Effects of Dietary Supplemented Amino Acids on Endogenous Hypercholesterolemia in Rats

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Summary  Effects of additions of amino acids to a 20% casein diet on serum cholesterol (Ch) were studied in hypothyroid and hepatoma-bearing rats with endogenous hypercholesterolemia as well as in normal rats. In normal Wistar rats, methionine (Met) was hypercholesterolemic at the “nutritional” level (0.2-0.4%), but hypocholesterolemic at the “excess” level (1.2-2.4%). In Wistar rats with hypothyroidism induced by thiouracil, the addition of excess (1.2%) Met to the 20% casein diet reduced an endogenous hypercholesterolemia due to hypothyroidism by suppressing an elevation in (VLDL+LDL)-Ch with no significant influence on HDL-Ch. In Donryu rats received a subcutaneous implantation of AH109A cells (an ascites hepatoma line), either 1.2% Met, 1.2% cystine (Cys), or 1.2% Met and 2.5% glycine (Gly) in combination improved a hepatoma-induced hypercholesterolemia and abnormal serum lipoprotein profiles by suppressing a hepatoma-induced increase in (VLDL + LDL)-Ch. From Ch turnover studies in hepatoma-bearing rats, an impaired catabolism of Ch in the liver was suggested to be one cause for the hepatoma-induced elevation in (VLDL + LDL)-Ch. One of the dietary manipulations, Met and Gly in combination (Met + Gly), was found to improve the impaired Ch catabolism, this leading to a reduction of the (VLDL + LDL)-Ch level by Met + Gly in hepatoma-bearing rats.

Key Words  Endogenous hypercholesterolemia, hyperlipidemia, serum lipoprotein, bile acid, hypothyroidism, hepatoma, methionine, cystine, glycine.

Certain diseases such as hypothyroidism, diabetes, nephrosis, and cancer are accompanied by endogenous hyperlipidemia in humans, and animal models corresponding to each of these diseases have been established (1). We have studied the effects of dietary amino acids, mainly sulfur-containing ones and glycine, on endogenous hypercholesterolemia as well as normocholesterolemia.

Before testing the effects of amino acids in animal disease models, the methionine (Met) effect on serum cholesterol (Ch) was investigated in normal (normocholesterolemic) rats. Male Wistar rats were fed for 3 weeks on a Ch-free, 20% casein diet containing sucrose as a sole carbohydrate source, and the effect of Met on normocholesterolemia was studied by varying added amounts (0.6-2.4%) of Met to the 20% casein diet. Methionine showed a hypocholesterolemic action going from 0.6 to 2.4% (2). In rabbits, however, Met was reported to increase serum Ch concentration when 0.2% of the amino acid was added to a 20.8% casein diet (3). So, we have next checked up on the effect of a small amount of supplemented Met on serum Ch, focusing our attention on added Met range of 0-0.6% (Fig. 1). Methionine significantly increased serum Ch in the range 0.2 to 0.4% Met. The serum Ch level was the highest at 0.3%, whereas the amino acid was hypocholesterolemic at the higher supplemented amount of 2.4% (Fig. 1A). Body weight gain was the highest at 0.3% and was not suppressed by Met addition up to 0.6%. On the other hand, Met suppressed the growth of rats at 2.4% (Fig. 1B). Therefore, it can be said that Met is hypercholesterolemic at the “nutritional” level, but hypocholesterolemic at the “excess” level in Wistar rats fed on the Ch-free, 20% casein diet under the experimental conditions employed here. On the basis of these data, we have tried to examine the effects of excess Met and other amino acids on endogenous hypercholesterolemia incident to hypothyroidism and cancer.
Hypothyroid rats were prepared by feeding the 20% casein diet containing 0.3% thiouracil to male Wistar rats for 3 weeks. Euthyroid (normal) rats were fed on the same diet without thiouracil for 3 weeks. In both the euthyroid and hypothyroid states, the Met effect on serum Ch was studied by adding 1.2% Met to the basal diets (4). At the same time, the effect of 1.2% cystine (Cys) was also examined. Serum total Ch was notably increased in hypothyroid rats. This elevation was not due to an elevation in HDL-Ch but due to that in (VLDL+LDL)-Ch. These changes in lipoprotein profile resulted in a striking elevation in the atherogenic index [(VLDL+LDL)-Ch/HDL-Ch]. Methionine reduced the hypercholesterolemia in hypothyroid rats by suppressing the elevation in (VLDL+LDL)-Ch with no significant reduction of HDL-Ch, leading to a notable reduction of atherogenic index. In normal rats, Met showed no significant influence on Ch distribution among lipoproteins and hence atherogenic index. This difference in Met action between normal and hypothyroid rats suggests that hypothyroid rats are more sensitive to dietary Met than are euthyroid (normal) rats. Another sulfur amino acid, Cys, induced hypercholesterolemia as a result of an increase in HDL-Ch in normal rats, while Cys no longer had such a significant effect in the hypothyroid state. Cholesterol biodynamics has been well documented in hypothyroid rats (5). In rats with hypercholesterolemia induced by an added excess Cys, 23% casein diet without Ch addition, almost all the changes in parameters involved in Ch turnover have been reported to couterbalance the parameter changes in hypothyroidism (6). Such an offset effect may account for the loss of the hypercholesterolemic action of Cys in hypothyroid rats. By contrast, Met is suggested to overcome the changes in parameters which induce hypercholesterolemia in the hypothyroid state. Studies concerning Ch biodynamics may clarify these aspects.

