Effect of Methionine Supplementation of a Soy Protein Isolate on Short-Term Nitrogen Balance in Young Women

Kayoko KANEKO, Kyoko NISHIDA, Junko YATSUDA, and Goro KOIKE

Kagawa Nutrition College Sakado, Saitama 350-02, Japan
(Received October 15, 1985)

Summary The effect of L-methionine supplementation on the utilization of a soy protein isolate (SPI) was evaluated by short-term nitrogen balance studies in young women. Thirteen female students were given SPI in an initial period and SPI supplemented with 1% methionine in a second period immediately after menstruation as the sole source of protein. After one day on protein-free diet, each subject received conventional low-protein diet for three days, and then low protein, semisynthetic diet containing 0.5 g/kg/day (seven subjects) or 0.3 g/kg/day (six subjects) of SPI or SPI supplemented with methionine for seven days. The energy intake was approximately of a maintenance level of 36.5 ± 3.8 kcal/kg/day.

The mean N balances of the subjects at an intake level of 0.5 g/kg/day in the SPI period and methionine supplemented period were -6.2 ± 12.6 mg N/kg and -9.8 ± 9.8 mg N/kg, respectively, while their N balances at an intake level of 0.3 g/kg/day were -17.8 ± 7.2 mg N/kg in the SPI period and -15.5 ± 3.0 mg N/kg in the methionine supplemented period. There was no significant difference between the values in the SPI and methionine supplemented periods at both levels of protein intake. Blood analyses were carried out before and after the SPI period and after the period of methionine supplementation. The urinary creatinine and urea excretions during these periods were not markedly affected.

Key Words soy protein isolate, methionine, limiting amino acid, nitrogen balance, protein utilization, human adults

Studies in growing (1–5) and adult rats (6) have shown that soy protein isolate (SPI) is of a lower quality than protein from animal sources, but that the quality can be improved by adding methionine or by a combination with rice or egg. However,
124 K. KANEKO, K. NISHIDA, J. YATSUDA, and G. KOIKE

some controversy exists over the nutritive value of SPI in human adults. Some investigators have reported lower efficiencies of SPI than with animal proteins (7–11) and improvements of the quality of soy protein by adding methionine (8–10) or by combination with beef (9,12) or fish (11). We previously observed a slightly lower efficiency of SPI than that of egg protein and a high nutritive value of SPI in combination with rice using the slope ratio method in female subjects (13). However, the deviations and the slopes or Y intercepts of the regression lines relating nitrogen balance to nitrogen intake for SPI alone and in combination with rice and egg protein were not significantly different. Moreover, Scrimshaw and Young (12) did not observe any difference in the protein qualities of SPI and milk protein, and Young et al. (14) recently reported that the N requirement of SPI for nitrogen equilibrium did not differ significantly from that of egg protein in male subjects and that supplementation of methionine had no effect on the nitrogen balance when the nitrogen intake level was 128 mg N/kg/day, but did so at a level of 82 mg N/kg/day. Thus, further investigations are required on the protein quality of SPI and its limiting amino acids in humans.

In the present work, we measured short-term nitrogen balances of subjects fed SPI and SPI supplemented with 1% L-methionine and compared the nitrogen utilization of SPI with or without methionine supplementation.

EXPERIMENTAL

Thirteen female students aged 18 to 22 years participated in two series of experiments using SPI and SPI supplemented with L-methionine. Table 1 summarizes the characteristics of the subjects. During the experiment they lived in a metabolic unit and continued their usual activities.

After one day on protein-free diet, the subjects received a conventional low-protein diet containing rice, egg, soy curd and vegetables for three days. They were then given a semi-purified, experimental diet containing soy protein isolate (Fujipro R; Fuji Oil Co., Osaka, Japan) as a sole source of protein for seven days. The

| Subj. No. | Age (yr) | Height (cm) | Weight (kg) | BMR (kcal/kg) | LBM (kg) | Fat (%) | Energy intake (kcal/kg) |
|----------|----------|-------------|-------------|--------------|----------|---------|------------------------|
| 75       | 7        | 20 ± 1.4    | 156 ± 4.7   | 50.1 ± 0.5   | 19.6 ± 3.2 | 36.1 ± 1.0 | 24.4 ± 2.8 | 37.3 ± 2.8 |
| 45       | 6        | 21 ± 0.4    | 154 ± 6.5   | 56.7 ± 0.5   | 24.2 ± 5.5 | 38.5 ± 4.4 | 31.9 ± 2.2 | 35.1 ± 4.5 |

*Mean ± SD. *Mean weight on day 22 of the experiment. *Measured during the experimental period. *Difference between body weight and body fat weight. *Calculated from the body density measured during the experimental period using the equation proposed by Brožek et al. (21).

