Interruption of the enterohepatic circulation of bile acids stimulates the esterification rate of cholesterol in human liver

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Abstract The activity of acyl CoA: cholesterol acyltransferase (ACAT), which catalyzes the esterification of cholesterol, was studied in liver microsomes obtained from cholestyramine-treated gallstone patients (n = 12) and patients with Crohn's disease who had undergone partial ileal resection (n = 11). Gallstone patients (n = 33) and gallstone-free subjects undergoing cholecystectomy because of polyps of the gallbladder (n = 8) served as controls. The mean levels of the ACAT activity were the same in the gallstone and the gallstone-free patient groups (6.0 ± 0.4 and 6.1 ± 1.1 pmol/min per mg protein, respectively). When exogenous cholesterol was added to the assay system the activities were increased four- to fivefold in both groups. The ACAT activity tended to be increased in the cholestyramine-treated patients (8.1 ± 1.8 pmol/min per mg protein), and was significantly enhanced ($P < 0.005$) in the ileal-resected patients (12.3 ± 2.3 pmol/min per mg protein). When the enzyme activity was determined with added exogenous cholesterol, it was significantly higher compared to the controls in both the cholestyramine-treated patients and the patients with ileal resection (57.9 ± 11.6 and 50.0 ± 10.3 pmol/min per mg protein, respectively). The content of free and esterified cholesterol in liver homogenates and microsomes was not significantly different between the patient groups. We conclude that ACAT activity is increased in patients with interruption of the enterohepatic circulation of bile acids, and speculate that this reflects a stimulated uptake of lipoprotein cholesterol and may indicate that more cholesteryl esters are incorporated into very low density lipoproteins. —**Ståhlberg, D., E. Reihnér, B. Angelin, and K. Einarsson.** Interruption of the enterohepatic circulation of bile acids stimulates the esterification rate of cholesterol in human liver. *J. Lipid Res.* 1991. **32:** 1409–1415.

Supplementary key words ACAT • ileal resection • cholestyramine treatment • cholesterol • bile acids • enterohepatic circulation

Bile acids are efficiently reabsorbed from the intestine, both by passive and active transport mechanisms (1). Treatment with anion-binding resins, such as cholestyramine, as well as the presence of ileal dysfunction or resection of the distal ileum, causes an interruption of the enterohepatic circulation of bile acids and an increased fecal loss of bile acids (2, 3). Since bile acid concentration is critical for micelle formation and thus the micellar solubility of cholesterol, the loss of bile acids may disturb the intestinal absorption of cholesterol (4, 5). Bile acid malabsorption also results in an increased conversion of cholesterol to bile acids in the liver (2, 6, 7), exerted by derepression of the activity of the rate-limiting enzyme, cholesterol 7α-hydroxylase (8, 9). As a response to the enhanced demand of cholesterol in the liver in this situation, both a stimulation of the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (9–11) and an induced expression of the low density lipoprotein (LDL) receptor have been demonstrated in the human liver (10, 11). From previous studies it is also known that interruption of the enterohepatic circulation of bile acids causes an increased secretion of triglyceride-rich very low density lipoproteins (VLDL) from the liver (12–15).

The influence of interruption of the enterohepatic circulation of bile acids on the cholesterol esterification process in the liver is not well explored. The formation of cholesteryl esters in the liver is catalyzed by the enzyme acyl CoA:cholesterol acyltransferase (ACAT), which is important for the regulation of the level of free cholesterol within the cell (16). Stone et al. (17) reported a decreased ACAT activity and a reduced cholesteryl ester content in liver microsomes of rats treated with cholestyramine. In contrast, Erickson et al. (18) and Strandberg, Telvis, and Miettinen (19) did not find any change in the hepatic ACAT activity during cholestyramine treatment of rats.

In the present work, the influence of interruption of bile acid enterohepatic circulation on the activity of ACAT

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

was studied in cholestyramine-treated gallstone patients undergoing cholecystectomy and in patients with Crohn's disease with partial ileal resection. Gallstone and gallstone-free patients undergoing cholecystectomy served as controls. In addition, the concentrations of free and esterified cholesterol in liver homogenates and microsomes were determined. The results indicate that interruption of the enterohepatic circulation of bile acids stimulates the esterification rate of cholesterol in the human liver.

