Effects of Addition of Sulfur-Containing Amino Acids and Glycine to Soybean Protein and Casein on Serum Cholesterol Levels of Rats

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(Received February 15, 1989)

Summary The effects of the supplementation of methionine (Met), cystine (Cys), and glycine (Gly) to soybean protein or casein on serum and liver lipid levels were studied in rats. Rats were fed cholesterol-free diets containing 25% soybean protein or casein supplemented with 0.75% Met, 2.5% Gly, or a combination of these two for 4 weeks. The addition of Met to soybean protein caused a significant increase in serum cholesterol and this was slightly ameliorated when Gly was given simultaneously. In rats fed casein diets, serum cholesterol tended to decrease when Gly, or Met and Gly were added. A simultaneous supplementation of Met and Gly to casein resulted in a reduction of hepatic cholesterol. Cystine added at the 0.6% level did not cause demonstrable changes in lipid concentrations except for a drop in serum triglyceride of the casein group. When 2.0% Gly was added to cholesterol-enriched diets containing 20% protein, serum cholesterol decreased significantly only when the protein source was casein and the level attained was comparable to that observed in rats fed soybean protein. Liver cholesterol was also markedly decreased by the addition of Gly to casein. The results suggest a possible role of Gly in the regulation of serum cholesterol levels by dietary protein.

Key Words soybean protein, casein, methionine, cystine, glycine, lysine, arginine, serum cholesterol

Dietary protein is one of the factors influencing serum cholesterol levels (1–4). The factors responsible for the hypocholesterolemic effect of dietary protein remain unclear. Kritchevsky (5, 6) suggested that the arginine/lysine (Arg/Lys) ratio of dietary protein is an important factor in the regulation of cholesterol metabolism, although several data conflicting with the importance of this ratio are available.

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when single amino acids, Lys or Arg, were added (7–9). In contrast, the ratio appears to determine cholesterol levels when proteins having the different ratios were fed to experimental animals (10, 11).

Sulfur-containing amino acids are the first limiting amino acid in various dietary proteins including soybean protein and casein. Casein contains twice as much Met as soybean protein does and is more hypercholesterolemic than soybean protein. In casein the most abundant sulfur-amino acid is Met, while in soybean protein Cys is the major sulfur-amino acid. The addition of Met to the cholesterol-enriched casein diet resulted in an elevation of the serum cholesterol level in rats, while the addition of Cys lowered it (12–14). In contrast, Seidel et al. (15) and Leveille and Sauberlich (16) reported a reduction of serum cholesterol by supplementation of Met to the casein diet. Supplementation of Met to soybean protein had no effect on serum cholesterol level of rabbits (7). Yagasaki et al. (17) also observed that Met is hypocholesterolemic and Cys is hypercholesterolemic in cholesterol-free casein diets. The effect of sulfur-containing amino acids appears to depend not only on the dietary casein level but also on the supplementary level of these amino acids (17).

On the other hand, Gly is known to alleviate Met toxicity by accelerating the metabolism of Met (18). Katan et al. (19) observed that addition of Gly to casein exhibited an extreme hypocholesterolemic effect in rabbits. The Gly content in casein is one-half that in soybean protein. Taurine (Tau), one of the metabolites of Met, also possesses a serum cholesterol-lowering action (12, 20). However, the supplementary effects of these amino acids on serum cholesterol levels of experimental animals fed soybean protein are not well known.

In the present study, we examined the effects of supplementation of Met, Cys, and Gly, alone or in combination, on lipid levels of serum and liver of rats fed cholesterol-free or -enriched diets containing soybean protein or casein. The effect of the ratio of Arg/Lys was also studied.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka) initially weighing approximately 100 g were used in three experiments. The animals had free access to semipurified diets for 4 weeks. The composition of the basal diet (weight percent) was as follows: protein 25 or 20, fat 1 or 5, mineral mixture (Harper) 4, vitamin mixture (Harper) 1, choline chloride 0.2, cellulose 2, and sucrose to 100. Isolated soybean protein (Fujipro R, Fuji Oil Co., Osaka) and casein (vitamin-free, ICN Pharmaceuticals Inc., Cleveland, OH) were used as the dietary protein source. In experiments 1 and 2, diets containing 25% protein and 1% corn oil were used; 0.75% Met and 2.5% Gly alone or in combination (Exp. 1) or 0.60% Cys (Exp. 2) were added (12–14, 17). In experiment 3, 2% Gly was added to cholesterol-enriched (1% cholesterol and 0.25% sodium cholate) diets containing 20% protein and 4% lard and 1% corn oil. In this trial the effect of addition of Lys (1.72%) to soybean protein or Arg (0.86%) to casein to approximate the Arg/Lys ratio of each other.
was also studied. Addition of amino acids (L-amino acids, Ajinomoto Co., Tokyo), cholesterol, and cholate was achieved at the expense of sucrose. Animals were killed by decapitation at around 1000 h after being deprived of diets for 4 h. Blood serum and liver were analyzed for lipid components as described elsewhere (8,21).

The data were analyzed for statistical significance using Duncan's multiple range test.

