

Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet

H. Yokogoshi¹ and H. Oda²

¹ School of Food and Nutritional Sciences, The University of Shizuoka, Shizuoka, Japan

² Department of Applied Biological Sciences, Nagoya University, Nagoya, Japan

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Summary. The effect of taurine on hypercholesterolemia induced by feeding a high-cholesterol (HC) diet (10 g/kg) to rats was examined. When taurine was supplemented to HC for 2 wk, serum total cholesterol significantly decreased and serum HDL-cholesterol increased compared with the HC diet group. In the hypercholesterolemic rats fed the HC diet, the excretion of fecal bile acids and hepatic cholesterol 7 α -hydroxylase (CYP7A1) activity and its mRNA level increased significantly, and the supplementation of taurine further enhanced these indexes, indicating an increase in cholesterol degradation. Agarose gel electrophoresis revealed that, in hypercholesterolemic rats fed the HC diet, the serum level of the heavier VLDL increased significantly, but taurine repressed this increase and normalized this pattern. Significant correlations were observed between the time-dependent increase of CYP7A1 gene expression and the decrease of blood cholesterol concentration in rats fed the HC diet supplemented with taurine. These results suggest that the hypocholesterolemic effects of taurine observed in the hypocholesterolemic rats fed the HC diet were mainly due to the enhancement of cholesterol degradation and the excretion of bile acid.

Keywords: Taurine – Cholesterol – Hypocholesterolemia – Cholesterol 7 α -hydroxylase (CYP7A1) – Rat

Taurine, 2-amino ethanesulfonic acid, is the major, free intracellular amino acid in many tissues producing oxidants and toxic substances, including the brain, retina, myocardium, skeletal muscle, liver, platelets and leukocytes (Chesney, 1985; Wright et al., 1986). Suggested biological and physiological functions of taurine include cell membrane stabilization (Pasantes-Morales et al., 1985), antioxidation (Nakamura et al., 1933), detoxification (Huxtable, 1992), osmoregulation (Thurston et al., 1980), neuromodulation (Bemardi, 1985; Kuriyama, 1980) and brain and retinal development (Sturman, 1986). Taurine is an essential nutrient for cats (Hayes et al., 1975), and formula-fed,

preterm infants are unable to maintain normal plasma and urinary taurine levels, although no functional impairment has been reported (Raiha et al., 1975). In lipid metabolism, the function of taurine is considered only because of its conjugation with bile acids in the liver (Danielsson, 1963), which increase the use of bile acids, the degradation products which participate in the formation of micelles that are used for fat absorption in the small intestine (Yamanaka et al., 1986). Numerous studies have been done that sought the effect of taurine on cholesterol metabolism (Cantafora et al., 1986; Candhi et al., 1992; Herrmann, 1959; Murakami et al., 1996; Petty et al., 1990; Sugiyama et al., 1989; Yan et al., 1993) in various species, including rats, guinea pigs, rabbits and cats, and almost all of the experiments have been conducted in animals with hypercholesterolemia induced by feeding a high-cholesterol diet. We also reported a hypocholesterolemic action of taurine in rats fed a high-cholesterol diet (Nanami et al., 1996), but the mechanism of its action is unclear. In rats fed a high cholesterol diet, cholesterol balance is dependent on the catabolism of cholesterol because cholesterol synthesis is abolished in these rats. Taurine is used for bile acid conjugation and may facilitate bile acids excretion in feces. Moreover, taurine itself may enhance the biotransformation of cholesterol to bile acids, and then increased bile acids might enhance the clearance of cholesterol out of the body. Therefore, we examined the hypocholesterolemic action of taurine by focusing on the degradation of circulating cholesterol. In the present study, we examined the mechanisms of the taurine-induced

reduction of serum cholesterol in hypercholesterolemic rats fed a high-cholesterol diet. We studied the degradation of cholesterol, which is associated with cholesterol 7 α -hydroxylase (CYP7A1).

