuously mixed with heparin, 100 IU/h, and the plasma separated through the sulbutan filter. The plasma was then passed through the first column of Liposorber, whereafter it was recombined with blood cells retained by the filter and reinfused into the patient through another antecubital vein.

The volume of the dead space in the system was about 400 ml and this was primed with about 900 ml of Ringer acetate solution containing 5 IU heparin per ml prior to treatment. The blood flow rate was adjusted to about 70–100 ml/min, and the plasma flow rate during treatment was 30–35 ml/min after absorption of 500 ml plasma into the first Liposorber column. The second column was used for treatment of another 600 ml plasma. Meanwhile, the first column was regenerated by rinsing with 0.7 mol/l NaCl and reloading with Ringer acetate. This procedure was then repeated for every 600 ml of plasma with the help of a computer program included in the MA-01 apparatus. Plasma volumes treated were 1008–2500 ml (mean 1707 ml). No complications were observed during the apheresis sessions, which lasted for two hours.

ANALYSIS OF PLASMA LIPOPROTEINS
The blood samples were immediately centrifuged at 3000 rpm for 20 minutes at 4°C. A portion of the plasma fraction in each sample was subjected to ultracentrifugation in a Beckman L5–65 ultracentrifuge equipped with a 40.3 Ti rotor at d=1.006 g/ml for 30 hours at 39 000 rpm in order to analyse the different lipoprotein fractions (high density lipoprotein (HDL), VLDL, and LDL). Cholesterol and triglyceride contents in plasma as well as in the supernatant and infranatant fractions were analysed using standard enzymatic methods (Boehringer-Mannheim, Germany). The HDL cholesterol level in the infranatant fractions was analysed after precipitation of apolipoprotein B containing particles with phosphotungstic acid.

ANALYSIS OF BILIARY LIPIDS
The biliary concentrations and outputs of bile acids, cholesterol, and phospholipids were measured in all patients. For determination of cholesterol and phospholipids, a portion of the bile samples obtained from the T tubes was extracted with 20 volumes of chloroform-methanol (2:1, vol/vol). Cholesterol was determined by an enzymatic method and phospholipids by the method of Rouser et al. The total bile acid concentration of one aliquot of the bile sample was determined using a 3a-hydroxysteroid dehydrogenase assay. Lipid composition of bile was expressed as molar percentage of cholesterol, bile acids, and phospholipids. The cholesterol percentage saturation of bile was calculated according to Carey.

MEASUREMENT OF BILIARY BILE ACID COMPOSITION
Biliary bile acid composition was determined in three patients. Bile samples were hydrolysed with 1 mol/l KOH in closed steel tubes at 110°C for 12 hours. The deconjugated bile acids were extracted with ethyl ether after acidification to pH 1 with hydrochloric acid, methylated, trimethyl silylated, and analysed with gas liquid chromatography using a 1% Hi-Eff BP8 column.

STATISTICAL ANALYSIS
The bile volumes, biliary lipids, and plasma lipids are given as mean (SEM). The statistical significance of differences in plasma lipoproteins was evaluated with the Student’s t test. Biliary lipids were evaluated by analysis of variance with repeated measures on one factor. Differences between levels of the within factor were evaluated by the post-hoc least significant difference test. The distributions for some variables were skewed. The data were log transformed in order to meet the requirements for an adequate analysis of variance. The mean values of biliary lipids given for every hour were based on the respective four 15 minute periods of bile sampling. The mean values of the four periods before plasma apheresis were considered as basal levels.

Results
PLASMA LIPIDS
Following apheresis plasma levels of cholesterol were reduced by an average of 27% and triglycerides by 52% (table 2). The degree of reduction was related to the amount of plasma that had been processed. Quantitative analysis of the different lipoprotein fractions (VLDL, LDL, and HDL) revealed that there

### TABLE 2
Effect of LDL apheresis on plasma lipoproteins (mmol/l)

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>LDL</th>
<th>HDL</th>
<th>VLDL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Triglycerides</td>
<td>Cholesterol</td>
<td>Triglycerides</td>
</tr>
<tr>
<td><strong>Before apheresis</strong></td>
<td>3.66 (0.33)</td>
<td>1.91 (0.22)</td>
<td>2.19 (0.29)</td>
<td>0.46 (0.07)</td>
</tr>
<tr>
<td><strong>Apheresis (1st hour)</strong></td>
<td>2.91 (0.21)**</td>
<td>0.95 (0.08)**</td>
<td>1.86 (0.19)*</td>
<td>0.32 (0.05)</td>
</tr>
<tr>
<td><strong>Apheresis (2nd hour)</strong></td>
<td>2.66 (0.22)**</td>
<td>0.91 (0.08)**</td>
<td>1.63 (0.17)*</td>
<td>0.30 (0.05)*</td>
</tr>
<tr>
<td><strong>After apheresis (1st hour)</strong></td>
<td>2.49 (0.22)**</td>
<td>1.16 (0.08)*</td>
<td>1.37 (0.20)**</td>
<td>0.26 (0.06)*</td>
</tr>
<tr>
<td><strong>After apheresis (2nd hour)</strong></td>
<td>2.58 (0.22)*</td>
<td>1.62 (0.11)*</td>
<td>1.41 (0.21)*</td>
<td>0.27 (0.06)</td>
</tr>
</tbody>
</table>

