Effects of Dietary Methionine, Cystine, and Glycine on Endogenous Hypercholesterolemia in Hepatoma-Bearing Rats

Kazumi YAGASAKI, Michie MACHIDA, and Ryuhei FUNABIKI

Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

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Summary The effects on hypercholesterolemia of dietary additions of cystine (Cys), methionine (Met), glycine (Gly), and a combination of Met and Gly to a 20% casein diet were studied in male Donryu rats subcutaneously implanted with an ascites hepatoma line of AH109A cells. The hepatoma-bearing rats fed the 20% casein diet lapsed into both endogenous hypertriglyceridemia and hypercholesterolemia when compared to hepatoma-free (normal) rats fed the same diet. The hypercholesterolemia was due to an elevation (3.2 fold) in the very low-density lipoprotein plus low-density lipoprotein (VLDL+LDL)-cholesterol (Ch) level. The high-density lipoprotein (HDL)-Ch level was slightly but significantly decreased. These lipoprotein changes in hepatoma-bearing rats resulted in a marked (4.5 fold) increase in the atherogenic index (AI, (VLDL+LDL)-Ch/HDL-Ch) in comparison with that of tumor-free rats. The dietary additions of 1.2% Met, 1.2% Cys, and a combination of 1.2% Met and 2.5% Gly significantly suppressed the hepatoma-induced increase in (VLDL+LDL)-Ch with no influence on the hepatoma-induced decrease in HDL-Ch, leading to a noticeable fall in AI. These results indicate that hepatoma-bearing rats are useful as an endogenously hyperlipidemic model and that some dietary amino acids are capable of improving hepatoma-induced hypercholesterolemia and abnormal serum lipoprotein profiles.

Key Words hypercholesterolemia, hypertriglyceridemia, hepatoma, methionine, cystine, glycine, serum cholesterol, lipoprotein

Hepatoma often induces hypercholesterolemia in humans (1, 2). Recently, rats subcutaneously implanted with an ascites hepatoma line of AH109A cells have been found to show a striking decrease in the high-density lipoprotein (HDL) fraction and an enormous increase in the very low-density lipoprotein plus low-density lipoprotein...
lipoprotein (VLDL + LDL) fraction with growth of the hepatoma (3). Thus, AH109A-bearing rats provide us with an endogenously high atherogenic model for patients with hepatoma, and the animals apparently differ from cholesterol (Ch)-loaded animals which have been widely used as an exogenously hypercholesterolemic model. Dietary sulfur amino acids and glycine (Gly) are known to regulate plasma/serum Ch concentration in animals fed Ch-free diets (4–8). We have recently reported different effects of cystine (Cys) and methionine (Met) on cholesterolemia in rats when added to a 20% casein diet without Ch supplementation: Met is hypocholesterolemic in both normal (6) and hypothyroid (7) rats, while Cys is hypercholesterolemic in normal rats but is of no effect on hypercholesterolemia in hypothyroid rats (7). These findings suggest the possibility that the effects of amino acids on cholesterolemia fluctuate depending on the physiological states or causes underlying hypercholesterolemia. This report describes the effects of Cys (5–7), Met (6, 7), Gly (4), and a combination of Met and Gly (8) on endogenous hypercholesterolemia and abnormal serum lipoprotein profiles, using hepatoma-bearing rats (3) as a model for actual human diseases with endogenous hyperlipidemia.

MATERIALS AND METHODS

Animals and diets. Male Donryu rats each weighing about 120 g received subcutaneous implantation of 500,000 AH109A cells2 per rat to produce a solid tumor as described previously (3). A sham implantation was performed in tumor-free (normal) rats. The animals were fed experimental diets and water ad libitum for 14 days in an air-conditioned room with a light period from 8 a.m. to 8 p.m. The composition of the basal diet was as follows: 20% casein,3 5% corn oil,4 17% sucrose,5 5% mineral mixture 1 (Oriental arrangement),3 1% vitamin mixture 1 (Oriental arrangement),3 2% cellulose powder,3 and α-corn starch4 to make up 100%. To the basal diet was added 1.2% of Cys,4 1.2% of Met,4 2.5% of Gly,6 or 1.2% Met plus 2.5% Gly at the expense of starch. Animals were deprived of food at 9 a.m. on the 14th day but allowed free access to water until sacrifice 4 h later by decapitation. Blood was collected, left to clot, and centrifuged to obtain serum. The liver and solid hepatoma were quickly removed, washed with 0.9% NaCl, blotted on filter paper, and weighed.