MATERIALS AND METHODS

Materials

[1-^{14}C]Oleoyl coenzyme A (sp act 57.8 mCi/mmol) and [1,2,6,7-^{3}H]cholesteryl oleate (sp act 82.7 Ci/mmol) were obtained from New England Nuclear Corp., Boston MA. Deuterium-labeled cholesterol was obtained from Larodan Fine Chemicals, Malmö, Sweden. Cholesteryl oleate, cholesterol, EDTA, Triton WR-1339, and human serum albumin were purchased from Sigma Chemicals Co., St. Louis, MO. Cholestyramine (Questran®) was obtained from Bristol-Myers, New York, NY.

Patients

Four different groups of patients were studied (**Table 1**). The first group included 33 (28 females, 5 males) untreated gallstone patients, aged 24–73 years, undergoing cholecystectomy. The second group consisted of 8 (5 females, 3 males) gallstone-free patients, aged 37–74 years, undergoing cholecystectomy because of adenomyoma or polyps of the gallbladder. The third group comprised 12 (11 females, 1 male) gallstone patients, treated with cholestyramine in a dose of 8 g twice daily for 2–3 weeks prior to surgery. Their ages ranged from 25 to 65 years. The fourth group included 11 (5 females, 6 males) patients with Crohn's disease subjected to resection of different lengths of the terminal ileum. The ages ranged from 20–66 years. These patients had undergone one or more partial ileal or ileocolic resections previously, and two of the patients had been cholecystectomized because of gallstone disease. Some of the patients were on medical treatment and one of the patients had been treated with total parenteral nutrition for 2 weeks prior to operation (**Table 2**).

All of the patients were normolipidemic with no evidence of diabetes mellitus, intestinal disease, or diseases affecting the liver, kidney, or thyroid functions.

Informed consent was obtained from each patient before operation. The ethical aspects of the study were approved by the Ethical Committee at Huddinge University Hospital.

Experimental procedure

After a 12-h fast, surgery was performed in the morning. After opening the abdomen a 2–4 g liver biopsy was cut from the left lobe of the liver. The biopsy was immediately placed in ice-cold homogenizing medium and the preparation of microsomes was started within 10 min. Blood samples were taken the same morning before the administration of premedication.

Determination of plasma lipids

Plasma cholesterol and triglycerides were analyzed by automated enzymatic techniques (Boehringer Mannheim Test Combination Cholesterol and Triglycerides, respectively).

Preparation of microsomes

An aliquot of the liver biopsy was minced and homogenized with a loose-fitting Teflon pestle in nine volumes of 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose and 1 mM EDTA. The homogenate was centrifuged at 20,000 *g* for 15 min. The supernatant solution was centrifuged at 100,000 *g* for 60 min. The pellet obtained was resuspended in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA and used for assay of the ACAT activity and determination of microsomal free and

TABLE 1. Basal clinical data of the patients

Patient Groups	Number	Sex	Age (range)	Relative Body Weight[a]	Serum Triglycerides	Serum Cholesterol
			yr	%	*mmol/l*	
Gallstone patients	33	28F, 5M	49 (24–73)	95 ± 2	1.2 ± 0.1	5.2 ± 0.2
Gallstone-free	8	5F, 3M	49 (37–74)	98 ± 6	1.5 ± 0.3	5.7 ± 0.4
Cholestyramine-treated	12	11F, 1M	42 (25–65)	96 ± 3	1.1 ± 0.1	4.2 ± 0.3[b]
Crohn's disease	11	5F, 6M	39 (20–66)	82 ± 4	1.3 ± 0.1	3.8 ± 0.3[c]

[a]Calculated as $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100$.

[b]The concentration of cholesterol after cholestyramine treatment. Before treatment the concentration was 5.2 ± 0.7 mmol/l.

[c]Significantly different from the groups of gallstone and gallstone-free patients, $P < 0.01$.

To convert mmol/l to mg/dl, multiply cholesterol by 38.7 and triglycerides by 88.5.

