Regulation of hepatic cholesterol metabolism in humans: stimulatory effects of cholestyramine on HMG-CoA reductase activity and low density lipoprotein receptor expression in gallstone patients

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Abstract To characterize the metabolic regulatory response to interruption of the enterohepatic circulation of bile acids, we examined the effects of cholestyramine treatment on the rate-limiting steps in cholesterol biosynthesis (HMG-CoA reductase) and bile acid production (cholesterol 7α-hydroxylase) as well as on the heparin-sensitive binding of low density lipoproteins (LDL) (reflecting LDL receptor expression) in human liver. Altogether, 18 normolipidemic patients with uncomplicated cholesterol gallstone disease were treated with cholestyramine (8 g b.i.d.) for 2–3 weeks prior to cholecystectomy, and another 34 cholesterol gallstone patients served as untreated controls. Cholestyramine treatment stimulated cholesterol 7α-hydroxylase more than sixfold, and increased both HMG-CoA reductase activity (552 ± 60 pmol/min per mg protein vs 103 ± 9 pmol/min per mg protein) and LDL receptor expression (6.1 ± 0.8 ng/mg protein; n = 6 vs 2.2 ± 0.3 ng/mg protein; n = 7). Moreover, there was a good correlation between HMG-CoA reductase activity and LDL receptor binding (r_s = +0.71; n = 13), suggesting a simultaneous stimulatory effect to compensate for the increased hepatic cholesterol catabolism due to bile acid depletion caused by cholestyramine. Further evidence for this assumption was the finding of a significant relationship between cholesterol 7α-hydroxylase activity and both LDL receptor expression (r_s = +0.77; n = 13) and HMG-CoA reductase activity (r_s = +0.76; n = 46). We conclude that in human liver a parallel stimulation of cholesterol synthesis and LDL receptor expression occurs in response to stimulation of bile acid synthesis. —Reihnér, E., B. Angelin, M. Rudling, S. Ewerth, I. Björkhem, and K. Einarsson. Regulation of hepatic cholesterol metabolism in humans: stimulatory effects of cholestyramine on HMG-CoA reductase activity and low density lipoprotein receptor expression in gallstone patients. *J. Lipid Res.* 1990. **31:** 2219–2226.

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The production of bile acids represents a major pathway for cholesterol excretion in humans (1–3). The pool of bile acids recirculates several times daily, only small amounts (≈ 1 mmol) being lost and excreted with the feces per day. This loss is compensated for by de novo synthesis, and the production rate is controlled by feedback regulation exerted on the rate-limiting enzyme, cholesterol 7α-hydroxylase (2, 4). Both the relative and absolute concentrations of individual bile acids returning to the liver in the portal vein are probably important in this regulation (5). Furthermore, the synthesis of bile acids is reduced with increasing age, resulting in a reduced pool size and an enhanced secretion of biliary cholesterol (6). Interruption of the enterohepatic circulation of bile acids by cholestyramine treatment increases the hepatic demand for cholesterol. Animal experiments have shown that the liver may respond to this situation in two different ways: by an increased cholesterol synthesis and by an enhanced uptake of lipoprotein cholesterol (3, 7).

The synthesis of cholesterol is regulated by the activity of the microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is determined both by the amount of enzyme protein present, and by the degree of activation (through dephosphorylation) of the enzyme (8, 9). The physiological importance in vivo of the latter mechanism is still not clear however (10, 11). As yet, there is no information in humans on the possible regulation of hepatic HMG-CoA reductase activity in response to interruption of the enterohepatic circulation. The other compensatory mechanism that may be activated by interruption of enterohepatic circulation is an induction of hepatic low density lipoprotein (LDL) receptors. In a recent study (12), we have demonstrated an up-regulation of the receptor in human liver by cholestyra-

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mine, thus explaining the increased clearance of LDL from the circulation during such therapy (13).

