Regulatory effects of dietary sterols and bile acids on rat intestinal HMG CoA reductase

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Abstract The specific activity (concentration) of microsomal HMG CoA reductase of intestinal crypt cells was studied in rats fed sterols and bile acids, either singly or in combination. It was found that the basal activity of the reductase was not suppressed by the administration of relatively large amounts of bile acid (taurocholate or taurochenodeoxycholate). Bile acids reduced the specific activity of the reductase only in rats in which the activity of the enzyme had first been enhanced by biliary diversion or by sitosterol feeding. In addition, bile acid feeding abolished the diurnal elevation of reductase activity that normally occurs between midnight and 2 a.m. In no case did bile acids reduce enzyme activity below basal levels. A pronounced (60%) reduction of intestinal HMG CoA reductase activity was observed in rats fed cholesterol and bile acid in combination. This reduction in activity could not be ascribed to an increase in intestinal bile acid flux but was associated with an increase in sterol concentration within the intestinal crypt cells. These results indicate that dietary sterols and bile acids both play a role in the regulation of intestinal HMG CoA reductase.

Supplementary key words   intestinal absorption · microsomal enzyme · intestinal crypt cells · taurocholate · taurochenodeoxycholate · sitosterol · rate-determining step · cholesterol biosynthesis · ileum · jejunum

STEROL SYNTHESIS in the intestinal tract is confined to the area of epithelial cell generation (the crypts), suggesting that it serves the function of providing structural cholesterol for newly forming cells (1). Nevertheless, there is some evidence that intestinal biosynthesis contributes significant quantities of cholesterol to the rapidly exchangeable cholesterol pool (2, 3), particularly under

conditions when hepatic cholesterogenesis is inhibited; for example, during cholesterol feeding.

According to information presently available, cholesterol synthesis in liver and intestine follows an identical pathway, and the rate-determining step is catalyzed in both tissues by the enzyme HMG CoA reductase (mevalonate:NADP oxidoreductase [acylating CoA] EC 1.1.1.34) (4). Superficially, the properties of the intestinal reductase resemble those of the hepatic enzyme. However, the regulation of the reductases appears to differ in at least one important respect: Dietschy (5) has reported that in the intestine the rate of cholesterogenesis from acetate varies as a function of the luminal bile acid concentration, whereas in the liver, the rate of cholesterol biosynthesis is controlled by cholesterol in intestinal chylomicrons reaching the liver via the blood stream (6). The regulatory effects of sterols and bile acids on cholesterol biosynthesis are probably due to their ability to affect the concentration (activity) of the rate-limiting enzyme, HMG CoA reductase (4). The detailed mechanism whereby these substances exert this effect is not known. Partially because of this lack of information, it cannot be concluded that cholesterol has no effect upon the activity of intestinal HMG CoA reductase and that bile acids do not function as regulators of the hepatic enzyme. Dietschy (5) has pointed out that bile acids may play a secondary function in the regulation of intestinal cholesterogenesis by facilitating the entry of cholesterol (the inhibitor) into the mucosal cells. Similarly, on the basis of direct enzyme assays and the results of balance studies, several investigators have suggested that bile acids exert regulatory effects upon hepatic cholesterol biosynthesis (7, 8).

The availability of a method for the assay of the microsomal HMG CoA reductase of intestinal crypt cells (9) has enabled us to observe changes in the activity of this enzyme under various conditions of sterol and

bile acid intake. The results obtained are in accord with the assumption that the activity of the enzyme is affected by both bile acid flux and intracellular sterol concentration, but they do not rule out the possibility that sterols are the primary regulators.

EXPERIMENTAL PROCEDURES

Materials

Cholesterol, U.S.P., was recrystallized from ethanol and used without further purification. The sitosterol was kindly provided by Eli Lilly and Co., Indianapolis, Ind., and contained 92% sitosterol, 7% campesterol, and less than 1% stigmasterol. Sodium taurocholate and sodium taurochenodeoxycholate were purchased from Calbiochem, Los Angeles, Calif., and from Maybridge, Tintagel, Cornwall, England. The conjugates were examined by semiquantitative TLC (10), and only those preparations containing less than 1–2% impurities were used. A sample of pure taurochenodeoxycholate kindly supplied by Dr. A. F. Hofmann yielded the same results as the commercial product.