Materials and methods

Animals and diets

Young male Wistar rats, weighing about 100 g (Japan SLC, Hamamatsu), were maintained at 24°C with a 12-h light (0700–1900 h) and dark cycle. To accustom the rats to the experimental conditions, they were initially given free access to a 20% casein diet, (control diet) for 2 d before being divided into groups. The compositions of the test diets are shown in Table 1. Animals were fed the control diet, a taurine-supplemented diet, a high-cholesterol (HC) diet, or a HC diet supplemented with taurine (HCT) for 2 wk. The HC diet contained 10 g cholesterol and 2.5 g sodium cholate per kg diet. Taurine was added to the control diet at the expense of carbohydrate. Rats were individually housed in stainless steel cages in a room with controlled temperature (23°C and humidity (55%)) and were given free access to the experimental diets and water. The experimental procedures used in this study met the guidelines of the Animal Care and Use Committee of the University of Shizuoka.

Experimental design

The effects of taurine on the serum and liver lipids, cholesterol degradation and lipoprotein distribution were examined in experiments 1 and 2. In both experiments, rats were fed one of the following diets for 2 wk: control diet, taurine-supplemented diet (50 g taurine/kg control diet), HC diet, or HCT diet (50 g taurine/kg HC). In experiment 1, we determined the concentrations of serum and liver lipids and the amount of fecal steroid excretion as assessed by cholesterol and bile acids in the feces. In experiment 2, we examined the serum lipoprotein distribution and determined the mRNA level of CYP7A1, the rate-limiting enzyme in the metabolic pathway of cholesterol to bile acids. Animals were killed at 1100 h. The activity and mRNA of CYP7A1 in the liver are known to exhibit a diurnal cycle and the highest level is observed during the dark period (Noshiro et al., 1990). Therefore, in experiment 3, animals were killed at 1100 h and 0100 h to examine the effects of taurine on the activity and mRNA level of CYP7A1. In this experiment, the correlation between serum total cholesterol concentration and the level of CYP7A1 mRNA was determined. Animals were fed the HC diet or the HCT diets (10 g taurine/kg diet) for 2 wk and were killed at 0100 h to measure the hepatic levels of CYP7A1 mRNA. Blood was collected from a cervical wound, and tissues were immediately removed, frozen in liquid nitrogen and stored at –8°C until assayed.

Biochemical analyses

Serum lipids (total cholesterol, HDL-cholesterol, triglycerides and phospholipids) were determined by using commercial kits (Cholesterol C-test, HDL-cholesterol-test, Triglyceride C-test and Phospholipids B-test, respectively; Wako Pure Chemical, Osaka). About 2 g of liver were homogenized, and lipids were extracted with a chloroform : methanol mixture (2 : 1, v/v) as described by Folch et al. (1957). Total lipids in the liver were determined gravimetrically. The concentration of liver cholesterol in the lipid extracts was measured enzymatically by using a kit (Cholesterol C-test; Wako Pure Chemical). Hepatic triglyceride concentration was determined by the acetyl acetone method (Fletcher, 1968). The amount of hepatic

phospholipids was calculated by subtracting the amount of liver cholesterol and triglycerides from the total lipid contents. The concentration of reduced glutathione in the liver was determined by using Ellman's reagent (Sedlak and Lindsay, 1968). Fecal bile acids were extracted by the mixture of chloroform and methanol (1 : 1, v/v) and were determined enzymatically by the method of Sheltawy and Losowsky (1975).