J. Nutr. Sci. Vitaminol.
METHIONINE SUPPLEMENTATION OF SPI

nitrogen intake was about 45 mg/kg in six subjects and 75 mg/kg in the other seven subjects throughout the periods on conventional and experimental diets. The composition of the experimental diet corresponded to that described elsewhere (13), except that the mineral mixture per day used daily in the present experiment consisted of CaPO₄·2H₂O 2 g, CaCO₃ 0.9 g, KH₂PO₄ 2 g, KHCO₃ 3.5 g, MgO 0.6 g, FeSO₄·7H₂O 60 mg, MnSO₄·4H₂O 3 mg, CuSO₄·5H₂O 8 mg, KI 0.2 mg, ZnCl₂ 5 mg, Na₂MoO₄·2H₂O 0.2 mg, Cr₂(SO₄)₃·15H₂O 1 mg, AlK(SO₄)₂·12H₂O 30 mg, and NaSeO₃ 0.008 mg. The powdery product of SPI was made into SPI cookies and gel with cornstarch, sugar, shortening and sodium chloride.

The approximate maintenance energy intakes of individuals were determined by detailed inquiries into food consumption before the experiment. When the body weight of a subject clearly decreased or increased during the early stage of the experiment, a certain amount of sucrose was added to or removed from the diet.

Immediately after the period on SPI diet, subjects were given a habitual diet containing a normal level of protein. Then, after the menstrual period, each subject was started on the next experimental diet of SPI supplemented with 1% L-methionine, because in female subjects urinary nitrogen excretion has been reported to vary with the menstrual cycle with constant intakes of energy and protein (15). For determination of the menstrual cycle, each subject measured her basal body temperature for more than three months before and during the experimental period. In the methionine supplemented period, subjects received one-third of their daily intakes of SPI as cookies or gel and that of crystalline methionine at three meals.

Throughout the experiment subjects were weighed daily before breakfast after voiding urine. Urine was collected in 24 h-samples, and feces in the period on test diet were pooled. The total nitrogen contents of SPI, urine and feces were analyzed by the semimicro-Kjeldahl method. The urinary excretions of creatinine (16), urea and ammonia (17) were determined. Blood samples were taken before and after the period on SPI diet and after the methionine supplemented period. Hemoglobin, hematocrit, serum protein (18), serum albumin (19), GOT and GPT activities (20) and the urea concentration (17) were analyzed. The basal metabolic rate was measured by indirect calorimetry on one day during the experimental period. The body fat content was calculated from the body density measured hydrometrically.

RESULTS

The mean values of energy intake of all subjects were 36.5 ± 3.6 kcal/kg BW and 1.75 ± 0.31 kcal/kcal basal metabolism, respectively. The mean body weights on the first and last days of the period on SPI diet were 56.9 ± 6.9 kg and 56.2 ± 6.5 kg, respectively, while those on the first and last days of the methionine supplemented period were 57.6 ± 7.2 kg and 56.1 ± 6.8 kg. The body weights decreased slightly as an effect of the low-protein diet. Urinary creatinine excretion was constant during the experimental period in all subjects.
Table 2. Nitrogen balance, urinary urea and ammonia N and creatinine excretion of individual subjects.