Animals and diets

Male rats of the Wistar strain, obtained from the Otisville laboratories of the New York City Health Department, were used in most of the experiments. For some of the studies, Sprague-Dawley-derived rats were purchased from the Charles River Breeding Laboratories, Wilmington, Mass. There was no difference between the two strains as far as intestinal HMG CoA reductase activity was concerned. The animals were kept in individual cages and were fed a stock diet consisting of ground Purina rat chow pellets supplemented with 5% corn oil. When required, sterols were added to this diet at a level of 2% and bile acids at a level of 1%. The sterols were incorporated into the diet from an ether solution, the bile acids from an aqueous alcohol solution.

The rats were fed the diets and drinking water ad lib. for 7 days. The animals were weighed at the start and at the end of the feeding period, and their food intake was measured daily. When at the end of the 7-day feeding period cumulative food intake or weight gain of an experimental animal differed by more than 10% from the average of the control group, that animal was excluded from the study. At the end of the feeding period, the animals were killed (between 9 a.m. and 11 a.m.) by cervical dislocation, the small intestines were removed, and the intestinal crypt cell layers were removed by a differential scraping technique (1). The microsomal fraction was prepared from jejunal and ileal crypts and used for assay of HMG CoA reductase activity as described previously (9). In all experiments not concerned

specifically with the diurnal rhythm of intestinal HMG CoA reductase activity, the rats were kept under the normal laboratory illumination. In the studies dealing with the rhythmic variations of reductase activity, groups of rats were kept in two identical dark rooms. Automatic timers were used to establish dark and light periods of equal duration (12 hr) (11). In some of the experiments, enzyme activity was studied at or near the maximum of the diurnal rhythm (12 midnight to 2 a.m.), i.e., 6–8 hr after the beginning of the dark cycle (11).

Sterol concentration in intestinal crypt cells

This determination was carried out as follows. The samples were hydrolyzed in 10% aqueous NaOH in an autoclave at 120°C for 3 hr. After allowing the samples to cool to room temperature, an equal volume of methanol was added, and the sterols were extracted with two equal volumes of n-hexane. The hexane solution was washed with water, dried over sodium sulfate, and evaporated in air at 60°C. A known volume of chloroform was added and an aliquot of this solution was analyzed by GLC, using a 180 cm × 4 mm glass column packed with 3% QF-1 on 80–100 mesh Gas-Chrom Q at a column temperature of 255°C.

Bile acid analysis

To compare the magnitude and the composition of the bile acid pool at the end of the feeding period, the rats were anesthetized with Diabutal (Diamond Laboratories, Des Moines, Iowa), and a cannula was inserted into the bile duct. Bile was collected for a period of 60 min; ether was used to maintain a light anesthesia. It was assumed for purposes of comparison that the rats secreted an approximately constant proportion of their bile acid pools during the 60-min period. The concentrations of cholesterol and bile acids were determined by a combination of TLC and GLC (12).

RESULTS

Experiments dealing with the effect of dietary bile acids on the activity of intestinal HMG CoA reductase are summarized in Table 1. The rats were fed the stock diet or the stock diet supplemented with 1% sodium taurocholate or sodium taurochenodeoxycholate for 1 wk. At the end of this period the animals were killed, and the activity of the microsomal HMG CoA reductase of jejunal and ileal crypts was determined. In all three groups, enzyme activity per milligram of protein was about 30% higher in the ileum than in the jejunum. This difference between ileal and jejunal enzyme activity was less pronounced in the present experiments than in a previous study in which the stock diet had not been

TABLE 1. Effect of dietary bile acids on intestinal HMG CoA reductase activity

Diet (1 wk)	No. of Rats	HMG CoA Reductase Activity	
		Jejunum	Ileum
		pmoles/mg/min[a]	
Stock[b]	8	86.7 (2.47)[c]	105.5 (3.18)
Stock + 1% sodium taurocholate	6	88.5 (1.67)	108.0 (3.32)
Stock + 1% sodium taurochenodeoxycholate	6	85.3 (1.41)	104.5 (2.68)

[a] Enzyme activity is reported throughout as pmoles mevalonolactone formed/mg microsomal protein/min.
[b] Ground Purina chow + 5% corn oil.
[c] Standard error of the mean in parentheses.

supplemented with 5% corn oil (9). Intestinal HMG CoA reductase activity was not affected by the ingestion of excess bile acid (approximately 200 mg/rat/day).

