Taurine increases bile acid pool size and reduces bile saturation index in the hamster[1]

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Abstract There is evidence that increased availability of taurine enhances the proportion of taurine-conjugated bile acids in bile. To explore the possibility that taurine treatment could also influence hepatic cholesterol and bile acid metabolism, we fed female hamsters for 1 week and measured both the biliary lipid content and the microsomal level of the rate-limiting enzymes of cholesterol and bile acid synthesis. In these animals the cholesterol 7α-hydroxylase activity was significantly greater in respect to controls ($P < 0.05$). The total HMG-CoA reductase activity, as well as that of the active form, was similarly increased. The stimulation of 7α-hydroxycholesterol synthesis was associated with an expansion of the bile acid pool size in taurine-fed animals. Taurine feeding was observed to induce an increase in bile flow as well as in the rate of excretion of bile acids, whereas the secretion rate of cholesterol in bile was decreased. As a consequence, the saturation index was significantly lower in taurine-fed animals ($P < 0.05$). The possible mechanisms through which taurine exhibits the modification of the enzyme activities and of the biliary lipid composition are discussed.—**Bellentani, S., M. Pecorari, P. Cordoma, P. Marchegiano, F. Manenti, E. Bosisio, E. De Fabiani, and G. Galli.** Taurine increases bile acid pool size and reduces bile saturation index in the hamster. *J. Lipid Res.* 1987. **28:** 1021–1027.

Supplementary key words bile acids • taurine • HMG-CoA reductase • cholesterol 7α-hydroxylase • biliary saturation index

Bile acids are conjugated with glycine and taurine before their secretion in bile. In the hamster and in humans, taurine is primarily of dietary origin (1) and normally the hepatic taurine conjugation of bile acids accounts for about 30–35% of the total bile acid pool in these species. Glycine and taurine bile acid derivatives possess different physicochemical properties which partly account for their functional and metabolic differences. Taurine-conjugated bile acids are more water-soluble and less toxic than glyco-conjugated bile acids (2). In rats, for example, the glyco-, but not the tauro-conjugated form of sulfolithocholate induces cholestasis (3). Furthermore, taurine feeding has been shown to prevent cholestasis induced by lithocholic acid sulfate in the guinea pig (4).

A reversal of glycine to taurine conjugation ratio (G/T ratio), which is easily obtained by prolonged feeding of taurine (5, 6), has been suggested to be particularly beneficial in humans during ursodeoxycholate treatment for cholesterol gallstone dissolution (7) inasmuch as the tauro-conjugate of ursodeoxycholic acid is more capable of solubilizing lipids than the glycine conjugate (8).

Evidence that taurine administration can also affect cholesterol and bile acid metabolism in humans has already been reported, but results were conflicting. Okamoto et al. (9) found an increase of bile salts and a decrease of cholesterol in the duodenal bile of taurine-supplemented preterm infants. On the other hand, Watkins et al. (10) showed that the rate of bile acid synthesis was unmodified in low-birth-weight infants. Finally, in the only study available in adult humans, Hardison and Grundy (6), by performing cholesterol balance studies in six volunteers fed taurine, did not find any remarkable modification of cholesterol and bile acid pool size, turnover and synthesis rate. The lack of the effect in this case may, however, depend on the fact that the dietary intake of taurine was not controlled; therefore, it is difficult to quantitate the exact amount of taurine consumed by those subjects.

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high pressure liquid chromatography; ALT, alanine transferase; AP, alkaline phosphatase; TCA, taurocholic acid; GCA, glycocholic acid; TCDCA, taurochenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; TDCA, taurodeoxycholic acid; GDCA, glycodeoxycholic acid; TLCA, taurolithocholic acid; GLCA, glycolithocholic acid; TUDCA, tauroursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid

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Due to these conflicting data, we thought it to be of interest to reconsider the problem by studying the effects of taurine dietary supplementation on cholesterol and bile acid metabolism under more controlled experimental conditions. The hamster was chosen because it shares some common features with man in the sterol and taurine metabolism, such as bile acid biliary composition (11), modulation of the rate-limiting enzymes of cholesterol and bile acid synthesis (12), hepatic bile acid conjugation (13), and taurine body distribution (14). Specifically, in relation to bile acid conjugation the hamster, as well as man, conjugates bile acids with both taurine and glycine, and taurine is generally the preferred substrate (13).