A point of major interest is the relative contribution of the two possible compensatory mechanisms, HMG-CoA reductase activity and LDL receptor expression, in the human liver. There is some indication from animal data that the two pathways may work independently, and that induction of lipoprotein receptors only takes place after a considerable increase in hepatic cholesterol production has occurred (14). In order to establish whether such a relationship exists also in humans, we undertook the present study. Specifically, we wanted to answer the questions: *i*) Does cholestyramine treatment increase the activity of HMG-CoA reductase, and, if so, is this achieved by enhancing the proportion of active enzyme? *ii*) Does the stimulation of LDL receptor expression occur independently or is it related to the changes induced in HMG-CoA reductase activity? and *iii*) Does age affect the capacity to increase cholesterol 7α-hydroxylase or HMG-CoA reductase activity in response to cholestyramine therapy? The results indicate that in human liver, a parallel stimulation of HMG-CoA reductase activity and LDL receptor expression occurs in response to interruption of the enterohepatic circulation of bile acids.

MATERIALS AND METHODS

Materials

[3-^{14}C]HMG-CoA (sp act 52 mCi/mmol) was obtained from DuPont Company Biotechnology Systems, Wilmington, DE, and diluted to a specific activity of 5.5 mCi/mmol. DL-[2-^{3}H]mevalonic lactone (sp act 125 mCi/mmol) was obtained from Radiochemical Center, Amersham, England. Unlabeled HMG-CoA, mevalonic acid lactone, EDTA, NADP, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO. Deuterium-labeled 7α-hydroxycholesterol was synthesized as described (15).

Durapore filters (0.45 μm) were obtained from Millipore Corp., Bedford, MA. Heparin (10,000 IU/ml, without preservatives) was a product of AB Kabi, Stockholm, Sweden. Human LDL (1.020–1.065 g/ml) were isolated by ultracentrifugation of serum from healthy volunteers and labeled with ^{125}I as described elsewhere (16); the specific activity ranged from 250 to 650 cpm/ng. [^{2}H$_7$]Cholesterol was obtained from Applied Sciences Laboratories Inc. State College, PA. [^{2}H$_3$]Lathosterol was synthesized as described elsewhere (17).

Patients and treatment

Altogether, 52 patients, 8 males, and 44 females with uncomplicated cholesterol gallstone disease were included in the study (**Table 1**). Before entering the study all patients were thoroughly examined. None of them had clinical or laboratory evidence of diabetes mellitus, hyperlipoproteinemia, or other disease affecting liver, thyroid, and kidney function, and they were all of normal weight. Eighteen of the patients were treated with cholestyramine (Questran®, Bristol-Myers) in a daily dose of 16 g (8 g b.i.d.) for 2–3 weeks prior to surgery. The serum cholesterol level in these patients was lowered by $\approx 20\%$ ($P < 0.01$). Two patients displayed slightly elevated liver enzyme tests during the treatment period, but these were normalized after the operation.

Informed consent was obtained from each patient before the operation, and the ethical aspects of the study were approved by the Ethical Committee at Huddinge University Hospital. Data on cholesterol 7α-hydroxylase activity in some of the patients have been included in a previous publication (5).

Experimental procedure

The patients were admitted to the hospital on the day before operation and were given the regular hospital diet. To prevent the possible influence of any diurnal variation in enzyme activity, cholecystectomy was always performed between 8 and 9 AM after a 12-h fast. Standar-

TABLE 1. Clinical data of the gallstone patients: means and range

| | Number Male/Female | Age | Relative Body Weight[a] | Plasma Concentrations[b] | | | |
| | | | | Cholesterol | | Triglycerides | |
				Before Treatment	After Treatment	Before Treatment	After Treatment
		yr	%	*mmol/l*			
Cholestyramine-treated group	1/17	48 (25–76)	98 (74–116)	5.5 (3.3–7.1)	4.4 (3.0–5.6)	0.9 (0.7–1.2)	1.2 (0.8–2.1)
Untreated control group	7/27	48 (22–73)	96 (64–119)	5.2 (3.6–7.3)		1.2 (0.3–2.3)	