The lack of an inhibitory effect of excess bile acid on the basal activity of ileal HMG CoA reductase was confirmed by the studies listed in Table 2. The upper part of the table illustrates experiments in four rats with intact enterohepatic circulation: intraduodenal infusion of taurocholate at a rate of 1 g/day for 2 or 3 days had no effect on intestinal reductase activity. Administration of this large amount of bile acid did produce severe diarrhea.

The lower part of Table 2 shows experiments with four bile fistula rats. After 2 or 3 days' biliary diversion, ileal reductase activity more than doubled. In two ad-

TABLE 2. Effect of sodium taurocholate, infused intraduodenally, on ileal HMG CoA reductase activity

Treatment[a]	Ileal HMG CoA Reductase Activity at End of:	
	2 days	3 days
	pmoles/mg protein/min	
Control[a]	95.8	105.0
Infused with sodium taurocholate[b]	102.9	107.3
Bile fistula[c]	209.1	231.5
Infused with sodium taurocholate[d]	101.2	118.0

[a] All animals were male Wistar rats (avg wt 280 g); they were kept in restraining cages and fed a semisynthetic liquid diet (12) via duodenal cannula at a rate of 30 kcal/day. Each number in this table represents the ileal HMG CoA reductase activity of a single animal. The methods employed have been described in detail in a previous publication (12).
[b] Identical treatment as controls except that sodium taurocholate was dissolved in the liquid diet and infused via the duodenal cannula at a rate of 14 mg/100-g rat/hr (equivalent to 1 g/day for a 300-g rat). These animals developed diarrhea.
[c] Bile fistula rats fed via duodenal cannula at a rate of 30 kcal/day.
[d] Bile fistula rats; sodium taurocholate dissolved in liquid diet and infused at a rate of 14 mg/100-g rat/hr to simulate the circulating bile acid pool (12).

TABLE 3. Effect of cholesterol and bile acid on intestinal HMG CoA reductase studied near the diurnal maximum of enzyme activity[a]

Diet (1 wk)	No. of Rats	Time of Activity Measurement	HMG CoA Reductase Activity	
			Jejunum	Ileum
			pmoles/mg protein/min	
Stock[b,c]	5	12 noon	85.7 (3.09)[d]	99.1 (2.12)
Stock[b]	4	12 midnight	139.5 (2.85)	166.3 (2.65)
Stock + 2% cholesterol	4	12 midnight	137.1 (2.54)	161.7 (2.39)
Stock + 1% sodium taurocholate	4	12 midnight	83.9[e] (3.12)	95.3[e] (3.03)
Stock + 1% sodium taurochenodeoxycholate	4	12 midnight	87.4[e] (2.61)	98.8[e] (2.75)

[a] At midnight.
[b] Ground Purina chow + 5% corn oil.
[c] These animals were studied at the diurnal minimum, 12 noon, to serve as additional controls.
[d] Standard error of the mean in parentheses.
[e] Differs significantly from group on stock diet studied at 12 midnight ($P < 0.01$).

ditional bile fistula rats infused with sodium taurocholate at a rate of 14 mg/100-g rat/hr to simulate the circulating bile acid pool, reductase activity was maintained at levels closely resembling those of animals with intact enterohepatic circulation.