Significance of change in relation to values before apheresis: *p<0.05; **p<0.01, Students t test.
was a substantial loss of LDL and VLDL during plasma apheresis, while HDL was unaffected. The plasma level of LDL cholesterol also continued to decrease in the first hour after apheresis. The plasma level of VLDL cholesterol, on the other hand, stabilised during the second hour of apheresis and increased after termination.

### Biliary Lipid Composition and Secretion Rates

LDL apheresis did not influence bile flow (table 3). The secretion rate of bile acids decreased by an average of 31% after two hours of plasma apheresis (table 3). There was no significant decrease in the secretion rate of cholesterol but phospholipid output was decreased by 19%. The effects of apheresis on the biliary lipid secretion rates were the same in the patients with elevated serum levels of alkaline phosphatase and/or aminotransferases as in those with normal levels.

Biliary bile acid composition was determined before, during, and after LDL apheresis (table 4). A 5:1 ratio between cholic acid and chenodeoxycholic acid was not changed during or after the apheresis. No detectable amounts of deoxycholic acid, ursodeoxycholic acid, or lithocholic acid were found.

### Discussion

The patients in the present study had biliary drainage via a T tube inserted in the common bile duct one week before plasma LDL apheresis was performed. The biliary drainage was completed overnight prior to plasma apheresis by inflating a distal balloon in the common bile duct. The complete interruption of the enterohepatic circulation was verified by the absence of the secondary bile acid, deoxycholic acid, from the bile as well as with a cholangiogram performed in the morning before the LDL apheresis was started.

Interruption of the enterohepatic circulation of bile acids by biliary drainage, ileal dysfunction, or resection of the distal ileum stimulates bile acid synthesis severalfold. This can be explained as an effect of interruption of the feedback regulation of bile acid synthesis by bile acids returning to the liver. In the present study the bile acid output before LDL apheresis was 131 (38) µmol/15 min, which represents a daily bile acid output of approximately 12 mmol. In our patients with a completely interrupted enterohepatic circulation, the output of bile acids should equal the new synthesis of bile acids in the liver. The normal daily synthesis of bile acids in humans has been determined to be about 1 mmol. This means that, due to drainage, the synthesis of bile acids was increased more than 10-fold in our patients.

The plasma levels of LDL cholesterol were already low before the start of LDL apheresis because the patients had undergone surgery and had had biliary drainage for about one week. As mentioned above, the synthesis of bile acids was increased severalfold, which implies a corresponding increase in the degradation of cholesterol. In this situation the liver may respond in two different ways: by an increased synthesis of cholesterol; and by an enhanced uptake of plasma lipoprotein cholesterol, especially LDL. It has been shown recently that the human liver uses both of these mechanisms. Thus cholestyramine treatment and ileal resection, both causing bile acid malabsorption, are associated with increased HMG CoA reductase activity and enhanced expression of LDL receptor activity. Upregulation of LDL receptors leads to increased clearance of LDL from the circulation and as a consequence reduced plasma levels of LDL cholesterol.

In the present study apheresis decreased the plasma level of LDL cholesterol by an average of 26%. An interesting finding was that the plasma level of LDL cholesterol continued to decrease after apheresis. This might be due to lack of VLDL as precursor or, less likely, further upregulation of LDL receptors to keep up the inflow of cholesterol to the liver and other organs. In the plasma apheresis carried out in this study, apolipoprotein B containing lipoproteins, mainly LDL but also VLDL, are sequestered from the plasma. As the patients were in a fasting state, the levels of VLDL were low from the start and further decreased through binding to the columns. An interesting finding was that the plasma level of VLDL cholesterol started to increase after LDL apheresis was terminated; in some of the