The aim of the present study was to answer the following questions. *1*) Does taurine feeding influence bile acid pool size and the rate of biliary secretion of cholesterol and bile acids? *2*)Are the changes related to a modification of cholesterol and bile acid synthesis in the liver?

MATERIALS AND METHODS

Compounds

[1,2-^{14}C]Taurine (sp act 97.5 mCi/mmol) was purchased from New England Nuclear Corp., Boston, MA. Taurine used as dietary supplement in this study was kindly supplied by Gipharmex S.p.A., Milano, Italy. All bile acids used as standards for HPLC were purchased from Calbiochem, La Jolla, CA, and they were 98–99% pure by HPLC,

The 3α-hydroxysteroid dehydrogenase used for the enzymatic assay of BA and the HMG-CoA were obtained from Sigma Chemical Co., St. Louis, MO. The kits for ALT, AP, cholesterol, and phospholipid assays were from Boehringer, Mannheim, FRG. All other chemicals and solvents were purchased from Carlo Erba, Milano, Italy, and were of analytical grade.

Animals and diets

Female Golden-Syrian hamsters (100–130 g body weight), obtained from Charles River, Calco Como, MI, Italy, were fed a commercial diet and maintained on a controlled light cycle from 8 AM to 8 PM. Animals were kept in metabolic cages and received taurine (0.063 g/100 ml of drinking water) for 1 week. This dosage was equivalent to an average consumption of 350–400 mg/kg body weight. Two sets of experiments were performed. In the first, at the end of the treatment, six couples of pair-matched animals (one control and one taurine-fed hamster) were anesthetized with Nembutal (10–12 mg/100 g body weight) and, after cholecystectomy, the bile duct was cannulated with a PE10 catheter. Bile was collected every 10 min for the first 2 hr for the determination of the bile flow and biliary lipid excretion rate. Each of the two hamsters was then put in a restraining cage and bile was collected for 14 hr for the measurement of the bile acid pool size. During the period of collection, body temperature was maintained at 37°C by the use of a heating lamp. In the second set of experiments, other animals (12 per each group) were killed by decapitation between 9 AM and 10 AM, blood was collected, and the liver was divided in three aliquots for taurine, HMG-CoA reductase, and cholesterol 7α-hydroxylase determination.

Biliary cholesterol, phospholipid, and bile acid assay

The concentration of cholesterol, phospholipids, and bile acids in the bile samples was measured using enzymatic methods as previously described (15–17). The cholesterol saturation index was calculated according to the formula of Thomas and Hofmann (18) based on the cholesterol saturation equilibrium of Hegardt and Dam (19), which has been shown to be valid for the range of total lipid concentration in the bile of hamsters (11).

Individual conjugated bile acids were analyzed by HPLC on an LC 5000 Varian instrument (Varian, Palo Alto, CA), a Varian column heater, and a Varian CDS 111L area integrator, using an RP-18, SPHERI-5 column 4.6 mm × 250 mm (Brownee Labs Inc., Santa Clara, CA), according to the method of Nakayama and Nakagaki (20). Bile samples were extracted with 10 volumes of methanol; the sample was dried, resuspended in the solvent system [acetonitrile–methanol–potassium phosphate buffer 30 mM (pH 3.40) 10:60:30 (v/v/v)] and filtered through a disposable Millipore HV filter, pore size, 0.45 μm. Testosterone acetate (100 μg/ml) was used as an internal standard. An aliquot of 20 μl of the filtered extract was injected onto the column. The flow rate was 1.0 ml/min with 2000 p.s.i. isobaric flow. Detection was made at 205 nm and temperature was kept at 40°C. Under these conditions all the conjugated bile acids were eluted within 40–45 min. A standard mixture of conjugated bile acids at a concentration of 100–120 μg/ml was injected each day. Peak areas were computed manually or with the use of an integrator. Recovery of each conjugated bile acid was similar to that reported by Nakayama and Nakagaki (19) and ranged between 95 and 100%.

Hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase determination

Liver aliquots were homogenized in buffer A or B, respectively, for HMG-CoA reductase or cholesterol 7α-hydroxylase. Buffer composition was as follows: A, 0.3 M sucrose, 10 mM mercaptoethanol, and 50 mM sodium fluoride; B, 0.25 M sucrose, 1 mM EDTA, and 50 mM sodium fluoride. Microsomes were isolated as previously described (20) by centrifuging the 10,000 *g* supernatant of the liver homogenates at 105,000 *g* for 1 hr. Microsomal pellets were washed and resuspended in the appropriate buffer as described for each enzyme determination. All the procedures for microsome preparation were performed

at 0–4°C. No further purification of the microsomal suspension was carried out. Microsomal suspensions were kept frozen at −80°C, until use. Protein concentration was determined according to Bradford (21), using albumin as a standard. Washed liver microsomes prepared as described above were incubated under the following different conditions. HMG-CoA reductase activity was determined with microsomes suspended in 300 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 50 mM phosphate buffer, pH 7.2. Assay conditions for the determination of both the fractions (active and total) of HMG-CoA reductase were as described by Cighetti, Galli, and Galli Kienle (22). Mevalonate formed during the incubation was measured by selected ion monitoring (23). The analyses of the extracts of samples after incubation were performed on a Varian Mat 112 S gas chromatograph-mass spectrometer as follows. Mevalonate, after its conversion to the lactone-trimethylsilyl derivative, was determined using an SPB 35 capillary column (Supelco, Bellefonte, PA), oven temperature 145°C, helium flow 0.8 ml/min; quantitation was carried out using deuterated mevalonic acid lactone as internal standard.

Cholesterol 7α-hydroxylase was assayed with microsomes suspended in 0.1 M phosphate buffer, pH 7.4 under the conditions previously described (24). 7α-Hydroxycholesterol formed from endogenous substrate was measured by selected ion monitoring (25). 7α-Hydroxycholesterol was determined as its trimethylsilyl derivative using an SP 2250 column (Supelco, Bellefonte, PA), 1 m length, oven temperature 280°C, helium flow 2 ml/min, using 5 α-cholestane as a reference compound. Each enzyme assay was performed in triplicate and the variation of the method was within the range of 10%.

Fecal extraction and analysis

Stool samples from the last 2 days of the study period were combined and homogenized with water. The procedures for the extraction of neutral sterols and bile acids in feces were according to Miettinen, Ahrens, and Grundy (26) and Grundy, Ahrens, and Miettinen (27). Neutral sterols were analyzed by gas-liquid chromatography (26) and sterol excretion was calculated as the sum of the amount of cholesterol and coprostanol. Bile acid content was analyzed by the Sterognost-3α enzymatic method (17). This method may give an overestimation of the amount of bile acids in feces due to the interference of other steroids that are substrates for the 3α-hydroxysteroid dehydrogenase. However, in the context of the present investigation, these interferences may not be so important, since the relative rather than the absolute value of excretion in the treated and control animals was the objective of the study.

Measurement of taurine

Taurine was measured in food, serum, urine, and liver of the animals. One g of the standard food pellet was homogenized in 5 ml of 6% sulfosalicylic acid and boiled at 110°C for 30 min. The solution was cooled, filtered, and centrifuged at 2000 *g* for 20 min. The supernatant was injected in an automated Kontron Liquimat III amino acid analyzer. The content of taurine in the standard diet was 0.905 mg/g of food pellet (mean of triplicate determinations).

Aliquots of liver (1 g) were homogenized in 4 ml of 0.25 M sucrose and taurine was extracted by the method described by Hardison and Proffitt (28). The extract was redissolved in 1 ml of water and 8 ml of acetone. After centrifugation to remove the inorganic salts, the supernatant was evaporated and the residue was dissolved in 1.2 ml of 6% sulfosalicylic acid. The solution was filtered through a 0.45-μm filter and 200 μl was used for amino acid analysis on the automated Kontron amino acid analyzer. Recovery of taurine was evaluated by addition of 1.2 μCi of [^{14}C]taurine to the food pellet and to the liver homogenates before the extraction. Taurine concentration in serum and urine was analyzed with the same procedure using 2 ml of the biological specimen.

Statistical analysis

Significance of differences between means was determined using the Student's *t* test (29).

