Effects of Cabbage Leaf Protein Concentrate on the Serum and Liver Lipid Concentrations in Rats

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Summary The effects of cabbage leaf protein concentrate (CLPC) on serum and liver lipid concentrations were determined in rats fed cholesterol-enriched and cholesterol-free diets. In rats fed the cholesterol-enriched diet with CLPC, total cholesterol, triacylglycerol and phospholipid concentrations in both the serum and liver, as well as the atherogenic index diet were significantly lower than those of the rats fed a casein diet. A supplement of methionine to the CLPC diet raised serum HDL-cholesterol and body weight gain, indicating that the addition of methionine to the CLPC diet is not only available to improve the nutritive value of CLPC but also to lower the atherogenic index. In rats fed the cholesterol-free diet, the liver total cholesterol and triacylglycerol concentrations of the CLPC-fed rats also showed lower values than those of the casein-fed rats, however, the serum total cholesterol concentration of the CLPC-fed rats did not differ from that of the casein-fed rats.

Key Words cabbage leaf protein, hypolipidemic effect

It has been reported that proteins derived from plant-origin, such as soybean, sunflower, rice, spinach and radish protein isolates (1-11), produce activity to maintain lower serum cholesterol concentrations as compared to those of animal proteins such as casein. These reports suggest that some other plant proteins may also have hypocholesterolemic activity; however, the influence of vegetable proteins which are consumed daily on the serum and liver lipid levels has not yet been fully examined. We have previously demonstrated that spinach leaf protein isolate, as compared to casein, had hypocholesterolemic and hypolipidemic activities, and that the supplement of methionine to the spinach leaf protein isolate diet improved the nutritive value without loss of the cholesterol-lowering effect (10,11).

In this study, the effects of cabbage leaf protein concentrate (CLPC) on the serum and liver lipid concentrations in rats fed cholesterol-enriched and cholesterol-free diets were examined. Since it is well known that sulfur-containing amino...
acids profoundly modify the lipid metabolism in rats (12–16), the effect of supplementing methionine to CLPC on the serum and liver lipid concentrations was also determined. Further, the serum amino acid concentration in CLPC-fed rats was compared to that of casein-fed rats to examine the relation between the serum concentration of each amino acid and serum cholesterol concentration.

Materials and methods

Protein concentrate. Cabbage, which had vein parts removed, was ground with approximately equal amounts (in weight) of aqueous 0.2% sodium hydroxide at 5°C in a Waring blender. The homogenate obtained was filtered through two pieces of gauze, and the filtrate was centrifuged at 15,000×g for 20 min at 5°C. The supernatant, filtered using No. 2 filter paper, was adjusted to a pH of around 4 with 5% acetic acid to produce a protein precipitate. The protein precipitate obtained by centrifuging the protein precipitate part was continuously washed with acetone, ethyl alcohol and diethyl ether in a glass filter until the filtrate became colorless. It was then dried in a vacuum desiccator, and used as the protein concentrate. The yield of protein concentrate was 4g from 1kg of fresh cabbage leaves.

Animals and diets. Five-week-old male Wistar strain rats (Japan SLC Inc., Hamamatsu, Japan), each weighing about 70g, were divided into 4 groups of 5 rats each for experiment 1, and 3 groups of 5–6 rats each for experiment 2. Each rat was housed in a stainless-steel cage with a screen bottom, and kept under controlled conditions with a 12-h light and 12-h dark cycle (06:00–18:00 light), temperature range of 22–24°C and relative humidity of about 55%.

The compositions of the diets for experiments 1 and 2 are given in Tables 1 and 2, respectively. In experiment 1, four kinds of cholesterol-enriched diet with a 15% level of protein were used: casein diet (15 Cas diet), cabbage leaf protein concentrate diet (15 CLPC diet), CLPC diet supplemented with L-methionine (15 CLPC+Met diet) and soybean protein isolate diet (15 SPI diet). CLPC and SPI were both added to the diet at the expense of sucrose until the nitrogen content was equal to that of casein. In the 15 CLPC+Met diet, methionine was added to the CLPC diet at the expense of sucrose to become equal to the casein diet in methionine content. In experiment 2, three kinds of cholesterol-free diets with a 20% level of protein were used: 20% casein, 20% CLPC and 20% CLPC+Met diets (20 Cas, 20 CLPC and 20 CLPC+Met diets, respectively). A 0.113% methionine content, which is half of the amount of methionine necessary to become equal to a 20% casein diet, was added to the 20 CLPC diet. This added amount was effective to increase body weight gain in the 20 CLPC group to equal that of the 20% casein group as described in the following results. Food and water were provided ad libitum for 14 days in each experiment.