[a]Calculated as weight (kg)/height (cm) – 100 × 100%.
[b]To convert mmol/l to mg/dl, multiply cholesterol by 38.7 and triglycerides by 88.5.

dized anesthesia was given during operation (11). A wedge biopsy weighing 2–4 g was taken from the left lobe of the liver immediately after opening the abdomen. A small portion of the tissue sample was sent for histological examination, whereas the remainder was immediately put into ice-cold homogenizing buffer and transported to the laboratory within 10 min. The cystic duct was clamped and gallbladder bile was obtained by aspiration. Cholecystectomy was then performed without complications.

Preparation of liver microsomes

Activation of inactive forms of HMG-CoA reductase may occur during the preparation of liver microsomes due to the effect of nonspecific phosphatases present in the cytosolic fraction (10, 11). This increase in enzyme activity can be prevented by isolation of the microsomal fraction in the presence of sodium fluoride (an inhibitor of phosphatases). Two aliquots of the liver biopsy (approximately 0.5–1 g) were weighed and placed into nine volumes (v/w) of cold 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose, 10 mM EDTA, 10 mM dithiothreitol, and 50 mM NaCl or 50 mM NaF, respectively.

The liver specimens were minced and homogenized in a Potter-Elvehjem homogenizer with a loosely fitting Teflon pestle. The homogenate was centrifuged at 20,000 g for 15 min at 4°C. The supernatant was centrifuged at 100,000 g for 60 min, and recentrifuged at 100,000 g (without dithiothreitol in the assay for cholesterol 7α-hydroxylase) for another 60 min as described previously (11). The protein concentrations of the microsomal fractions were determined by the method of Lowry et al. (18).

Assay of microsomal HMG-CoA reductase activity

Microsomal fractions (20–110 μg of protein) were preincubated for 15 min at 37°C, and the HMG-CoA reductase assay was then initiated by the addition of [3-¹⁴C]HMG-CoA. After incubation for 15 min, the reaction was stopped by addition of 6 M HCl, and [³H]mevalonic acid was added as internal standard. After lactonization, the mevalonic acid lactone formed was isolated by thin-layer chromatography and counted in a liquid scintillation spectrometer. (For further details about the method, see ref. 11.)

Assay of microsomal cholesterol 7α-hydroxylase activity

The activity of cholesterol 7α-hydroxylase was assayed as described (5, 19). Microsomal fractions (0.25–1.1 mg protein) were incubated for 15 min at 37°C. After the reaction was stopped, deuterium-labeled 7α-hydroxycholesterol was added as internal standard. The amount of 7α-hydroxycholesterol formed was determined by combined gas–liquid chromatography–mass spectrometry and was expressed as pmol/min per mg protein. Endogenous cholesterol was used as substrate for the reaction, and no exogenous cholesterol was added.

Binding assay for hepatic LDL receptors

The liver samples (0.25–0.5 g, wet weight) were homogenized on ice in 2.4 ml buffer (50 mM NaCl, 20 mM Tris-HCl, 1.0 mM CaCl₂, pH 7.5). The homogenates were filtered through a nylon net and thereafter stored at −20°C. All samples available (from 13 patients altogether) were assayed on one occasion. The protein content of the homogenates was determined according to Lowry et al. (18). The binding reactions with ¹²⁵I-labeled LDL (50 μg/ml) were performed in an albumin-containing buffer exactly as described in detail elsewhere (12). The heparin-sensitive binding of ¹²⁵I-labeled LDL, previously shown to represent receptor-mediated LDL binding (12, 20, 21), was calculated by subtracting the radioactivity of filters on which homogenate particulates had been incubated in the presence of heparin (heparin-resistant binding) from the radioactivity of filters on which homogenate particulates had been incubated in the absence of heparin (total binding). Nonspecific (heparin-resistant) binding was about 55% of total, and the coefficient of variation of the assay was 9%.

