Effect of Taurine Levels on Liver Lipid Metabolism: An *In Vivo* Study in the Rat (43516)

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Abstract. **Previous studies using guinea pigs and cats have shown that liver lipid composition is affected by intrahepatic taurine levels. The purpose of the present study was to determine whether this sulfonated amino acid could also affect lipid metabolism in the rat, an animal capable of synthesizing substantial amounts of taurine and used extensively in studies on lipid metabolism. Wide variations in the hepatic taurine content were induced by administering either 1% taurine or 1% guanidinoethane sulfonate in the drinking water for 2 weeks. These treatments increased and decreased taurine liver content, respectively, but did not affect either food or water intakes, or growth rates. The plasma concentrations of the major lipid classes in treated animals did not show any significant alteration in comparison to control animals, except for nonesterified fatty acid levels that were significantly lowered by guanidinoethane sulfonate administration. Taurine supplementation did cause a significant decrease in total hepatic lipid content that was attributable to the reduction of free and esterified cholesterol, triglyceride, and phosphatidylethanolamine hepatic concentrations. This same treatment slightly increased both bile flow and secretion of taurine-conjugated primary bile salts. In particular, the proportion of tauro-β-muricholate significantly increased, whereas that of taurodeoxycholate greatly decreased. The administration of guanidinoethane sulfonate reduced both the bile flow and the secretion of taurine-conjugated bile salts and caused a significant alteration in the ratio between glycine- and taurine-conjugated bile salts. This did not occur after the treatment with taurine. Interestingly, we observed an inverse correlation between hepatic taurine levels and the proportion of either cholesteryl ester in hepatic lipids or taurochenodeoxycholate in biliary bile salts. These facts suggest that taurine hepatic levels influence mostly hepatic steroid metabolism, but they also affect the metabolism of other lipid classes.** [P.S.E.B.M. 1993, Vol 202]

Taurine, considered to be an essential nutrient for infants and cats, is distributed extensively in mammalian cells and tissues (1, 2). Its only recognized metabolic function in liver is conjugation with bile acids, which is important for bile secretion and lipid absorption (3). Nevertheless, this compound also exerts nonmetabolic functions and has beneficial effects on the liver that include prevention and treatment of cholestasis and prevention of liver damage due to toxic chemicals (4–6).

In past decades, much attention has been paid to the effects of taurine on bile acid metabolism. Conversely, few studies concerning the role of taurine on the other liver lipids have been reported, even though liver is the central organ of lipid metabolism and, in most animal species, liver is also the major site of taurine biosynthesis. Previous *in vitro* studies with human hepatoblastoma cells showed that cellular levels of taurine were associated with the rate of bile acid synthesis, the reduction of free cellular cholesterol concentration, and the expression of high affinity, low density lipoprotein (LDL) receptors (7). Similarly, *in vivo* studies with hamster (8), guinea pig (9), and mouse (10) showed that supplementation with taurine influenced the activity of 7α-cholesteryl hydroxylase and the HMG-CoA reductase activity. Thus, it appears that taurine is involved not only in biliary lipid secretion, but also in lipid metabolism. This possibility is supported by our previous reports on taurine-related changes in liver lipid composition of guinea pigs (11) and cats (12).

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Received March 23, 1992. [P.S.E.B.M. 1993, Vol 202]
Accepted June 23, 1992.

0037-9727/93/2021-0088$3.00/0

In the present report, we extend the study of taurine effects on liver lipid metabolism to the rat, a species commonly used in lipid metabolism studies and one that can actively synthesize taurine. We induced large variations in the intrahepatic concentrations of this sulphur-containing amino acid by administering either taurine or guanidinoethane sulfonate (GES) in the drinking water. Administration of GES, a structural analog of taurine, has been used to effectively and rapidly reduce taurine levels in rat tissues (13, 14). Although its mechanism of action has not been completely elucidated, the histologic changes caused by GES administration in many rat tissues (e.g., retina, heart, and central nervous system) were similar to those observed in cats chronically fed a taurine-free diet (15, 16). Therefore, rats treated with GES have been suggested as an animal model for studying taurine function *in vivo* (15). In this study, the lipid composition of liver, blood, and bile in animals treated with either taurine or GES has been compared with that of untreated animals used as controls. This comparison showed that taurine hepatic levels affected not only the bile steroid metabolism, but also the liver lipid metabolism.

