Effect of Dietary Taurine on Cholesterol 7α-Hydroxylase Activity in the Liver of Mice Fed a Lithogenic Diet

Yumiko NAKAMURA-YAMANAKA,* Keisuke TSUJI, and Tomio ICHIKAWA1

Division of Applied Food Research, National Institute of Nutrition, Toyama, Shinjuku-ku, Tokyo 162, Japan

(Received January 22, 1987)

Summary The effect of dietary taurine on hepatic cholesterol 7α-hydroxylase activity was investigated in mice. At first, male ICR strain mice were fed a commercial non-purified diet for 4 weeks and killed at 01:00 h (midnight) or 13:00 h (daytime) and the cholesterol 7α-hydroxylase activity in the hepatic microsomal fraction was measured. The enzyme activity was 5.9-fold higher at midnight than in the daytime. Next, to investigate the effect of dietary taurine on the activity of this enzyme, male ICR strain mice were fed semi-purified diets for 5 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). All mice were killed at midnight and cholesterol 7α-hydroxylase activity was measured. The enzyme activity of the mice fed the lithogenic diet was about 20% that of mice fed the standard diet. Dietary taurine increased the activity by 1.9-fold. Therefore, it was concluded that the inhibitory effect of dietary taurine on cholesterol gallstone formation was related to increased bile acid synthesis as reflected by stimulation of cholesterol 7α-hydroxylase activity.

Key Words dietary taurine, cholesterol 7α-hydroxylase, diurnal variation, enzyme activity, cholesterol gallstone

Fujihara et al. (1) reported that gallstone formation in mice was inhibited almost completely by ingestion of taurine with a lithogenic diet. We previously reported on the decrease in the incidence of gallstone formation and hepatic cholesterol mass (2, 3) and the increase in fecal bile acid excretion (4) effected by

---

1 中村（山中）優美子，辻 靖介，市川寛夫

* Present address: Division of Foods, National Institute of Hygienic Sciences, Osaka Branch, Hoenzaka, Higashi-ku, Osaka 540, Japan.
dietary taurine in hypercholesterolemic mice. These results suggested that the protective effect of dietary taurine against cholesterol gallstone formation is related to the stimulation of bile acid synthesis (4).

To verify this hypothesis, the effect of taurine on the activity of cholesterol 7α-hydroxylase [EC 1.14.13.17], the rate-limiting enzyme in bile acid synthesis, was studied in mice fed a lithogenic diet.

EXPERIMENTAL

Male ICR strain mice (Nihon Clea Inc., Tokyo), 4 weeks old, were used in the experiment. They were kept in an air-conditioned room (23 ± 1°C, 50-60% humidity) lighted for 12 h a day (07:00 h to 19:00 h).

First, diurnal variation of cholesterol 7α-hydroxylase activity was studied. Eight mice were fed the commercial non-purified diet (CE-2, Nihon Clea Inc., Tokyo) for 4 weeks. Four mice were then killed at 01:00 h (midnight) and the others were killed at 13:00 h (daytime). Livers were immediately excised for the measurement of enzyme activity.

Next, the effect of dietary taurine on cholesterol 7α-hydroxylase activity was investigated. Mice from 3 groups (each group contained 3 or 8 mice) were fed on the 3 purified diets indicated in Table 1 for 5 weeks. All mice were killed between 00:00 h and 02:00 h and livers were excised immediately following the observation of gallstone formation with the naked eye.

The hepatic microsomal fraction was obtained and cholesterol 7α-hydroxylase activity was determined by measuring the hydroxylation of [4-14C]cholesterol (6, 7). A microsomal fraction corresponding to 1 g of fresh liver was suspended in 0.1 M potassium phosphate buffer (pH 7.4) containing nicotinamide (30 mM) and MgCl2 (5 mM). The standard assay mixture consisted of 0.1 M potassium phosphate buffer (pH 7.4), MgCl2 (5 mM), NADPH (10 mM), [4-14C]cholesterol, and 0.3–0.5 mg of

Table 1. Composition of purified diets.