The activity of CYP7A1 was determined as described previously (Oda et al., 1989). In brief, the liver microsome was incubated in the reaction buffer containing 100 mmol potassium phosphate/L (pH 7.4), 0.1 mmol EDTA/L, 50 mmol NaF/L, 2 mmol NADPH/L, 20 mmol cysteamine/L, 0.2 mmol cholesterol/L, 1.5 g Tween 80/L, 222 kBq [7 α -³H] cholesterol. After the termination of the reaction with trichloroacetic acid, the supernatant fraction was extracted with chloroform two times. The radioactivity of the upper, aqueous phase was counted with a liquid scintillation counter (Aloka, Tokyo). Total RNA was isolated according to the method described by Chomczynski and Sacchi (1987), and 10 μ g of total RNA was subjected to Northern blot hybridization. The cDNA clones of rat CYP7A1 (Noshiro et al., 1989) were labeled with Megaprime DNA labeling system (Amersham, Tokyo) and used for hybridizations. Specific hybridization was quantified with an image analyzer (BAS 2000; Fuji Film, Tokyo).

Agarose gel electrophoresis of serum lipoproteins

Agarose gel electrophoresis was carried out by using Corning Universal Film from Corning (Palo Alto, CA). After the agarose gel was running at 90 V for 1 h, lipoprotein-cholesterol was stained with Co-Cholest-A (Nippon Chemiphar, Tokyo).

Statistics

The means and SEM of 4–8 rats per group are reported. In experiment 4, significance of differences among values was analyzed by one-way ANOVA. When treatment was significant, Duncan's multiple range test was performed (Duncan, 1955). In experiments 1–3, significance of differences among values was analyzed by two-way ANOVA (Oda et al., 1991). When interaction was significant, Student's t-test was performed (Snedecor and Cochran, 1967). P values of <0.05 were considered significance. All statistical analyses were performed using the Statistical Analysis System (SAS/STAT Version 6, SAS Institute, Cary, NC).

Results

Effect of taurine on lipid concentrations in serum and liver and the degradation of cholesterol (experiments 1 and 2)

Body weight gain and food intake of rats did not differ among the groups, in spite of taurine supplementation (Table 2). Liver weight of rats fed the HC diet was significantly greater than that of rats fed the control diet, and taurine supplementation significantly suppressed this increase of liver weight. Dietary taurine significantly reduced the serum triglycerides concentration in rats fed either the control or the HC diet. Taurine tended to decrease the concentration of serum phospholipids in rats fed the HC diet. The

Table 1. Composition of control and high cholesterol (HC) diet

Ingredient	Control	HC
	<i>g/kg</i>	
Casein ¹	200	200
Corn starch ¹	425.7	417.3
Sucrose ¹	212.8	208.7
Corn oil	50	50
AIN-93G mineral mixture ²	50	50
AIN-76™ vitamin mixture ²	10	10
Choline chloride	1.5	1.5
Cellulose	50	50
Cholesterol	0	10
Sodium cholate	0	2.5

¹ Supplied by Oriental Yeast, Tokyo. ² Supplied by Nihon Nosan K. K., Yokohama (AIN 1976 and 1993)

concentrations of the lipids, such as cholesterol, triglycerides, phospholipids as well as total lipids were unaltered in the liver of rats fed the taurine-supplemented, control diet. The concentrations of these lipids in the liver were significantly elevated by feeding the HC diet, and dietary taurine significantly reduced the increasing extent in these hepatic lipids. The concentration of reduced glutathione in the liver was significantly increased by the supplementation of taurine in rats fed either the control or the HC diet. The excretion of fecal cholesterol was significantly greater in rats fed the HC diet than in those fed the control diet. Taurine significantly enhanced the excretion of bile acids in rats fed the HC diet, but not in rats

Table 2. Effect of taurine on lipid concentrations in serum and liver, and the degradation of cholesterol in rats fed control (C), C supplemented with taurine (T), high cholesterol (HC) and HC supplemented with taurine (HCT) diets (experiments 1 and 2)¹