Materials and Methods

Animals and Experimental Design. Twenty-five male Wistar rats (Charles River Italiana, Calco-Como, Italy) with body weights ranging from 120 to 150 g were randomly divided into the following groups: taurine (10 animals), control (10 animals), and GES (five animals). The rats were maintained for 2 weeks in a room with controlled temperature and humidity (20–22°C and 40–60%, respectively) under a 12:12-hr light:dark cycle. All the rats were fed *ad libitum* with the same semisynthetic regimen for rodents containing 18.5% protein, 3.0% fat, 6.0% fiber, 6.0% salt, and vitamin mixture (4RF21 Formula by Mucedola s.r.l., Settimo Milanese). The taurine content in this diet was about 0.02%, as determined by the automatic amino acid analyzer (see below). Control rats drank tap water. Taurine- and GES-treated animals had a 1% addition of taurine or GES in tap water. The drinking water was replenished daily. The food and water consumption of each animal was recorded daily. At the end of treatment, after an overnight fasting, each animal had the common bile duct cannulated under ether anesthesia, and the bile was collected for 2 hr. The animals were then sacrificed by decapitation under light ether anesthesia. The blood was collected and the liver was excised.

Lipid Analyses. The blood samples were centrifuged at 3000 rpm for 10 min. Plasma levels of free cholesterol (FCH), cholesteryl esters, triglyceride, and nonesterified fatty acid were measured using enzymatic commercial kits purchased from Boehringer Mannheim Italia (Milano, Italy). Total phospholipid content was determined by inorganic phosphorus assay after digestion with perchloric acid (17). Plasma lipoproteins were separated by gradient ultracentrifugation with a discontinuous gradient into very low density lipoprotein plus low density lipoprotein (VLDL + LDL), high density lipoprotein 1 (HDL1), and high density lipoprotein 2 plus high density lipoprotein 3 (HDL) fractions, as described previously (18). In each lipoprotein fraction, FCH, cholesteryl esters, triglyceride, and phospholipid were determined with the same methods used for plasma.

Aliquots of liver were homogenized and the lipids were extracted with a chloroform:methanol (2:1, v/v) mixture, according to Folch *et al.* (19). Neutral lipid and phospholipid subclasses were separated by thin-layer chromatography precoated silica gel plates (E. Merck, Darmastadt, Germany) with the solvent mixtures *n*-hexane:diethyl-ether:acetic acid (70:30:1, by vol) and chloroform:methanol:acetic acid:water (25:15:4:2, by vol), respectively. The lipid classes separated on the plate were revealed with iodine vapor. The bands identified by comparison with standard lipid mixtures as cholesteryl esters, triglyceride, nonesterified fatty acid, diglyceride, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine plus phosphatidylinositol, sphingomyelin, and lysophosphatidylcholine, were scraped off the plate. The fatty acid composition and content of each band were measured by gas-liquid chromatography using methyl heptadecanoate as internal standard (12).

Total bile salt concentration in the bile was determined by an enzymatic procedure based on the use of 3α-hydroxysteroid dehydrogenase (20). Bile salt composition of bile samples was analyzed by high pressure liquid chromatography, as reported previously (21).

The taurine determination was carried out on aliquots of plasma and liver homogenates after their deproteinization with 10% perchloric acid and centrifugation at 10,000 rpm for 20 min by an automatic amino acid analyzer (Beckman amino acid analyzer, model 6300).

Taurine was purchased from Sigma Chemical Co. GES was synthesized and purified as described by Huxtable *et al.* (13). Its residual content of taurine was below 0.1%, as verified with the amino acid analyzer.

Statistical Analyses. The results are reported as means ± SD. Statistical evaluation of differences between treatment groups was made by using a one-way analysis of variance at probability level ≥95%. Means were separated by a protected Fisher's least significant difference test. Analyses were performed with the SOLO statistical system by BMDP Statistical Software, Inc. (Los Angeles, CA).

Results

During the study, neither food nor water intake showed any significant differences between controls and

treated groups. Water and food intakes were 35.4 ± 3.0 ml/day, 36.1 ± 3.4 ml/day, and 35.9 ± 2.3 ml/day and 29.4 ± 3.5 g/day, 30.2 ± 1.7 g/day, and 27.1 ± 3.4 g/day in taurine, control, and GES groups, respectively. Similarly, there were no significant differences between treated and control groups in growth rate values (25.8 ± 6.3 g/day, 26.0 ± 3.6 g/day, and 24.6 ± 1.6 g/day in taurine, control, and GES groups, respectively). However, there were divergent changes in plasma and liver taurine levels in response to taurine and GES treatments. Both plasma and liver taurine concentrations were significantly increased by taurine treatment and decreased by GES treatment (Fig. 1). Besides the variations in liver taurine levels, changes in liver lipid composition were also observed (Table I). In brief, the animals treated with 1% taurine in drinking water for 2 weeks had total lipid concentrations in the liver that were much lower than the other two groups. This reduction was attributable mostly to a significant decrease in the FCH, triglyceride, cholesteryl ester, and phosphatidylethanolamine classes and to a slight decrease in the phosphatidylcholine fraction. GES-treated animals did not show any significant difference when compared with the control group. The concentrations of individual lipid classes in rat liver did not show any correlation with intrahepatic taurine content, except for the cholesteryl ester percentage, which was inversely related to liver taurine levels (Fig. 2).

