Stimulation of Chenodeoxycholic Acid Excretion in Hypercholesterolemic Mice by Dietary Taurine

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Summary The effects of dietary taurine on fecal steroid excretion and bile acid pool size were investigated in Jcl: ICR strain mice. The mice were fed on semi-purified diets for five weeks: a cholesterol-free diet (Standard), a lithogenic diet containing 0.5% cholesterol and 0.25% sodium cholate (C-CA) and a lithogenic diet supplemented with 5% taurine (C-CA+5% taurine). The changes in fecal steroid excretion were studied as a function of time and the bile acid pool size was estimated. Dietary taurine affected fecal bile acid excretion both quantitatively and qualitatively. No change in bile acid pool size was observed. The fecal excretion of bile acids increased in taurine-supplemented mice. The increase in the fecal neutral steroid excretion was less than that in C-CA fed mice. The proportion of chenodeoxycholic acid (CDCA) and the related bile acids to total bile acids increased both in the fecal bile acids and in the bile acid pool. Therefore, the protective effect of dietary taurine against cholesterol gallstone formation may be related to the stimulation of bile acid synthesis, especially of CDCA and related compounds.

Key Words taurine, cholesterol gallstones, chenodeoxycholic acid, bile acid pool, bile acid excretion

Taurine is a sulfur amino acid that conjugates with bile acids in the liver (1). It is well known that dietary taurine markedly increases the proportion of taurine-conjugated bile acids to biliary bile acids (2).

Fujihara et al. (3) reported that the gallstone formation was inhibited almost completely by ingestion of taurine with a lithogenic diet and that cholesterol levels in the serum, liver and bile were significantly low in the taurine-supplemented C57BL/6 mice.

We reported that dietary taurine inhibited the cholesterol gallstone formation in Jcl: ICR mice fed a lithogenic diet (4) and that the serum HDL cholesterol level was slightly elevated (4). Fecal bile acid excretion significantly increased in the
taurine-supplemented mice (5). We previously reported that a decrease in hepatic cholesterol mass and in cholesterol gallstone formation was observed after the 3rd week in mice fed the lithogenic diet, and these findings suggested that the inhibitory effect of dietary taurine on cholesterol gallstone formation was related to a decreased hepatic cholesterol content (6). In the present study, the bile acid pool size and fecal steroid excretion were investigated as a function of feeding time to clarify the effect of dietary taurine on bile acid metabolism in mice.

**METHODS**

Thirty-one Jcl: ICR strain male mice (Nihon Clea Inc., Tokyo) were used in this study. Mice (four weeks old) were kept in an air-conditioned room (23±1°C, 50–60% humidity) lighted for 12 h a day (07:00 to 19:00). After acclimating on a commercial CE-2 chow for 5 days, they were divided into 3 groups and maintained individually. Mice were fed on the semi-purified diets shown in Table 1 for five weeks. Diet and water were provided ad libitum.

Feces was collected individually for two days every week. After being air-dried in an oven at 60°C overnight, it was ground with a mortar and pestle, and fecal steroids were analyzed by gas-liquid chromatography (Ohkura Gas Chromatograph Model 103). To about 0.2 g of the fine powder were added 3 ml of 4N KOH solution and 2 ml of ethanol, and the mixture was saponified at 70°C for 1 h. Neutral steroids were extracted three times with n-hexane. Cholesterol and coprostanol were determined quantitatively by the GLC method on a Diasolid ZS column using 5α-cholestan as an internal standard (8). To the bile salt fraction (water phase) was added distilled water to yield a final concentration of 1.2 N KOH. The mixtures were autoclaved at 121°C for 3 h and acidified to pH 1 with concentrated HCl. The bile acids were extracted three times with diethylether. The

| Table 1. Composition of semi-purified diets. |
|---------------------------------------------|
| Constituents (%) | Standard | C-CA | C-CA +5% Taurine |
|------------------|----------|------|-----------------|
| Casein           | 22       | 22   | 22              |
| Mineral mixturea | 3.5      | 3.5  | 3.5             |
| Vitamin mixturea | 1.2      | 1.2  | 1.2             |
| Choline chloride | 0.15     | 0.15 | 0.15            |
| Celluloseb       | 3        | 3    | 3               |
| Cholesterol      | —        | 0.5  | 0.5             |
| Sodium cholate   | —        | 0.25 | 0.25            |
| Soybean oil      | 10       | 10   | 10              |
| Taurine          | —        | —    | 5               |
| Sucrose          | 60.15    | 59.4 | 54.4            |

*AIN-76™ mixture (7). bSolka-floc.
hexafluoroisopropyl ester-trifluoroacetyl derivatives of bile acids (9) were prepared and analyzed by the GLC method on a 2% QF-1 column using 5β-cholanic acid as an internal standard (5). Similarly, the methyl ester of bile acids was prepared with acetyl chloride–methanol (1:20, v/v) (10) and their trifluoroacetyl derivatives were quantified by GLC on a 1.5% AN-600 column (11, 12).