RESULTS

As shown in **Table 1**, taurine administration induced a 25–30% increase of water intake and an equivalent decrease of food consumption in the treated animals, although these differences were not statistically significant. However, it is likely that the nutritional status of the two groups of animals was similar at the end of treatment, because neither the body nor the liver weights were influenced (Table 1). The amount of taurine derived from the diet in control animals corresponded to 13 ± 1 mg/day. On the basis of the combined food and water intake, the amount of taurine ingested by the treated hamsters was 35–50 mg/day, thus equivalent to three- to fourfold the normal average dietary intake.

Biochemical parameters such as serum cholesterol, ALT, AP, and taurine levels were also similar **(Table 2).**

TABLE 1. Effects of taurine on food consumption, water intake, and body and liver weights at the end of treatment[a]

Parameter	Control	Taurine-Treated
Food consumption (g)	14.2 ± 2.2	9.6 ± 1.9
Water intake (ml)	46.2 ± 18.2	65.2 ± 28.7
Body weight (g)	122 ± 34	132 ± 12
Liver weight (g)	4.3 ± 1.0	4.1 ± 0.4

[a]Means ± SD; six animals per group.

TABLE 2. Effects of taurine on serum cholesterol, alanine transferase (ALT), alkaline phosphatase (AP), and taurine levels[a]

Serum Biochemical Parameters	Control	Taurine-Treated
Cholesterol (μmol/ml)	4.3 ± 0.5	4.3 ± 0.9
ALT (U/ml)	100 ± 36	84 ± 33
AP (U/ml)	150 ± 53	202 ± 24
Taurine (μmol/ml)	0.25 ± 0.04	0.29 ± 0.08

[a]Means ± SD; six animals per group.

The urine and hepatic content of taurine was significantly greater in taurine-treated animals than in controls **(Fig. 1).**

Taurine induced an evident choleresis, as bile flow during the first hour of bile collection was 2.9 ± 0.27 μl/min per g of liver in treated hamsters versus 1.7 ± 0.38 μl/min per g of liver in controls ($P < 0.05$). The choleretic effect was associated with a higher total bile acid output in the taurine-treated animals during the first hour of bile collection **(Table 3).** Similarly, the amount of total bile acids in the bile collected after 14 hr from the taurine-fed animals was higher than in controls as well, indicating that taurine feeding expanded the total bile acid pool (Table 3). On the other hand, the biliary cholesterol output was decreased in the taurine-supplemented animals and, consequently, the saturation index was significantly lower than in control animals (Table 3). No difference in the daily sterol and bile acid fecal excretion between the two groups was found (Table 3).

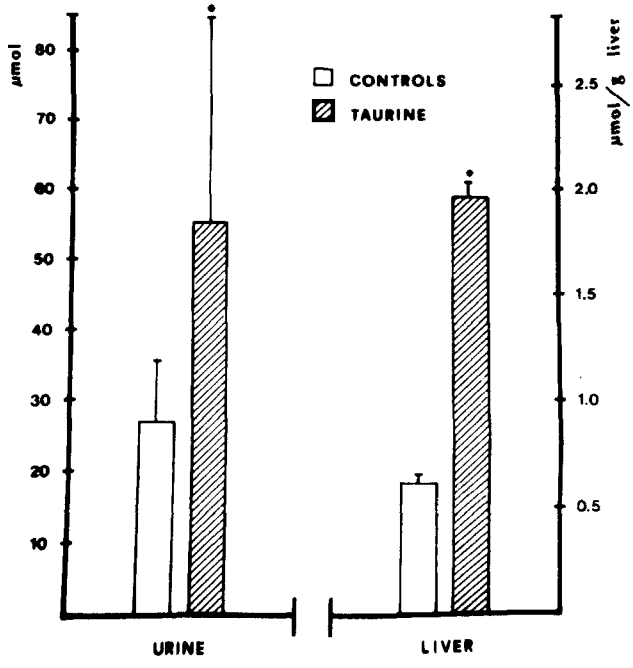

Fig. 1. Urinary and hepatic content of taurine in control and taurine-fed hamsters (mean ± SD of six animals per group). *$P < 0.001$ versus controls (unpaired t-test).

Taurine did not cause any significant modification of the content of the main bile acids in the bile or of the proportion of tauro- to glyco-conjugated bile acids **(Table 4).** G/T ratio was, however, statistically significant in respect to controls only after 15 days of treatment (1.08 ± 0.3 vs. 1.8 ± 0.5, $P < 0.05$, mean ± SD of six experiments). Bile samples of taurine-fed animals contained unidentified, more hydrophilic bile acids, which were not present in the bile of control hamsters (Table 4).