The feces of each rat were collected during feeding from days 10 to 13 and freeze-dried prior to extracting lipids. At the end of the feeding period, the rats were anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka, Japan)
Table 1. Composition of the cholesterol-enriched diet (%) (Experiment 1).

| Ingredient                      | 15 Cas | 15 CLPC | 15 CLPC+Met | 15 SPI |
|---------------------------------|--------|---------|-------------|--------|
| Casein                          | 15.00  | —       | —           | —      |
| Cabbage leaf protein concentrate| —      | 15.81   | 15.81       | —      |
| Soy protein isolate             | —      | —       | —           | 15.08  |
| Sucrose                         | 67.175 | 66.365  | 66.191      | 67.095 |
| Corn oil                        | 10.0   | 10.0    | 10.0        | 10.0   |
| Mineral mixture\(^2\)           | 4.0    | 4.0     | 4.0         | 4.0    |
| Vitamin mixture\(^3\)           | 1.0    | 1.0     | 1.0         | 1.0    |
| Cellulose                       | 2.0    | 2.0     | 2.0         | 2.0    |
| Cholesterol                     | 0.5    | 0.5     | 0.5         | 0.5    |
| Na-cholate                      | 0.125  | 0.125   | 0.125       | 0.125  |
| Choline chloride                | 0.2    | 0.2     | 0.2         | 0.2    |
| Methionine                      | —      | —       | 0.174       | —      |

Cas, casein; CLPC, cabbage leaf protein concentrate; Met, methionine; SPI, soy protein isolate. 181.3% protein. \(^2\)AIN-93G-MX and \(^3\)AIN-93-VX (36) were obtained from Oriental Yeast Co., Tokyo, Japan.

Table 2. Composition of the cholesterol-free diets (%) (Experiment 2).

| Ingredient                      | 20 Cas | 20 CLPC | 20 CLPC+Met |
|---------------------------------|--------|---------|-------------|
| Casein                          | 20.00  | —       | —           |
| Cabbage leaf protein concentrate| —      | 21.22   | 21.297      |
| \(\alpha\)-Cornstarch            | 49.0   | 48.2    | 48.06       |
| Sucrose                         | 24.5   | 24.1    | 24.03       |
| Corn oil                        | 2.0    | 2.0     | 2.0         |
| Mineral mixture\(^2\)           | 3.5    | 3.5     | 3.5         |
| Vitamin mixture\(^3\)           | 1.0    | 1.0     | 1.0         |
| L-Methionine                    | —      | —       | 0.113       |

Cas, casein; CLPC, cabbage leaf protein concentrate; Met, methionine. 181.3% protein. \(^2\)AIN-93G-MX and \(^3\)AIN-93-VX (36) were obtained from Oriental Yeast Co., Tokyo, Japan.

after 12 h of starvation, and bled by cardiac puncture. The liver excised from each of the rats was stored at \(-20^\circ\)C until the lipid analyses. The serum was separated by centrifuging the blood at 1,000\(\times\)g for 15 min. Liver lipids were extracted and purified by the method of Folch et al. (17).

Lipid analyses. The total amounts of cholesterol, triacylglycerol and phospholipids in the serum were measured enzymatically with commercial kits (cholesterol E-test, triglyceride E-test and phospholipid B-test, respectively; Wako Pure Chemical Ind., Osaka, Japan) (18–20). Serum HDL-cholesterol was enzymatically
measured in the supernatant obtained after heparin-Mn precipitation of the other lipoproteins (21) using a commercial kit (HDL-cholesterol test, Wako Pure Chemical Ind.).

The total amount of cholesterol in the liver lipid extract was determined enzymatically using a commercial kit (Mono-test cholesterol, Boehringer Mannheim Yamanouchi Co., Tokyo, Japan) (22). The kits used for determining the serum lipids were also used to determine other liver lipids.