Lipid analyses. From the liver and solid hepatoma (ca. 0.5 g), total lipids were extracted according to the procedure of Folch et al. (9). After aliquots of the chloroform phase had been dried, the Ch (10) and triglyceride (TG) (11) levels were determined as described previously (12). The serum TG level was also determined (11).

Lipoprotein separation and cholesterol determination. The serum lipoproteins

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2 Provided by the Sasaki Institute, Tokyo. 3 Oriental Yeast Co., Ltd., Tokyo. 4 Ajinomoto Co., Inc., Tokyo. 5 Mitsui Sugar Co., Ltd., Tokyo. 6 Yoneyama Chemical Ind., Ltd., Osaka.
were separated into HDL and VLDL+LDL fractions by a precipitation method as described previously (3). The total Ch contents of unfractionated serum (S-Ch) and HDL (HDL-Ch) were enzymatically determined with a commercial kit, and the difference between S-Ch and HDL-Ch was regarded as (VLDL+LDL)-Ch.

Statistical method. Statistical analysis was carried out using Duncan's multiple-range test (13).

RESULTS

Food intake, growth, and relative weights of the liver and hepatoma are shown in Table 1. The hepatoma implantation caused significant decreases in food intake, body weight gain, and relative liver weight (N vs. C). None of the amino acids added to the 20% casein diet exerted a significant influence on food intake and body weight gain when compared to the control group (C vs. CC, CM, CG, and CMG). Food intake and body weight gain of the Met group were the lowest among the groups and significantly differed from those of the Cys and Gly groups (CM vs. CC and CG). The addition of Cys significantly increased the relative liver weight (C vs. CC), whereas the addition of Met alone or Gly alone and the concomitant addition of Met and Gly showed no significant effect on the liver weight (C vs. CM, CG and CMG). Dietary-supplemented Cys significantly suppressed hepatoma growth (C vs. CC). While Met alone or Gly alone showed no significant effect on hepatoma growth (C vs. CM and CG), the combination of Met and Gly significantly suppressed hepatoma growth when compared to Met alone and Gly alone as well as

Table 1. Effects of dietary additions of amino acids on food intake, body weight gain, and liver and hepatoma weights in hepatoma-bearing rats.

| Diet (group) | Hepatoma | Food intake (g/14 days) | Body wt gain | Relative liver wt | Relative hepatoma wt (% of body wt) |
|--------------|----------|-------------------------|--------------|-------------------|-----------------------------------|
| 20C (normal, N) | - | 206 ± 19<sup>a</sup> | 70 ± 5<sup>a</sup> | 4.14 ± 0.14<sup>a</sup> | — |
| 20C (control, C) | + | 158 ± 10<sup>b,c</sup> | 38 ± 5<sup>b,c</sup> | 3.46 ± 0.06<sup>b</sup> | 19.5 ± 1.9<sup>a</sup> |
| 20C + 1.2Cys (CC) | + | 174 ± 8<sup>a,b</sup> | 55 ± 8<sup>a,b</sup> | 4.13 ± 0.09<sup>a</sup> | 14.2 ± 2.2<sup>b</sup> |
| 20C + 1.2Met (CM) | + | 135 ± 9<sup>c</sup> | 28 ± 10<sup>c</sup> | 3.45 ± 0.14<sup>b</sup> | 18.7 ± 1.2<sup>a</sup> |
| 20C + 2.5Gly (CG) | + | 180 ± 12<sup>a,b</sup> | 55 ± 12<sup>a,b</sup> | 3.77 ± 0.11<sup>b</sup> | 19.3 ± 0.9<sup>a</sup> |
| 20C + 1.2Met (CMG) + 2.5Gly | + | 150 ± 7<sup>b,c</sup> | 31 ± 7<sup>b,c</sup> | 3.66 ± 0.14<sup>b</sup> | 13.0 ± 0.8<sup>b</sup> |

Each value represents the mean of five rats ± SE. Values not sharing a common letter are significantly different at p<0.05.