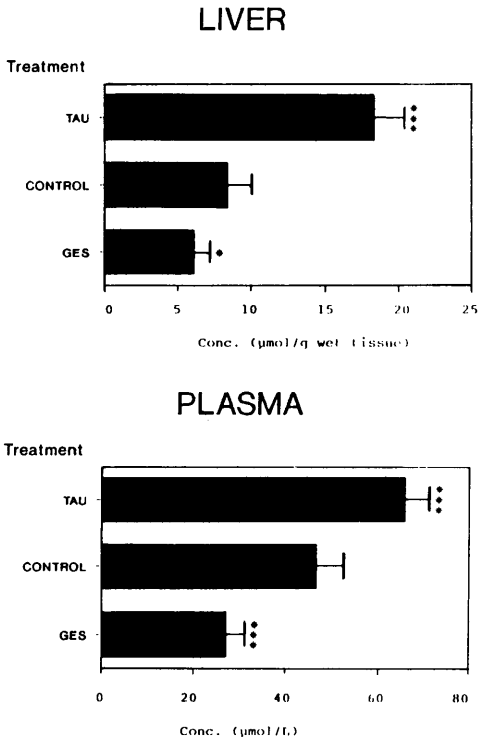

Figure 1. Plasma and hepatic taurine concentrations in rats treated with either 1% taurine (n = 8) or 1% GES (n = 5) in drinking water for 2 weeks in comparison with control animals (n = 8). $^{*}P < 0.05$ and $^{***}P < 0.001$, treatment versus control group.

The plasma concentrations of FCH, cholesteryl esters, triglyceride, and phospholipid (Table II) were not affected by the different treatments. However, rats given 1% GES for 2 weeks showed a significant decrease in plasma nonesterified fatty acid levels when compared with control animals. In keeping with these results, the total lipid concentrations of isolated lipoprotein fractions did not show any significant difference induced by the treatments. The total lipid concentrations of VLDL + LDL fractions were 75.6 ± 6.0 mg/dl, 74.8 ± 6.5 mg/dl, and 69.8 ± 6.2 mg/dl in the taurine, control, and GES groups, respectively. In HDL1 and HDL fractions, the lipid concentrations were 64.2 ± 2.6 mg/dl, 63.7 ± 2.4 mg/dl, and 59.7 ± 4.8 mg/dl and 197.6 ± 5.2 mg/dl, 194.4 ± 6.1 mg/dl, and 202.6 ± 7.2 mg/dl in taurine, control, and GES groups, respectively. The three groups showed significant differences in percentage of distribution of some of the lipid classes that were measured in each lipoprotein fraction (Fig. 3). This is shown in Figure 3 by separate panels for each lipoprotein class. Treatment with GES increased the proportion of phospholipid and decreased that of triglyceride in the VLDL + LDL fraction. This same treatment increased the proportion of cholesteryl esters in both the HDL1 and HDL fractions and decreased the proportion of triglyceride and proteins in HDL1 and HDL, respectively. Treatment with taurine induced an increase in phospholipid and cholesteryl ester levels of HDL and HDL1 fractions, respectively. Protein level was reduced by taurine treatment, but only in the HDL fraction.

Table III shows the relationship between taurine liver levels and bile flow and biliary lipid secretion. The animals supplemented with taurine showed a slight increase in bile flow. This increase was accompanied by a slight increase in secretion of biliary steroids (both FCH and bile salt). Conversely, GES treatment induced a slight decrease in bile flow and a significant decrease in the secretion of the major bile lipids.