The experiments summarized in Tables 1 and 2 indicate that in rats with intact enterohepatic circulation, large increases in intestinal bile acid flux do not reduce the activity of intestinal HMG CoA reductase below the basal level observed in intact animals. However, when the formation of the enzyme had first been stimulated by biliary diversion, administration of bile acid for 2 or 3 days restored the basal activity. Similar effects of bile

TABLE 4. Effect of dietary plant sterol plus bile acid on intestinal HMG CoA reductase activity

Diet (1 wk)	No. of Rats	HMG CoA Reductase Activity	
		Jejunum	Ileum
		pmoles/mg protein/min	
Stock[a]	7	85.4 (2.48)[b]	99.1 (2.71)
Stock + 2% sitosterol	4	94.2[c] (1.69)	114.3[c] (2.42)
Stock + 2% sitosterol + 1% sodium taurocholate	4	88.1 (1.91)	100.5 (2.15)
Stock + 2% sitosterol + 1% sodium taurochenodeoxycholate	4	83.9 (2.13)	96.8 (2.68)

[a] Ground Purina chow + 5% corn oil.
[b] Standard error of the mean in parentheses.
[c] Differs significantly from control group on stock diet ($P < 0.01$).

TABLE 5. Inhibition of intestinal HMG CoA reductase activity by dietary cholesterol plus bile acid

Diet (1 wk)	No. of Rats	HMG CoA Reductase Activity	
		Jejunum	Ileum
		pmoles/mg protein/min	
Stock[a]	7	85.9 (2.46)[b]	99.2 (2.67)
Stock + 2% cholesterol	5	87.1 (2.51)	96.7 (2.95)
Stock + 2% cholesterol + 1% sodium taurocholate	4	39.5[c] (2.23)	38.6[c] (2.41)
Stock + 2% cholesterol + 1% sodium taurochenodeoxycholate	4	37.7[c] (1.98)	37.4[c] (2.17)

[a] Ground Purina chow + 5% corn oil.
[b] Standard error of the mean in parentheses.
[c] Differs significantly from control group on stock diet ($P < 0.01$).

TABLE 6. Bile composition of rats fed sterols and bile acids[a]

Diet (1 wk)	No. of Rats	Biliary Cholesterol Concentration	Total Taurine-conjugated Bile Acids
		mg/ml	mg/100-g rat/hr
Stock	7	0.201 (0.011)[b]	7.35 (0.208)
Stock + 1% sodium taurocholate	6	0.194 (0.013)	13.48[c] (0.629)
Stock + 1% sodium taurochenodeoxycholate	6	0.191 (0.011)	12.72[c] (0.522)
Stock + 2% cholesterol	6	0.179 (0.011)	7.24 (0.262)
Stock + 2% cholesterol + 1% sodium taurocholate	6	0.198 (0.007)	12.82[c] (0.341)
Stock + 2% cholesterol + 1% sodium taurochenodeoxycholate	4	0.180 (0.009)	12.68[c] (0.409)

[a] Bile was collected for 1 hr at the end of the 1-wk feeding period and was analyzed for cholesterol and bile acids as described in Experimental Procedures. On the average, the animals used in this study weighed 250–300 g and secreted 0.8–1.2 ml of bile/hr.
[b] Standard error of the mean in parentheses.
[c] Differs significantly from control group on stock diet ($P < 0.01$).

acids were observed in rats studied at midnight, when intestinal HMG CoA reductase activity has reached the maximum of its diurnal variation. The data summarized in Table 3 demonstrate that at midnight ileal reductase activity is nearly 70% greater than the basal activity measured at noon. The rhythmic increase of HMG CoA reductase activity persisted in cholesterol-fed rats but was abolished in animals fed 1% taurocholate or taurochenodeoxycholate for 1 wk.

The ability of dietary bile acids to maintain HMG CoA reductase activity at the basal level is further apparent from the experiments with sitosterol summarized in Table 4. The administration of 2% plant sterol for 1 wk consistently produced a stimulation of reductase activity in both jejunal and ileal crypt cells. This increase was not observed in rats fed sitosterol and bile acid in combination.

Feeding 2% cholesterol for 1 wk had no effect on the activity of intestinal HMG CoA reductase (Table 5). However, when cholesterol and bile acid (taurocholate or taurochenodeoxycholate) were fed in combination, HMG CoA reductase activity in jejunum and ileum was reduced by nearly 60%. The inhibitory effect of cholesterol plus bile acid is not ascribable to an increased bile acid flux. It is true that in the groups fed cholesterol and bile acid in combination, bile acid secretion was approximately doubled in comparison with the stock diet controls and the cholesterol-fed group (Table 6). However, the animals receiving either 1% taurocholate or 1% taurochenodeoxycholate exhibited the same increase in bile acid secretion as the groups fed these bile acids in combination with cholesterol, yet reductase activity in the former was not decreased below the control value.

