Rapid Paper

Anti-Obesity Effect of Cholest-4-en-3-one, an Intestinal Catabolite of Cholesterol, on Mice

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Summary An anti-obesity effect was observed for cholest-4-en-3-one (cholestenone) which is an intestinal catabolite of cholesterol. Body weight gain and body fat accumulation of CDF1 mice were inhibited by 0.5% dietary exposure to this chemical. Dose response for the effect of cholestenone was found to increase as the dose rose from 0.1 to 0.3% and 0.5%. No obvious anomaly due to consumption of cholestenone was detected by necropsy and clinical observation. The mechanism of this effect of cholestenone is not known at present, but it was not due to anorexia.

Key Words cholest-4-en-3-one (cholestenone), intestinal cholesterol catabolite, anti-obesity (body-weight loss), adipose tissue, serum lipids, lipoprotein, CDF1 mouse

Much attention (1,2) is currently focused on the role of dietary fat in food intake regulation and obesity. If fat is over-accumulated in the body, physical activity is restricted, a burden is placed on the internal organs, and associated diseases such as hypertension, heart disease, and diabetes mellitus develop with a resulting increase in mortality. Various treatments are applied to prevent obesity, but there are many difficulties involved. Fats such as triglyceride and cholesterol are absorbed from the intestine or synthesized in the liver, and then incorporated into lipoproteins and transported to adipose tissue of the body.

Cholesterol is catabolized by intestinal bacteria into various substances. There are two main pathways by which cholesterol is catabolized by intestinal bacteria as follows (3): (a) cholesterol→cholest-4-en-3-one (cholestenone)→5β-cholestan-3-one (coprostanone)→5β-cholestan-3-ol (coprostanol); and (b) cholesterol→cholestenone→5α-cholestan-3-one (cholestanone)→5α-cholestanol (cholestanol). Direct conversion of cholesterol to coprostanol is thought to be the uncommon pathway (4,5). Once cholesterol is catabolized to coprostanone and coprostanol, they are no longer reabsorbed from the intestine (3).

We studied the biological effects of intestinal catabolites of cholesterol, and
found that cholestenone markedly inhibits body fat accumulation. This chemical prevented obesity in CDF1 mice and maintained normal body weights without clinical abnormalities and carcinogenicity.

Experimental. CDF1 (BALB/c × DBA) mice obtained from Charles River Japan Inc. (Kanagawa) were used in this experiment since they have the characteristics of small variations in body weight and a low incidence of spontaneous malignant tumors. Three hundred mice (150 females and males each), 4 weeks of age, were divided into three groups for experiment 1 as follows: 1) cholestenone-added feed, 2) cholesterol-added feed, and 3) untreated controls. Animals were fed ad libitum pelletized diet with 0.5% cholestenone (Aldrich, USA) added, 0.5% cholesterol (Aldrich, USA) added, or no additives. Commercial feed (type CMF, Oriental Yeast Co., Tokyo; calories 1,549 kJ; fat 8.7%; protein, 29.4%; nitrogen-free extract [NFE], 43.6%) was used for the basal diet.

To examine the dose response of cholestenone and feed intake (experiment 2), 100 male CDF1 mice, 4 weeks of age, were divided into five groups of 20 mice each as follows: 1) 0.1% cholestenone-added feed, 2) 0.3% cholestenone-added feed, 3) 0.5% cholestenone-added feed, 4) controls, and 5) stock feed. A modified feed (calories, 1,599 kJ; fat, 9.8%; protein, 23.5%; NFE, 50%) was used as the basal diet in this experiment. It was prepared by supplementing commercial stock feed (type MF, Oriental Yeast Co.; calories, 1,498 kJ; fat, 5.6%; protein, 24.6%; NFE, 52.3%) with soy-bean oil in a 95.6:4.4 ratio.