The distribution of bilary bile salt in each group is shown in Table IV. The proportions of primary taurine-conjugated bile acid were 69.58 ± 5.45%, 46.39 ± 6.50%, and 49.04 ± 3.91% in taurine, control, and GES groups, respectively. The difference between taurine and control groups was highly significant ($P < 0.001$). In taurine-treated rats, the proportion of tauro-β-muricholate dramatically increased, whereas those of taurochenodeoxycholate and taurodeoxycholate decreased when compared with control animals. In GES-treated rats, the proportion of taurodeoxycholate decreased and that of glycocholate increased when compared with the control group. This fact caused a great increase in the molar ratio of glycine and taurine conjugates that was 0.11, 0.10, and 0.38 in taurine, control, and GES groups, respectively. In all the animals, the liver con-

Table I. Liver Lipid Concentrations (μmol/g wet tissue) in Rats Treated with Either 1% Taurine or 1% GES for 2 Weeks in Comparison with Control Animals

	Taurine ($n = 10$)	Control ($n = 10$)	GES ($n = 5$)
FCH[a]	1.35 ± 0.42[b,c]	2.03 ± 0.48	1.82 ± 0.13
TG	5.30 ± 3.06[b,c]	8.82 ± 3.39	8.16 ± 4.59
FFA	1.19 ± 0.22[b]	1.43 ± 0.30	1.28 ± 0.46
CE	0.93 ± 0.31[d,e]	1.56 ± 0.35	1.78 ± 0.20
DG	0.44 ± 0.14	0.48 ± 0.18	0.45 ± 0.24
PE	7.24 ± 1.36[d,e]	10.96 ± 2.27	10.86 ± 0.79
PC	12.88 ± 1.49	14.98 ± 3.32	13.40 ± 3.00
PS	2.82 ± 0.51[b,c]	3.71 ± 1.03	3.46 ± 0.81
LPC	0.48 ± 0.12	0.47 ± 0.10	0.47 ± 0.10
SM	1.15 ± 0.21[c]	1.37 ± 0.33	1.38 ± 0.13
Total concentration	33.92 ± 4.21[d,e]	45.93 ± 5.85	43.05 ± 4.89

[a] Abbreviations used in table: FCH, free cholesterol; TG, triglyceride; FFA, free fatty acids; CE, cholesteryl ester; DG, diacylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; LPC, lysophosphatidylcholine; SM, sphingomyelin.
[b] Different from control ($P \leq 0.05$).
[c] Different from GES ($P \leq 0.05$).
[d] Different from control ($P \leq 0.01$).
[e] Different from GES ($P \leq 0.01$).

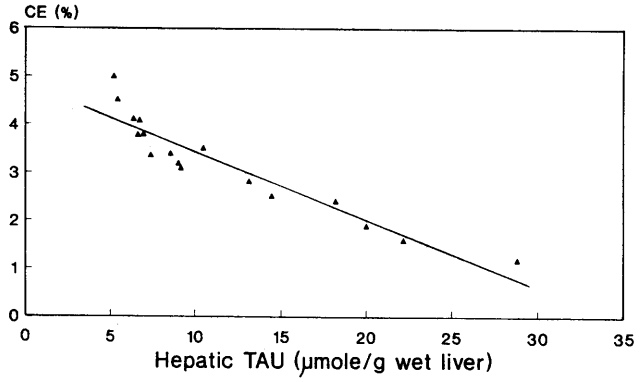

Figure 2. The inverse correlation between hepatic taurine concentration and the percentage of cholesteryl ester (CE) in liver lipid (Pearson $R = -0.934$, $n = 17$).

Table II. Plasma Concentrations (μmol/liter) of the Major Lipid Classes in Rats Treated with Either 1% Taurine or 1% GES in Drinking Water for 2 Weeks in Comparison with Control Animals[a]

	Taurine ($n = 8$)	Control ($n = 8$)	GES ($n = 5$)
FCH	43.4 ± 9.0	47.8 ± 7.8	43.4 ± 8.5
CE	127.2 ± 21.0[b,c]	108.3 ± 19.4	87.2 ± 23.9
TG	54.1 ± 8.1	51.2 ± 4.3	47.8 ± 7.6
PL	356.8 ± 16.3	356.0 ± 18.9	355.5 ± 24.1
FFA	139.8 ± 30.1[c]	137.7 ± 30.1	81.8 ± 26.5[d]

[a] See Table I for abbreviations.
[b] Different from control ($P \leq 0.05$).
[c] Different from GES ($P \leq 0.01$).
[d] Different from control ($P \leq 0.01$).

centration of taurine was inversely related to the proportion of taurochenodeoxycholate in bile (Fig. 4).