The activity of HMG-CoA reductase and cholesterol 7α-hydroxylase was higher in the taurine-treated animals compared to controls **(Fig. 2).**

DISCUSSION

Female hamsters were fed an amount of taurine that was three- to fourfold the normal intake, and many effects were observed. The hepatic content of taurine increased more than threefold. Bile acid pool size, directly measured by draining the bile over 14 hr (30), expanded by 30%; the biliary secretion rate of cholesterol decreased while bile acid output increased and, therefore, the lithogenic index was reduced. The activity of HMG-CoA reductase and cholesterol 7α-hydroxylase was stimulated. Fecal excretion of sterols and bile acids did not change significantly, nor did the biliary conjugation pattern of bile acids. Under our conditions, the change of G/T ratio was not apparent, probably because of the short time of treatment, since when animals were fed taurine for 15 days, the G/T ratio was significantly different from that of the controls.

There are three possible reasons to explain the increased pool size of bile acids: decreased loss, increased synthesis, or decreased turnover rate. We found that the bile acid fecal excretion in the taurine-fed hamsters was lower than that of controls even though the difference was not statistically significant. This could be consistent with the hypothesis that bile acid turnover is diminished. However, in contrast to these findings, the biliary bile acid secretion rate measured during the first hour of bile diversion, which in part can reflect the bile acid cycling frequency, was constantly and significantly higher in respect to controls. This suggests that the bile acid turnover rate could be either unchanged or expanded (as other authors have previously shown in other species) (31, 32). An in vivo measure of the daily fecal loss of a preinjected dose of ^{14}C-labeled cholic acid could have probably helped in discriminating between decreased, unchanged, or increased bile acid turnover rate. Since we did not perform this balance study, any of these possibilities can presently be excluded. We also measured cholesterol 7α-hydroxylase activity in isolated microsomes, which in most instances is directly correlated with the rate of bile acid synthesis in vivo (33), and we found that it was also 30% higher than

TABLE 3. Biliary and fecal lipid excretion and bile acid pool size in control and taurine-treated animals[a]

Parameter	Control	Taurine-Treated
Bile acid output (4)[b] (μmol/1st hr per kg)	45.8 ± 18.0	75.0 ± 16.6[c]
Cholesterol output (4)[d] (μmol/hr per kg)	1.38 ± 0.6	0.82 ± 0.37[c]
Phospholipid output (4)[d] (μmol/hr per kg)	3.6 ± 0.9	4.5 ± 1.6
Bile acid pool size (4) (μmol/kg)	174 ± 58	250 ± 81[c]
Saturation index (4)	1.21 ± 0.4	0.59 ± 0.3[c]
Fecal neutral sterol excretion (6) (mg/day)	5.1 ± 1.4	3.7 ± 0.6
Fecal bile acid excretion (6) (mg/day)	3.7 ± 1.2	2.4 ± 0.7

[a]Means ± SD; number of determinations in parentheses.
[b]Values refer to average output during the first hour of bile collection.
[c]$P < 0.05$ versus controls (paired t-test).
[d]Values refer to average output during the 14 hr of bile collection.

in controls. Therefore, we might assume that the increased cholesterol 7α-hydroxylase activity supports the hypothesis that the expansion of the bile acid pool in the taurine-fed animals is sustained by an increased hepatic bile acid synthesis.

The biliary output of cholesterol in taurine-fed animals was lower than in controls. The secretion of cholesterol into bile depends on the rate of cholesterol synthesis and on the amount of lipoprotein cholesterol entering the liver. It has been shown that, in the hamster, biliary cholesterol secretion is dissociated from the rate of hepatic cholesterol synthesis (30). Biliary cholesterol derives predominantly from a preformed source and only 2–5% comes directly from the newly synthesized sterol (30). As it can be argued from the higher activity of HMG-CoA reductase in taurine-fed animals, hepatic cholesterol synthesis was probably increased to provide for the increased need of new cholesterol for bile acid synthesis. Since hepatic uptake of cholesterol is inversely correlated to hepatic cholesterol synthesis (34), the hepatic uptake of cholesterol from the circulation was probably reduced in taurine-fed animals, due to the modulation of low density lipoprotein receptors by newly synthesized cholesterol. This would mean a reduced availability of cholesterol to be delivered to bile and a consequent reduction of biliary cholesterol output. The possibility that HMG-CoA reductase and cholesterol 7α-hydroxylase activities could be stimulated via an increased loss of bile acids, as occurs with bile salt sequestrants (24), is excluded by the fact that fecal excretion of sterols and bile acids is unchanged.

