Differing effects of cholesterol and taurocholate on steady state hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels in the rat

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Abstract We investigated the effects of cholesterol, cholestyramine, and taurocholate feeding on steady state specific activities and mRNA levels of hepatic 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase and cholesterol 7α-hydroxylase in the rat. Interruption of the enterohepatic circulation of bile acids (cholestyramine feeding) increased total HMG-CoA reductase activity 5-fold. Cholesterol and taurocholate administration suppressed total microsomal HMG-CoA reductase activities 87% and 65%, respectively. HMG-CoA reductase mRNA levels increased 3-fold with cholestyramine, did not decrease significantly with cholesterol feeding, but were markedly decreased after taurocholate treatment. Cholesterol 7α-hydroxylase activity increased 4-fold with cholestyramine and 29% during cholesterol feeding, but decreased 64% with taurocholate. Cholesterol 7α-hydroxylase mRNA levels rose 150% and 50% with cholestyramine and cholesterol feeding, respectively, but decreased 73% with taurocholate. The administration of cholesterol together with taurocholate prevented the decline in cholesterol 7α-hydroxylase mRNA levels, but inhibition of enzyme activity persisted (−76%). Hepatic microsomal cholesterol concentrations increased 2-fold with cholesterol feeding but did not change with taurocholate or cholestyramine treatment. These results demonstrate that mRNA levels of HMG-CoA reductase are controlled by the hepatic taurocholate flux, whereas mRNA levels of cholesterol 7α-hydroxylase are controlled by the cholesterol substrate supply. These end products, cholesterol and bile acids, exert post-transcriptional regulation on HMG-CoA reductase and cholesterol 7α-hydroxylase, respectively.—**Shefer, S., L. B. Nguyen, G. Salen, G. C. Ness, I. R. Chowdhary, S. Lerner, A. K. Batta, and G. S. Tint**. Differing effects of cholesterol and taurocholate on steady state hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels in the rat. *J. Lipid Res.* 1992. **33:** 1193–1200.

Supplementary key words HMG-CoA reductase activity • HMG-CoA reductase mRNA • cholesterol 7α-hydroxylase activity • cholesterol 7α-hydroxylase mRNA • cholestyramine • bile acids

Cholesterol is the obligate precursor of bile acids in all mammalian species (1). During bile acid synthesis, the nonpolar cholesterol molecule is transformed into a water-soluble bile acid. The key reaction in this pathway (**Fig. 1**) is the 7α-hydroxylation of cholesterol that is catalyzed by the enzyme cholesterol 7α-hydroxylase (EC 1.14.13.17). According to current information, this reaction is rate-limiting (2, 3). The preferred substrate pool for cholesterol 7α-hydroxylase is newly synthesized cholesterol (4), and therefore, bile acid synthesis is controlled, not only by the activity of cholesterol 7α-hydroxylase, but also by the supply of the substrate, cholesterol. Thus, bile acid synthesis is coordinately regulated with cholesterol synthesis, which is controlled by HMG-CoA reductase (EC 1.1.1.23). Both HMG-CoA reductase and cholesterol 7α-hydroxylase activities vary diurnally (5–7) and are believed to be under the feedback control of the hepatic cholesterol pool and the flux of bile acids through the liver (8–14). Recently, HMG-CoA reductase and cholesterol 7α-hydroxylase have been purified and their cDNA sequences have been elucidated so that probes are available to quantitate mRNA levels for each enzyme under various experimental conditions (6, 11–18). In this study, we have determined HMG-CoA reductase and cholesterol 7α-hydroxylase activities and relative levels of mRNA in

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; TLC, thin-layer chromatography.