	C	T	HC ²	HCT ²	ANOVA ³		
					Chol	Tau	Interaction
Experiment 1							
Body weight gain, g/14 d	75.7 ± 3.7	65.1 ± 3.6	73.1 ± 2.9	63.5 ± 4.7	NS	NS	NS
Final body weight, g	223 ± 7.5	219 ± 7.0	225 ± 6.3	215 ± 7.9	NS	0.05	NS
Liver weight, g	6.73 ± 0.86	6.37 ± 0.24	8.53 ± 0.69***	7.01 ± 0.15### ⁵	0.01	0.01	0.05
Serum lipids, mmol/L							
Total cholesterol	3.06 ± 0.12	3.07 ± 0.36	3.97 ± 0.42*	2.31 ± 0.19#	NS	0.01	0.01
HDL-cholesterol	1.39 ± 0.06	1.27 ± 0.21	0.71 ± 0.09	0.81 ± 0.07	0.01	NS	NS
Triglyceride	2.29 ± 0.15	1.69 ± 0.14*	1.56 ± 0.14	1.16 ± 0.18	0.01	0.01	NS
Phospholipids	2.19 ± 0.14	2.04 ± 0.20	1.90 ± 0.20	1.46 ± 0.12	0.05	NS	NS
Liver lipids, μ mol/g liver							
Cholesterol	11.8 ± 0.9	11.1 ± 0.4	30.1 ± 2.1**	22.2 ± 1.7#	0.01	0.01	0.05
Triglyceride	15.9 ± 1.8	12.3 ± 1.2	47.6 ± 6.6**	21.2 ± 1.9##	0.01	0.01	0.01
Phospholipids	45.9 ± 1.4	46.6 ± 1.1	131 ± 11**	63.9 ± 2.7##	0.01	0.01	0.01
Liver reduced glutathione, μ mol/g liver	6.05 ± 0.41	6.94 ± 0.27	5.30 ± 0.39	6.64 ± 0.44	NS	0.01	NS
Fecal steroid excretion, μ mol/3 d							
Cholesterol	40.7 ± 3.8	53.1 ± 7.3	273 ± 14**	286 ± 16	0.01	NS	0.01
Bile acids	27.6 ± 1.1	38.2 ± 2.4	156 ± 15**	248 ± 16##	0.01	0.01	0.01
Experiment 2							
Body weight gain, g/14 d	56.9 ± 2.3	54.4 ± 1.6	56.9 ± 1.2	54.6 ± 1.6	NS	NS	NS
Final body weight, g	155 ± 2.8	152 ± 2.6	154 ± 1.7	151 ± 1.7	NS	NS	NS
Liver weight, g	5.02 ± 15%	4.97 ± 0.12	6.42 ± 0.09**	5.62 ± 0.11##	0.01	0.05	0.05
Serum lipids, mmol/L							
Total cholesterol	3.08 ± 0.15	3.18 ± 0.13	7.87 ± 0.73**	2.95 ± 0.16##	0.01	0.01	0.01
HDL-cholesterol	0.84 ± 0.06	1.08 ± 0.05	0.45 ± 0.06	0.56 ± 0.03	0.01	0.01	NS
Triglyceride	2.48 ± 0.09	2.40 ± 0.24	1.32 ± 0.12	1.14 ± 0.04	0.01	NS	NS
Phospholipids	2.64 ± 0.19	2.54 ± 0.13	2.66 ± 0.17	1.96 ± 0.08	NS	0.05	NS
mRNA concentration (arbitrary unit)							
CYP7A1	1.00 ± 0.28	1.20 ± 0.27	1.70 ± 0.17	3.32 ± 0.47##	0.01	0.01	0.05
Apo A-I	1.00 ± 0.08	0.99 ± 0.09	0.57 ± 0.03	0.81 ± 0.03##	0.01	NS	0.05

¹ Values are means ± SEM, n = 5–8

² HC, high cholesterol diet group; HCT, taurine supplemented high cholesterol diet group

³ Statistical significance of difference among values were analyzed two-way ANOVA. When the interaction was significant, Student's t-test was performed. NS, not significant (P > 0.05)

⁴ *, ** These values differed significantly (P < 0.01, P < 0.001, respectively) from the value of the Control group

⁵ #, ## These values differed significantly (P < 0.01, P < 0.001, respectively) from the value of the HC group

fed the control diet. In experiment 2, serum lipids were affected by taurine in the same manner as in experiment 1 (Table 2). The concentrations of hepatic mRNA of CYP7A1 were increased significantly by feeding the HC diet, and further increase in the CYP7A1 mRNA level was seen in rats fed the HCT diet.

