Effect of Feeding Peptic Digest of Soy Protein Isolate on Rat Serum Cholesterol

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Summary Growing rats were fed ad libitum soy protein isolate (SPI) or its peptic (SPI-P) or tryptic digest (SPI-T) for a month and their sera were examined for cholesterol and triglyceride levels and enzyme activities such as cholinesterase, glutamate-pyruvate transaminase (GPT) and alkaline phosphatase. The rats fed SPI-P or SPI-T were inferior in growth to those fed SPI. Similarly, the serum glyceride level was lower in the SPI-P and SPI-T groups than in the SPI group. On the other hand, a significant difference was found in the serum cholesterol level between the SPI-P and SPI-T groups but not between the SPI and SPI-T groups. A similar tendency was observed for serum GPT and alkaline phosphatase activities, although there were no significant differences among dietary groups in small intestinal enzyme activities. As for the atherogenic index being a risk factor inducing atherosclerosis, the order of its value was SPI-P < SPI < SPI-T.

Key Words soy protein isolate, peptic digest, tryptic digest, serum cholesterol, serum triglyceride, rat growth

Hypercholesterolemia is generally believed to be one of the atherosclerosis-inducing risk factors (1). In recent years there has been considerable interest with vegetable proteins relative to animal ones, which may have anti-hypercholesterolemic effects (2). Although there are a large number of reports in this connection (3–6), the mechanism by which feeding vegetable proteins, especially soy protein isolate, lowers the serum cholesterol level has not necessarily been elucidated in detail. Huff et al. (7) have previously described how the serum cholesterol level in rabbits receiving low fat, cholesterol-free diets is elevated to some extent by...
the substitution of SPI for its corresponding amino acid mixture but not by the substitution of the protein for its enzymatic hydrolysate. This and another observations (8) suggest that post-digestion residual peptides rather than amino acid composition may play a crucial role in lowering the serum cholesterol level, because of incomplete digestion by protease used to prepare the hydrolysate. The present investigation was undertaken to re-examine the effect of feeding peptic or tryptic digest of SPI on the serum cholesterol level in rats.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats weighing 50 to 60 g were housed in an environmentally controlled room (temp. 22 ± 1°C; humidity 60%) with a 12 h light/dark cycle (lighted at 9:00–21:00), where they were fed ad libitum cholesterol-free diets containing casein and SPI and its peptic and tryptic digests as protein sources, respectively, for a month. The basal composition of these diets is shown in Table 1. The peptic and tryptic digests of SPI were prepared from Fuji-pro R (Fuji Oil Co.) as follows: For example, 1.5 kg of SPI was suspended in 10 liters of distilled water, adjusted to pH 1.6 with HCl, and incubated at 37°C together with one-hundred amounts of porcine pepsin (Sigma Co.). Pepsin digestion reached a plateau in about 2 h. Then, the reaction was terminated by adjustment to pH 4.0, followed by lyophilization. In a similar scale, SPI was treated at pH 8.0 with bovine trypsin (Sigma Co.), followed by lyophilization. The bulk of these digests were composed of peptides with molecular weights of 1,000–10,000. Dried SPI-digest powders thus obtained were directly used as protein sources in the experimental diets.

Cholesterol and triglyceride determinations. All rats previously fasted on drinking water overnight were sacrificed by decapitation one month after the start of the experiment, from which blood was drawn and serum was separated by

| Ingredients            | % |
|------------------------|---|
| Protein source¹        | 20|
| α-Corn starch          | 45|
| Sucrose                | 15|
| Oil mixture²           | 7 |
| Mineral mixture³       | 4 |
| Vitamin mixture³       | 1 |
| Cellulose powder       | 8 |

¹ Each of casein, SPI and its digestive products was added to make up 20% of the diet.  
² Soybean oil/cod liver oil (4:1).  
³ Vitamin mix (in mg/100 g diet): thiamin chloride (0.51), riboflavin (0.51), nicotinic acid (2.5), calcium pantothenate (2.0), vitamin K³ (0.053), biotin (0.0085), folic acid (0.017), vitamin B₁₂ (0.0015), inositol (10), ascorbic acid (5.0), choline chloride (150), and lactose (829.4).
EFFECT OF PEPTIC DIGEST OF SPI

centrifugation at 1,000 × g for 15 min. The serum was analyzed for total- and HDL-cholesterol and triglyceride contents by use of commercially available reagents kits (Kokusai Shiyaku Co.).