### Table 3: Bile flow and biliary lipids before, during and after LDL apheresis

<table>
<thead>
<tr>
<th></th>
<th>Before 1 hour</th>
<th>During 1st hour</th>
<th>During 2nd hour</th>
<th>After 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile flow (ml/15 min)</td>
<td>4.9 (0.8)</td>
<td>4.6 (0.6)</td>
<td>4.6 (0.6)</td>
<td>4.6 (0.6) NS</td>
</tr>
<tr>
<td>Bile acid output (µmol/15 min)</td>
<td>131 (38)</td>
<td>98 (17)</td>
<td>90 (16)*</td>
<td>93 (17)* p=0.045</td>
</tr>
<tr>
<td>Phospholipid output (µmol/15 min)</td>
<td>36 (7)</td>
<td>32 (5)</td>
<td>29 (4)*</td>
<td>28 (4)* p=0.007</td>
</tr>
<tr>
<td>Cholesterol output (µmol/15 min)</td>
<td>7.2 (1.9)</td>
<td>7.2 (2.2)</td>
<td>7.0 (2.0)</td>
<td>6.9 (2.0) NS</td>
</tr>
<tr>
<td>Bile acid concentration (µmol/l)</td>
<td>25 (3)</td>
<td>21 (3)</td>
<td>20 (3)*</td>
<td>20 (3)* p=0.046</td>
</tr>
<tr>
<td>Phospholipid concentration (µmol/l)</td>
<td>7.3 (0.6)*</td>
<td>7.0 (0.8)</td>
<td>6.5 (0.7)*</td>
<td>6.1 (0.6)* p=0.007</td>
</tr>
<tr>
<td>Cholesterol concentration (µmol/l)</td>
<td>1.5 (0.4)</td>
<td>1.5 (0.4)</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.3) NS</td>
</tr>
<tr>
<td>Bile acids (molar %)</td>
<td>73 (2)</td>
<td>71 (1)</td>
<td>71 (1)</td>
<td>72 (2) NS</td>
</tr>
<tr>
<td>Phospholipids (molar %)</td>
<td>23 (1)</td>
<td>24 (1)</td>
<td>24 (1)</td>
<td>23 (1) NS</td>
</tr>
<tr>
<td>Cholesterol (molar %)</td>
<td>4.4 (1.1)</td>
<td>4.8 (1)</td>
<td>5.2 (1)</td>
<td>5.2 (1) NS</td>
</tr>
<tr>
<td>Cholesterol saturation (%)</td>
<td>85 (16)</td>
<td>92 (18)</td>
<td>103 (18)</td>
<td>102 (20) NS</td>
</tr>
</tbody>
</table>

*In relation to values before apheresis. Analysis of variance with post-hoc least significant difference test.

### Table 4: Biliary bile acid composition before, during, and after plasma LDL apheresis

<table>
<thead>
<tr>
<th></th>
<th>Before 1 hour</th>
<th>During 1 hour</th>
<th>After 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA (%)</td>
<td>84.9 (4.9)</td>
<td>82.8 (5.1)</td>
<td>83.6 (3.6)</td>
</tr>
<tr>
<td>CDCA (%)</td>
<td>15.1 (5.0)</td>
<td>17.2 (5.1)</td>
<td>16.4 (3.6)</td>
</tr>
</tbody>
</table>

CA, cholic acid; CDCA, chenodeoxycholic acid. Mean (SEM) values per hour for three of the patients.
patients it had already started to increase during the later part of the apheresis. A possible explanation might be increased formation and secretion of VLDL from the liver to compensate for the lipoprotein removal by the columns.

An important finding was that LDL apheresis caused a decrease of about 31% in biliary bile acid output. There was also a decrease in the secretion of phospholipids. Surprisingly, however, the cholesterol output was unchanged. In humans, the biliary secretion of phospholipids depends on bile acid secretion, and therefore the secretion rates of bile acids and phospholipids usually correlate with each other. The decrease in phospholipid output can therefore be considered to be due to the reduced bile acid output.

The decreased biliary output of bile acids during LDL apheresis indicates that the hepatic uptake of LDL cholesterol is of importance for bile acid synthesis. As mentioned above, however, the plasma level of VLDL cholesterol also decreased during LDL apheresis, probably stimulating formation of VLDL particles in the liver and enhancing secretion of VLDL cholesterol to the plasma. Therefore, it cannot be excluded that the decrease in bile acid output–synthesis might be due not only to the decrease in plasma LDL cholesterol but also to the fall in plasma VLDL cholesterol and the concomitant increase in VLDL secretion. During apheresis, the plasma level of LDL cholesterol continuously decreased, whereas that of HDL cholesterol did not change. As mentioned above, the biliary output of cholesterol was not influenced by the apheresis, which may indicate that the biliary output of cholesterol is not related to the hepatic uptake of plasma LDL cholesterol. Provided that there is a metabolic compartmentalisation of hepatic cholesterol in man, which has been suggested, it could be hypothesised that HDL cholesterol, probably taken up by the liver via a docking receptor, is more related to the biliary secretion of cholesterol than that of bile acids. In support of that view, evidence for a relationship between HDL cholesterol and biliary cholesterol in man has been presented previously.

In conclusion, this study has shown that LDL apheresis, which lowers plasma levels of LDL and also VLDL cholesterol, reduces the biliary output of bile acids and phospholipids but does not affect the biliary output of cholesterol in patients with complete biliary drainage. This provides indirect evidence that in patients with cholesterol gallstone disease and under the present experimental conditions, LDL cholesterol and possibly also VLDL cholesterol are of greater importance for the metabolism of cholesterol to bile acids than for the biliary secretion of cholesterol.

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