Various cancers including hepatoma are well known to induce abnormal serum lipid metabolism. Rats bearing transplantable hepatomas such as AH109A (7) or Morris hepatoma 7288C (8) have been reported to show alterations in serum (plasma) lipoprotein profiles. When Donryu rats received subcutaneous implantation of an ascites hepatoma line of AH109A cells in the back, endogenous hypercholesterolemia and highly atherogenic lipoprotein profiles in the serum, characterized by an enormus elevation in (VLDL+LDL)-Ch and a decrease in HDL-Ch, were induced with growth of the solid tumor (7). We examined the effects of dietary supplemented Met, Cys and glycine (Gly) on AH109A-induced, endogenous hypercholesterolemia (9). Hepatoma-free (normal) or hepatoma-bearing rats were fed on a basal diet (20% casein,
Table 1. Cholesterol absorption, hepatic Ch synthesis and fecal bile acid excretion in normal and hepatoma-bearing rats given a 20% casein diet without or with concomitant addition of 1.2% Met and 2.5% Gly.

| Diet              | Hepatoma state | Intestinal Ch absorption | Hepatic Ch synthesis | Bile acid excretion |
|-------------------|----------------|--------------------------|----------------------|---------------------|
|                   |                | days 5–7                 | days 12–14            |
| 20C (normal)      | free           | 100^a                    | 100^a                | 100^a               |
| 20C + 1.2Met + 2.5Gly | free           | 111^a                    | 127^a                | 179^b               |
| 20C (control)     | bearing        | 102^a                    | 206^b                | 108^b               |
| 20C + 1.2Met + 2.5Gly | bearing        | 120^a                    | 235^b                | 154^b               |

Each value represents the percentage of normal (100%). Values not sharing a common letter are significantly different at p < 0.05. ^1 Intestinal Ch absorption of the normal group was 54.1 ± 6.3% (n = 5). ^2 Hepatic Ch synthesis of the normal group was 0.63 ± 0.05 μmol H₂O/h/100 g body weight (n = 5). ^3 Bile acid excretion of the normal group was 7.2 ± 1.3 (days 5–7) or 13.2 ± 1.4 (days 12–14) μmol/rat/2 days (n = 6).

5% corn oil, 17% sucrose, 50% α-corn starch, 5% mineral mixture, 1% vitamin mixture, and 2% cellulose powder (10), as a normal or hepatoma-bearing, control group. Other hepatoma-bearing rats were given the basal diet supplemented with either 1.2% Met, 1.2% Cys, 2.5% Gly, or 1.2% Met and 2.5% Gly in combination. Animals were fed on experimental diets for 2 weeks. The dietary addition of either Met, Cys, or Met + Gly to the 20% casein diet improved the hepatoma-induced hypercholesterolemia and abnormal serum lipoprotein profiles by suppressing the hepatoma-induced increase in (VLDL + LDL)-Ch (9). The addition of Gly alone showed no effect on Ch distribution and hence atherogenic index (9). The suppressive effect of Met on endogenous hypercholesterolemia in hepatoma-bearing rats is in good agreement with that in hypothyroid rats. In contrast, the Cys effect on endogenous hypercholesterolemia is inconsistent, that is, Cys was hypocholesterolemic in the hepatoma-bearing state, while it had no effect on hypercholesterolemia in the hypothyroid state. So, the effect of dietary supplemented Cys on cholesterol seems to depend on the causes underlying the hypercholesterolemia and/or dietary conditions.

We next tried to understand the mechanism for the induction of hypercholesterolemia by AH109A and its improvement by amino acids by studying Ch turnover, selecting the combination of Met and Gly (11, 12). Normal and AH109A-bearing rats were given either the 20% casein diet or the 20% casein diet supplemented with 1.2% Met and 2.5% Gly for 2 weeks. Cholesterol absorption from the intestine was estimated by the dual-isotope serum ratio method, Ch synthetic rate was measured on 14th day of feeding using [³H]water, and bile acid excretion was determined enzymatically after extraction from feces individually collected on days 5–7 and 12–14. Results are summarized in Table 1. Cholesterol absorption was not significantly different among the 4 groups. The Ch synthetic rate in the liver of hepatoma-bearing rats increased to twice that of normal rats. Dietary supplemented Met and Gly had no significant effect on the hepatic cholesterogenesis in both normal and hepatoma-bearing rats. By the hepatoma implantation, bile acid excretion was unaffected on days 5–7, but was significantly suppressed on days 12–14. In general, the addition of Met and Gly caused a stimulatory effect on bile acid excretion in both the normal and hepatoma-bearing states. The concomitant addition of Met and Gly reduced the (VLDL + LDL)-Ch level in both normal and hepatoma-bearing rats (12). From these results, an increased cholesterogenesis in the host liver and an impaired catabolism of Ch are suggested to be responsible for the hepatoma-induced increase in (VLDL + LDL)-Ch. Dietary supplemented Met and Gly in combination may enhance Ch catabolism in the liver, thus leading to a reduction of the (VLDL + LDL)-Ch level in normal and hepatoma-bearing rats. The mechanisms for the induction of hypertriglyceridemia, the decrease in HDL-Ch and the stimulation of hepatic cholesterogenesis in hepatoma-bearing rats are to be clarified. Lipoprotein lipase (13) and tumor
necrosis factor (14) seem the most attractive clues to such subjects, as discussed elsewhere (1).

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