RESULTS

Addition of methionine and glycine to cholesterol-free diet (Exp. 1)

Table 1 shows body weight gain, food intake, and liver weight. The addition of Met (0.75%) to casein but not soybean protein caused a slight retardation of growth and food intake, but a simultaneous addition of Gly (2.5%) alleviated them. Amino acid supplementation to both proteins did not largely alter liver weight.

Concentrations of serum and liver lipids are shown in Fig. 1. The antihypercholesterolemic effect of soybean protein was evident. The addition of Met to soybean protein resulted in a significant elevation of the serum cholesterol level. Glycine alone did not show any effect on the serum cholesterol level, but when added in combination with Met, it partly interfered with an increase by Met in serum cholesterol. In rats fed casein diets the concentration of serum cholesterol tended to decrease by the addition of Gly or a simultaneous addition of Met and Gly (p<0.10). The effect of amino acid administration on serum triglyceride was rather variable when soybean protein was a protein source, but it tended to decrease when casein was fed. The serum phospholipid level was not altered by the addition of amino acids (data not shown).

The concentration of liver cholesterol tended to decrease by the addition of

Table 1. Effect of supplementation of methionine and glycine on body weight gain, food intake and liver weight (Exp. 1).

| Groups                        | Body weight gain (g/4 weeks) | Food intake (g/day) | Liver weight (g/100 g body wt.) |
|-------------------------------|-------------------------------|---------------------|---------------------------------|
| Soybean protein               | 244 ± 8ae                    | 21.9 ± 0.7ae        | 4.50 ± 0.12                     |
| Soybean protein + Met         | 237 ± 9ae                    | 21.1 ± 0.5ae        | 4.90 ± 0.08                     |
| Soybean protein + Gly         | 244 ± 7ae                    | 22.6 ± 0.4ab        | 4.59 ± 0.17                     |
| Soybean protein + Met + Gly   | 238 ± 5ae                    | 21.1 ± 0.3ae        | 4.67 ± 0.16                     |
| Casein                        | 224 ± 13ae                   | 20.2 ± 1.0ae        | 4.73 ± 0.22                     |
| Casein + Met                  | 213 ± 7ab                    | 19.5 ± 0.3e         | 4.84 ± 0.12                     |
| Casein + Gly                  | 252 ± 7c                     | 21.3 ± 0.4ae        | 4.74 ± 0.13                     |
| Casein + Met + Gly            | 241 ± 6ce                    | 20.9 ± 0.4ae        | 4.75 ± 0.22                     |

Mean ± SE of 6 rats per group. Values bearing different letters are significantly different (p<0.05).
Fig. 1. Effect of supplementation of methionine and glycine to cholesterol-free diets on serum and liver lipid levels (Exp. 1). Mean ± SE of 6 rats per group. Different superscript letters indicate significant difference ($p<0.05$). S, Soybean protein; C, casein; M, methionine; G, glycine.

amino acids in both protein diets ($p<0.10$), especially the simultaneous administration of Met and Gly to casein exerted a significant reduction. Hepatic cholesterol levels in the soybean protein groups were significantly lower than those in the corresponding casein groups except for the Met and Gly supplementary groups.

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Fig. 2. Effect of supplementation of cystine to cholesterol-free diets on serum and liver lipid levels (Exp. 2). Mean ± SE of 8 rats per group. Different superscript letters indicate significant difference (p<0.05). S, Soybean protein; C, casein.

Soybean protein lowered the triglyceride concentration as compared to casein, but the difference was not significant. No significant supplementary effects of Met or Gly could be observed. The effect of amino acid supplementation on liver phospholipid was marginal in both groups of rats (data not shown).

Addition of cystine to cholesterol-free diet (Exp. 2)

Addition of Cys (0.60%) to soybean protein or casein diet had no significant effects on body weight gain, food intake, and relative liver weight (data not shown). With both proteins, supplementary Cys did not cause any changes in serum cholesterol levels, while it caused an extreme reduction of serum triglyceride in the casein group (Fig. 2). Liver lipid levels were not altered by Cys.

Addition of glycine to cholesterol-enriched diet (Exp. 3)

When rats were fed cholesterol-enriched diets, growth and food intake were not influenced either by the difference in dietary proteins or by the addition of Gly (2.0%) (Table 2). An increase in liver weight on the casein diet was significantly lowered by supplementation of either Gly or Arg (0.86%). Lysine (1.72%) sup-
Table 2. Effect of supplementation of glycine, lysine, and arginine to cholesterol-enriched diets on body weight gain, food intake and liver weight (Exp. 3).

| Groups              | Body weight gain (g/4 weeks) | Food intake (g/day) | Liver weight (g/100 g body wt.) |
|---------------------|------------------------------|---------------------|----------------------------------|
| Soybean protein     | 203 ± 11                     | 20.4 ± 0.7          | 6.04 ± 0.15                     |
| Soybean protein + Lys| 192 ± 11                     | 19.5 ± 0.8          | 5.46 ± 0.17                     |
| Soybean protein + Gly| 199 ± 6                      | 20.3 ± 0.6          | 6.01 ± 0.19                     |
| Casein              | 227 ± 5                      | 19.5 ± 0.4          | 6.85 ± 0.11                     |
| Casein + Arg        | 207 ± 13                     | 18.3 ± 0.8          | 6.22 ± 0.11                     |
| Casein + Gly        | 213 ± 12                     | 18.7 ± 0.8          | 6.11 ± 0.11                     |

Mean ± SE of 5 to 6 rats per group. Values bearing different letters are significantly different (p < 0.05).