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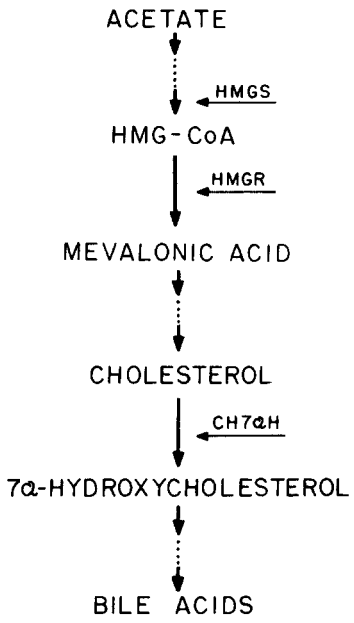

Fig. 1. Key reactions in the cholesterol and bile acid biosynthetic pathways. HMGS, hydroxymethylglutaryl-CoA synthase; HMGR, hydroxymethylglutaryl-CoA reductase; CH7αH, cholesterol 7α-hydroxylase.

livers from rats fed diets supplemented with cholesterol, cholestyramine, taurocholate, or the combination of cholesterol and taurocholate. Our objective was to elucidate the role of cholesterol and bile acids as regulators of these rate-controlling enzymes.

MATERIALS AND METHODS

Animals and experimental design

Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY) that weighed 150–180 g were fed rat chow (Purina Mills Inc., Saint Louis, MO) and water ad lib. After 1 week the rats were divided into five groups of six to ten rats each and fed rat chow diets that were supplemented with: 2% cholesterol, 2.5% cholestyramine, 1% taurocholate, or the combination of 1% taurocholate and 2% cholesterol. A control group received only rat chow. All treatment groups ate the same amounts of food and gained similar weight during the feeding period. When food intake or weight gain of an experimental animal differed by more than 10% from the average of the control group, the animal was excluded from the study. The diets were continued for 7 days after which five rats from each group were anesthetized with sodium pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, IA), common bile ducts were cannulated, and bile was collected for a period of 30 min. All rats were then killed by decapitation and their livers were excised, weighed, and stored at −70°C until used for sterol, enzymatic, and mRNA determinations. To minimize diurnal variations in cholesterol and bile acid synthesis (19, 20), the animals were killed at about 10 AM. The research protocol was approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ.

Materials

Cholesterol (Sigma Chemical Co., St. Louis, MO) and taurocholate (Calbiochem Co., La Jolla, CA) were >98% pure. Cholestyramine was a gift from Merck Sharp and Dohme Research Laboratories, Rahway, NJ. [3-^{14}C]HMG-CoA (Amersham, Arlington Heights, IL) was diluted with unlabeled HMG-CoA to a specific activity of 50 dpm/pmol. [4-^{14}C]Cholesterol (Du Pont Co., New England Nuclear Research Products, Boston MA) was diluted to a specific activity of 2×10^4 dpm/nmol and purified by chromatography on a silicic acid column (21). The purified cholesterol contained less than 0.06% 7α-hydroxycholesterol. A mixture of 7α- and 7β-hydroxycholesterol, used as reference standards for TLC, was obtained by the reduction of 7-ketocholesterol (Schwarz/Mann, Orangeburg, NY) with sodium borohydride (22). The cDNA probes for hamster HMG-CoA reductase (pRED 227) and human catalase (pCAT 10) were purchased from American Type Culture Collection (Rockville, MD). The probe for rat liver cholesterol 7α-hydroxylase (7α6) was obtained from Dr. John Y. L. Chiang (Northeastern Ohio Universities College of Medicine, Rootstown, OH).

Assays for total HMG-CoA reductase and cholesterol 7α-hydroxylase activities

Hepatic microsomes were prepared by differential ultracentrifugation (23), and the protein was determined according to Lowry et al. (24). The assay for HMG-CoA reductase activity was carried out as described previously (25). Cholesterol 7α-hydroxylase activity was measured by the isotope incorporation method of Shefer, Salen, and Batta (23). In cholesterol- and taurocholate-fed rats where hepatic cholesterol concentrations increased, cholesterol 7α-hydroxylase activity was assayed in a reconstituted system after removal of endogenous cholesterol by acetone treatment (26, 27) and compared to similarly assayed control microsomes. This method eliminates the confounding effect of endogenous cholesterol, measures cholesterol 7α-hydroxylase activity with the enzyme fully saturated with substrate (zero-order kinetics), and gives results comparable to intact microsomes (26, 27).

Isolation and quantitation of mRNA

Total RNA was isolated from 1-g pieces of rat liver by a low temperature modification of the guanidinium thiocyanate extraction procedure (28) with the addition of a lithium chloride extraction step to remove glycogen (29).