TABLE 2. Clinical data of patients with Crohn's disease

Patient No.	Sex	Age	Relative Body Weight[a]	Length of Resected Part of Ileum	Extension of Disease	Medical Treatment	Other Clinical Disorders
		yr	*%*	*cm*			
1. AL	F	35	95	20	Dist. ileum		Cholecystectomy
2. RB	M	24	82	30	Ileocolic	Prednisolon Azathioprine	
3. LÅE	M	35	81	35	Dist. ileum		
4. HF	F	32	65	35	Dist. ileum	Prednisolon Sulphasalazine	
5. SS	F	60	89	40	Dist. ileum		
6. AG	M	66	100	60	Ileocolic		
7. US	F	46	69	105	Ileocolic		
8. IK	M	40	92	110	Ileocolic		
9. CW	F	33	74	120	Ileocolic	TPN[b] Prednisolon	Cholecystectomy
10. IC	M	39	89	120	Ileocolic		
11. SE	M	20	68	150	Ileocolic	Prednisolon	

[a]Calculated as $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100$.

[b]TPN, total parenteral nutrition.

esterified cholesterol. The protein concentrations of the microsomes and the liver homogenates were determined by the method of Lowry et al. (20).

Assay of ACAT activity

The ACAT activity was assayed both in the absence and the presence of exogenous cholesterol (21). The assay system contained 0.1 ml of the microsomal preparation and 1 mg of fatty acid-free bovine serum albumin in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA to give a final volume of 1.0 ml. The mixture was preincubated as such for 5 min at 37°C or for 20 min at 37°C after the addition of 50 nmol unlabeled cholesterol, dissolved in 600 μg of Triton. The reaction was initiated by the addition of 25 nmol (1.45 μCi) of [1-^{14}C]oleoyl coenzyme A. After 6 min the assay was stopped by the addition of 10 ml chloroform–methanol 2:1 (v/v). Tritium-labeled cholesteryl oleate (0.01 μCi) was added as an internal standard to estimate recovery followed by 1 ml 0.9% (w/v) NaCl. The chloroform phase was collected and evaporated to dryness under N_2. The residue was resuspended in chloroform–methanol 2:1 (v/v) and subjected to thin-layer chromatography together with unlabeled cholesteryl oleate as a marker. The chromatogram was developed in hexane–ethyl acetate 95:5 (v/v). The cholesteryl oleate zone was visualized with iodine vapor and scraped off into a counting vial and analyzed for radioactivity.

Determination of liver cholesterol

The concentrations of total cholesterol in the liver homogenates and the microsomal fractions were determined by isotope dilution–mass spectrometry after addition of deuterium-labeled cholesterol as an internal standard as described previously (22, 23). Free cholesterol was determined by the same method but the hydrolysis step was omitted. The concentration of esterified cholesterol was calculated as the difference between the total and the free cholesterol in the same sample.

Statistical analysis

Data are presented as means ± SEM (standard error of the mean). The significance of differences was evaluated using the Mann-Whitney test or Wilcoxon signed-rank test.

RESULTS

Interruption of the enterohepatic circulation of bile acids by treatment with cholestyramine resulted in a decrease in the serum cholesterol level by 19% ($P < 0.05$). In patients with Crohn's disease and partial ileal resection the serum cholesterol concentration was almost 30% lower ($P < 0.01$) than in gallstone and gallstone-free patients (Table 1). The concentrations of serum triglycerides were about the same in all the patient groups. The activities of cholesterol 7α-hydroxylase and HMG-CoA reductase, reported previously in references 8, 9 and 11, are shown in **Table 3**. In the cholestyramine-treated patients and in the patients with ileal resection the activities of cholesterol 7α-hydroxylase were increased about sixfold and threefold, respectively, compared to the control groups. The activity of HMG-CoA reductase was more than fourfold increased in the cholestyramine-treated group. The HMG-CoA reductase activity was 267 ± 79

TABLE 3. Activities of HMG-CoA reductase and cholesterol 7α-hydroxylase in the different patient groups

Patient Group	Cholesterol 7α-Hydroxylase	n	HMG-CoA reductase	n
	pmol/min/mg protein		*pmol/min/mg protein*	
Gallstones	8.2 ± 1.1	25	120 ± 18	27
Gallstone-free	7.6 ± 4.0	5	138 ± 35	6
Cholestyramine-treated	51.9 ± 8.1[a]	12	582 ± 86[a]	12
Mb Crohn (gallstone-free)	22.5 ± 3.9[b]	8	267 ± 79	8

Data represent mean ± SEM; data from references 8, 9, and 11.
[a]Significantly different from gallstone patients, $P < 0.0001$.
[b]Significantly different from gallstone-free patients, $P < 0.05$.

pmol/min per mg protein in the patients with Crohn's disease, compared to 138 ± 35 pmol/min per mg protein in the gallstone-free patients. This difference did not reach statistical significance, however.