Serum lipoprotein profiles (experiment 3)

In the hypercholesterolemic rats fed the HC diet, pre- β lipoprotein cholesterol markedly increased (Fig. 1). The pre- β lipoprotein migrated somewhat slowly like β -VLDL (Mahley et al., 1980). The addition of taurine to the HC diet drastically repressed the increase in the amount of VLDL cholesterol and thus normalized the lipoprotein profile. These results indicate that dietary taurine specifically reduces the molecular form of VLDL that was increased in quantity by feeding the HC diet.

Time-dependent changes of the activity and mRNA of CYP7A1 of rats fed high-cholesterol diet supplemented with taurine (experiment 3). The activity and mRNA of CYP7A1 in the liver are known to exhibit a diurnal rhythm, and the highest level is observed during the dark period (Noshiro et al., 1990). We showed that CYP7A1 mRNA in the liver was induced by dietary taurine when rats were killed during the light period (Table 2). In experiment 3, we investigated the effect of taurine on the activity and mRNA level of CYP7A1 in the liver during both the light period (daytime) and the dark period (nighttime). As shown above, the increase in the serum cholesterol induced by the HC diet was completely abolished by dietary taurine during both the light and dark periods (Fig. 2A), and the cholesterol-lowering effect of taurine was more explicit during the dark period. Both the activity and mRNA of CYP7A1 during the dark period were higher than those during the light period (Fig. 2B and C). During both periods, dietary taurine increased the activity and mRNA level of CYP7A1, and the variations of the activity of CYP7A1 were in parallel with those of the level of CYP7A1 mRNA. The highest activity and mRNA level of CYP7A1 were observed in HCT group during the dark period, the group that showed the lowest level of serum cholesterol. There was a significant negative correlation between serum level of cholesterol and the mRNA level (Fig. 2D, $P < 0.01$) and activity of CYP7A1 ($P < 0.05$, data not shown).

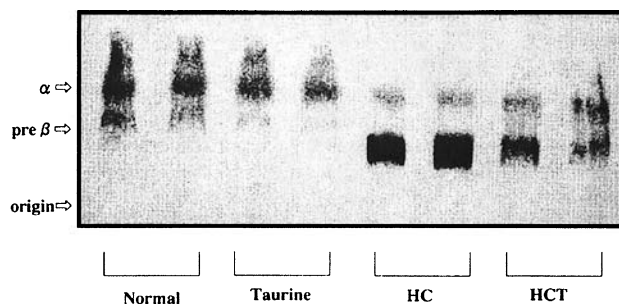


Fig. 1. Agarose gel electrophoresis of lipoproteins from control diet-fed rats (normal), taurine-fed rats, high-cholesterol diet-fed rats (HC), and taurine supplemented HC diet-fed rats (HCT). Lipoprotein-cholesterol was stained enzymatically by using Co-Cholest-A, α , HDL; β , VLDL

Discussion

We reported previously that supplementation of taurine to the high-cholesterol diet significantly reduced the serum total cholesterol and increased the HDL-cholesterol concentrations in rats (Nanami et al., 1996). Tsuji et al. (1980) also reported that taurine had a hypocholesterolemic effect in experimentally induced hypercholesterolemic animal models. However, the mechanism of the hypocholesterolemic action of taurine was not clear. In this study, we examined the hypocholesterolemic action of taurine by focusing on the changes in time-dependency, distribution of lipoproteins and the degradation of circulating cholesterol. Hypercholesterolemia caused by feeding the HC diet was significantly reversed by the supplementation of taurine. According to the agarose gel electrophoresis (Fig. 1), the density of the band, which represents mainly HDL-cholesterol, was significantly reduced, while heavier VLDL fractions were remarkably enriched by feeding the HC diet.