The metabolic route through which taurine exerts its effect on HMG-CoA reductase and cholesterol 7α-hydroxylase is presently under investigation. Among the hypotheses, the one which takes into account the activation of the enzymes induced by thiol compounds was considered. Several authors have reported that the activities of the two enzymes are modulated by sulfhydryl compounds (35–40), and reduced glutathione appears to be the physiological modulator. Taurine could indirectly act on the metabolic pathway of sulphur-amino acids, since it is biosynthesized in the liver from cysteine. Administration of taurine could therefore enhance the availability of cysteine, which is the limiting precursor in glutathione

TABLE 4. Bile acid composition and ratio between glyco- and tauro-conjugated bile acid (G/T ratio) of hepatic bile[a]

Bile Acid	Controls	Taurine-Treated
TUDCA	2 ± 0.6	2 ± 0.7
GUDCA	4 ± 3.5	2 ± 1.0
TCA	28 ± 15	21 ± 10
GCA	35 ± 10	38 ± 10
TCDCA	7 ± 4.0	5 ± 2.8
GCDCA	9 ± 6.1	10 ± 5.6
TDCA	3 ± 1.3	5 ± 4.0
GDCA	5 ± 2.5	8 ± 3.0
TLCA	2 ± 1.8	2 ± 1.4
GLCA	5 ± 3.6	2 ± 1.4
Others[b]		5 ± 2.5
G/T ratio	1.8 ± 0.5	1.6 ± 0.6

[a]Means ± SD; six animals per group.
[b]Unidentified bile acids with higher polarity.

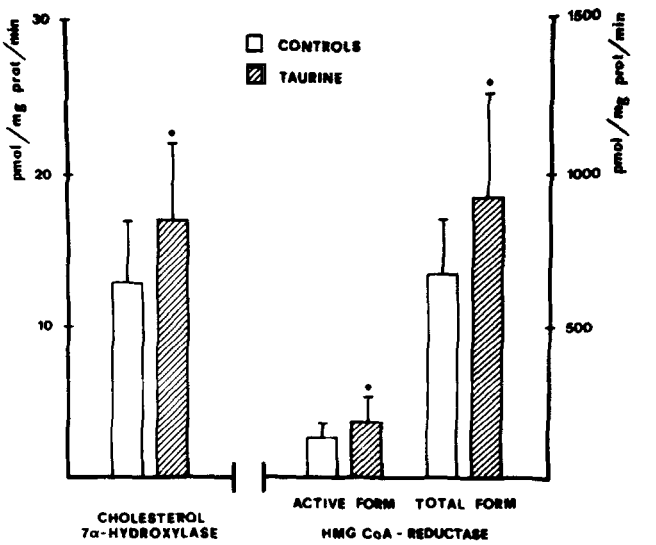

Fig. 2. Activity of HMG-CoA reductase and cholesterol 7 α-hydroxylase in control and taurine-fed hamsters (mean ± SD of 12 animals per group). *$P < 0.05$ versus controls (unpaired t-test).

synthesis (40). Such an effect on the bile acid enzyme regulatory system could not be specific for taurine and could be evident also for other sulfur-amino acids, as was recently demonstrated for L-cysteine (40). At present, we cannot make a firm conclusion on this matter. However, results of a preliminary experiment show that, in glutathione-depleted animals treated with taurine, the levels of hepatic glutathione (1.45 ± 0.25 μmol/g tissue, mean $\pm$ SD of three determinations) are higher than in untreated animals (0.73 ± 0.23 μmol/g tissue, $P < 0.02$). These data are in favor of the hypothesis that a modification of the thiol status of the liver may account for the enhancement of the enzymatic activities observed in taurine-fed animals.