<sup>7</sup> Wako Pure Chemical Ind., Ltd., Osaka.

Vol. 32, No. 6, 1986
As illustrated in Fig. 1, the serum TG and S-Ch levels were significantly elevated by AH109A implantation (N vs. C), indicating that the hepatoma endogenously induced both hypertriglyceridemia and hypercholesterolemia. None of the supplemented amino acids showed any significant effect on hepatoma-induced hypertriglyceridemia, although the mean TG values were lower in the Cys, Met, and Met+Gly groups than in the control group (C vs. CC, CM, and CMG). Cystine significantly suppressed the hepatoma-induced hypercholesterolemia (C vs. CC), and the S-Ch level was even higher than normal (N vs. CC). Methionine alone, Gly alone, and a combination of Met and Gly exerted no significant influences on the S-Ch level (C vs. CM, CG, and CMG).

Fig. 1. Effects of dietary additions of amino acids on serum lipid levels in hepatoma-bearing rats. Each value represents the mean of five rats. Vertical bars indicate standard errors. For the abbreviations of groups, see Table 1. Values not sharing a common letter are significantly different at $p<0.05$. 

J. Nutr. Sci. Vitaminol.
Changes in Ch distribution among serum lipoproteins and the atherogenic index (AI, \(\text{VLDL}+\text{LDL}-\text{Ch}/\text{HDL}-\text{Ch}\)) are illustrated in Fig. 2. The hepatoma-induced hypercholesterolemia was due to an elevation (3.2 fold) in the \(\text{VLDL}+\text{LDL}-\text{Ch}\) level but was not due to that in the \(\text{HDL}-\text{Ch}\) level which was conversely decreased, resulting in a striking increase (4.5 fold) in AI (N vs. C). The dietary additions of Cys, Met, and Met+Gly significantly suppressed the hepatoma-induced increase in \(\text{VLDL}+\text{LDL}-\text{Ch}\) with no significant influence on the hepatoma-induced decrease in \(\text{HDL}-\text{Ch}\), leading to a notable fall in AI (C vs. CC, CM, and CMG). However, the \(\text{VLDL}+\text{LDL}-\text{Ch}\) level and AI of the Cys, Met, and Met+Gly groups were still higher than those of the normal group (N vs. CC, CM and CMG). The addition of Gly alone showed no effect on Ch distribution and hence on AI (C vs. CG).

Vol. 32, No. 6, 1986
Table 2. Effects of dietary additions of amino acids on liver and hepatoma lipid levels in hepatoma-bearing rats.

| Diet (group)       | Liver lipid level (mg/g liver) | Hepatoma lipid level (mg/g hepatoma) |
|--------------------|--------------------------------|--------------------------------------|
|                    | TG                             | Ch                                  | TG                           | Ch                           |
| 20C (normal, N)    | 20.6 ± 2.5a                    | 2.52 ± 0.10a                        | 33.0 ± 1.4a                  | 1.83 ± 0.13a                 |
| 20C (control, C)   | 12.0 ± 2.6a                    | 2.78 ± 0.06ab                       | 34.9 ± 0.8a                  | 1.76 ± 0.03ab                |
| 20C + 1.2Cys (CC)  | 14.3 ± 2.1a                    | 2.86 ± 0.05ab                       | 33.0 ± 0.7a                  | 1.59 ± 0.06b                 |
| 20C + 1.2Met (CM)  | 9.0 ± 2.5a                     | 2.84 ± 0.08ab                       | 35.5 ± 1.4a                  | 1.81 ± 0.10a                 |
| 20C + 2.5Gly (CG)  | 11.3 ± 2.7a                    | 3.02 ± 0.21b                       | 33.6 ± 2.8a                  | 1.81 ± 0.06a                 |
| 20C + 1.2Met (CMG) | 15.0 ± 6.9a                    | 2.86 ± 0.13ab                       | —                            | —                            |

Each value represents the mean of five rats ± SE. Values not sharing a common letter are significantly different at p < 0.05.