Discussion

The well-known involvement of hepatic taurine in bile acid metabolism has led to specific investigations on the role of this sulfur-containing amino acid in steroid metabolism of many animal species (8–10, 22–25). In animals that depend on dietary taurine intake, these investigations included the effects of taurine on other lipid classes (11, 12, 26). This information has been lacking for the rat, whose liver has been used in numerous studies on lipid metabolism. This is quite surprising because large variations in hepatic taurine levels, derived from either biosynthesis or diet, have been demonstrated to affect liver lipid metabolism in animals dependent on dietary taurine (12). To begin our studies, we needed to be able to induce large variations in taurine liver levels without causing pathologic changes to the liver. The administration of either 1% taurine or 1% GES in the drinking water for 2 weeks allowed a 200% variation in average taurine levels (13). This variation, certainly above the physiologic range, was an effective way to determine the metabolic role of taurine in the absence of major pathologic alterations. This was indicated by the lack of significant differences between control and treated animals in histologic observations, hepatic function tests (results not shown), food and water intake, and growth rates.

This study unequivocally confirms that lipid metabolism in rat liver is affected by taurine levels. In particular, the large increase due to treatment with taurine, more than the lower decrease due to treatment with GES, induced the most significant variations in

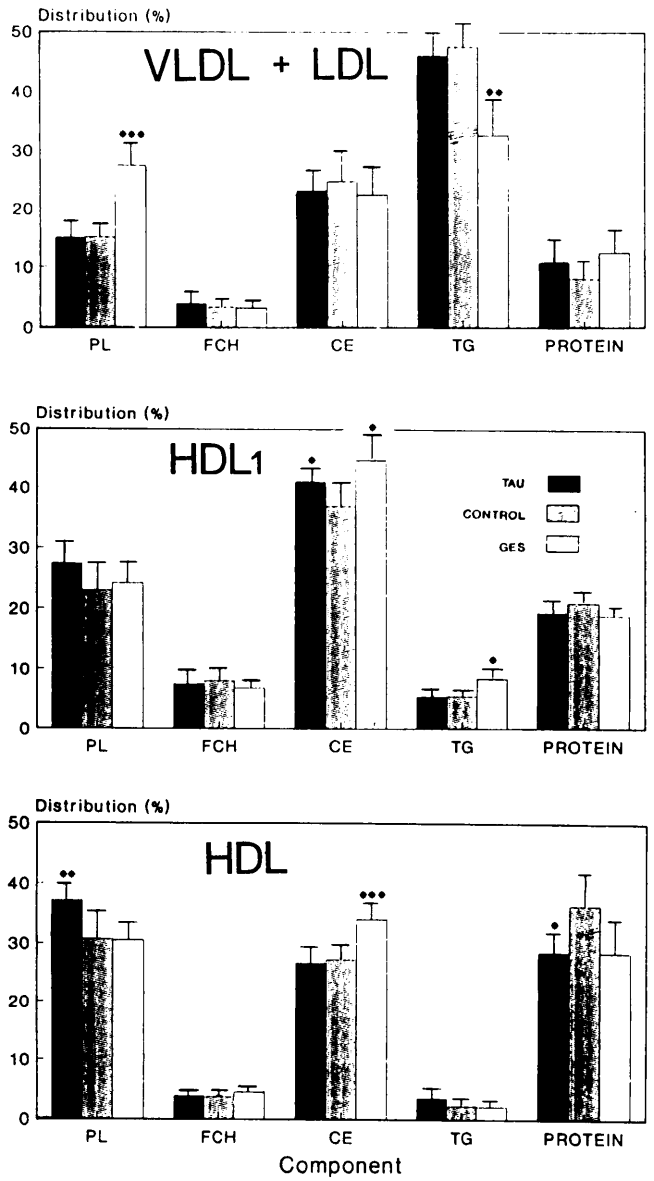

Figure 3. The distributions of the major components in the lipoprotein fractions isolated from plasma of rats treated with either 1% taurine (■, $n = 7$) or 1% GES (□, $n = 5$) in drinking water for 2 weeks in comparison with control animals (▨, $n = 7$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, treatment versus control group. PL, phospholipid; FCH, free cholesterol; CE, cholesteryl ester; TG, triglyceride.

liver lipid composition (table I). The decrease in liver concentration of both FCH and cholesteryl esters after taurine treatment was likely to be due to an increased output of bile steroids, as indicated by the increased proportion of primary taurine-conjugated bile salts found in taurine-treated animals. Treatment with GES, which reduced liver taurine levels, also had the opposite effect of taurine on bile flow and bile salt secretion (Table III). The enhanced steroid metabolism induced by high taurine levels led to a decreased hepatic cholesteryl ester content in taurine-treated animals (Fig. 2). In this case, taurine, by modulating the enzyme activities involved in cholesteryl ester synthesis and degradation (acyl cholesterol acyltransferase and cholesteryl ester hydrolase), seemed to be able to mobilize the hepatic cholesterol stores. This possibility is supported by the linear correlation between hepatic taurine concentration and the percentage of cholesteryl esters in hepatic lipids (Fig. 2).