Analysis of hepatic cholesterol

To 20 μl of homogenized liver suspension or microsomes were added 2.0 μg [²H₇]cholesterol and chloroform-methanol 2:1 (v/v). The chloroform phase was then evaporated and the residue was either hydrolyzed with 0.5 M KOH, extracted with hexane and converted into trimethylsilyl ether (total cholesterol), or directly converted into trimethylsilyl ether (free cholesterol) before analysis by gas–liquid chromatography–mass spectrometry (22).

Analysis of serum lipids

Cholesterol and triglycerides were assayed using standard enzymatic techniques (Boehringer Mannheim, FRG). Unesterified lathosterol and cholesterol in serum were determined by gas–liquid chromatography–mass spectrometry after addition of [²H₃]lathosterol and [²H₇]cholesterol as internal standards as described (17, 22).

Analysis of biliary lipids and bile acid composition

Gallbladder bile was extracted with chloroform-methanol 2:1 (v/v), and the chloroform phase was analyzed with respect to cholesterol (23) and phospholipids (24). Total bile acid concentration was determined by an enzymatic method (25) in a separate portion of bile. The relative concentrations of cholesterol, bile acids, and phospholipids were expressed as molar percentages of total biliary lipids. The cholesterol saturation of bile (%) was calculated according to Carey (26).

Bile acid composition was determined in portions of bile hydrolyzed in 1 M KOH at 110°C for 12 h. The deconjugated bile acids were extracted with ethyl ether

after acidification to pH 1 with HCl. Trimethylsilyl ether derivatives of the extracted bile acids were prepared and analyzed by gas–liquid chromatography using 1% Hi-Eff BP8 as the stationary phase (27).

Statistical analysis

Data are given as means ± SEM. The statistical significance of differences was evaluated using the Mann-Whitney test or Wilcoxon matched-pair test. Correlations were tested by calculating Spearman's rank-order correlation coefficient, r_s.

RESULTS

As observed previously (5), treatment with cholestyramine resulted in a more than sixfold increase in microsomal cholesterol 7α-hydroxylase (48.1 ± 5.7 vs 7.7 ± 1.0 pmol/min per mg protein in controls; $P<0.001$; **Fig. 1A**). There was no influence of sex or body weight on enzyme activity, and when untreated females younger than 45 years (n = 12) were compared to those older than 55 years (n = 8), no significant difference was seen (7.6 ± 1.8 vs 6.7 ± 1.2 pmol/min per mg protein). Although the enzyme activity tended to be lower in the older cholestyramine-treated females (37.0 ± 6.0 pmol/min per mg protein, n = 6) compared to the younger treated group (56.6 ± 10.0 pmol/min per protein, n = 9) the difference was not statistically different.

The presence of a stimulated cholesterol synthesis during cholestyramine treatment could be demonstrated directly by the assay of microsomal HMG-CoA reductase activity in vitro. The enzyme activity was increased more than fivefold in the cholestyramine-treated gallstone patients (552 ± 60 pmol/min per mg protein) compared to the untreated controls (103 ± 9 pmol/min per mg protein; $P<0.001$; Fig. 1B). Mean HMG-CoA reductase activity analyzed in NaF-prepared microsomes was 34 ± 3% of the activity in NaCl-prepared microsomes in nontreated patients (n = 20). This "expressed" enzyme activity was not significantly changed in cholestyramine-treated subjects, 36 ± 5% (n = 12), and the activities of microsomes prepared in the absence of fluoride correlated well with those of microsomes prepared with fluoride (r_s = +0.88; n = 32; $P<0.001$). This suggests that the large variation of enzymatic activity seen is predominantly the consequence of variation of the amount of enzyme protein.

Further support for the association of the change in HMG-CoA reductase activity with a stimulation of cholesterol production in vivo was gained from the analysis of serum lathosterol levels in four patients. Thus, the concentration of serum lathosterol, which has been shown to reflect whole-body cholesterol synthesis in humans (28, 29), was increased by 131% (from 2.0 ± 0.3 to 4.7 ± 0.9 μg/ml) during cholestyramine treatment; the ratio between free lathosterol and free cholesterol was threefold higher after treatment (data not shown).