Feces were extracted with ethyl alcohol under reflux conditions as described by Sautier et al. (23) and used to determine neutral steroids and bile acids. The amounts of neutral steroids were determined by gas chromatography as previously reported (24). The amounts of bile acids dissolved in isopropyl alcohol after evaporating the ethyl alcohol extracts were measured enzymatically by the procedure of Mashige et al. (25), using a Wako commercial kit (bile acids test).

**Amino acid analysis.** Amino acid compositions of casein and CLPC were determined by an amino acid analyzer using a procedure previously reported (26). To determine serum-free amino acid concentrations, a 0.5 mL aliquot of the serum was mixed with 0.06 mL of 20% sulfosalicylic acid, left overnight and centrifuged. The supernatant obtained was analyzed for amino acid on an ion exchange column (Li+ form) using an Atto MLC-703 amino acid analyzer.

**Statistical analysis.** Data were statistically analyzed by Duncan's multiple-range test after one-way analysis of variance (ANOVA), and significant differences in the means were inspected at p < 0.05.

**Results and discussion.** The nitrogen contents of CLPC used in experiments 1 and 2 were 12.14 and 12.26% on a dry-matter basis, respectively. These nitrogen contents were a little less than that of soybean protein isolate (13.01%). As shown in Table 3, glycine and arginine, which are considered to be amino acids that lower serum cholesterol concentration (15,27–29) were higher in the rats fed CLPC than in those fed casein, suggesting the possibility that CLPC may have hypocholesteremic activity.

Table 4 shows the effects of dietary CLPC on food intake, body weight gain, serum and liver lipid concentrations, and on the fecal excretion of steroids in the rats fed a cholesterol-enriched diet. Although food intake was not statistically different among the 15 Cas, 15 CLPC and 15 SPI diet groups, body weight gain was less in the 15 CLPC and 15 SPI groups than in the 15 Cas group. As the feed efficiency was almost equal between the 15 CLPC and 15 SPI groups (Table 4), the nutritive value of CLPC may be almost equal to that of SPI. The improvement of feed efficiency in the rats fed the 15 CLPC diet supplemented with methionine indicates that methionine supplement to a CLPC diet is capable of improving the nutritive value of CLPC. However, as the 15% protein level in the diet is not sufficient to obtain the maximum gain in body weight in growing rats, it may also be necessary to make a comparison with a higher protein level.
Table 3. Amino acid composition of casein and cabbage leaf protein concentrate (CLPC) and the concentrations of serum free amino acids in the rats fed casein, CLPC and CLPC+Met diets without cholesterol (Experiment 2).