We therefore examined the possibility that the inhibition of intestinal HMG CoA reductase in the groups fed cholesterol plus bile acid was related to changes in the cholesterol content of the intestinal crypt cells. The data

summarized in Table 7 demonstrate that in animals fed the stock diet, or the stock diet containing 1% taurocholate or 2% cholesterol, the cholesterol concentrations of ileal crypt cells were approximately identical, ranging from 1.62 to 1.73 mg/g wet wt. In the two groups receiving dietary cholesterol plus bile acids, the concentration of cholesterol in the crypt cells was increased by 20–30% in comparison with the cholesterol-fed group.

DISCUSSION

The present studies were carried out in the expectation that the direct assay of microsomal HMG CoA reductase (which catalyzes the rate-determining step of intestinal cholesterol synthesis) might provide more

TABLE 7. Cholesterol content of ileal crypt cells

Diet (1 wk)	No. of Rats	Cholesterol
		mg/g wet wt
Stock[a]	6	1.61 (0.072)[b]
Stock + 1% sodium taurocholate	5	1.67 (0.092)
Stock + 2% cholesterol	7	1.73 (0.096)
Stock + cholesterol + 1% sodium taurocholate	8	2.28[c] (0.21)
Stock + cholesterol + 1% sodium taurochenodeoxycholate	4	2.11[c] (0.119)

[a] Ground Purina chow + 5% corn oil.
[b] Standard error of the mean in parentheses.
[c] Differs significantly from control group on stock diet ($P < 0.01$).

precise measurements of synthetic rates than previous estimates based on acetate incorporation. In preliminary studies, we had attempted to determine rates of cholesterogenesis by measuring the incorporation of labeled acetate into cholesterol in slices or homogenates of rat intestine but had found it difficult to obtain reliable, reproducible results. In addition, even accurate measurements based on acetate incorporation may lead to erroneous conclusions because apparent changes of synthetic rates may be attributed to changes in the magnitude of the acetate pool, or pools of other intermediates (13). The improved precision and accuracy inherent in direct measurements of intestinal HMG CoA reductase have now enabled us to demonstrate that the activity of this enzyme (and, presumably, the rate of intestinal cholesterogenesis) does not bear a simple inverse relationship to the quantity of bile acid passing through the intestinal mucosa per unit time (5). The experiments summarized in Table 1 show that the specific activity (concentration) of intestinal HMG CoA reductase, measured at its diurnal minimum, was not affected by the ingestion of 1% taurocholate or 1% taurochenodeoxycholate for 1 wk. This lack of an inhibitory effect was observed with both of the primary bile acids of the rat and applied to both jejunal and ileal reductase activity. The rats used in these experiments weighed about 300 g and ingested 20–25 g of food per day, so that their daily bile acid intake was 200–250 mg. Bile acid secretion in the bile acid-fed animals increased about twofold (from 7 to 13 mg/100 g-rat/hr)(Table 6). If we assume that the circulation rate of the bile acid pool in all three groups of animals shown in Table 1 was approximately equal, we can conclude that a 100% increase in intestinal bile acid flux failed to inhibit intestinal HMG CoA reductase activity. This conclusion applies to rats with intact enterohepatic circulation, when enzyme activity was studied between 9 a.m. and 12 noon, i.e., during the period of the diurnal minimum. When the dose of bile acid was increased even further (by intraduodenal infusion of sodium taurocholate at a rate of approximately 1000 mg/day), the rats developed diarrhea, indicating that intestinal bile acid flux had reached or exceeded toxic levels. Nevertheless, even this excessive amount of administered bile acid had no effect on intestinal HMG CoA reductase activity (Table 2).