Supplementation to soybean protein resulted in the lowest liver weight.

Concentrations of serum and liver lipids are shown in Fig. 3. The hypocholesterolemic effect of soybean protein was not necessarily evident when rats were fed the cholesterol-enriched diet. The addition of Gly to the soybean protein diet did not modify the serum cholesterol level, but the addition to the casein diet caused a significant reduction of serum cholesterol to a level comparable to that of the soybean protein groups. As in the case of cholesterol-free diets, addition of Gly to cholesterol-enriched diets did not affect the serum triglyceride level, although it was considerably higher in rats fed casein as compared to those fed soybean protein. Glycine again caused a reduction of hepatic cholesterol only when it was added to the casein diet, whereas the liver triglyceride level tended to be reduced when Gly was added to the soybean protein diet.

Lysine addition to soybean protein tended to elevate, although not significantly, while Arg addition to casein did not largely influence the serum cholesterol level. Arginine exerted a remarkable reduction of serum triglyceride concentration. No effect was observed on liver cholesterol, but liver triglyceride decreased slightly when Arg was added to the casein diet (p < 0.10).

**DISCUSSION**

Methionine is the first limiting amino acid in a number of dietary proteins and is also an amino acid that retards growth when given at a high level (18, 22, 23). No unequivocal evidence is available on whether or how Met influences the serum cholesterol level. On the basis of studies involved in the metabolites of Met, Sugiyama et al. (24) reported that the methyl group of Met is responsible for its cholesterol-elevating effect. However, there is still controversy regarding the influence of Met on serum cholesterol. In fact, Met was hypercholesterolemic when the casein diet enriched with cholesterol was fed (12–14), while it had no effect as in

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Fig. 3. Effect of supplementation of glycine, lysine and arginine to cholesterol-enriched diets on serum and liver lipid levels (Exp. 3). Mean ± SE of 5 to 6 rats per group. Different superscript letters indicate significant difference (p<0.05). S, Soybean protein; C, casein; L, lysine; G, glycine; A, arginine.

The present study or was even hypocholesterolemic (17) when the cholesterol-free casein diet was fed. Alternatively, the addition of Met to soybean protein resulted in an elevation of the serum cholesterol level of rats fed the diet free from cholesterol. Thus, it is still obscure whether Met itself really has a hypercholesterolemic action or not.

Addition of Gly to the cholesterol-enriched casein diet but not the soybean protein diet caused a reduction of serum cholesterol. Katan et al. (19) and Sugiyama et al. (13, 14) reported that dietary Gly reduces casein-induced hypercholesterolemia in rabbits and rats, respectively. The latter authors presumed that the hypocholesterolemic action of Gly is due to the stimulation of the metabolism of Met and consequently to the augmentation of the formation of the hypocholesterolemic
metabolites such as Cys or Tau. When given simultaneously with Met, Gly produced a reduction of serum cholesterol in rats fed the casein diet. This was not the case when rats were fed the soybean protein diet.

Sautier et al. (25) reported that the content of Cys in dietary protein is negatively correlated with the serum cholesterol concentrations. This was recently confirmed by Sugiyama et al. (26) using several dietary proteins containing different levels of Cys. However, in the present study, addition of Cys to soybean protein and casein did not exert any effects on concentrations of serum and liver cholesterol. Although it seems likely that both the content and metabolic rate of Met may play a role in the determination of the serum cholesterol level (24), the effect that a single amino acid exerts on the serum cholesterol level appears to be indeed complicated as previously presumed (27). In the cholesterol-free diet, the addition of Gly to soybean protein did not affect the serum cholesterol level, whereas the addition of Gly to casein tended to lower it. The same pattern of response was observed in both dietary protein groups when rats were fed cholesterol-enriched diets. If one assumes that the level attained with the soybean protein is the lower limit for serum cholesterol of these types of study, then the Gly content may be a common factor regulating cholesterol metabolism. This line of consideration cannot be applied for Met.

In rats fed cholesterol-enriched diets, as well as in those fed cholesterol-free diets (8), addition of Lys to soybean protein or Arg to casein did not influence serum cholesterol concentration. It seems that the Arg/Lys ratio of dietary protein is not necessarily an important factor in the regulation of the serum cholesterol level.

In summary, although the present study suggested the possibility that the content of Gly is involved in the regulation of serum cholesterol level, the situation seems to be very complicated as generally presumed. The amino acid sequence of proteins could possibly influence their cholesterolemic effect. The event encountered in the intestinal tract, production of specific peptide fractions should be taken into consideration (28–32).

The authors thank Dr. R. Chanderbhan (George Washington University, Washington, D.C.) for his important suggestions during the preparation of the manuscript, and Dr. I. Ikeda for statistical analysis.

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