Enzyme assays. The small intestine was removed immediately after exsanguination and divided into 4 equal parts. The second segment from pyrolys was homogenized in a Teflon-glass homogenizer with 3 volumes of 0.9% saline and centrifuged at 6,000 × g for 15 min. An aqueous solution containing 10% sodium deoxycholate was added to the supernatant fluid in a volume ratio of 1:20. After being allowed to stand overnight at 4°C, the mixture was again centrifuged at 10,000 × g for 10 min, and the supernatant was used for the enzyme assay. The activities of γ-glutamyltransferase, leucine aminopeptidase and alkaline phosphatase were measured with synthetic substrates, i.e. L-γ-glutamyl-p-nitroanilide, L-leucine-p-nitroanilide and p-nitrophenyl phosphate, respectively, as previously described (9–11). One unit of enzyme was defined as μmol of p-nitroaniline or p-nitrophenol released per min at 37°C under the respective routine assay systems. Similarly the activities of cholinesterase (12), glutamate-pyruvate transaminase (13) and alkaline phosphatase in the serum were determined with their corresponding reagent kits (Kokusai Shiyaku Co.) and expressed in terms of international units per liter.

Statistic analysis. Data were evaluated by analysis of variance; when F-tests were significant (p<0.05), means were compared using the Least Significance Difference test to assess which pair of means were significantly different at p<0.05 (14).

RESULTS AND DISCUSSION

Rat growth, feed intake and tissue weight

Table 2 shows body weight gains, daily feed intakes and tissue weights (liver, small intestine and caecum) of rats fed the experimental diets containing casein and SPI and its digestive products for a month. The rats fed either SPI or its digestive product were inferior in growth to those fed casein. The degree of growth

Table 2. Effect of feeding SPI and its digestive products on rat growth, feed intake and tissue weight.

| Dietary groups | Body weight gain (g/month) | Feed intake (g/day) | Liver (g/100 g BW) | Small intestine (g/100 g BW) | Caecum (g/100 g BW) |
|----------------|---------------------------|---------------------|-------------------|-----------------------------|---------------------|
| Casein         | 167.9 ± 3.1a              | 15.4 ± 0.2a         | 4.4 ± 0.1a        | 4.1 ± 0.1a                  | 1.2 ± 0.1a          |
| SPI            | 134.7 ± 3.5b              | 15.0 ± 0.2b         | 4.3 ± 0.1a        | 4.0 ± 0.1a                  | 1.1 ± 0.1a          |
| SPI-P          | 115.9 ± 3.3c              | 14.0 ± 0.3b         | 3.7 ± 0.1b        | 3.7 ± 0.2a                  | 1.1 ± 0.1a          |
| SPI-T          | 113.2 ± 3.8b              | 13.5 ± 0.4b         | 4.1 ± 0.1ab       | 3.8 ± 0.1a                  | 1.0 ± 0.1a          |

Means ± SE for 8 animals without a common superscript in the same column differ significantly at p<0.05.

Vol. 32, No. 4, 1986
Table 3. Effect of feeding SPI and its digestive products on serum cholesterol and triglyceride levels.

| Dietary groups | Serum level (mg/100ml) |  |
|----------------|------------------------|---|
|                | Total CHOL         | HDL-CHOL | Triglyceride |
| Casein         | 94.6 ± 2.5^a        | 60.7 ± 3.1^a | 240 ± 14^a   |
| SPI            | 85.4 ± 1.2^b        | 58.3 ± 3.3^a | 224 ± 15^a   |
| SPI-P          | 68.5 ± 3.0^c        | 53.7 ± 3.7^a | 130 ± 12^b   |
| SPI-T          | 87.6 ± 2.0^ab       | 57.2 ± 1.6^a | 133 ± 13^b   |

^a,b,c The same as footnote in Table 2.

nearly corresponded to the amount of feed intake during the feeding period. In other words, feeding on pepsin- or trypsin-treated SPI led to a decrease in the feed intake relative to casein feeding. This decreased feed intake may have been caused by gastric inflation due to high osmotic pressure of the proteolytic product or by formation of bitter-tasting peptides. It is worth noting that a significant difference has been found between SPI-P and other dietary groups in liver weight (g per 100 g body weight) but not in small intestine and caecum weights, in relation to serum cholesterol level hereinafter mentioned.