The administration of bile acids reduced the specific activity of intestinal HMG CoA reductase only when the activity of this enzyme had first been raised to values greater than those found in intact animals under basal conditions. Elevation of intestinal HMG CoA reductase activity was observed in the present study under three different conditions:

(1) In bile fistula rats, the specific activity of the enzyme was increased by more than 100% (Table 2).

Intraduodenal infusion of sodium taurocholate at a rate of 14 mg/100-g rat/hr, to simulate the circulating bile acid pool (12), reduced HMG CoA reductase activity to that observed in intact controls (Table 2).

(2) Rats fed a diet with 2% sitosterol exhibited significant increases in intestinal HMG CoA reductase activity (Table 4). We are unable at present to account for this stimulatory effect of the plant sterol. It is clear, however, that the enhancement of enzyme activity was no longer observed when the plant sterol was fed in combination with bile acid.

(3) Intestinal HMG CoA reductase activity possesses a diurnal rhythm that parallels that of the liver but has a considerably lower amplitude (11). The specific activity of the intestinal enzyme increases from the diurnal minimum (between 9 a.m. and 12 noon) to the diurnal maximum (12 midnight to 2 a.m.) by 60–70% (Table 3). The rhythmic variation persisted in cholesterol-fed animals, but in rats receiving dietary taurocholate or taurochenodeoxycholate, the characteristic peak of enzyme activity near midnight was abolished.

This finding implies that bile acid feeding had a profound effect on the rate of intestinal cholesterol synthesis when measured over a 24-hr period. Evidently, at night, when the bulk of intestinal cholesterol synthesis occurs, there was a 70% reduction during the administration of bile acids. Consequently, the net result of bile acid feeding is a profound drop in the daily rate of cholesterol biosynthesis, as determined by measurements of HMG CoA reductase activity.

These observations on "stimulated" reductase appear to be in accord with the hypothesis that the activity of the enzyme is regulated by the concentration of bile acids in the intestinal lumen or, better, by intestinal bile acid flux (1, 5). However, two experimental findings suggest that this view of the regulation of intestinal cholesterogenesis is probably an oversimplification. First, we have already shown that even very large increases of intestinal bile acid flux failed to reduce enzyme activity when the latter had reached a certain basal level (Table 2). Second, the activity of intestinal HMG CoA reductase was reduced to 30–40% of the basal level when cholesterol and bile acids were fed in combination (Table 5). This inhibition of enzyme activity does not appear to be a function of intestinal bile acid flux: bile acid secretion rates were nearly identical in the groups fed cholesterol plus bile acid and those fed bile acid alone (Table 6). It seems more likely that the observed inhibitory effects can be ascribed to increased cholesterol concentrations in the intestinal crypt cells of the animals fed cholesterol and bile acid in combination (Table 7). Similar correlations between the sterol content of the crypt cells and inhibitory effects on HMG CoA reductase activity were observed whenever absorbable sterols (cholestanol, campesterol) were

fed in combination with taurocholate or taurochenodeoxycholate.[1]

Superficially, the experiments reported here appear to be more closely in accord with the hypothesis that intestinal HMG CoA reductase is regulated by sterols, as well as by bile acids, a hypothesis that has also been proposed for hepatic HMG CoA reductase (4, 8). We cannot rule out the possibility, however, that bile acids exert their regulatory effect indirectly, by facilitating the entry of the inhibitor (cholesterol) into the cell. The failure of dietary cholesterol, or of infused micellar cholesterol (5), to inhibit intestinal cholesterogenesis might then be attributable to the inability of the sterol to reach the interior of the crypt cell, either directly or via the blood stream. A similar hypothesis was proposed by Harry, Morris, and McIntyre (14), who explained the apparent absence of negative feedback control of cholesterol synthesis by cholesterol in hepatoma cells in terms of the impermeability of the tumor to sterols or to sterol metabolites.

Clearly, additional data must be obtained before it can be decided whether sterols or bile acids, or both, are primary regulators of cholesterol synthesis by intestinal crypt cells. It will be necessary to measure both the fluxes and the concentrations of sterols and bile acids in intestinal crypt cells under a variety of conditions. These findings will have to be correlated with parallel measurements of HMG CoA reductase activity and, perhaps, with rates of epithelial cell turnover (1, 15).

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