There was no significant relation between HMG-CoA reductase activity and age or body weight in either group of patients. Furthermore, the enzyme activity was not significantly different in untreated females older than 55 years (91.0 ± 9.4 pmol/min per mg protein, n = 8) compared to females younger than 45 years (112 ± 17 pmol/min per mg protein, n = 15). Cholestyramine treatment raised the enzyme activity to about the same extent in females >55 years (488 ± 52 pmol/min per mg protein, n = 6) and <45 years (633 ± 111 pmol/min per mg protein, n = 9).
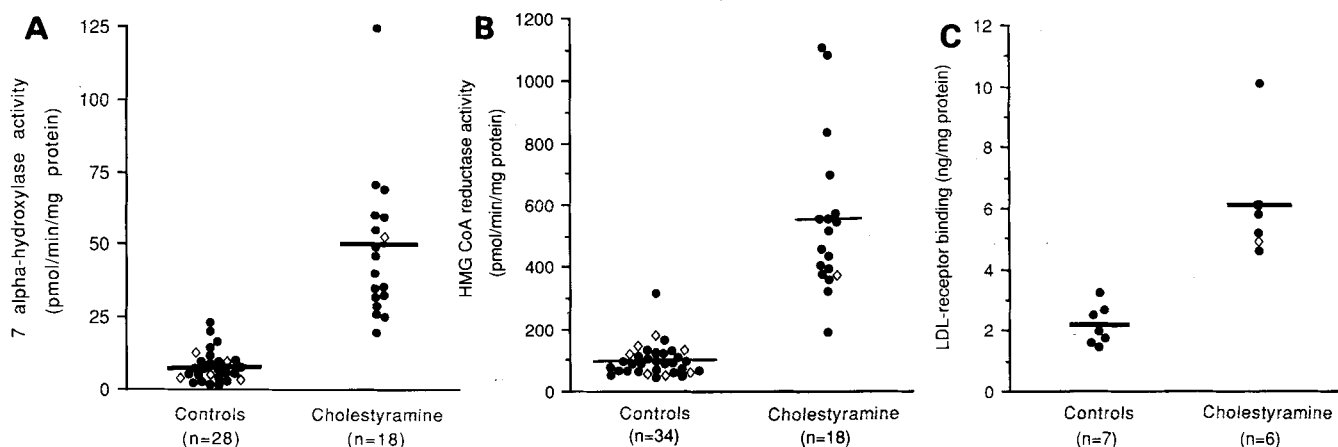

Fig. 1. (A) Hepatic microsomal cholesterol 7α-hydroxylase activity, (B) hepatic microsomal HMG-CoA reductase activity, and (C) hepatic LDL-receptor binding activity in untreated and cholestyramine-treated patients with cholesterol gallstones. Open symbols, males. Horizontal bars indicate means within each group. Data on cholesterol 7α-hydroxylase from some of the patients have been included in a previous report (5).

As can be seen in Fig. 1C, there was an almost threefold rise in LDL receptor activity in the six treated patients compared to the seven untreated controls (6.1 ± 0.8 ng/mg protein vs 2.2 ± 0.3 ng/mg protein, $P<0.005$). The absolute values for binding obtained in the present study were slightly lower than those previously reported by us (12). The variation between individuals was smaller, however, presumably because all assays were performed simultaneously.

No significant differences in hepatic cholesterol concentrations were seen between the two groups of patients, neither in microsomes nor in homogenates (**Table 2**). Furthermore, there were no significant correlations between hepatic cholesterol levels and enzyme activities or LDL receptor binding (data not shown).