Table 2 shows changes in the lipid levels of the host liver and solid hepatoma. The liver TG level was, although not significantly, decreased by tumor implantation (N vs. C). The liver TG level in hepatoma-bearing rats was not affected by dietary amino acids added (C vs. CC, CM, CG, and CMG). The liver Ch level showed a subtle elevation due to the hepatoma (N vs. C). In hepatoma-bearing rats, dietary additions of amino acids tested showed no significant effect on the liver Ch level (C vs. CC, CM, CG, and CMG). The liver Ch level in the Gly group was significantly higher than that in the normal group (N vs. CG). The hepatoma TG level was nearly constant among the groups and was 2 to 3 fold higher than the liver TG level. None of the amino acids except for Met exerted any influence on the hepatoma Ch level (C vs. CC, CG, and CMG). The lowest level of the Met group was significantly different from those of the control, Gly, and Met + Gly groups (CM vs. C, CG, and CMG). In general, the hepatoma Ch level was lower than the liver level.

DISCUSSION

The present results clearly showed that in improving hepatoma-induced, endogenous hypercholesterolemia and abnormal serum lipoprotein profiles, dietary Cys, Met, and Met + Gly were comparable to a drug (3) which has been shown to reduce hypertriglyceridemia (14) and hypercholesterolemia (12) and to inhibit both fatty acid (FA) (15) and Ch (16) biosyntheses.

Food intake and body weight gain of the Met group were the lowest among the amino acid-supplemented groups. However, the decreases in the Met group were not significant when compared to the control (no addition) group. There is thus little possibility that the hypolipidemic action of Met is related to depressed food...

J. Nutr. Sci. Vitaminol.
intake and hence growth. The suppressive effect of Met on endogenous hypercholesterolemia in hepatoma-bearing rats is in good agreement with that in hypothyroid rats (7). In contrast, the effect of Cys on endogenous hypercholesterolemia is variable: The amino acid was hypocholesterolemic in the present study, whereas it had no effect on hypercholesterolemia in hypothyroid rats (7). These findings suggest that the effect of dietary-supplemented Cys on cholesterolemia depends on hormonal states, the causes underlying hypercholesterolemia, and/or dietary conditions. Casein diets supplemented with Gly have been reported to reduce exogenous hypercholesterolemia in rats (4) and casein-induced, endogenous hypercholesterolemia in rabbits (17). In the present study, Gly alone exerted no influence on hepatoma-induced hypercholesterolemia. The reason for this discrepancy is not known at present, but differences in hypercholesterolemic models may be a possible explanation for the ambivalent effects of Gly. Glycine, however, showed a reductive effect on hepatoma-induced hypercholesterolemia by improving abnormal serum lipoprotein profiles when Met was concomitantly supplemented. The concomitant addition of Met and Gly also has been found to reduce the (VLDL + LDL)-Ch level without depressing the HDL-Ch level in normal (tumor-free) rats fed a 10% casein diet without the addition of Ch (8). Supplemental Gly has been shown to nearly alleviate the toxicity (depressed food intake and growth) due to excess Met (18). Thus, the combination of Met and Gly seems preferable to Met alone to improve hepatoma-induced hypercholesterolemia, although depressed food intake and growth due to excess Met were not fully abolished by Gly in the present study.

Littman et al. (19) were able to show that reduction of the availability of Ch, either by restriction in the diet or by administration of hypocholesterolemic drugs, retarded the growth and development of a number of transplantable animal tumors. Schneider et al. (20) have reported that estrone, which inhibits Ch biosynthesis and lowers rat plasma Ch, prolongs survival of subcutaneous hepatoma-bearing rats and inhibits hepatoma growth. They also have demonstrated that there is a significant and positive correlation of lower tumor weight and prolonged animal survival with lower plasma Ch and lower Ch content of certain (VLDL and LDL) lipoprotein fractions (20). In the present study, Cys alone and a combination of Met and Gly significantly retarded solid hepatoma growth and lowered the (VLDL + LDL)-Ch concentration, while Met failed to inhibit tumor growth despite the fact that the host (VLDL + LDL)-Ch concentration was significantly decreased by the amino acid. Malignant rodent cells and some malignant human cell lines have been shown to absolutely require Met for growth in culture, unlike normal human and rodent cell lines that in all cases can grow adequately in the presence of homocysteine instead of Met (21). Provided that AH109A cells, a malignant rodent cell line, do require Met for their growth, a sufficient supply of Met to the hepatoma cells may cancel growth inhibition of the tumor due to a reduction of the host (VLDL + LDL)-Ch concentration. On the other hand, concurrent addition of Gly with Met to the 20% casein diet may cause a reduction of available Met for
hepatoma growth, since Gly has been thought to augment Met metabolism (18). Thus, a combination of Met and Gly is likely to succeed in retarding hepatoma growth and lower the (VLDL+LDL)-Ch concentration.