The ACAT activities are shown in **Table 4**. No difference in the ACAT activity was noted between the gallstone and the gallstone-free patients (6.0 ± 0.4 and 6.1 ± 1.1 pmol/min per mg protein, respectively). When exogenous cholesterol was added to the assay system, there was a four- to fivefold increase of the ACAT activity in both groups of patients.

Cholestyramine treatment did not cause any significant change in ACAT activity in the absence of exogenous cholesterol. In the presence of exogenous cholesterol in the assay system, the activity of the enzyme was twice as high in the cholestyramine-treated patients compared to the untreated group (57.9 ± 11.6 and 28.6 ± 3.0 pmol/min per mg protein, respectively; $P < 0.01$). In the patients with ileal resection, two gallstone and nine gallstone-free subjects, the activity of ACAT was doubled compared to the combined control group of gallstone and gallstone-free patients (12.3 ± 2.3 and 6.0 ± 0.4 pmol/min per mg protein, respectively; $P < 0.005$). When exogenous cholesterol was added there was a more than fourfold increase of the ACAT activity, and the activity was still significantly higher in the patients with ileal resection ($P < 0.05$). No correlation was obtained between the ACAT activity and the resection length of ileum in the patients with Crohn's disease. The concentrations of free and esterified cholesterol in liver homogenates and microsomes were not significantly different among the patient groups (**Table 5**).

DISCUSSION

ACAT activity was examined in patients with interrupted enterohepatic circulation of bile acids. Both in patients with Crohn's disease with partial ileal resection and in the cholestyramine-treated patients the ACAT activities were increased compared to the controls, particularly when determined with exogenous cholesterol added to the assay system. This indicates that the total capacity to esterify cholesterol was increased in patients with interruption of the enterohepatic circulation of bile acids. In the patients with ileal resection, the ACAT activity assayed without exogenous cholesterol was increased compared to the controls as well. In the cholestyramine-treated patients, on the other hand, the ACAT activity was only slightly, but not significantly, increased when assayed without exogenous cholesterol. Our findings contradict results from studies of rats treated with cholestyramine, where the ACAT activity was decreased (17) or unchanged (18, 19). As there are many disparities between the metabolism of cholesterol in rat and human liver, this difference is most probably a species variation.

TABLE 4. The ACAT activity in the different patient groups, assayed without and with exogenous cholesterol

Patient Groups	ACAT Activity	n	ACAT Activity + Exogenous Cholesterol	n
	pmol/min/mg protein		*pmol/min/mg protein*	
Gallstone patients	6.0 ± 0.4	33	28.6 ± 3.0	21
Gallstone-free	6.1 ± 1.1	8	33.2 ± 5.4	5
Gallstone + gallstone-free	6.0 ± 0.4	41	29.5 ± 2.6	26
Cholestyramine-treated	8.1 ± 1.2	12	57.9 ± 11.6[a]	12
Mb Crohn	12.3 ± 2.3[b]	11	50.0 ± 10.3[c]	11

Data represent mean ± SEM.
[a]$P < 0.01$; significantly different from gallstone patients.
[b]$P < 0.005$; [c]$P < 0.05$; significantly different from the combined group of gallstone and gallstone-free patients.

TABLE 5. Cholesterol levels in liver homogenates and liver microsomes in the different patient groups

Patient Group (Number of Subjects)	Total Cholesterol	Free Cholesterol	Esterified Cholesterol	Ester
		nmol/mg protein		*%*
Liver microsomes				
Gallstone (n = 30)	90.2 ± 5.9	72.6 ± 4.8	15.2 ± 2.9	15.6 ± 1.8
Gallstone-free (n = 8)	94.0 ± 8.3	77.3 ± 7.3	17.1 ± 3.6	17.9 ± 3.0
Cholestyramine-treated (n = 12)	91.0 ± 5.6	73.5 ± 5.1	17.5 ± 3.5	18.7 ± 3.5
Mb Crohn (n = 10)	89.9 ± 9.8	79.7 ± 9.3	10.2 ± 0.7	11.9 ± 0.9
Liver homogenate				
Gallstone (n = 24)	39.8 ± 2.0	32.1 ± 1.7	7.7 ± 0.6	19.2 ± 1.2
Gallstone-free (n = 7)	43.4 ± 4.0	32.2 ± 2.8	11.2 ± 2.5	24.7 ± 4.5
Cholestyramine-treated (n = 9)	35.3 ± 2.8	27.4 ± 2.3	7.8 ± 1.2	22.4 ± 3.2
Mb Crohn (n = 11)	48.1 ± 4.2	36.5 ± 3.0	11.6 ± 1.6	23.8 ± 1.7