| Amino acid composition¹ (g/16 g N) | Serum amino acid concentration (nmol/0.1 mL of serum) | Diet: 20 Cas  | 20 CLPC  | 20 CLPC+Met |
|-----------------------------------|-----------------------------------------------------|---------------|----------|-------------|
| Tau                               |                                                      | 49.1±4.1ᵃ     | 37.6±3.0ᵇ | 60.5±3.5ᶜ   |
| Asp                               |                                                      | 1.6±0.1ᵃ      | 2.0±0.2ᵃ  | 1.8±0.1ᵃ    |
| Asn                               |                                                      | 12.1±1.0ᵃ     | 14.2±1.2ᵇ | 13.0±0.7ᵇ   |
| Thr                               |                                                      | 15.4±0.5ᶜ     | 28.7±1.7ᵃ  | 21.6±1.2ᵇ   |
| Ser                               |                                                      | 13.6±0.8ᵇ     | 20.7±0.6ᵇ  | 15.0±0.9ᵇ   |
| Glu                               |                                                      | 9.9±0.7ᵃ      | 10.5±0.7ᵃ  | 10.4±0.3ᵃ   |
| Gln                               |                                                      | 36.5±3.7ᵃ     | 40.8±4.1ᵃ  | 40.0±3.5ᵃ   |
| Pro                               |                                                      | 7.6±0.6ᵃ      | 8.4±0.5ᵃ   | 8.3±0.4ᵃ    |
| Gly                               |                                                      | 9.6±0.5ᵃ      | 13.4±0.3ᵇ  | 15.6±0.4ᵇ   |
| Ala                                |                                                      | 21.4±1.5ᵃ     | 20.5±0.5ᵃ  | 18.7±0.5ᵃ   |
| Val                                |                                                      | 7.4±0.8ᵃ      | 8.6±1.5ᵃ   | 7.8±0.8ᵇ    |
| Cys                                |                                                      | 2.4±0.3ᵃ      | 2.2±0.1ᵇ   | 2.4±0.2ᵇ    |
| Met                                |                                                      | 2.8±0.2ᵃ      | 2.9±0.1ᵇ   | 2.6±0.1ᵇ    |
| Ile                                |                                                      | 6.7±0.4ᵃ      | 6.4±0.3ᵃ   | 6.3±0.2ᵇ    |
| Leu                                |                                                      | 5.6±0.5ᵃ      | 4.2±0.1ᵇ   | 3.6±0.4ᵃ    |
| Tyr                                |                                                      | 2.9±0.2ᵃ      | 3.3±0.5ᵃ   | 3.0±0.1ᵇ    |
| Phe                                |                                                      | 0.49±0.01ᵃ    | 0.50±0.01ᵃ | 0.50±0.01ᵃ  |
| Lys                                |                                                      | 2.1±0.2ᵃ      | 5.4±1.4ᵃ   | 3.9±0.9ᵃ    |
| His                                |                                                      | 2.4±0.1ᵃ      | 2.4±0.1ᵇ   | 2.5±0.1ᵇ    |
| Arg                                |                                                      | 18.3±1.4ᵃ     | 17.7±1.2ᵇ  | 16.6±0.9ᵃ   |
| Trp                                |                                                      | 1.34          | 1.63      |             |
| Total                              |                                                      | 111.4         | 83.75     |             |

¹Data for casein were cited from a previous paper (10). ²The amounts are expressed as that of Asp or Glu obtained by hydrolyzing protein with 6N HCl. Serum amino acid concentrations are M±SE of 5 to 6 rats per group. 20 Cas, 20% casein diet; 20 CLPC, 20% cabbage leaf protein concentrate diet; 20 CLPC+Met, 20 CLPC diet with added methionine.

The concentrations of the serum total and free cholesterols, triacylglycerol and phospholipid in the 15 CLPC group were lower than those in the control group (15 Cas group); moreover, the declines resembled those of the 15 SPI group. The addition of methionine to the 15 CLPC diet significantly increased the serum HDL-cholesterol and decreased the atherogenic index observed in rats fed the 15 CLPC diet, suggesting that the addition of methionine to CLPC is more effective in strengthening the preventive effect of CLPC for atherosclerosis. The decrease in serum total cholesterol and increase in HDL-cholesterol by supplementing methionine in the CLPC diet were similar to the results observed when 0.3% methionine was added to a 10% casein diet (30).

The liver total cholesterol and phospholipid concentrations decreased in both
Table 4. Effects of cabbage leaf protein concentrate on the growth, food intake, liver weight, serum and liver lipid concentrations and fecal excretion of steroids in rats fed a cholesterol-enriched diet (Experiment 1).

| Diet                        | 15 Cas  | 15 CLPC | 15 CLPC+Met | 15 SPI |
|-----------------------------|---------|---------|-------------|--------|
| Initial body weight (g)     | 65 ± 3a | 68 ± 1a | 68 ± 1a     | 68 ± 1a|
| Food intake (g/14 days)     | 149 ± 4b| 149 ± 5b| 163 ± 1a    | 142 ± 4b|
| Body weight gain (g/14 days)| 47 ± 3b | 30 ± 5c | 58 ± 1a     | 26 ± 2c |
| Feed efficiency<sup>1</sup> | 0.32 ± 0.01<sup>c</sup> | 0.20 ± 0.01<sup>c</sup> | 0.36 ± 0.01<sup>a</sup> | 0.18 ± 0.01<sup>c</sup> |
| Liver weight (% of body weight) | 5.2 ± 0.1<sup>a</sup> | 4.9 ± 0.1<sup>bc</sup> | 5.0 ± 0.1<sup>ab</sup> | 4.6 ± 0.1<sup>c</sup> |