Both the HMG-CoA reductase activity and the LDL receptor binding correlated well with cholesterol 7α-hydroxylase activity in the combined group of untreated and cholestyramine-treated patients (r_s = + 0.76, n = 28, $P<0.001$, and r_s = + 0.77, n = 13, $P<0.01$, respectively; **Fig. 2**). Moreover, there was a close correlation between the LDL receptor expression and the HMG-CoA reductase activity (r_s = + 0.71, n = 13, $P<0.01$; **Fig. 3**).

There were no significant differences between the two groups of patients in molar percentage of cholesterol or cholesterol saturation of gallbladder bile. Bile acid composition was significantly changed, however, with an increased proportion of the trihydroxy bile acid, cholic acid, compared to the dihydroxy bile acids, chenodeoxycholic acid and deoxycholic acid (**Table 3**).

TABLE 2. Cholesterol concentration in liver homogenate and microsomes in untreated (controls) and cholestyramine-treated gallstone patients (mean ± SEM)

	Controls	Cholestyramine
	nmol/mg protein	
Homogenate[a]	(n = 20)	(n = 14)
Total cholesterol	41.8 ± 2.5	38.9 ± 2.7
Free cholesterol	34.1 ± 2.2	31.4 ± 2.4
Microsomes[a]	(n = 27)	(n = 16)
Total cholesterol	91.4 ± 7.8	95.1 ± 5.8
Free cholesterol	73.7 ± 5.9	79.6 ± 5.7

[a]To convert nmol to μg, multiply by 0.387.

DISCUSSION

Cholestyramine binds to bile acids in the intestinal tract, thereby interfering with their normal enterohepatic circulation. The resin has a higher affinity for dihydroxy than for trihydroxy bile acids, and their relative amount in the portal venous blood is reduced (5, 30). This results in an induction of the hepatic cholesterol 7α-hydroxylase activity (5), and, in consequence, in a compensatory increase in bile acid synthesis (31–33). These efficient regulatory mechanisms result in conservation of the total bile acid pool, although, as confirmed here, the contribution of the dihydroxy bile acids is reduced (32, 33). In the present work, a detailed analysis of the metabolic regulatory response to interruption of the enterohepatic circulation of bile acids in man could be achieved by utilizing a
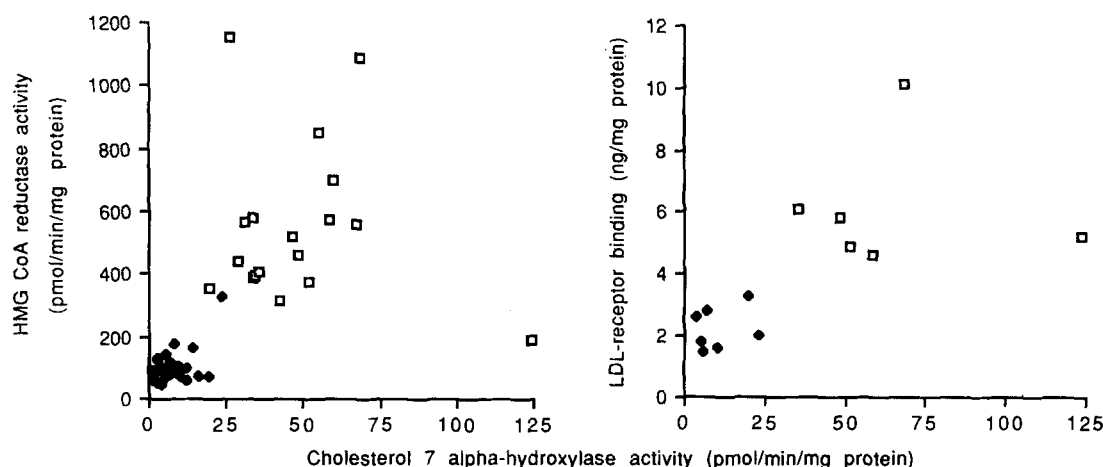