Poly A$^+$ RNA was isolated by oligo (dT) cellulose chromatography (30). Ten-μg aliquots of poly A$^+$ RNA were denatured and electrophoresed in 1% agarose gels containing 0.02 M borate, pH 8.3, 0.2 mM ethylene diamine tetraacetic acid, and 3% formaldehyde (31). The separated RNAs were transferred to Gene Screen Plus membranes by capillary blotting and the RNA was fixed by baking under vacuum for 2 h at 80°C. The cDNA probes were labeled with ^{32}P to specific activities ranging from 2×10^7 to 4×10^9 cpm/μg by nick translation. The hybridizations were carried out as previously described (32), except that a hybridization incubator from Robbins Scientific equipped with 38 × 300 mm glass screw-cap tubes was used. Typically, 5 ml of hybridization solution (32) containing $2-4 \times 10^8$ cpm and 1–2 μg of ^{32}P-labeled cDNA probe were incubated with a 9 × 14 cm membrane in a glass screw-cap tube at 42°C overnight, being careful not to overlap the membrane. Washing conditions were as previously described (33). The washed membranes were exposed to Kodak X Omat AR film with Cronex intensifying screens at −70°C for times ranging from 2 to 16 h. The autoradiograms were scanned with an LKB Ultrascan laser densitometer to determine relative mRNA levels. The values for catalase mRNA were used as internal controls. All values are presented as means ± SEM.

Determination of hepatic total and microsomal cholesterol

For the determination of free and esterified cholesterol in the liver, aliquots of whole liver homogenates (50–200 mg in 0.5 ml) were extracted with 20 volumes of chloroform–methanol 2:1 (vol/vol) after the addition of [^{3}H]cholesterol and [^{3}H]cholesteryl oleate (1.1×10^6 dpm each) as recovery standards. The extract was separated by TLC on silica gel G plates with hexane–ethyl ether–acetic acid 85:15:0.5 (vol/vol/vol). The bands corresponding to free and esterified fractions (R_f = 0.09 and 0.87, respectively) were scraped and eluted with ethyl acetate–methanol 85:15 (vol/vol). The esterified cholesterol fractions were hydrolyzed at 60°C for 1 h in 10% ethanolic KOH and extracted with n-hexane. Both free and esterified cholesterol fractions were counted and analyzed by capillary gas–liquid chromatography as the trimethylsilyl ether derivatives using 5α-cholestane as internal standard (34).

For the determination of total cholesterol concentrations in the microsomes, aliquots of microsomal suspensions (2–5 mg) were saponified in 10% ethanolic KOH at 60°C for 2 h with 30,000 dpm [^{3}H]cholesterol as internal recovery standard. Cholesterol was extracted with n-hexane and quantitated by capillary gas–liquid chromatography as described above.

Determination of biliary bile acids

Bile acid composition was determined in bile specimens obtained at the end of each treatment period, using tauroursodeoxycholic acid as internal recovery standard (9, 35). The bile acids were quantitated as the methyl ester trimethylsilyl ether derivatives by capillary gas–liquid chromatography on a wall-coated open tubular fused silica column (0.22 mm × 25 m), coated with a 0.12-μm film of CP Sil 5 CB (Chrompak, Inc. Bridgewater, NJ). The chromatograph was operated at a column temperature of 265°C and a helium flow of 1.3 ml/min. The retention times of the bile acid methyl ester trimethylsilyl ethers relative to 5α-cholestane (13.1 min) were: deoxycholic acid, 1.49; chenodeoxycholic acid, 1.54; α-muricholic acid, 1.56; cholic acid, 1.57; ursodeoxycholic acid, 1.63; and β-muricholic acid, 1.83.

Statistical analysis

Data were analyzed statistically by comparing the 95% and 99% confidence intervals for the means of the various treatment groups according to Altman and Gardner (36).

RESULTS

In **Fig. 2A** are presented measurements of total HMG-CoA reductase activities during the various treatments. After cholesterol and taurocholate administration, total HMG-CoA reductase activities declined 87% and 65%, respectively. When cholesterol was combined with taurocholate, HMG-CoA reductase activity was reduced 93%. In contrast, cholestyramine treatment stimulated HMG-CoA reductase activity 5-fold.

