Short Communication

Effects of Methionine, Cystine and Taurine on Plasma Cholesterol Level in Rats Fed a High Cholesterol Diet

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Currently it is recognized that the plasma cholesterol concentration is significantly influenced by the kind and amount of dietary protein in experimental animals and humans.^{1,2)} Earlier experiments demonstrated that sulfur-containing amino acids (SAA) such as methionine, cysteine, and cystine have cholesterol-lowering effects in rats fed a low protein diet containing cholesterol.³⁾ However, the effects of dietary proteins on plasma cholesterol levels cannot be explained simply by the amount of total SAA contained in the proteins, because, for instance, the plasma cholesterol concentration of animals fed soy protein diets is always lower than that of animals fed milk casein diets⁴⁾ although the casein contains more total SAA than soy protein. Milk casein is a unique protein among many dietary proteins since a large portion of the total SAA is methionine and the cystine content is extremely low.⁵⁾

These facts suggest that cholesterol metabolism might be affected by methionine and cystine in dietary protein in a distinct manner. This communication describes the effects of methionine, cystine, and taurine added to a cholesterol-containing casein diet on plasma and liver cholesterol levels in rats.

Male albino rats of the Wistar strain weighing about 80 g were fed the experimental diets for 3 weeks. The basal diet contained 25% casein, 15% sucrose, 15% lard, 2% corn oil,

5% salt mixture, 1% vitamin mixture, 0.2% choline chloride, 1% cholesterol, 0.25% sodium cholate, and α -starch to make 100%. Sulfur-containing amino acids (L-methionine, L-cystine, and taurine) were added to the basal diet at the expense of starch. Room temperature was kept at $24 \pm 1^\circ\text{C}$ with a 12 hr cycle of light (0600 to 1800) and dark. At the end of the feeding period, food was removed at 2400, and the animals were killed between 1100 and 1200. Liver lipids were extracted by the method of Folch *et al.*,⁶⁾ and used for the determination of cholesterol and total lipids. The cholesterol concentration of plasma and liver was determined by the method of Zak.⁷⁾ High density lipoprotein (HDL) was separated by the heparin-Mn precipitation method⁸⁾ followed by the enzymatic determination of cholesterol with a commercial kit (Wako Pure Chemical Ind. Ltd., Osaka). The difference between the total cholesterol and HDL-cholesterol was assumed to be low density lipoprotein (LDL)- and very low density lipoprotein (VLDL)-cholesterol.

Body weight gain, food intake and liver weight of experimental animals are summarized in Table I. Growth and food intake were depressed as the amount of methionine added to the basal diet was increased, but the depression was not severe. Liver weight was invariant except for the group fed a diet supplemented with 0.63% taurine.

Figure 1 shows the effects of dietary addition of methionine, cystine, and taurine on the cholesterol concentration of plasma and liver. High levels of cystine and taurine reduced the concentration of total plasma cholesterol. Dietary methionine, on the contrary, greatly increased the plasma cholesterol concentration. On the other hand, the liver cholesterol concentration was decreased slightly, but significantly, by the addition of methionine, cystine, and taurine at higher levels. The effects of methionine, cystine, and taurine on total liver lipids showed a tendency similar to the liver cholesterol concentration (data not shown). Table II shows the distribution of plasma cholesterol between HDL- and