Fig. 2. (A) Relationship of the microsomal HMG-CoA reductase activity and the microsomal cholesterol 7α-hydroxylase activity in human liver tissue in 28 untreated (◆) and 18 cholestyramine-treated (□) gallstone patients (r_s = 0.76, $P<0.001$). (B) Relationship of the heparin-sensitive binding activity of LDL and the cholesterol 7α-hydroxylase activity in human liver tissue in seven untreated (◆) and six cholestyramine treated (□) gallstone patients (r_s = 0.77, $P<0.01$).
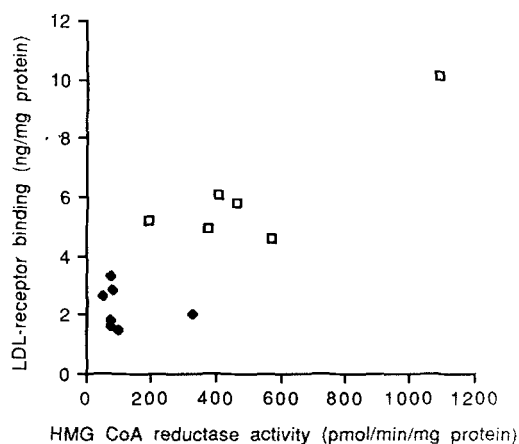
Fig. 3. Relationship between the heparin-sensitive binding activity of LDL and HMG-CoA reductase activity in human liver tissue in seven untreated (♦) and six cholestyramine-treated (□) gallstone patients (r_s = 0.71, $P<0.01$).

standardized operative model. For the first time we have been able to examine in humans the two regulatory steps of cholesterol supply, HMG-CoA reductase activity and LDL receptor binding, in relation to cholesterol 7α-hydroxylase activity. Several new and important conclusions relevant to human physiology may be drawn from the results of this study.

Our results establish that cholestyramine treatment considerably stimulates the HMG-CoA reductase activity in human liver. In spite of a more than sixfold variation in enzyme activity, however, the proportion of initially active enzyme (NaF-prepared microsomes) was the same in untreated and cholestyramine-treated gallstone patients. This observation, which is in agreement with previous animal work (10), supports the concept that the proportion of active enzyme is relatively constant in a steady-state situation, and that the physiological variation in enzyme activity is due to varying amounts of enzyme protein (10, 11). This may be the result of both stimulation of enzyme synthesis and inhibition of enzyme degradation (8, 9).

Another important finding of the present study was that the human liver may respond to an enhanced demand of cholesterol by expressing more LDL-receptors. Cholestyramine-treatment stimulated the LDL receptor binding activity about threefold. This observation is in accordance with our previous study (12), and explains the results of Shepherd et al. (13) and Thompson et al. (34), who reported a doubling of LDL catabolism via the receptor pathway during cholestyramine treatment. It has previously been suggested from animal experiments that the rate of cholesterol synthesis and receptor-dependent LDL-uptake in the liver are regulated independently, and that the primary response to a change in cholesterol

availability is an alteration of cholesterol synthesis (14). However, as shown in Fig. 3, our data suggest that the human liver cells respond to an enhanced demand of cholesterol by a concomitant increase of cholesterol synthesis and LDL receptor activity. This view is further supported by a recent study in human hepatoma cells (HepG2), where a coordinate regulation of the levels of mRNA for LDL receptor and HMG-CoA reductase was demonstrated (35).

It is of major interest that in response to an increase in cholesterol 7α-hydroxylase activity, the stimulation of HMG-CoA reductase activity and LDL receptor expression occurred in parallel. As is evident from Fig. 2, however, the degree of stimulation was greater for HMG-CoA reductase (average increase, 5.4-fold) than for the LDL-receptor (average increase, 2.8-fold). This indicates that, in the human liver, the capacity of the response is larger for the synthesis of HMG-CoA reductase than for the synthesis of LDL receptors. The fact that the human liver has the possibility to meet the increased demand for cholesterol by increases of both HMG-CoA reductase activity and LDL receptor expression has several clinical implications. The large dependence on stimulated cholesterol synthesis probably explains why a relatively moderate lowering of plasma LDL cholesterol is sometimes observed during treatment with bile acid-binding resins (36, 37). Particularly, it provides an explanation for the dramatic effect on LDL cholesterol levels that may be achieved in hyperlipidemic patients by the addition of a cholesterol synthesis inhibitor to ongoing medication with a resin (38–40). In this situation, blocking of the compensatory increase in cholesterol synthesis probably results in a maximal stimulation of LDL receptor expression in the liver, as has been demonstrated in animals (41).