**Cholesterol and triglyceride levels in rat serum**

Table 3 compares serum total- and HDL-cholesterol and triglyceride levels in rats a month after feeding casein-, SPI- and its peptic or tryptic digest-based diets. The serum cholesterol level was not so high in rats fed SPI and its digests as was the case with rats fed casein. With respect to SPI and its digestive products, the peptic digest was most effective in lowering the serum cholesterol level and the tryptic one following it. A similar tendency was observed with the HDL-cholesterol level, although not significantly. Using these values, the atherogenic indices

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\text{Atherogenic index} = \frac{\text{Total CHOL} - \text{HDL CHOL}}{\text{HDL CHOL}}
\]

were obtained as 0.56, 0.46, 0.28 and 0.53 for casein, SPI, SPI-P and SPI-T groups, respectively. Noteworthily, the index in the SPI-P group was half that of the casein group and less than both the SPI and SPI-T groups. Such a significant difference between the groups of rats given intact and post-digestion proteins was also reflected in the serum triglyceride level. Feeding SPI-P was seemingly favorable for hypocholesterolemia unless appetite depression was considered.

**Enzyme activities in serum and small intestine**

When liver receives functional injuries, certain enzymes are known to leak out abnormally from hepatocytes. sGPT, cholinesterase and alkaline phosphatase are

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Table 4. Effect of feeding SPI and its digestive products on enzyme activities in serum.

| Dietary groups | Enzyme activity (IU/liter) |      |          |
|----------------|---------------------------|------|----------|
|                | Cholinesterase            | Glutamate-pyruvate transaminase | Alkaline phosphatase |
| Casein         | 91.8 ± 5.2*a              | 34.4 ± 2.3*a                    | 72.0 ± 5.2*a         |
| SPI            | 83.8 ± 3.2*a              | 35.2 ± 1.9*a                    | 63.6 ± 5.0*a         |
| SPI-P          | 91.7 ± 3.9*a              | 26.2 ± 1.2*b                    | 39.6 ± 3.1*b         |
| SPI-T          | 86.6 ± 2.5*a              | 35.5 ± 1.1*a                    | 73.8 ± 9.7*a         |

*a,b,c The same as footnote in Table 2.

Table 5. Effect of feeding SPI and its digestive products on enzyme activities in the small intestine.

| Dietary groups | Enzyme activity (units/mg protein) |      |          |
|----------------|-----------------------------------|------|----------|
|                | γ-Glutamyltransferase              | Leucine aminopeptidase | Alkaline phosphatase |
|                | (× 10⁻²)                          | (× 10⁻²)               |                      |
| Casein         | 2.05 ± 0.31*a                     | 3.07 ± 0.34*a          | 1.93 ± 0.26*a        |
| SPI            | 1.83 ± 0.31*a                     | 3.63 ± 0.29*a          | 1.94 ± 0.28*a        |
| SPI-P          | 2.28 ± 0.18*a                     | 3.22 ± 0.27*a          | 2.49 ± 0.46*a        |
| SPI-T          | 2.27 ± 0.45*a                     | 3.57 ± 0.45*a          | 2.10 ± 0.41*a        |

*a,b,c The same as footnote in Table 2.

involved in such enzymes and their activity levels in serum are well adapted for the diagnosis of liver functions. The results of activity measurements are summarized in Table 4. There were no significant differences in cholinesterase activity among 4 dietary groups but there were in sGPT and alkaline phosphatase activities between SPI-P and other dietary groups. Decreases in the serum levels of these enzymes are attributable to a lowering of protein synthesis within the hepatocyte. The small intestine as well as the liver plays an important role in apolipoprotein synthesis closely related to cholesterol metabolism. The activities of a few enzymes in small intestinal mucosa were measured to roughly look at how feeding with various diets affects intestinal function. Apparently, as shown in Table 5, differences in specific activity among 4 dietary groups could not be regarded as significant. It therefore seems reasonable to assume that the function of the small intestine is very little, if any, influenced by feeding pepsin- or trypsin-treated SPI.

In this experiment, all rats were given free access to drinking water and dietary supplement throughout the month of feeding, so that a significant difference in growth and serum cholesterol was not only observed between casein and SPI groups
but also with SPI and SPI-P groups. On the other hand, the rats in the SPI-P group were almost equal to those of the SPI-T group in growth; nevertheless, the former were distinct from the latter in serum cholesterol. This may be explained in terms of differences in quality and quantity of residual peptides following luminal digestion (15). It thus seems likely that the effect of SPI-P feeding on serum cholesterol may have been caused by factors affecting cholesterol metabolism in the small intestine and/or liver, although there is no information indicating what kinds of peptides are responsible. A recent report (16) describes that the serum cholesterol level tends to be more lowered by restricted feeding than by ad libitum feeding, when casein or SPI is administered to growing rats. If this is valid, then in our experiment, the difference in serum cholesterol between the SPI and SPI-P groups may be related to that in body weight gain. The small size of the liver in the SPI-P group may partly account for the malfunction in cholesterol metabolism there. In any case, these problems remain further to be resolved.

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