The degradation products of cholesterol are bile acids, and the conjugates of bile acids with taurine or glycine contribute in the solubilization and excretion of cholesterol. There is a species difference in the conjugation of bile acids, and in rats, the conjugates with taurine predominate. When rats were fed the HC diet, fecal excretion of cholesterol and bile acids increased significantly compared to those of rats fed the test diet without cholesterol, and taurine further increased the bile acids excretion. We found that, in the HC diet group, the content of hepatic mRNA of CYP7A1 significantly increased, and it was also further elevated by taurine.

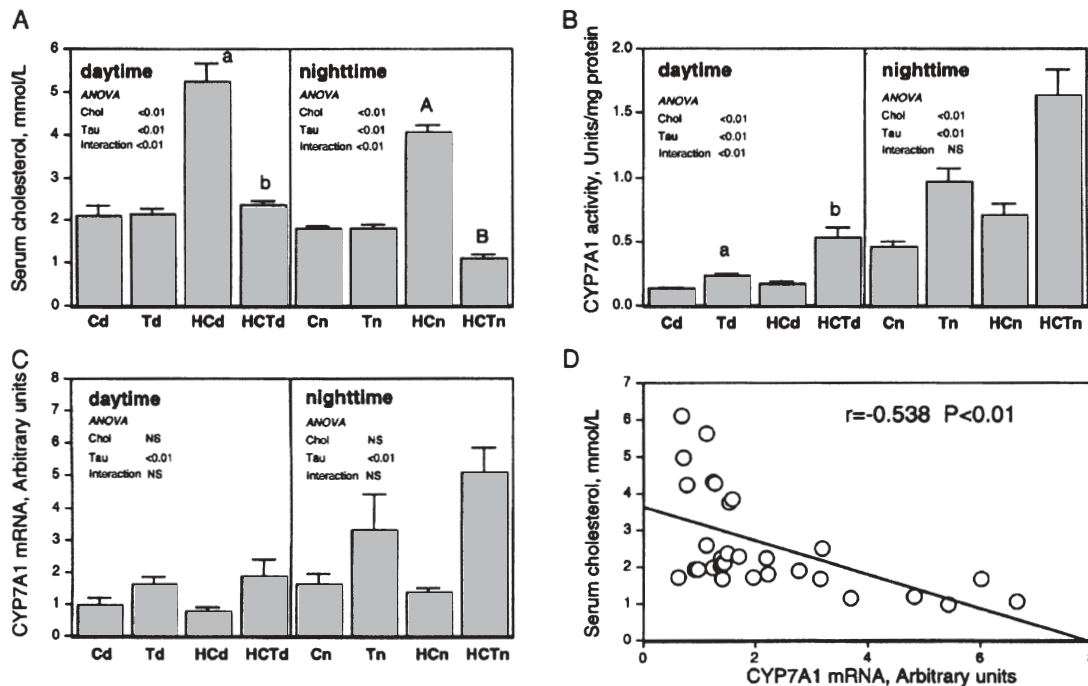


Fig. 2. Effect of dietary taurine on (A) serum cholesterol and (B) the activity and (C) mRNA of CYP7A1 in rats fed a high cholesterol diet (expt. 3). Animals were killed at 1300 h and 0100 h after 4 h of fasting. Values are means and SEM for four rats in each dietary group. C. T. HC and HCT indicate control diet group, taurine supplemented control diet group, high cholesterol diet group, and taurine supplemented high cholesterol diet groups, respectively, killed during daytime (1300 h) and during nighttime (0100 h). Significant differences among values were analyzed by two-way ANOVA. When the interaction (Chol $\times$ Tau) was significant, Student's t-test was performed. Results of ANOVA are inset in each graph. Superscripts a and b indicate that these values differed significantly ($P < 0.05$) from Cd group and from HCd group, respectively, Student's t-test. Superscripts A and B indicate that these values differed significantly ($P < 0.05$) from Cn group and from HCn group, respectively, Student's t-test. D Correlation between serum level of cholesterol and hepatic level of CYP7A1 mRNA

Taurine concentrations were significantly and dramatically decreased by feeding the HC diet, and taurine supplementation significantly increased the taurine concentrations in the liver (at 10 g taurine/kg) (data are not shown). The decreased level of serum taurine concentration caused by the intake of the HC diet was gradually restored by the administration of taurine in a dose-dependent manner. Similar elevation of the taurine concentration was obtained in the liver in animals fed the HCT diet (data are not shown).