Serum lipids (mmol/L)

|                        | 15 Cas  | 15 CLPC | 15 CLPC+Met | 15 SPI |
|------------------------|---------|---------|-------------|--------|
| Total cholesterol      | 6.77 ± 0.28<sup>a</sup> | 4.30 ± 0.28<sup>b</sup> | 3.42 ± 0.41<sup>ab</sup> | 3.39 ± 0.26<sup>b</sup> |
| HDL-cholesterol        | 0.82 ± 0.05<sup>ab</sup> | 0.65 ± 0.06<sup>b</sup> | 0.93 ± 0.04<sup>a</sup> | 0.80 ± 0.07<sup>ab</sup> |
| Free-cholesterol       | 0.90 ± 0.01<sup>a</sup> | 0.60 ± 0.04<sup>b</sup> | 0.54 ± 0.06<sup>b</sup> | 0.52 ± 0.03<sup>b</sup> |
| Triacylglycerol        | 0.64 ± 0.06<sup>c</sup> | 0.44 ± 0.02<sup>b</sup> | 0.48 ± 0.08<sup>ab</sup> | 0.38 ± 0.04<sup>b</sup> |
| Phospholipid           | 0.67 ± 0.01<sup>a</sup> | 0.54 ± 0.02<sup>b</sup> | 0.60 ± 0.04<sup>ab</sup> | 0.56 ± 0.03<sup>b</sup> |
| Atherogenic index<sup>2</sup> | 7.3 ± 0.4<sup>a</sup> | 5.7 ± 0.6<sup>b</sup> | 2.7 ± 0.4<sup>c</sup> | 3.3 ± 0.3<sup>c</sup> |

Liver lipids (μmol/g of liver)

|                        | 15 Cas  | 15 CLPC | 15 CLPC+Met | 15 SPI |
|------------------------|---------|---------|-------------|--------|
| Total cholesterol      | 113 ± 5<sup>a</sup> | 81.2 ± 2.7<sup>b</sup> | 80.6 ± 4.8<sup>b</sup> | 83.0 ± 4.0<sup>b</sup> |
| Triacylglycerol        | 32.6 ± 3.0<sup>a</sup> | 28.7 ± 2.5<sup>a</sup> | 26.7 ± 3.2<sup>a</sup> | 26.3 ± 3.5<sup>a</sup> |
| Phospholipid           | 29.9 ± 2.1<sup>b</sup> | 23.7 ± 1.0<sup>b</sup> | 20.7 ± 1.0<sup>b</sup> | 21.6 ± 1.2<sup>b</sup> |

Fecal steroids (μmol/4 days)

|                        | 15 Cas  | 15 CLPC | 15 CLPC+Met | 15 SPI |
|------------------------|---------|---------|-------------|--------|
| Neutral steroids<sup>3</sup> | 213 ± 25<sup>a</sup> | 236 ± 21<sup>a</sup> | 241 ± 15<sup>a</sup> | 268 ± 10<sup>a</sup> |
| Bile acids             | 78.7 ± 7.4<sup>b</sup> | 92.5 ± 3.1<sup>ab</sup> | 105 ± 5<sup>a</sup> | 106 ± 3<sup>a</sup> |

Values are M±SE of 5 rats per group. Values within the same row and not sharing a common superscript letter are significantly different at p<0.05. 15 Cas, 15% casein diet; 15 CLPC, 15% cabbage leaf protein concentrate diet; 15 CLPC+Met, 15 CLPC diet with added methionine; 15 SPI, 15% soybean protein isolate diet. 1 Body weight gain (g)/food intake (g). 2 (Total cholesterol−HDL-cholesterol)/HDL-cholesterol. 3 Cholesterol+coprostanol.

The 15 CLPC and 15 SPI groups as compared to the 15 Cas group, indicating that the 15 CLPC has almost the same hypolipidemic effects as those of SPI. As the amounts of fecal neutral steroids and bile acids in the 15 CLPC group were not significantly different from those of the control group, lower serum and liver cholesterol concentrations in the CLPC-fed rats may, in part, be due to the influence of CLPC on the endogenous cholesterol metabolism. However, the precise mechanism for the cholesterol-lowering effect of CLPC remains to be clarified.