Hepatomas are of particular interest, since they have been shown to be capable of synthesizing serum lipoproteins in vitro like their parent tissue, and to release over 90% of the Ch produced by hepatomas into the blood circulation (22). Nonhepatic tumors apparently do not have this ability (23). It has been well documented that hepatomas show a loss of normal inhibition of Ch biosynthesis in vivo by Ch feeding (24, 25) and a defect in the dietary regulation of FA biosynthesis (24, 26, 27). Thus, an overproduction of VLDL and hence LDL by AH109A cells as well as the host liver may be a possible explanation for the increase in the VLDL + LDL fraction, and hence for hypertriglyceridemia. In addition to overproduction of VLDL by either host liver or hepatoma, hypertriglyceridemia in AH109A-bearing rats might be induced by a reduction of serum TG hydrolytic activities which is considered to be a cause of hypertriglyceridemia (14). Although the participation of Cys, Gly, and taurine in bile acid conjugation has been discussed in Ch-loaded animals (28), the mechanism for the hypocholesterolemic actions of sulfur amino acids and Gly in hepatoma-bearing rats is not known. Studies concerning the mechanisms for the induction of hypercholesterolemia by hepatoma and its improvement by dietary amino acids are now in progress from the aspect of Ch turnover.

REFERENCES

1) Alsabti, E. A. K. (1979): Serum lipids in hepatoma. *Oncology*, **36**, 11–14.
2) Hirayama, C., Yamanishi, Y., and Irisa, T. (1979): Serum cholesterol and squalene in hepatocellular carcinoma. *Clin. Chim. Acta*, **91**, 53–57.
3) Irikura, T., Takagi, K., Okada, K., and Yagasaki, K. (1985): Effect of KCD-232, a new hypolipidemic agent, on serum lipoprotein changes in hepatoma-bearing rats. *Lipids*, **20**, 420–424.
4) Katan, M. B., Vroomen, L. H. M., and Hermus, R. J. J. (1982): Reduction of casein-induced hypercholesterolemia and atherosclerosis in rabbits and rats by dietary glycine, arginine and alanine. *Atherosclerosis*, **43**, 381–391.
5) Sérougne, C., and Rukaj, A. (1983): Plasma and lipoprotein cholesterol in rats fed L-amino acid-supplemented diets. *Ann. Nutr. Metab.*, **27**, 386–395.
6) Yagasaki, K., Aoki, T., and Funabiki, R. (1986): Serum and liver lipid responses to methionine and cystine in rats fed diets with different casein levels. *Nutr. Rep. Int.*, **34**, 59–66.
7) Yagasaki, K., Aoki, T., Machida, M., and Funabiki, R. (1986): Effects of dietary methionine and cystine on endogenous hypercholesterolemia in hypothyroid rats. *Agric. Biol. Chem.*, **50**, 2785–2789.
8) Yagasaki, K., Ohsawa, N., and Funabiki, R. (1986): Effects of dietary amino acids on, and role of thyroid hormone in, methionine-induced endogenous hypercholesterolemia. *Nutr. Rep. Int.*, **33**, 321–328.
9) Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957): A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.*, **226**, 497–509.

*J. Nutr. Sci. Vitaminol.*
10) Zak, B. (1957): Simple rapid microtechnic for serum total cholesterol. *Am. J. Clin. Pathol.*, **27**, 583–588.