Taurine-treated animals showed a marked decrease in hepatic concentration of triglyceride as well as of some phospholipid classes, principally phosphatidylethanolamine and phosphatidylcholine. It is likely that the enhanced lipoprotein secretion contributed to lower hepatic levels of both triglyceride and phospholipid, although the increase in these circulating lipids was not high enough to account for most of these changes in liver metabolism. We did not observe any changes in plasma triglyceride in the taurine group. However, in the GES group, triglyceride concentrations of the VLDL + LDL lipoprotein fraction were markedly decreased (Fig. 3). The reduction in triglyceride liver concentration after taurine treatment was likely due to taurine inhibition of diacylglycerol:acyl-CoA acyltransferase, the key enzyme in hepatic triglyceride synthesis (27). This idea is supported by the observation that the level of diacylglycerol, the rate-limiting substrate in triglyceride biosynthesis, was not affected by taurine treatment (Table I).

The phosphatidylethanolamine level in the taurine group was much lower than that in the control. The phosphatidylcholine level in the taurine group showed a slight, although not significant, decrease (Table I). These observations, which are in partial agreement with previous findings in guinea pig and cat (11, 12, 26), may be explained as follows. Hepatic phosphatidylcholine has two major pathways for biosynthesis. One is the stepwise methylation of phosphatidylethanolamine, with *S*-adenosylmethionine as the methyl donor, which accounts for 20% to 40% of liver phosphatidylcholine biosynthesis. The other is the reaction between diacylglycerol and CDP-choline (27, 28). It has been reported recently by Hamaguchi *et al.* (29) that taurine inhibited myocardial *N*-transmethylating activity in the rat. However, it also has been demonstrated by Ridgway *et al.* (30) that the activity of phosphatidylethanolamine methyltransferase in hepatocytes from choline- and methionine-deficient rats was strongly correlated with the concentration of phosphatidylethanolamine. Thus, the decrease in hepatic phosphatidylcholine of the taurine group may be directly associated with the inhibition of *N*-transmethylation of phosphatidylethanolamine induced by high intracellular taurine content, although the decrease in hepatic phosphatidylethanolamine certainly had an important role in lowering phosphatidylcholine levels. A possible explanation of the reduction of intrahepatic levels of phosphatidylethanolamine may be derived from the observations of Huxtable *et al.* (31), who showed the existence of a

Table III. Bile Flow, Biliary Lipid Secretion, Biliary Concentration, and Biliary Lipid Distribution in Rats Treated with Either 1% Taurine or 1% GES in Drinking Water for 2 Weeks in Comparison with Control Animals[a]

	Taurine ($n = 5$)	Control ($n = 5$)	GES ($n = 5$)
Bile flow (ml/hr)	1.16 ± 0.17[b]	1.03 ± 0.28	0.75 ± 0.31
Bile concentration (μmol/ml)			
FCH	0.76 ± 0.14	0.87 ± 0.20	0.65 ± 0.29
PL	4.82 ± 0.31[c]	6.57 ± 1.24	4.50 ± 1.1[c]
BS	27.10 ± 4.70[d]	28.20 ± 4.71	14.61 ± 5.22[e]
Bile secretion (μmol/hr)			
FCH	0.92 ± 0.08[d]	0.86 ± 0.21	0.54 ± 0.07[c]
PL	5.61 ± 0.94[d]	6.74 ± 1.30	3.38 ± 1.10[c]
BS	31.18 ± 4.41[d]	27.60 ± 5.26	11.10 ± 3.68[e]
Bile lipid distribution (%)			
FCH	2.13 ± 0.44[d]	2.54 ± 0.45	3.29 ± 0.41[c]
PL	14.06 ± 1.13[d,e]	20.01 ± 2.12	22.77 ± 2.13
BS	83.81 ± 1.00[d]	77.54 ± 2.49	73.89 ± 3.58[c]

[a] Abbreviations used in table: FCH, free cholesterol; PL, phospholipid; BS, bile salt.
[b] Different from GES ($P \leq 0.05$).
[c] Different from control ($P \leq 0.05$).
[d] Different from GES ($P \leq 0.01$).
[e] Different from control ($P \leq 0.01$).