Food intake, body weight gain and serum and liver lipid concentrations in rats fed the cholesterol-free diets containing casein, CLPC and CLPC with methionine are shown in Table 5. Although the food intake among the three groups was equal, body weight gain in the 20 CLPC group was significantly lower than that of the control group (20 Cas group). Supplementation of methionine to the diet fed to the 20 CLPC group raised the body weight gain to the level of the control group. Serum total cholesterol, HDL-cholesterol and phospholipid concentrations were
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Table 5. Effects of cabbage leaf protein concentrate on the growth, food intake, liver weight, serum and liver lipid concentrations, and fecal excretion of lipids in rats fed a cholesterol-free diet (Experiment 2).

| Diet                          | Cas       | CLPC      | CLPC+Met  |
|-------------------------------|-----------|-----------|-----------|
| Initial body weight (g)       | 72 ± 1a   | 72 ± 2a   | 71 ± 2a   |
| Food intake (g/14 days)       | 165 ± 1a  | 165 ± 1a  | 163 ± 2a  |
| Body weight gain (g/14 days)  | 50 ± 1a   | 42 ± 2b   | 50 ± 2a   |
| Liver weight (% of body weight)| 4.4 ± 0.1a| 4.2 ± 0.1a| 4.2 ± 0.2a|

Serum lipids (mmol/L)

|                      | Cas       | CLPC      | CLPC+Met  |
|----------------------|-----------|-----------|-----------|
| Total cholesterol    | 1.75 ± 0.08a| 1.79 ± 0.08a| 1.76 ± 0.09a|
| HDL-cholesterol      | 1.23 ± 0.07a| 1.28 ± 0.07a| 1.25 ± 0.05a|
| Triacylglycerol       | 0.63 ± 0.10a| 0.53 ± 0.06ab| 0.32 ± 0.05b|
| Phospholipid         | 1.79 ± 0.11a| 1.76 ± 0.07a| 1.62 ± 0.04a|

Liver lipids (μmol/g of liver)

|                      | Cas       | CLPC      | CLPC+Met  |
|----------------------|-----------|-----------|-----------|
| Total cholesterol    | 5.21 ± 0.08a| 4.63 ± 0.12b| 5.32 ± 0.05a|
| Triacylglycerol       | 10.8 ± 1.6a | 6.03 ± 0.49b| 5.32 ± 0.39b|
| Phospholipid         | 17.6 ± 0.7a | 15.2 ± 0.1a | 16.3 ± 0.1a |

Values are M±SE of 5 to 6 rats per group. Values within the same row and not sharing a common superscript letter are significantly different at p < 0.05.

not different among the three groups. Serum triacylglycerol concentration tended to decrease in the 20 CLPC group as compared to that of the control group, and tended to decrease further when methionine was added to the 20 CLPC diet. Since there was a report describing that the serum triacylglycerol concentration of SPI-fed rats was not affected by adding methionine to the SPI diet (15), this phenomenon remains to be further clarified.

The liver triacylglycerol concentrations in the 20 CLPC and 20 CLPC+Met groups were significantly lower than that of the control group. The decrease of triacylglycerol in the 20 CLPC group may be due to a decrease in the fatty acid synthesis in the CLPC-fed rats, because it has been reported that SPI, which is a plant protein like CLPC, lowers the liver triacylglycerol level through the inhibition of fatty acid synthesis in rats fed cholesterol-free diets (14, 30, 31). Since Iritani et al. suggested that lower serum and liver triacylglycerol levels are related to the quality of dietary protein (31, 32), the lower triacylglycerol concentration of the CLPC-fed rats as compared to that of casein-fed rats may be attributed to the characteristics of CLPC, such as differences in amino acid composition from that of casein or due to unknown compounds included in the CLPC.