11) Van Handel, E. (1961): Suggested modifications of the micro determination of triglycerides. *Clin. Chem.*, 7, 249–251.

12) Yagasaki, K., Okada, K., Takagi, K., and Irikura, T. (1984): Effect of 4-(4’-chlorobenzyloxy)benzyl nicotinate (KCD-232) on cholesterol metabolism in rats fed an amino acid imbalance diet. *Agric. Biol. Chem.*, **48**, 1417–1423.

13) Duncan, D. B. (1955): Multiple range and multiple F tests. *Biometrics*, **11**, 1–42.

14) Irikura, T., Takagi, K., Okada, K., and Yagasaki, K. (1984): Reduction of fructose-induced hypertriglyceridemia and fatty liver in rats by 4-(4’-chlorobenzyloxy)benzyl nicotinate (KCD-232). *Agric. Biol. Chem.*, **48**, 977–983.

15) Yagasaki, K., Okada, K., Mochizuki, T., Takagi, K., and Irikura, T. (1984): Effect of 4-(4’-chlorobenzyloxy)benzyl nicotinate (KCD-232) on triglyceride and fatty acid metabolism in rats. *Biochem. Pharmacol.*, **33**, 3151–3163.

16) Okada, K., Yagasaki, K., Mochizuki, T., Takagi, K., and Irikura, T. (1985): Effect of 4-(4’-chlorobenzyloxy)benzyl nicotinate (KCD-232) on cholesterol metabolism in rats. *Biochem. Pharmacol.*, **34**, 3361–3367.

17) Hermus, R. J. J., and Dallinga-Thie, G. M. (1979): Soya, saponins, and plasma-cholesterol. *Lancet*, **II**, 48.

18) Steele, R. D., Barber, T. A., Lalich, J., and Benevenga, N. J. (1979): Effects of dietary 3-methylthiopropionate on metabolism, growth and hematopoiesis in the rat. *J. Nutr.*, **109**, 1739–1751.

19) Littman, M. L., Taguchi, T., and Mosbach, E. H. (1966): Effect of cholesterol-free, fat-free diet and hypocholesterolemic agents on growth of transplantable animal tumors. *Cancer Chem. Rep.*, **50**, 25–45.

20) Schneider, P. D., Chan, E. K., Guzman, I. J., Rucker, R. D., Varco, R. L., and Buchwald, H. (1980): Retarding Novikoff tumor growth by altering host rat cholesterol metabolism. *Surgery*, **87**, 409–416.

21) Cooper, A. J. L. (1983): Biochemistry of sulfur-containing amino acids. *Annu. Rev. Biochem.*, **52**, 187–222.

22) Narayan, K. A., and Morris, H. P. (1972): *In vitro* synthesis of rat serum lipoproteins and proteins by Morris hepatoma 7777. *FEBS Lett.*, **27**, 311–315.

23) Narayan, K. A. (1970): Serum lipoproteins of rats fed an essential fatty acid-deficient diet and N-2-fluorenylacetamide. *Cancer Res.*, **30**, 1185–1191.

24) Sabine, J. R., Abraham, S., and Chaikoff, I. L. (1967): Control of lipid metabolism in hepatoma: Insensitivity of fatty acid and cholesterol synthesis by mouse hepatoma BW7756 to fasting and to feedback control. *Cancer Res.*, **27**, 793–799.

25) Beirne, O. R., and Watson, J. A. (1976): Comparison of regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in hepatoma cells grown *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci., U.S.A.*, **73**, 2735–2739.

26) Sabine, J. R., Abraham, S., and Morris, H. P. (1968): Defective dietary control of fatty acid metabolism in four transplantable hepatomas: Numbers 5123C, 7793, 7795, and 7800. *Cancer Res.*, **28**, 46–51.

27) Majerus, P. W., Jacobs, R., and Smith, M. B. (1968): The regulation of fatty acid biosynthesis in rat hepatomas. *J. Biol. Chem.*, **243**, 3588–3595.

28) Jackson, J. A., and Burns, M. J. (1974): Effects of cystine, niacine and taurine on cholesterol concentration in the Japanese quail with comments on bile acid metabolism. *Comp. Biochem. Physiol.*, **48A**, 61–68.