In Fig. 2B are presented measurements of cholesterol 7α-hydroxylase activities. Cholestyramine treatment increased cholesterol 7α-hydroxylase activity 4-fold as compared to a 29% increase when cholesterol was administered. In contrast, taurocholate inhibited cholesterol 7α-hydroxylase activity 64%, while the combination of taurocholate plus cholesterol resulted in a 76% decline. Thus, the hepatic bile acid flux appears to be a potent down-regulator of HMG-CoA reductase and cholesterol 7α-hydroxylase activities, while cholesterol inhibits the activity of HMG-CoA reductase but stimulates slightly the activity of cholesterol 7α-hydroxylase.

Estimates of relative steady state mRNA levels for HMG-CoA reductase, determined by densitometric scanning and corrected for catalase mRNA recovery (25, 37), are presented in **Table 1**. Taurocholate virtually abolished HMG-CoA reductase mRNA as compared with an insignificant decrease observed with cholesterol feeding (37). When taurocholate was combined with cholesterol, HMG-CoA reductase mRNA was also barely detected. In contrast, cholestyramine treatment increased HMG-CoA reductase mRNA levels over 2-fold. Thus, hepatic bile acid depletion stimulates HMG-CoA reductase mRNA levels while taurocholate virtually eliminates the message for enzyme protein synthesis. Interestingly, cholesterol

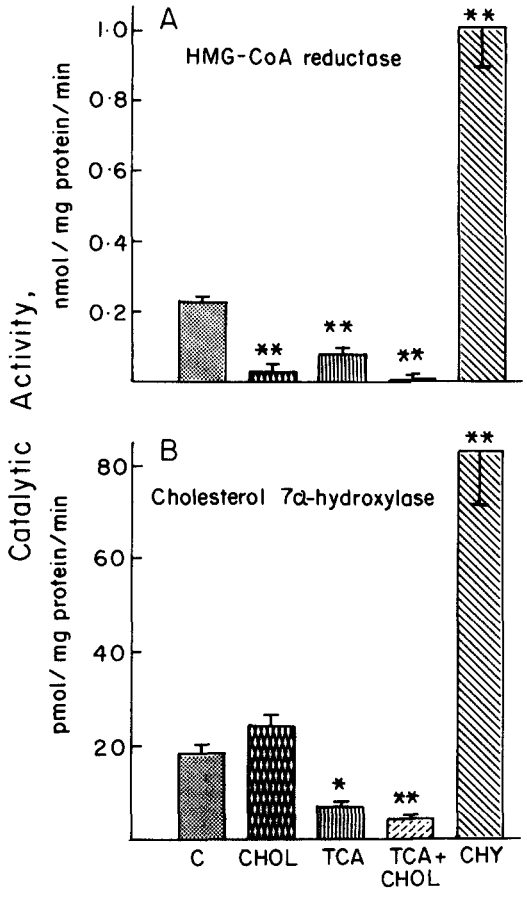

Fig. 2. Catalytic activities of HMG-CoA reductase (A) and cholesterol 7α-hydroxylase (B) in rats treated for 7 days with the following dietary supplements: C, control (n = 9); CHOL, 2% cholesterol (n = 5); TCA, 1% taurocholate (n = 5); CHOL + TCA, 2% cholesterol + 1% taurocholate (n = 6); CHY, 2.5% cholestyramine (n = 5). Bars represent means ± SEM; *, $P < 0.05$; **, $P < 0.01$.

feeding, which markedly inhibits enzyme activity (Fig. 2A), is associated with an insignificant decrease in HMG-CoA reductase mRNA levels.