The liver total cholesterol concentration was significantly lower in the 20 CLPC group than in the control group. The addition of methionine to the 20 CLPC diet elevated the liver cholesterol concentration to the level of the control group, indicating that improvement of nutritional condition by supplementing methionine to the 20 CLPC diet did not accompany the cholesterol-lowering effect, which was observed in the case of the cholesterol-enriched diet.
The serum concentrations of glycine, threonine and serine, in which the latter two amino acids may be produced from glycine in vivo, were significantly higher in the 20 CLPC group than in the control group (Table 3). Although it is generally pointed out that an increase in the serum glycine concentration accompanies a lower serum cholesterol concentration (27), the lowering of serum cholesterol was not observed in the 20 CLPC-fed rats that showed a higher serum glycine concentration. The relationship between serum glycine concentration and serum cholesterol concentration may need to be further examined. As the serum concentrations of threonine, serine and taurine are known to be influenced by supplementing methionine to a diet (33–35), decreases in the serum concentrations of threonine and serine, and increase in the serum taurine concentration in the 20 CLPC+Met group, as compared with the amino acids concentrations in the 20 CLPC group, may be attributed to the methionine added to the 20 CLPC diet.

In this study, it was demonstrated that CLPC activities include lowering serum and liver cholesterol as well as triacylglycerol in rats fed a cholesterol-enriched diet, and lowering both liver triacylglycerol and total cholesterol concentrations in rats fed a cholesterol-free diet.

REFERENCES

1) Huff MW, Carrol KK. 1980. Effects of dietary proteins and amino acid mixtures on plasma cholesterol levels in rabbits. J Nutr 110: 1676–1685.
2) Mokady S, Lienier IE. 1982. Effects of plant proteins on cholesterol metabolism in growing rats fed atherogenic diets. Ann Nutr Metab 26: 138–144.
3) Sautier C, Dieng K, Flamant C, Doucet C, Suquet JP, Lemonnier D. 1983. Effects of whey proteins, casein, soya-bean and sunflower proteins on the serum, tissue and faecal steroids in rats. Br J Nutr 49: 313–319.
4) Barth CA, Pfeuffer M, Hahn G. 1984. Influence of dietary casein or soy protein on serum lipids and lipoproteins of monkeys (Macaca fascicularis). Ann Nutr Metab 28: 137–143.
5) Sugano M, Ishiwaki N, Nakasima K. 1984. Dietary protein-dependent modification of serum cholesterol level in rats. Ann Nutr Metab 28: 192–199.
6) Sautier C, Flamant C, Doucet C, Suquet JP. 1986. Effects of eight dietary proteins and their amino acid on serum, hepatic and fecal steroids in rats. Nutr Rep Int 34: 1051–1061.
7) Kuyvenhoven MW, West CE, Hakkert BC, Mensink CMM, Beynen AC. 1987. Digestibility of dietary proteins and serum cholesterol in rats. Nutr Rep Int 36: 537–549.
8) Schrijver RD. 1990. Cholesterol metabolism in mature and immature rats fed animal and plant protein. J Nutr 120: 1624–1632.
9) Cho YS, Horigome T, Sakaguchi E, Uchida S. 1988. Effects of feeding of leaf protein on serum cholesterol concentration in rats. Nippon Eiyo Shokuryo Gakkaishi (J Jpn Soc Nutr Food Sci) 41: 121–132.
10) Satoh T, Gotoh M, Igarashi K. 1993. Effects of protein isolates from radish and spinach leaves on serum lipids levels in rats. J Nutr Sci Vitaminol 39: 627–633.
Hypolipidemic Activity of Cabbage Protein

11) Satoh A, Hitomi M, Igarashi K. 1995. Effects of spinach leaf protein concentrate on the serum cholesterol and amino acid concentrations in rats fed a cholesterol-free diet. *J Nutr Sci Vitaminol* 41: 563–573.

12) Sugiyama K, Akai H, Muramatsu K. 1986. Effects of methionine and related compounds on plasma cholesterol level in rats fed a high cholesterol diet. *J Nutr Sci Vitaminol* 32: 537–549.

13) Yagasaki K, Aoki T, Machida M, Funabiki R. 1986. Effects of dietary methionine and cystine on endogenous hypercholesterolemia in hypothyroid rats. *Agric Biol Chem* 50: 2785–2789.

14) Ide T, Murata M, Sunada Y. 1992. Soybean protein-dependent changes in triacylglycerol synthesis and concentrations of diacylglycerol in the liver microsomes of fasted-refed rats. *Ann Nutr Metab* 36: 87–96.

15) Tanaka K, Sugano M. 1989. Effects of addition of sulfur-containing amino acids and glycine to soybean protein and casein on serum cholesterol levels of rats. *J Nutr Sci Vitaminol* 35: 323–332.