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REFERENCES

1. Gaull, G. E., H. Pesantes-Morales, and E. Charles. 1985. Taurine in human nutrition: overview. *Prog. Clin. Biol. Res.* **179:** 3–21.
2. Hofmann, A. F., and A. Roda. 1984. Physicochemical properties of bile acids and their relationship to biological properties: an overview of the problem. *J. Lipid Res.* **25:** 1477–1489.
3. Yousef, I. M., B. Tuchweber, R. J. Vonk, D. Mass, M. Audet, and C. C. Roy. 1981. Lithocholate cholestasis. Sulfated glycolithocholate-induced intrahepatic cholestasis in rats. *Gastroenterology.* **80:** 233–241.
4. Dorvil, N. P., I. M. Yousef, B. Turchweber, and C. C. Roy. 1983. Taurine prevents cholestasis induced by lithocholic acid sulfate in guinea pigs. *Am. J. Clin. Nutr.* **37:** 221–231.
5. Sjövall, J. 1959. Dietary glycine and taurine effects on bile acid conjugation in man: bile acids and steroids. 75.*Proc. Soc. Exp. Biol. Med.* **100:** 676–678.
conjugation in man. *Proc. Soc. Exp. Biol. Med.* **100:** 676–678.
6. Hardison, W. G. M., and S. M. Grundy. 1983. Effect of bile acid conjugation pattern on bile acid metabolism in normal humans. *Gastroenterology.* **84:** 617–620.
7. Batta, A. K., and G. Salen. 1981. Taurine increases the effectiveness of ursodeoxycholic acid as gallstone dissolving agent. *Hepatology.* **1:** 494 (abstract).
8. Igimi, H., and M. C. Carey. 1981. Cholesterol gallstone dissolution in bile: dissolution kinetics of crystalline (anhydrate and monohydrate) cholesterol with chenodeoxycholate, ursodeoxycholate, and their glycine and taurine conjugates. *J. Lipid Res.* **22:** 254–270.
9. Okamoto,E., D. K. Rassin, C. L. Zucker, G. S. Salen, and W. C. Heird. 1984. Role of taurine in feeding the low-birthweight infant. *J. Pediatr.* **104:** 936–940.
10. Watkins, J. B., A. L. Jarvenpaa, P. Sczepanik-Van Leeuwen, P. D. Klein, D. K. Rassin, G. Gaull, and N. C. R. Raiha. 1983. Feeding the low-birth-weight infant. V. Effects of taurine, cholesterol and human milk on bile acid kinetics. *Gastroenterology.* **85:** 793–800.
11. Handelsman, B., G. Bonorris, and G. W. Marks. 1982. Enrichment of bile with tauroursodeoxycholic acid and biliary cholesterol saturation in hamsters. *Am. J. Physiol.* **243:** G424–G427.
12. Bosisio, E., G. Cighetti, M. Galli Kienle, R. Tritapepe, and G. Galli. 1984. HMG-CoA reductase and cholesterol 7α-hydroxylase in human liver. *Life Sci.* **34:** 2075–2081.
13. Hardison, W. G. M. 1983. Relation of hepatic taurine pool size to bile acid conjugation in man and animals. *In* Sulfur Amino Acids: Biochemical and Clinical Aspects. Alan R. Liss Inc., New York. 407–417.
14. Jacobsen, J. G., and L. H. Smith, Jr. 1968. Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* **48:** 424–511.
15. Fromm, H., P. Amin, H. Klein, and I. Kupke. 1980. Use of a simple enzymatic assay for cholesterol analysis in human bile. *J. Lipid Res.* **21:** 259–261.
16. Gurantz, D., M. F. Laker, and A. F. Hofmann. 1981. Enzymatic measurement of choline-containing phospholipids in bile. *J. Lipid Res.* **22:** 373–376.
17. Talalay, P. 1960. Enzymatic analysis of steroid hormones. *Methods Biochem. Anal.* **8:** 119–143.
18. Thomas, P. J., and A. F. Hofmann. 1973. A simple calculation of lithogenic index of bile: expressing biliary lipid composition on rectangular coordinates. *Gastroenterology.* **65:** 698–700.
19. Hegardt, F. G., and H. Dam. 1971. The solubility of cholesterol in aqueous solution of bile salts and lecithin. *Z. Ernaehrungswiss.* **10:** 239–243.
20. Nakayama, F., and M. Nakagaki. 1980. Quantitative determination of bile acids in bile with reversed phase high performance liquid chromatography. **183:** 287–293.
21. Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72:** 248–254.
22. Cighetti, G., G. Galli, and M. Galli Kienle. 1983. A simple method for studies on the regulation of cholesterol synthesis using freshly isolated hepatocytes. *Eur. J. Biochem.* **133:** 573–578.
23. Cighetti, G., E. Santariello, and G. Galli. 1981. Evaluation of 3-hydroxy-3-methylglutaryl-CoA reductase activity by multiple selection monitoring. *Anal. Biochem.* **110:** 153–158.
24. Cighetti, G., E. Bosisio, G. Galli, and M. Galli Kienle. 1983. The effect of cholestyramine on liver HMG-CoA reductase and cholesterol 7 alpha-hydroxylase in various laboratory animals. *Life Sci.* **33:** 2483–2488.
25. Sanghvi, A., E. Grassi, C. Bartman, R. Lester, M. Galli Kienle, and G. Galli. 1981. Measurement of cholesterol-7α-hydroxylase activity with selected ion monitoring. *J. Lipid Res.* **22:** 720–724.
26. Miettinen, T. A., E. H. Ahrens, and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
27. Grundy, S. M., E. H. Ahrens Jr., and T. D. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
28. Hardison, W. G. M., and J. H. Proffitt. 1977. Influence of hepatic taurine concentration on bile acid conjugation with taurine. *Am. J. Physiol.* **232:** E75–E79.
29. Glantz, S. A. 1977. Primer of Biostatistics. McGraw-Hill Book Co., New York. 63–90.
30. Turley, S. D., D. K. Spady and J. M. Dietchy. 1983. Altera-