Steady state cholesterol 7α-hydroxylase mRNA levels are presented in Table 1. Both cholestyramine and cholesterol feeding increased cholesterol 7α-hydroxylase mRNA levels 150% and 50%, respectively. In contrast, taurocholate reduced cholesterol 7α-hydroxylase mRNA 73%. However, the decrease of cholesterol 7α-hydroxylase mRNA in the taurocholate-fed animals was prevented when the taurocholate-containing diet was supplemented with cholesterol (Table 1). **Fig. 3** shows the Northern blots of cholesterol 7α-hydroxylase in controls and during treatments with taurocholate alone and with taurocholate in combination with cholesterol. Three prominent mRNA signals for cholesterol 7α-hydroxylase are seen in controls, as reported previously by Jelinek et al. (18). In contrast, taurocholate virtually eliminated mRNA for cholesterol 7α-hydroxylase, an effect that was negated by the addition of cholesterol to the taurocholate diet. Thus, similar to HMG-CoA reductase, cholesterol 7α-hydroxylase mRNA is increased by depletion of the hepatic α-hydroxylated bile acid flux and decreased by replacement with taurocholate. However, feeding cholesterol increased cholesterol 7α-hydroxylase mRNA, a rise that persisted after taurocholate was added to the cholesterol diet. It is important to emphasize that the 30-min bile collection produced no effect on HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels as compared to the intact rats.

In **Table 2** are listed hepatic cholesterol concentrations during the various treatments. Cholesterol feeding resulted in a significant increase in both the microsomal and total hepatic cholesterol concentrations. In contrast, despite a 2-fold increase in hepatic cholesterol, microsomal cholesterol did not change after taurocholate treatment. The addition of taurocholate to the cholesterol-supplemented diet further increased total and microsomal cholesterol concentrations. Cholestyramine treatment did not change either total or microsomal cholesterol levels.

Table 3 lists biliary bile acid outputs and composition in the various groups. Bile acid malabsorption (cholestyramine treatment) or cholesterol administration did not change biliary bile acid outputs or composition. In contrast, the ingestion of taurocholate increased biliary bile acid secretion and shifted the composition of the bile acid pool from 68 to 88% taurocholate. The addition of cholesterol to taurocholate did not change biliary bile acid

TABLE 1. Relative mRNA levels of hepatic HMG-CoA reductase and hepatic cholesterol 7α-hydroxylase

Treatment	HMG-CoA Reductase mRNA	Cholesterol 7α-Hydroxylase mRNA
	peak area/μg RNA[a]	
Control	4.0 ± 0.3 (10)	6.3 ± 1.3 (8)
Cholesterol	3.2 ± 0.4 (9)	9.4 ± 0.3[b] (5)
Taurocholate	<0.1[b] (5)	1.7 ± 0.3[b] (8)
Taurocholate + cholesterol	<0.1[b] (6)	8.4 ± 1.2 (4)
Cholestyramine	10.2 ± 0.6[b] (5)	15.7 ± 0.9[b] (5)

Values are given as mean ± SEM; number of animals in parenthesis.
[a]Corrected for recovery of catalase mRNA (37).
[b]Significantly different from control, $P < 0.01$.

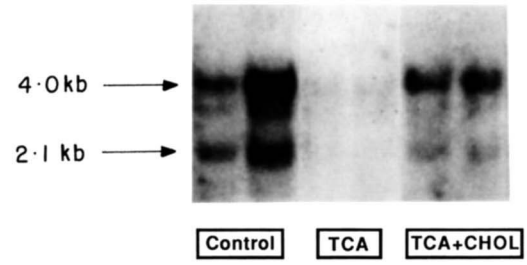

Fig. 3. Northern blots of cholesterol 7α-hydroxylase mRNA. Three bands between 2.1 and 4.0 kb are detected in the controls. Cholesterol 7α-hydroxylase mRNA was markedly decreased by taurocholate (TCA) feeding, an effect that was prevented when cholesterol (CHOL) was combined with TCA.

outputs or the proportion of taurocholate secreted into the bile. Both α-muricholic acid and chenodeoxycholic acid were reduced, but the β-muricholic acid remained constant.

DISCUSSION

The results of this investigation demonstrate steady state feedback regulation of the rate-controlling enzymes of cholesterol and bile acid synthesis, HMG-CoA reductase, and cholesterol 7α-hydroxylase. The supply of cholesterol and the flux of taurocholate control mRNA levels and catalytic activities of both enzymes. However, these two regulators apparently modulate each enzyme at specific but different locations.