After being maintained for five weeks on experimental diets, the mice were anesthetized with diethylether and bled by decapitation. Gallstone formation (13) was observed with the naked eye. The liver, gall bladder and small intestine with its contents were immediately excised and then their bile acid pool size was estimated. They were saponified completely with ethanolic KOH and bile acids were quantified by the method described above. Serum total cholesterol (14) and serum HDL-cholesterol (15) were analyzed by enzymatic methods using commercial kits (Kyowa Hakko Co., Ltd., Tokyo).

Data were analyzed by Student’s t-test and p values of less than 0.05 were considered statistically significant.

RESULTS

Body weight, food intake and fecal dry weight

The changes in body weight, food intake and fecal dry weight are shown in Fig. 1. Body weight did not differ significantly among the three groups. Food intake tended to decrease with time in all the groups. Fecal dry weight was reduced to a level 20% of that in chow-fed mice when the diets were changed, but did not differ significantly among the three groups.

Gallstone incidence and serum cholesterol concentration

The gallstone incidence and serum cholesterol concentration are shown in Table 2. Gallstone formation was observed in 8 out of 11 mice fed on the C-CA diet, but in only 3 out of 10 mice in the taurine-supplemented group. No stones were observed in mice in the standard group. The grade of gallstone formation (13) was grade I or grade II.

Dietary taurine significantly suppressed the elevation of total serum cholesterol level. The serum HDL cholesterol concentration was slightly higher in the taurine-supplemented mice than in the C-CA-fed mice but was not significantly different. Taurine efficiently lowered the atherogenic index.

Bile acid pool size

The size and the composition of the bile acid pool are shown in Table 3 and Fig. 2. Bile acid pool size increased several times in mice fed on the lithogenic diet containing cholesterol and sodium cholate. Dietary taurine did not change the total pool size. In the bile acid composition, the main bile acids were cholic acid (CA), β-muricholic acid (βMCA) and chenodeoxycholic acid (CDCA). The proportion of CA and deoxycholic acid (DCA) to total bile acids increased in mice fed the
Fig. 1. Body weight, food intake and fecal dry weight. Each point is the mean for ten or eleven mice and vertical extension indicates SEM. * Differs significantly from C-CA-fed group ($p<0.05$).

Table 2. Gallstone incidence and serum cholesterol concentration.

|                      | Standard | C-CA | C-CA + 5% Taurine |
|----------------------|----------|------|-------------------|
| Gallstone incidence  | 0/10     | 8/11 | 3/10              |
| Serum cholesterol concentration (mg/100 ml) |          |      |                   |
| Total cholesterol    | 168 ± 10$^{*}$ | 310 ± 18 | 169 ± 11$^{*}$ |
| HDL cholesterol      | 143 ± 7$^{*}$  | 113 ± 8  | 126 ± 9           |
| Atherogenic index$^{b}$ | 0.18 ± 0.06$^{*}$ | 1.80 ± 0.16 | 0.31 ± 0.04$^{*}$ |

$^a$ Results are represented as mean ± SEM for ten or eleven mice.

$^b$ Atherogenic index = $\frac{\text{Total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$

* Differs significantly from C-CA-fed group ($p<0.05$).
Table 3. Bile acid pool size and fecal steroid excretion.

|                             | Standard | C-CA    | C-CA +5% Taurine |
|-----------------------------|----------|---------|------------------|
| Bile acid pool size (μmol/animal) |          |         |                  |
|                             | 1.07 ± 0.28* | 4.03 ± 0.74 | 3.43 ± 0.55      |
| Fecal neutral steroids (μmol/2 days) |     |         |                  |
| Initial                     | 10.3 ± 0.56* |         |                  |
| 1st week                    | 4.5 ± 0.2*  | 29.9 ± 2.6 | 36.2 ± 1.8*      |
| 2nd                         | 4.1 ± 0.5*  | 48.9 ± 4.4 | 47.4 ± 4.4       |
| 3rd                         | 4.3 ± 0.3*  | 59.5 ± 3.8 | 49.1 ± 4.5*      |
| 4th                         | 4.1 ± 0.5*  | 66.2 ± 2.3 | 54.6 ± 3.5*      |
| 5th                         | 4.3 ± 0.6*  | 95.4 ± 4.7 | 65.9 ± 7.1*      |
| Fecal bile acids (μmol/2 days) |     |         |                  |
| Initial                     | 0.5 ± 0.1  |         |                  |
| 1st week                    | 5.5 ± 0.7*  | 22.2 ± 3.6 | 34.3 ± 3.8*      |
| 2nd                         | 5.4 ± 0.6*  | 15.2 ± 1.0 | 24.1 ± 3.2*      |
| 3rd                         | 6.7 ± 1.1*  | 20.0 ± 2.0 | 43.9 ± 5.8*      |
| 4th                         | 4.9 ± 0.5*  | 14.7 ± 2.4 | 35.3 ± 4.8*      |
| 5th                         | 4.3 ± 0.5*  | 16.8 ± 2.7 | 40.2 ± 4.5*      |

*Results are represented as mean ± SEM for ten or eleven mice. *Differs significantly from C-CA-fed group (p < 0.05).