It was reported that the supplementation of taurine increased the activity of hepatic microsomal CYP7A1 in hamsters (Bellentani et al., 1987) and guinea pigs (Kibe et al., 1980). This increase of microsomal CYP7A1 activity might indicate that taurine promotes the conjugation of bile acids with taurine, leading to a reduction of the concentration of serum cholesterol increased by feeding the HC diet. Sugiyama et al. (1989) reported that taurine increased the activity of CYP7A1 in rats fed a HC diet, but glycine administra-

tion did not alter this enzyme activity, in spite of the reduction of serum cholesterol. Spady et al. (1995) demonstrated that the overexpression of exogenous CYP7A1 genes effectively reduced the plasma level of cholesterol in hamsters fed a low- or high-fat, western diet. There is a diurnal rhythm in the activity and mRNA of CYP7A1 (Noshiro et al., 1990). Therefore, in the present experiments, to elucidate the relationship between serum level of cholesterol and CYP7A1, time-dependent effects of dietary taurine on serum cholesterol and CYP7A1 were investigated in rats fed the HC diet. As shown in Figs. 2, supplementation of taurine reduced the blood level of cholesterol and induced CYP7A1 activity and CYP7A1 gene expression. Moreover, serum cholesterol level was negatively correlated with the CYP7A1 mRNA level. Our results presented in this paper suggest that dietary taurine has a strong hypocholesterolemic action through the induction of CYP7A1 gene expression. Stephan et al. (1987) demonstrated that taurine enhanced LDL

receptor activity in HepG2 cells. Although lipoprotein receptor activity was not determined in this study, the increased catabolism of cholesterol might result in enhancement of VLDL uptake in the liver from serum.

The addition of taurine to the diet did not reduce serum level of cholesterol in control rats (Table 2 and Fig. 2) (Mochizuki et al., 1998). Even in these rats fed a control diet, CYP7A1 was induced by taurine (Fig. 2). We speculated that the loss of steroid induced caused by taurine would be compensated for by the synthesis of cholesterol. We thought that this would explain why hypocholesterolemic action of taurine observed only in rats fed the HC diet.

There is no information as to how CYP7A1 gene expression is induced by dietary taurine, so far. The CYP7A1 mRNA and protein levels are regulated principally at the transcriptional level. Lavery and Schibler (1993) observed a close correlation between the CYP7A1 transcription rate and the level of D larger binding protein (DBP), one of the liver-enriched transcription factors. It can be speculated that there is a putative taurine responsive element in CYP7A1 genes. However, there are some technical problems in investigating the effect of taurine on CYP7A1 gene expression. Hepatoma cell lines and even primary hepatocytes lose some liver-specific functions and normal regulation of some gene expression. It was reported that CYP7A1 gene expression was rapidly lost in primary cultured hepatocytes after disaggregation (Hylemon et al., 1992). We recently demonstrated that CYP7A1 mRNA was maintained in hepatocytes cultured on EHS-gel (Oda et al., 1997). We are now investigating how taurine regulates CYP7A1 gene expression on a molecular level. The alternative possibility that enhancement of steroid excretion in feces by taurine indirectly induce CYP7A1 gene expression should be examined.

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Authors' address: Dr. Hidehiko Yokogoshi, School of Food and Nutritional Sciences, The University of Shizuoka, Shizuoka 4228526, Japan, E-mail: yokogosi@u-shizuoka-ken.ac.jp