TABLE I. BODY WEIGHT GAIN, FOOD INTAKE AND LIVER WEIGHT

Diet	No. of animals	Body wt gain (g/21 days)	Food intake (g/21 days)	Liver wt (% of body wt)
25% Casein (25C)	7	115.1 ± 2.7 ^a	268.7 ± 4.5	6.30 ± 0.10
25C + 0.25% Met	5	120.4 ± 2.9	262.8 ± 3.7	6.27 ± 0.11
25C + 0.50% Met	5	109.7 ± 1.3	249.6 ± 3.0*	6.07 ± 0.09
25C + 0.75% Met	5	93.8 ± 4.7*	228.9 ± 6.0*	6.08 ± 0.12
25C + 0.20% Cys	5	114.6 ± 3.4	250.1 ± 5.3*	6.37 ± 0.13
25C + 0.40% Cys	5	111.7 ± 3.6	252.7 ± 5.5*	6.04 ± 0.26
25C + 0.60% Cys	5	119.5 ± 2.5	260.1 ± 3.7	6.22 ± 0.16
25C + 0.21% Tau	5	114.0 ± 3.6	260.5 ± 6.2	5.94 ± 0.16
25C + 0.42% Tau	5	115.5 ± 3.0	261.0 ± 4.0	6.02 ± 0.11
25C + 0.63% Tau	5	115.6 ± 4.4	264.4 ± 7.2	5.73 ± 0.06*

^a Values are the means ± SEM.* Significantly different from 25C at $p < 0.05$.

FIG. 1. Effects of Dietary Addition of Methionine, Cystine and Taurine on Plasma (A) and Liver (B) Cholesterol Levels.

L-Methionine (○), L-cystine (●) and taurine (▲) were added to the basal diet at a constant level of inorganic sulfur in the corresponding groups. Each point and its bar represent a mean ± SEM. Asterisk indicates a significant difference from the value for the group on the basal diet.

TABLE II. DISTRIBUTION OF PLASMA CHOLESTEROL BETWEEN HDL- AND (LDL + VLDL)-FRACTIONS

Diet	Plasma cholesterol concn. (mg/100 ml)		
	Total chol	HDL-Chol	(LDL + VLDL)-Chol
25% Casein (25C)	233 ± 10 ^a	29.0 ± 2.5	204 ± 10
25C + 0.75% Met	438 ± 18*	51.1 ± 5.8*	382 ± 17*
25C + 0.60% Cys	179 ± 17*	30.6 ± 0.6	148 ± 17*
25C + 0.63% Tau	167 ± 11*	27.0 ± 2.1	140 ± 11*

^a Values are the means ± SEM.* Significantly different from 25C at $p < 0.05$.

(LDL + VLDL)-fractions. The data indicate that alterations in the concentration of total plasma cholesterol are mainly attributable to the changes in the (LDL + VLDL)-cholesterol.

The findings presented here, that the concentration of plasma cholesterol is influenced in an opposing manner by dietary methionine and cystine, appear to offer a basis for the reason why the plasma cholesterol level of rats fed a casein diet is higher than those of rats fed diets containing plant proteins such as soy protein⁴⁾ and wheat gluten⁹⁾ in the presence of dietary cholesterol, since methionine comprises the most part of the total SAA of casein, whereas both soy protein and wheat gluten contain less methionine and more cystine as compared with casein. To confirm this assumption detailed studies are now in progress using various dietary proteins.

In our preliminary experiments (data not shown), the addition of 0.75% methionine to the 10% casein diet containing cholesterol, in contrast to the 25% casein level, promoted the growth of the animals and resulted in a significant reduction in their plasma cholesterol levels. These findings are consistent with the observations of Seidel *et al.*³⁾ However, since the addition of 1.5% methionine to the 10% casein diet which caused a growth depression elevated the plasma cholesterol level, it is likely that the action of methionine in cholesterol metabolism is essentially hypercholesterolemic, at least under the experimental conditions in which cholesterol is added to the diet.

It is not known, at present, how dietary

methionine increases the plasma cholesterol concentration. Since methionine supplementation depressed growth and food intake, a possibility is that the increase in plasma cholesterol level in these groups may be in part related to the depressed growth and food intake. However, it was reported that poor growth and depressed food intake did not necessarily result in a higher plasma cholesterol concentration.⁹⁾ The metabolic formation of cystine and taurine is enhanced when methionine-excess diets are administered to rats.¹⁰⁾ Consequently, the dietary supplementation with methionine should result in a strong cholesterol-elevating efficacy which overcomes the cholesterol-lowering actions by cystine and taurine. The detailed mechanisms of these actions remain to be elucidated.

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