Table IV. Percentage of Distribution of Biliary Bile Salts Determined by High Pressure Liquid Chromatography Analysis of Bile Samples of Rats Treated with Either 1% Taurine or 1% GES for 2 Weeks in Comparison with Control Animals

	Taurine ($n = 5$)	Control ($n = 5$)	GES ($n = 5$)
Tauro-α-muricholate	3.98 ± 2.48	3.06 ± 1.05	3.20 ± 0.91
Tauro-β-muricholate	35.14 ± 10.16[a,b]	12.96 ± 9.11	16.35 ± 1.58
Taurochenodeoxycholate	3.25 ± 1.44[a,b]	8.35 ± 2.14	11.81 ± 2.18[c]
Taurocholate	27.21 ± 8.46[d]	23.02 ± 7.95	17.68 ± 2.36
Taurodeoxycholate	19.93 ± 9.11[a]	44.47 ± 14.38	23.81 ± 1.33[a]
Glycocholate	10.50 ± 2.35[b]	8.08 ± 3.20	27.14 ± 1.72[a]

[a] Different from control ($P \leq 0.01$).
[b] Different from GES ($P \leq 0.01$).
[c] Different from control ($P \leq 0.05$).
[d] Different from GES ($P \leq 0.05$).

fairly linear relationship between the phosphatidylethanolamine to phosphatidylcholine ratio of the synaptosomal P2B fraction of developing rat brain and taurine content. This is consistent with our finding of a strong correlation between hepatic taurine concentration and hepatic molar ratio between phosphatidylethanolamine and phosphatidylcholine (Pearson $R = -0.886$, $n = 14$).

Detailed analysis of fatty acid composition of the major lipid classes showed that the phospholipids in the taurine group had a higher proportion of saturated fatty acids and a lower proportion of both polyunsaturated and monounsaturated fatty acids (Fig. 5). This modification of the fatty acid profile was probably due to taurine inhibition of microsomal enzymes used for desaturating and elongating the acyl chains. This is not surprising, given that many dietary factors, including the content of cholesterol, the degree of unsaturation of fats, the type of protein, and the carbohydrate intake, have been demonstrated to affect the desaturating activities in rat liver microsomes (32–35).

We observed that free fatty acid levels in both liver and plasma were increased by taurine and decreased by GES treatments. (The free fatty acid percentages relative to total lipid concentration were 3.5%, 3.1%, and 3.0% in liver and 8.3%, 8.6%, and 5.5% in plasma of

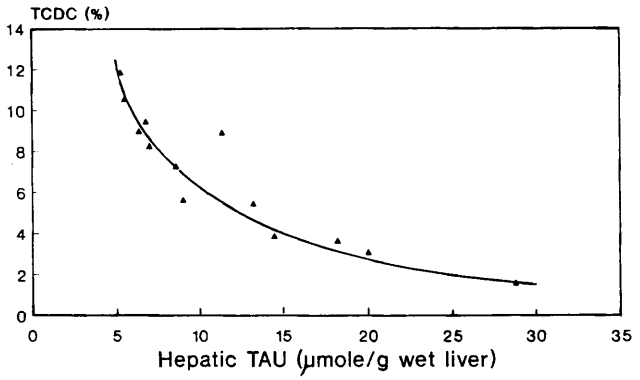

Figure 4. The correlation between hepatic taurine concentration and the percentage of taurochenodeoxycholate (TCDC) in bile acids.

taurine, control, and GES groups, respectively.) This is in agreement with, and may explain, the findings of Harada *et al.* (36), who found a decrease in long chain saturated fatty acyl-CoA in the hearts of rats with taurine deficiency induced by β-alanine administration.

It is generally accepted that both the availability of taurine and the relative affinity of conjugating enzymes for taurine and glycine influence the bile acid conjugation pattern (37). Apart from species differences, hepatic taurine content appears to play a decisive role in the formation of taurine conjugates of bile salts. In this study, taurine supplementation significantly increased the proportion of primary taurine-conjugated bile salts, especially tauro-β-muricholate, but did not affect the glycine to taurine ratio. On the other hand, GES treatment, which induced a 20% decrease in hepatic taurine concentration, caused a reduction in taurine conjugation and, consequently, increased the glycine to taurine ratio. Nevertheless, in all groups, most bile salts were taurine-conjugated (89.51%, 91.92%, and 72.86% in taurine, control, and GES groups, respectively). These findings are consistent with the fact that bile salt-conjugating enzyme in rat liver has an affinity for taurine 50–100 times greater than for glycine (37), and that there was a high taurine availability in both the taurine and control groups.