Fig. 2. Composition of bile acid pool. Abbreviations: CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; KLCA, ketolithocholic acid; KDCA, ketodeoxycholic acid. Data shown are the means of ten or eleven mice.
Fig. 3. Apparent excretion rates of cholesterol and cholic acid. Each point is the mean for ten or eleven mice and vertical extension indicates SEM. * Differs significantly from C-CA-fed group ($p<0.05$).

Apparent excretion rate [%] (cholesterol or cholic acid)

$$\text{Apparent excretion rate} = \frac{\text{fecal excretion (containing its metabolites)}}{\text{food intake}} \times 100.$$
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Fig. 4. Composition of fecal bile acids. Abbreviations: CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid; UCA, ursodeoxycholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; KLCA, ketolithocholic acid; KDCA, ketodeoxycholic acid.

Fecal bile acid excretion was elevated several times in mice in the C-CA- or taurine-fed groups compared to the standard group. The ratio of CA and related bile acids increased in mice fed on the lithogenic diet. Based on the data in Table 3 and Fig. 4, the fecal excretion of CDCA and β-MCA increased quantitatively as well as qualitatively in taurine-supplemented mice. Although CA excretion also increased, the proportion of CDCA and related bile acids was increased more by dietary taurine (Fig. 4). The composition of fecal bile acids did not change greatly in
any group during the experimental period. The excretion rate of CA did not change with time (Fig. 3).

DISCUSSION

In general, the fundamental defect in cholesterol gallstone disease is the secretion of abnormal bile supersaturated with cholesterol (16). The physical state of bile is determined by its relative bile salt, lecithin and cholesterol contents (17). It has been reported that cholesterol synthesis increased, bile acid formation decreased (18–20) and the bile acid pool size diminished (21) in some patients with cholesterol gallstones.

Fujihara et al. (3) demonstrated that biliary cholesterol levels were significantly lower and the biliary bile acid level was significantly higher in mice fed on a lithogenic diet supplemented with taurine compared to those fed a lithogenic diet alone, but that the biliary phospholipid levels did not differ between the two groups.

We obtained results indicating that dietary taurine inhibited cholesterol gallstone formation (Table 2) and that the fecal excretion of bile acid increased (Table 3) while the bile acid pool size did not change (Table 3). The latter coincides with the results of Kibe et al. (22) which also demonstrated that there was no significant increase in bile acid pool size due to dietary taurine supplementation.

In this experiment, the fecal excretion of cholesterol was relatively less while that of bile acids was significantly higher in the taurine-supplemented group than in C-CA-fed mice (Table 3). The apparent excretion rate of cholesterol increased with time both in C-CA-fed mice and in taurine-supplemented mice while the excretion rate of CA did not change with time (Fig. 3). Beher et al. (23) reported that the bile acid pool size was 5.62 ± 1.09 mg and that the fecal digitonide-precipitable steroids totaled 5.54 ± 0.65 mg/day in mice fed on a diet supplemented with 1% cholesterol and 0.25% CA. The reason for the difference in the profiles between the apparent excretion rate of CA and that of cholesterol is not known. Transformation of cholesterol to coprostanol was significantly depressed in both the C-CA- and taurine-supplemented groups. Tsuji et al. (8) previously reported that in the rat, transformation of cholesterol to coprostanol was significantly depressed by dietary CA. It is suggested that dietary CA may change the intestinal microflora. Fecal CA excretion increased, but this fact does not necessarily show that dietary taurine stimulates CA synthesis because CA is supplemented in lithogenic diets. Therefore the findings that the content of CDCA and related bile acids increased both in the bile acid pool and in the fecal bile acids (Figs. 2 and 4) are very important.

Schoenfield et al. (24) demonstrated that CA inhibited CDCA synthesis by feedback inhibition. Their results supported ours that the ratio of CDCA and related bile acids in mice in the C-CA-fed group was significantly lower than in the standard group (Fig. 3). Dietary taurine increased the content of CDCA and related bile acids in bile acid pool and feces (Figs. 2 and 3). Therefore, it is suggested that dietary taurine stimulates the fecal excretion of bile acids, especially that of CDCA.
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and related bile acids.

In general, active transport and passive non-ionic diffusion are quantitatively of the greatest importance and they account for the net movement of bile acids across the gastrointestinal tract (25). In the two primary bile acids, CA and CDCA, CA is transported much more efficiently than CDCA (26–30). Therefore, it is suggested from our results that dietary taurine stimulates the catabolism of cholesterol to bile acids, especially CDCA, in the liver. These results are comparable with previous results (6) indicating the reduction of hepatic cholesterol content by dietary taurine. These facts may show one of the important mechanisms for the protective effect of dietary taurine against cholesterol gallstone formation.

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