The content of cholesterol in the diet was 0.07% (CMF) and 0.075% (MF). Other sterols may be present in trace amounts in the diet. Cage and bedding were changed twice a week. Mice were housed in aluminum cages (22 × 33 × 11 cm) with 5 animals per cage and maintained at 24±1°C and 50±5% relative humidity with a 12 h/12 h light/dark schedule. The experimental diet was produced every 2 months at Oriental Yeast Co. and stored at 4°C before use. Body weights of the animals were determined every month. Feed intake was calculated from the weekly residue of feed and expressed as the mean cumulative feed intake per mouse for each experimental group.

Animals were fed for 17 months in experiment 1 and for 5 months in experiment 2. After feeding, the animals in experiment 1 were dissected, whole blood was collected from the vena cava for lipid measurements and organs were necropsied. In animals free from lesions, the weights of the brain, lung, heart, liver, kidneys, spleen, testes (or ovaries), pituitary gland, adrenal glands, and abdominal adipose tissues as well as the concentration of serum lipid components were measured. Serum lipids were measured using an autoanalyzer (Hitachi 736) by the method of Allain et al. (6) for total cholesterol, Eggstein et al. (7) for triglyceride, Takayama et al. (8) for phospholipids, and Scholnick et al. (9) for lipoprotein. Data were subjected to statistical analysis using two-way analysis of variance for changes in body weight and feed intake, and one-way analysis of variance for the concentration of serum lipid components. Animal care and the experiments were carried out in accordance with the guidelines for animal experimentation of the

Results. In experiment 1, control and cholesterol-fed mice grew rapidly as shown in Fig. 1. They showed signs of obesity after consumption of either the control or cholesterol diet for 4 months in males or 6 months in females. However, mice fed cholestenone did not show any signs of obesity even when fed the diet for 17 months. The differences between the body weight of the cholestenone group and the other groups were statistically significant at the <0.001 level. In this case, body weights in cholestenone-fed male mice were almost equal to values obtained for male CDF1 mice fed a standard stock feed (type CE-2, CLEA Japan Inc.; calories, 1,435 kJ; fat, 4.4%; protein, 25.1%; NFE, 50.9%) for 4 months reported by Morisada et al. (10). Body weights of mice fed cholesterol were similar to those of the control mice. Body weights of the cholestenone-fed mice were 85% (males) and 87% (females) of those of the control mice at 8 months of age. Cholestenone fed mice did not show any clinical signs of abnormalities such as coat roughness, weakness, or diarrhea.

As shown in Fig. 2, the amounts of adipose tissue in the abdominal cavity of the cholestenone fed mice were about 1/3 (males) or 1/2 (females) of those of the control and cholesterol-fed mice. A preliminary examination also showed major

![Graph of body weights over time](image)

Fig. 1. Time-course of body weights of CDF1 mice. Animals were fed 0.5% cholestenone-added feed, 0.5% cholesterol-added feed, or basal diet. Values represent M±SD for 50 mice of each group. □, cholestenone; ○, cholesterol; ■, control. Reference data (▲) from Morisada et al. (10).
Fig. 2. Weight of adipose tissue in the abdominal cavity of 18-month-old CDF1 mice. Adipose tissues were surgically collected from the abdominal cavity. The mesenterium was included in the adipose tissues. Values represent M±SD for 10 mice. A, cholestenone; B, cholesterol; C, control.

Fig. 3. Serum lipid concentration of 18-month-old CDF1 mice. Values represent M±SD for five mice. A, cholestenone; B, cholesterol; C, control.

Fig. 4. Serum lipoprotein concentration of 18-month-old CDF1 mice. Values represent M±SD for five mice. LDL, low density lipoprotein; VLDL, very low density lipoprotein; CM, chylomicron. A, cholestenone; B, cholesterol; C, control.
differences between the thickness of the abdominal subcutaneous fat in the cholest-
enone group and in the other groups.

Serum lipid components of 18-month-old mice are shown in Figs. 3 and 4. Total cholesterol and phospholipid concentrations of the cholestenone-fed mice were significantly higher than those of the control mice for female mice, but not for male mice. A decreasing tendency in the amounts of triglyceride and lipoproteins, especially chylomicron, was observed in the cholestenone group. Survival rates and the tumor incidences in 18-month-old mice were not affected by the feeding of cholestenone and cholesterol. All animals showed high incidences of small intestinal polyposis at levels of 37% in females and 54% in males. There were few differences in the weights of the brain, lung, heart, liver, kidneys, spleen, testes (or ovaries), pituitary gland, and adrenal glands among the groups (data not shown).