Consistent with earlier reports on HMG-CoA reductase (11, 12), bile acid malabsorption (induced by cholestyramine) increased both mRNA levels (3-fold) and enzyme activities (5-fold). Apparently, the depletion of the hepatic bile acid flux was responsible for enhanced synthesis of a more active enzyme. Conversely, when taurocholate was fed, HMG-CoA reductase activity decreased and there was virtually no HMG-CoA reductase mRNA (Table 1). Recently, Spady and Cuthbert (38) and Duckworth et al. (14) showed that feeding unconjugated cholic acid also reduced hepatic mRNA for HMG-CoA reductase, albeit to a more modest degree. The disappearance of HMG-CoA reductase mRNA after treatment with taurocholate cannot be attributed to increased cholesterol absorption (39) and/or hepatic cholesterol accumulation. Cholesterol feeding increased hepatic total and microsomal cholesterol concentrations far more than taurocholate (Table 2) but did not reduce HMG-CoA reductase mRNA levels significantly (Table 1). Recently, Jelinek et al. (18) have shown that cholate and chenodeoxycholate (α-hydroxylated bile acids) depressed hepatic mRNA levels for HMG-CoA synthase, an enzyme that catalyzes the formation of the substrate for HMG-CoA reductase (Fig. 1). Therefore, other enzymes in cholesterol synthesis, i.e., HMG-CoA synthase, may also be coordinately regulated with HMG-CoA reductase by α-hydroxylated bile acids (12).

Of considerable interest is that cholesterol feeding, which markedly suppressed hepatic HMG-CoA reductase activity (8, 14, Fig. 2), did not significantly decrease (−20%) the abundance of HMG-CoA reductase mRNA (Table 1). Similar results have been noted previously by Ness, Keller, and Pendleton (37) and Spady and Cuthbert (38) in rat liver, and by Nakanishi, Goldstein, and Brown (40) in Chinese hamster ovary cells, although Duckworth et al. (14) reported a larger decrease of hepatic HMG-CoA reductase mRNA with cholesterol feeding. Our results suggest that the enzyme is modulated post-transcriptionally by cholesterol, although it is unclear whether this control involves the formation and/or degradation of the enzyme protein, as has been suggested by Nakanishi et al. (40). In earlier studies, Liscum et al. (11) failed to detect HMG-CoA reductase mRNA in the livers from either normal or cholesterol-fed rats. It is important to emphasize that the cDNA probe used in the hybridizations in our study contained greater amounts of ^{32}P, which increased sensitivity of the Northern blotting analysis (37). Thus, it appears that regulation of cholesterol biosynthesis takes place at two locations: bile acids (or metabolite) control the enzyme at the level of transcription and cholesterol modulates translational efficiency or catalytic activity.

With respect to cholesterol 7α-hydroxylase, feeding of

TABLE 2. Hepatic cholesterol concentrations

Treatment	Whole Liver: Total Cholesterol (Esterified)	Microsomes: Total Cholesterol
	μmol/g liver (%)	*nmol/mg protein*
Control	5.8 ± 1.0 (29)	48.7 ± 8.7
Cholesterol	18.1 ± 0.2[a] (66)	103.7 ± 6.8[a]
Taurocholate	11.0 ± 1.6[b] (54)	55.5 ± 9.9
Taurocholate + cholesterol	39.5 ± 1.1[a] (89)	172.9 ± 19.4[a]
Cholestyramine	6.7 ± 0.1 (18)	52.5 ± 5.9

Mean ± SEM; five to seven animals in each group.
[a]Significantly different from control, $P < 0.01$.
[b]Significantly different from control, $P < 0.05$.

TABLE 3. Biliary bile acid secretion and composition

Treatment	Bile Acids					
	Total[a]	CA	CDCA	α-MCA	β-MCA	DCA
	μg/100 g rat/h			%		
Control	6.2 ± 1.0	67.5	12.5	7.2	7.0	5.0
Cholesterol	6.6 ± 0.8	68.8	11.3	8.3	8.1	3.5
Taurocholate	14.1 ± 0.6[b]	88.0	0.8	0.6	3.8	6.9
Taurocholate + cholesterol	13.0 ± 3.2[c]	87.0	2.3	1.5	5.3	3.9
Cholestyramine	7.2 ± 1.6	68.7	15.1	7.5	5.7	3.0

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; DCA, deoxycholic acid.