We observed a reverse correlation between hepatic taurine concentration and taurochenodeoxycholic acid percentage in bile (Fig. 4). This is in partial agreement with the results obtained in mice (38) and normal humans (39), but not in hamsters (8). This correlation may be due to the conversion of chenodeoxycholate into either α- or β-muricholate (40, 41). In fact, taurine-treated animals showed an increase in β-muricholate and a decrease in chenodeoxycholate, which indicates that a taurine-induced activation of six hydroxylating enzymes occurs in rat liver microsomes.

Biliary phospholipids are composed mainly of phosphatidylcholine, whose intracellular site of origin is still controversial. However, under normal physiologic conditions, the secretion rate of phosphatidylcholine into bile depends on both the type and concentration of bile salts secreted (42). In taurine-conjugated bile salts, the relatively hydrophobic taurodeoxycholate stimulates biliary phosphatidylcholine secretion more than relatively hydrophilic bile salts, such as tauroursodeoxycholate and tauromuricholate (43–46). The infusion of taurocholate at low doses and for short infusion times stimulates biliary phosphatidylcholine secretion, but the output of phosphatidylcholine declines progressively during the infusion (47). Thus, we propose that decreased phospholipid secretion in GES-treated

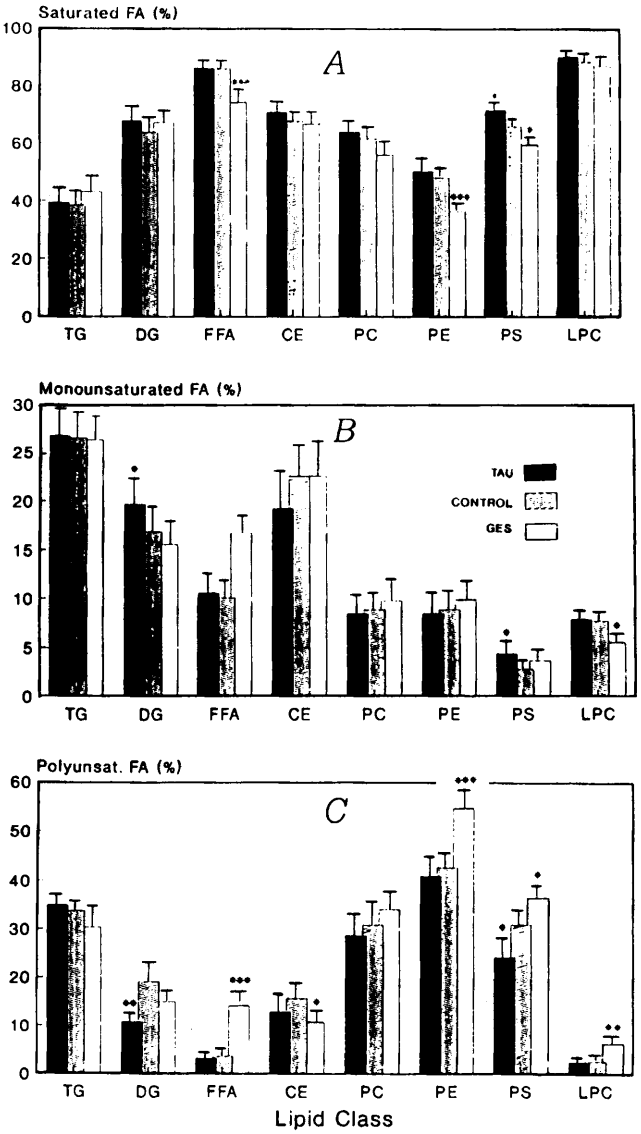

Figure 5. The distributions of (A) saturated fatty acids, (B) monounsaturated fatty acids, and (C) polyunsaturated fatty acids in the major lipid classes isolated from livers of rats treated with either 1% taurine (■, $n = 10$) or 1% GES (□, $n = 5$) in drinking water for 2 weeks in comparison with control animals (▨, $n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, treatment versus control group. FA, fatty acid; TG, triglyceride; DG, diacylglycerol; FFA, free fatty acid; CE cholesteryl ester; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; LPC, lysophosphatidylcholine.

rats results from both lower bile salt secretion (Table III) and taurodeoxycholate content. The decrease in biliary phosphatidylcholine secretion after taurine administration appears to be related mostly to the relevant changes in bile salt composition, including a reduction in taurodeoxycholate and an increase in β-muricholate proportion (Table IV).

This investigation was partially supported by the National Research Council of Italy, Progetto Finalizzato Invecchiamento (Grant 92.00399.PF40, Inv. 92.3.198). The expert assistance of Dr. Giorgio Pinto is gratefully acknowledged.

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