16) Oda H, Fukui H, Hitomi Y, Yosida A. 1991. Alteration of serum lipoprotein metabolism by polychlorinated biphenyls and methionine in rats fed a soybean protein diet. *J Nutr* 121: 925–933.

17) Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497–509.

18) Allain CC, Poon LC, Chan CSG, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470–475.

19) Spayd RW, Bruschi B, Burdick BA, Dappen GM, Elkenberry JN, Eaders TW, Figueras J, Goodhue CT, LaRossa DD, Nelson RW, Rand RN, Wu T-W. 1978. Multilayer film elements for clinical analysis: Applications to representative chemical determinations. *Clin Chem* 24: 1343–1350.

20) Takayama M, Itoh S, Nagasaki T, Tanimizu I. 1977. A new enzymatic method for determination of serum choline-containing phospholipids. *Clin Chim Acta* 79: 93–98.

21) Burstein M, Scholnick HR, Morfin R. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyaniions. *J Lipid Res* 11: 583–595.

22) Stähler F, Gruber W, Stinshoff K, Röschlau P. 1977. Eine praxisgerechte enzymatische cholesterin-bestimmung. *Med Lab* 30: 29–37.

23) Sautier C, Dieng K, Flamant C, Doucet C, Suquet JP, Lemonnier D. 1983. Effects of whey protein, casein, soya-bean and sunflower proteins on the serum, tissue and fecal steroids in rats. *Br J Nutr* 49: 313–319.

24) Igarashi K, Ohnuma M. 1995. Effects of isorhamnetin, rhamnetin, and quercetin on the concentrations of cholesterol and lipoperoxide in the serum and liver and on the blood and liver antioxidative enzyme activities of rats. *Biosci Biotechn Biochem* 59: 595–601.

25) Mashige F, Tanaka N, Maki A, Kamei S, Yamanaka M. 1981. Direct spectrophotometry of total bile acids in serum. *Clin Chem* 27: 1352–1356.

26) Igarashi K, Kamiyama H, Tezuka M, Yasui T. 1985. Changes in amino acid composition during sun-curing of leaves and stems of red clover (*Trifolium pratense* L.). *Jpn J Zootech Sci* 56: 566–570 (in Japanese).

27) Horigome T, Cho Y-S. 1992. Dietary casein and soybean protein affect the concentra-
tions of serum cholesterol, triglyceride and free amino acids in rats. J Nutr 122: 2273–2282.

28) Kritchevsky D, Tepper SA, Czarneski SK, Klurfeld DM. 1982. Atherogenecity of animal and vegetable proteins. Influence of the lysine to arginine ratio. Atherosclerosis 41: 429–431.

29) Sugano M, Ishiwaki N, Nagata Y, Imaizumi K. 1982. Effects of arginine and lysine addition to casein and soya-bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. Br J Nutr 48: 211–221.

30) Seidel JC, Harper AE. 1960. Diet and cholesterolemia: V. Effects of sulfur-containing amino acids and protein. J Lipid Res 1: 474–481.

31) Iritani N, Nagashima K, Fukuda H, Katsurada A, Tanaka T. 1988. Effects of dietary proteins on lipogenic enzyme in rat liver. J Nutr 116: 120–197.

32) Iritani N, Suga A, Fukuda H, Katsurada A, Tanaka T. 1988. Effects of dietary casein and soybean protein on triglyceride turnover in rat liver. J Nutr Sci Vitaminol 34: 309–315.

33) Mizuno T, Yamada K. 1994. Effects of dietary methionine on the levels of plasma cholesterol and free amino acids in rat. Nippon Kaseigaku Kaishi (J Home Economics Jpn) 45: 375–383 (in Japanese).

34) Van der Meer R, Beynen AC. 1987. Species-dependent responsiveness of serum cholesterol to dietary proteins. J Am Oil Chem Soc 64: 1172–1177.

35) Sugiyama K, Kushima Y, Muramatsu K. 1984. Effects of methionine, cystine and taurine on plasma level in rats fed a high cholesterol. Agric Biol Chem 48: 2897–2899.

36) Reeves PG, Nielsen FH, Fahey GC. 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 123: 1939–1951.