The dose response for the body weight of CDF1 mice is shown in Fig. 5. Proportional inhibition of body weight by cholestenone feeding was observed with the 0.1, 0.3, and 0.5% doses. The variance among values in the experimental groups was statistically significant ($p<0.001$). As shown in Table 1, no significant difference was obtained for cumulative feed intake per mouse in each experimental group. All animals showed healthy growth without clinical abnormalities.

Discussion. The results of our study showed that cholestenone, an intestinal catabolite of cholesterol, prevents diet-induced obesity by dietary exposure without any clinical abnormalities. Dose response was recognized for this anti-obesity effect of cholestenone. Since weights of major internal organs and the hormonal glands, survival rate, tumor incidence, and clinical observations generally showed little change after consumption of cholestenone for 17 months, it appeared that cholest-

![Fig. 5. Body weight of male CDF1 mice fed different doses of cholestenone. Values represent means for 20 mice of each group. ○, cholestenone 0.1%; †, cholestenone 0.3%; □, cholestenone 0.5%; ■, control; ○, stock feed.](image-url)
Table 1. Cumulative feed intake of male CDF1 mice fed different doses of cholestenone.

<table>
<thead>
<tr>
<th>Group</th>
<th>Feeding period (month)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cholestenone 0.1%</td>
<td>85.0&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholestenone 0.3%</td>
<td>93.3</td>
</tr>
<tr>
<td>Cholestenone 0.5%</td>
<td>111.6</td>
</tr>
<tr>
<td>Control</td>
<td>95.0</td>
</tr>
<tr>
<td>Stock feed</td>
<td>76.3</td>
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</tbody>
</table>

<sup>1</sup>Values represent the mean cumulative intake per mouse.

Cholestenone had very weak toxicity and carcinogenicity in test animals. The high incidence of small intestinal polyposis in all experimental mice was thought to be inherited since Mizutani et al. (11) reported that BALB/c mice, a maternal line of CDF1 mice, showed high incidences of small intestinal polyposis at levels of 37% in females and 51% in males at 12 months of age. It is obvious that the effect of cholestenone on body-weight loss of mice was not due to taste aversion for feed as observed from the feed intake and spillage of test mice. This is also evident from the characteristics of this chemical, i.e., insoluble in water and forms stable, achromatic, tasteless, and odorless crystals at normal temperature (12).

The above data regarding the anti-obesity effect of cholestenone are still not sufficient to indicate the mode of action of the chemical, but several speculations exist on why cholestenone feeding leads to inhibition of body fat accumulation. First, it is accepted that cholesterol and cholestenone are typical representatives of membrane active and inactive steroids, respectively (13). If cholestenone hinders the formation of lipoprotein membrane in an antagonistic manner to cholesterol, triglyceride transportation with lipoprotein may be reduced. Secondly, cholestenone feeding to rats results in adrenal hypertrophy and suppression of adrenal steroid secretion (14). This suppression of corticosteroids should then be reflected in inhibition of synthesis of fatty acids in the liver. However, our experiment using mice showed no hypertrophy of the adrenal glands in spite of long-term feeding of cholestenone.

Further work is necessary to substantiate the mechanism of the effect of cholestenone. Analysis of cholesterol and cholestenone contents in lipoprotein membrane and corticosteroids concentration in the adrenal glands should provide information on the mode of action of this cholesterol metabolite. Measurement of fecal lipid for excreted energy content and histological examination of the intestinal villi and the liver for accumulation of lipid should also indicate whether there is inhibition of fat absorption or transportation. Moreover the rate of absorption and distribution in the organs of labeled cholestenone after oral administration is necessary to assess the toxicity of this chemical.
ANTI-OBESITY EFFECT OF CHOLESTENONE

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