tion of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed newly synthesized cholesterol. *Gastroenterology.* **84:** 253–264.
31. Kibe, A., C. Wake, T. Kuramoto, and T. Hoshita. 1980. Effect of dietary taurine on bile acid metabolism in guinea pigs. *Lipids.* **15:** 224–229.
32. Yamanaka, Y., K. Tsuji, T. Ichikawa, Y. Nakagawa, and M. Kawamura. 1985. Effect of dietary taurine and cholesterol gallstone formation and tissue cholesterol contents in mice. *J. Nutr. Sci. Vitaminol.* **31:** 225–232.
33. Bosisio, E. 1987. Dietary and pharmacological control of cholesterol 7alpha-hydroxylase. Proceedings of the IX International Symposium on Drugs Affecting Lipid Metabolism. Springer Verlag, Heidelberg. In press.
34. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
35. Ziegler, D. M. 1985. Role of reversible oxidation–reduction of enzyme thiols-disulphides in metabolic regulation. *Annu. Rev. Biochem.* **54:** 305–329.
36. Roitelman, J., and J. Shechter. 1984. Regulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. Evidence for thiol-dependent allosteric modulation of enzyme activity. *J. Biol. Chem.* **259:** 870–877.
37. Kalles, I., and K. Wikvall. 1981. Role of sulfhydryl groups in catalytic activity of purified cholesterol 7 alpha-hydroxylase system from rabbit and rat liver microsomes. *Biochem. Biophys. Res. Commun.* **100:** 1361–1369.
38. Danielsson, H., I. Kalles, and K. Wikvall. 1984. Regulation of hydroxylations in biosynthesis of bile acids. Isolation of a protein from rat liver cytosol stimulating reconstituted cholesterol 7 alpha-hydroxylase activity. *J. Biol. Chem.* **259:** 4258–4262.
39. Andersson, S., and H. Boström. 1984. Effect of disulfiram on rat liver cholesterol 7alpha-hydroxylase. *Biochem. Pharmacol.* **33:** 2930–2932.
40. Hassan, A. S., J. J. Hackley, and E. H. Jeffrey. 1984. Role of glutathione in the regulation of hepatic cholesterol 7alpha-hydroxylase, the rate-limiting enzyme of bile acid biosynthesis. *Steroids.* **44:** 373–380.
41. Higashi, T., N. Tateishi, and Y. Sakamoto. 1983. Liver glutathione as a reservoir of L-cysteine. *In* Sulfur Amino Acid: Biochemical and Clinical Aspects. Alan R. Liss Inc., New York. 419–434.