[a]Mean ± SEM; five animals in each group.

[b]Significantly different from control, $P < 0.01$.

[c]Significantly different from control, $P < 0.05$.

cholesterol increased steady state mRNA levels 50% with insignificant change in catalytic activity. We have previously suggested (9) that cholesterol 7α-hydroxylase exists in two different states of catalytic efficiency (activity per unit enzyme mass). The administration of mevalonolactone (a cholesterol precursor) in combination with taurocholate to bile fistula rats induced the formation of abundant enzyme mass with low catalytic efficiency. The present findings support this contention since mRNA levels, but not enzyme activity, increased with cholesterol feeding. It was suggested that feeding large amounts of cholesterol may induce bile acid malabsorption, which can result in an increase in cholesterol 7α-hydroxylase mRNA. However, our experimental design mitigates this possibility as we administered taurocholate, a conjugated bile acid, which is absorbed in the ileum while cholesterol is absorbed in the jejunum. Indeed, bile acid outputs from taurocholate- and taurocholate plus cholesterol-fed rats were almost identical (Table 3), which indicated that bile acid malabsorption did not occur. In addition, the fact that cholesterol 7α-hydroxylase activity was markedly inhibited by taurocholate, even when administered in combination with cholesterol, further refutes the possibility that cholesterol produced bile acid malabsorption.

Bile acid malabsorption (induced by cholestyramine feeding) stimulated both cholesterol 7α-hydroxylase activity and abundance of mRNA. Similar results have been recently reported in rats fed 4% cholestyramine for 10–14 days (41), and in a rat model with chronic biliary divertion (42). Conversely, treatment with taurocholate inhibited cholesterol 7α-hydroxylase activity and decreased steady state mRNA levels. However, it is important to remember that bile acid depletion stimulates and taurocholate replacement inhibits HMG-CoA reductase mRNA, thereby varying the substrate supply for cholesterol 7α-hydroxylase. Thus, the changes in cholesterol 7α-hydroxylase mRNA levels caused by cholestyramine and taurocholate may relate to the availability of cholesterol. Indeed, when cholesterol was added to the taurocholate-supplemented diet, the decrease in cholesterol 7α-hydroxylase mRNA levels was prevented (Table 1, Fig. 3), although the catalytic activity remained inhibited (Fig. 2B). It is noteworthy that the low cholesterol 7α-hydroxylase activities in these animals were not due to an underestimation of enzyme activity because of dilution of the radioactive cholesterol substrate by endogenous sterols which have been removed prior to assay (26, 27). In a recent report, Spady and Cuthbert (38) also showed that the decrease in cholesterol 7α-hydroxylase mRNA levels induced by feeding unconjugated cholic acid was completely prevented by adding cholesterol to the diet.

Our results suggest that the cholesterol substrate supply controls the amount of cholesterol 7α-hydroxylase mRNA by either modulating formation or degradation. In distinction, α-hydroxylated bile acids regulate the catalytic activity of the enzyme. In recent studies, Pandak et al. (42), using taurocholate, and Jelinek et al. (18), using cholate and chenodeoxycholate, have suggested that these bile acids regulate cholesterol 7α-hydroxylase transcriptionally, a conclusion that is completely different from the idea presented in this study. However, the powerful inhibitory effect of taurocholate on HMG-CoA reductase mRNA, which interrupts the supply of newly synthesized cholesterol, may not have been considered. As a consequence, the decrease in cholesterol 7α-hydroxylase mRNA attributed to taurocholate may, in fact, reflect the deficient supply of cholesterol. Only the experiments where cholesterol was added to taurocholate (Table 1) help clarify this important regulatory mechanism.

In summary, these experiments demonstrate that steady state mRNA levels of HMG-CoA reductase, the rate-controlling enzyme of cholesterol biosynthesis, are controlled by the hepatic taurocholate flux and its activity, post-transcriptionally, by cholesterol. In contrast, mRNA levels of cholesterol 7α-hydroxylase, the rate-controlling enzyme of bile acid synthesis, are regulated by the cholesterol substrate supply while catalytic activity is modulated by the hepatic taurocholate flux.

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