<table>
<thead>
<tr>
<th>Constituents (%)</th>
<th>Standard</th>
<th>C-CA</th>
<th>C-CA + 5% taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Mineral mixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Cellulose&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>—</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Taurine</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>60.15</td>
<td>59.4</td>
<td>54.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> AIN-76™ mixture (5). <sup>b</sup> Solka-floc.
microsomal protein. [4-14C]Cholesterol (Amersham International plc; specific activity 55 mCi/mmol) was purified before being used for thin-layer chromatography. The assay was started by the addition of [4-14C]cholesterol (150 nmol, specific activity 3.5 $\times$ 10^3 dpm/nmol) to the incubation mixture. All enzyme assays were carried out for 20 min with air as the gas phase at 37°C with constant shaking, with care being taken to avoid unnecessary exposure to light. A boiled enzyme control was run with each experiment. The incubation was terminated by adding 20 volumes of chloroform–methanol 2:1 (v/v), and the sterols were extracted after the addition of 3 ml of water. The organic solvent layer was removed and evaporated to dryness under N$_2$ at 40°C. The sterol fraction was dissolved in 40 μl of acetone containing 20 μg of 7α-hydroxycholesterol and applied on a 0.25 mm-thick silica gel plate (Kieselgel 60, Merck). The plates were developed with ether at 5°C, and the spots were made visible by spraying lightly with 3.5% phosphomolybdic acid in isopropanol. The pertinent spots were removed by suction, transferred to scintillation vials containing 0.5% (w/v) 2,5-diphenyloxazole in toluene and counted in a Packard TRI-CARB Liquid Scintillation Spectrometer. Microsomal protein was estimated by the method of Lowry et al. (8). The results were corrected for the non-enzymatic oxidation of cholesterol by subtracting values obtained for the boiled enzyme blanks and were expressed in terms of pmoles of 7α-hydroxycholesterol formed per mg protein per min.

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

RESULTS AND DISCUSSION

The 7α-hydroxylation of cholesterol is the rate-limiting step in the conversion of cholesterol into bile acids. The enzyme system of cholesterol 7α-hydroxylase, which catalyzes this reaction, plays a significant role in the regulation of bile acid synthesis (9). This activity has been investigated in various animals (10), and shows diurnal rhythmicity that is high at midnight and low in the daytime in mice (11) as well as in rats (12). We therefore investigated the effect of dietary taurine on this enzyme activity by killing the mice at midnight.

The results are shown in Table 2. The hepatic 7α-hydroxylase activity was 5.9-fold higher at midnight than in the daytime in mice fed the non-purified diet. Van Cantfort and Gielen (11) measured this enzyme activity in various strains of mice and rats, and indicated that the activity was 3.53 to 7.66 at 10 a.m. and 7.67 to 12.32 pmol $\times$ min$^{-1}$ $\times$ mg protein$^{-1}$ at 10 p.m. and that the magnitude of the rhythm was much weaker in mice compared to rats. Cholesterol 7α-hydroxylase activity in mice fed the lithogenic diet was about 20% of that in mice fed the standard diet. Dietary taurine increased the enzyme activity by 1.9-fold, but this was still lower than that in mice fed the standard diet. The reduction of gallstone incidence by dietary taurine coincides with previous results (2–4).

Schoenfield et al. (13) reported that administration of cholic acid significantly
Table 2. Activity of hepatic microsomal cholesterol 7α-hydroxylase in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mice</th>
<th>Gallstone incidence (%)</th>
<th>Cholesterol 7α-hydroxylase activity (pmol of 7α-hydroxycholesterol formed per min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE-2a (01:00h)</td>
<td>4</td>
<td>—</td>
<td>20.03 ± 3.63$^b$</td>
</tr>
<tr>
<td>CE-2 (13:00h)</td>
<td>4</td>
<td>—</td>
<td>3.39 ± 0.36$^*$</td>
</tr>
<tr>
<td>Standard</td>
<td>3</td>
<td>0</td>
<td>11.53 ± 0.29$^{**}$</td>
</tr>
<tr>
<td>C-CA</td>
<td>8</td>
<td>87.5</td>
<td>2.25 ± 0.52</td>
</tr>
<tr>
<td>C-CA +5% taurine</td>
<td>8</td>
<td>25.0</td>
<td>4.33 ± 0.76$^{**}$</td>
</tr>
</tbody>
</table>

$^a$ Commercial non-purified diet (Nikon Clea Inc., Tokyo). $^b$ Values are means ± SEM. ** Differs significantly from the CE-2 (01:00h) group ($p<0.05$).

inhibits 7α-hydroxylase activity. Thus, the reduction of this enzyme in mice fed the lithogenic diet (Table 2) may be due to dietary cholic acid. The 1.9-fold increase in cholesterol 7α-hydroxylase activity due to dietary taurine corresponds to the report of Kibe et al. (14), who observed a 2.4-fold increase due to dietary taurine in guinea pigs.

The reduction in gallstone incidence and the increase in the activity of cholesterol 7α-hydroxylase due to dietary taurine are also comparable with the results of Fujihara et al. (1), who reported a twofold reduction in the cholesterol level accompanying a twofold increase in the bile acid level in the bile of mice that ingested taurine compared to non-treated mice. Hepner and Quarfordt (15) reported that cholesterol 7α-hydroxylase activity was reduced in cholesterol gallstone patients.

The results of the present study seem to confirm the previous hypothesis that the protective effect of dietary taurine against cholesterol gallstone formation is related to the stimulation of bile acid synthesis. Whether taurine increases the synthesis of specific bile acid is still an open question.

REFERENCES