Finally, it is of interest to note that, within the age span studied, the stimulatory response to cholestyramine

TABLE 3. Biliary lipid and bile acid composition of gallbladder bile in untreated (controls) and cholestyramine-treated gallstone patients (mean ± SEM) ·

	Controls	Cholestyramine
Biliary lipids	(n = 23)	(n = 10)
Cholesterol (mol %)	7.5 ± 0.6	7.0 ± 1.4
Bile acids (mol %)	69.6 ± 1.0	72.1 ± 1.9
Phospholipids (mol %)	22.8 ± 0.7	20.5 ± 0.8
Cholesterol saturation (%)	122 ± 13	121 ± 21
Bile acids[a]	(n = 22)	(n = 10)
Cholic acid (%)	44.6 ± 2.4	83.5 ± 2.5[b]
Chenodeoxycholic acid (%)	33.3 ± 1.7	16.1 ± 2.0[c]
Deoxycholic acid (%)	21.8 ± 2.6	4.4 ± 0.9[c]

[a]Minor amounts (<2%) of ursodeoxycholic acid and lithocholic acid were sometimes observed.
[b,c]Significantly different from controls, [b]$P < 0.01$; [c]$P < 0.001$.

as regards the activities of cholesterol 7α-hydroxylase and HMG-CoA reductase was not blunted with increasing age in the group of female patients. This indicates that the capacity for bile acid synthesis and hepatic cholesterol production may be maintained also with increased age. In normal subjects, the formation of bile acids is reduced with increasing age (6). Although gallstone patients may not be fully representative, it is tempting to speculate that this reduced production of bile acids is the consequence of metabolic down-regulation of synthesis, and not of loss of synthetic capacity. Due to the relatively limited number of patients studied, it was not possible to look for any relationship between age and LDL receptor binding, but such studies should be of interest, considering the known association between increased plasma LDL cholesterol levels and age (42).

The availability of substrate may to some extent limit the rate of 7α-hydroxylation of cholesterol under conditions of accelerated bile acid biosynthesis (43). If so, it is tempting to suggest that the stimulation of HMG-CoA reductase activity and LDL receptor expression is secondary to a reduced critical pool of cholesterol, which may be the direct regulator. The reduction of this pool of cholesterol may be secondary to the stimulation of the cholesterol 7α-hydroxylase. The fact that there was no reduction in the content of free or total microsomal cholesterol in the livers of patients treated with cholestyramine would not be in conflict with this concept. Thus, a tight control of cholesterol concentration would actually preclude the detection of such effects. In fact, Jelinek et al. (44) have recently described a parallel induction of cholesterol 7α-hydroxylase and HMG-CoA reductase mRNA levels in rat liver during cholestyramine feeding. Another possible mechanism for an augmented hepatic cholesterol synthesis is a reduced amount of reabsorbed cholesterol (45). All our patients were studied after an overnight fast, however, and took the last dose of cholestyramine more than 12 h before the operation. We therefore consider the latter mode of regulation of the enzyme to be of minor importance in the present study.

In conclusion, we have demonstrated that the increased demand for cholesterol that occurs in the human liver during stimulation of bile acid synthesis may be met by two adaptive responses: an enhanced activity of HMG-CoA reductase and an increased binding activity of LDL receptors. The two responses are triggered concomitantly, and it is speculated that they react to a common regulatory agent. Further studies will be needed to explore this mechanism in more detail. ▮▮

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