Data represent mean ± SEM.

From previous studies it is known that interruption of the enterohepatic circulation of bile acids causes an increased conversion of cholesterol to bile acids, by stimulation of the cholesterol 7α-hydroxylase (8, 9), and a compensatory induction of the synthesis of cholesterol (9–11) and a stimulation of the uptake of low density lipoproteins by the liver via the LDL-receptor pathway (10, 11, 24). The increase of the ACAT activity in our study was surprising and not easily explained. One possibility is that the induced LDL receptor expression results in an enhanced uptake of lipoprotein cholesterol which stimulates ACAT activity in analogy with the situation in up-regulated cultured fibroblasts (25). Another explanation could be that the ACAT activity is linked to the increased production rate of triglycerides and the enhanced secretion of VLDL observed in patients with interrupted enterohepatic circulation of bile acids (12, 13, 15). Witztum, Schonfeld, and Weidman (26) have shown that treatment with the bile acid sequestering agent, colestipol, causes a transient increase of both VLDL-triglycerides and VLDL-cholesterol; transitory changes in VLDL size take place simultaneously (14, 26). Clifton-Bligh, Miller, and Nestel (27) found an increased flux of newly synthesized cholesterol into plasma VLDL, and after 8–9 weeks of colestipol treatment the plasma concentrations of VLDL triglycerides and free and esterified cholesterol were increased. The elevation of esterified cholesterol in VLDL was explained as a result of an increased activity of plasma lecithin:cholesterol acyltransferase (LCAT) (27).

In spite of an enhanced esterification rate of cholesterol, particularly in the patients with ileal resection, the concentration of esterified cholesterol in liver homogenates and microsomes was unchanged in the patients with bile acid malabsorption. This finding may indicate that cholesteryl esters formed under these circumstances are incorporated into VLDL secreted from the liver to plasma in order to maintain a constant ratio of triglyceride to cholesterol in the core of lipoprotein particle. Such a correlation between hepatic ACAT activity and the secretion of VLDL has indeed been observed in the rat. When the ACAT activity in cultured rat hepatocytes was stimulated by the addition of 25-hydroxycholesterol or mevalonolactone, Drevon, Engelhorn, and Steinberg (28) found an increase of the esterified cholesterol in VLDL while the amount of free cholesterol decreased or remained unchanged. Stone et al. (29) showed that the administration of a bolus of cholesterol-rich lipoprotein to the perfused rat liver resulted in an increased ACAT activity, an elevation of hepatic cholesteryl ester content, and an enhanced hepatic VLDL secretion without change in the composition of VLDL. It has been suggested, though, that nascent VLDL particles from human liver contain mainly free cholesterol, which is then converted to cholesteryl esters in plasma by the LCAT reaction (30). In this context, it is interesting to note that interruption of the enterohepatic circulation of bile acids is associated with an increased LCAT activity (31–34), particularly in those patients whose plasma triglyceride levels are raised by the treatment. A similar mechanism could thus be responsible for stimulating the ACAT activity.

In summary, the increased ACAT activity in our patients with interrupted enterohepatic circulation of bile acids could be a concomitant effect of the increased synthesis of triglycerides, known to occur in these patients (12–15). As the concentration of cholesteryl esters in the liver remains unchanged, an "overproduction" of cholesteryl esters might be incorporated and secreted in plasma VLDL to maintain constant the ratio of triglycerides/cholesterol in the VLDL. From previous studies of hyperlipidemic patients it has been suggested that an increased formation of plasma triglycerides, monitoring very low density lipoprotein synthesis, is linked to an enhanced degradation of cholesterol to bile acids and that there is an integrated regulation of the metabolism of these two parameters (35–37).

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