| Subj. code | SPI N intake (mg/kg/day) | Fecal Nitrogen balance (%) | Urinary Urea N (%) | Ammonia N (%) | Creatinine (mg/day) | SPI + Met N intake (mg/kg/day) | Fecal Nitrogen balance (%) | Urinary Urea N (%) | Ammonia N (%) | Creatinine (mg/day) |
|------------|--------------------------|-----------------------------|-------------------|---------------|--------------------|-----------------------------|-----------------------------|-------------------|---------------|-------------------|
| 11         | 45.2 48.9 9.2 -12.9 77   | 4.6 1,290 ± 74             | 45.3 55.0 7.1 -16.7 73 | 4.6 1,290 ± 74 | 45.3 55.0 7.1 -16.7 73 |
| 12         | 45.6 60.0 14.7 -29.0 75   | 4.3 1,073 ± 37             | 44.9 49.0 8.4 -13.2 73 | 4.3 1,073 ± 37 | 44.9 49.0 8.4 -13.2 73 |
| 13         | 46.1 46.7 7.3 -7.9 76     | 3.2 892 ± 37               | 45.8 51.5 12.4 -18.1 76 | 3.2 892 ± 37 | 45.8 51.5 12.4 -18.1 76 |
| 14         | 46.5 52.6 11.1 -17.1 74   | 3.3 862 ± 70               | 46.9 46.8 11.2 -11.1 74 | 3.3 862 ± 70 | 46.9 46.8 11.2 -11.1 74 |
| 15         | 46.2 52.1 15.3 -21.2 77   | 3.7 986 ± 60               | 45.7 48.9 11.8 -15.0 76 | 3.7 986 ± 60 | 45.7 48.9 11.8 -15.0 76 |
| 16         | 45.7 54.7 9.6 -18.6 73    | 4.4 1,132 ± 158            | 47.3 55.4 10.9 -17.0 68 | 4.4 1,132 ± 158 | 47.3 55.4 10.9 -17.0 68 |
| Mean       | 45.9 52.5 11.2 -17.8 75   | 3.9 1,039                  | 46.0 51.2 10.3 -15.5 72 | 3.9 1,039 | 46.0 51.2 10.3 -15.5 72 |
| SD         | 0.5 4.6 3.2 7.2 1.3       | 0.5 160                    | 0.9 3.4 2.1 3.0 3.2 | 0.5 160 | 0.9 3.4 2.1 3.0 3.2 |

*Average for the last 4 days of the experimental period. b Percentage of the total urinary nitrogen. c Mean ± SD in the 11 days of the experimental period. d Significant difference between the values in the SPI period and methionine supplemented period (p<0.05).
The means of urinary nitrogen excretion, the fecal nitrogen, nitrogen intake and apparent nitrogen balance in the last 4 days of each experimental period are shown in Table 2. There was no significant difference in the urinary nitrogen excretions, fecal nitrogen excretions or nitrogen balances in the SPI period and methionine supplemented period. The apparent nitrogen balances in the period on SPI diet and methionine supplemented diet were $-6.2 \pm 12.6$ and $-9.8 \pm 9.8\, \text{mg N/kg}$ at an intake level of $75\, \text{mg N/kg}$. Two subjects (05 and 06) showed an improvement in nitrogen balance, but the other five subjects did not. At an intake of $45\, \text{mg N/kg}$, the apparent nitrogen balance was $-17.8 \pm 7.2\, \text{mg N/kg}$ in the SPI period and $-15.5 \pm 3.0\, \text{mg N/kg}$ in the methionine supplemented period. Thus 3 of 6 subjects showed an improved nitrogen balance at this low level of protein intake. The urinary excretions of urea and creatinine did not change significantly on addition of methionine to SPI (Table 2).

Table 3 shows the result of blood analyses. Some constituents changed slightly during the experimental period, but no marked changes were observed. The blood urea concentration increased slightly on supplementation of SPI with methionine.

Net protein utilization and digestibility were calculated based on values for the obligatory urinary and fecal nitrogen losses in Japanese women(22). The mean digestibility was calculated as $95 \pm 5.6\%$ for SPI and $97 \pm 4.3\%$ for SPI supplemented with methionine. The NPUs of SPI at intake levels of $45$ and $75\, \text{mg N/kg}$ were $54 \pm 13$ and $51 \pm 15$, respectively, while the NPUs of SPI supplemented with methionine at intake levels of $45$ and $75\, \text{mg N/kg}$ were $64 \pm 17$ and $45 \pm 13$, respectively. There was no significant difference in the values for the digestibility or NPU of SPI and SPI supplemented with methionine.
DISCUSSION

The present study confirmed the high nutritional value of isolated soy protein reported previously (11). Results showed that the digestibility and NPU of SPI were comparable with those of egg protein reported previously by us (23) and others (24).

No beneficial effect of methionine supplementation was observed, although a submaintenance nitrogen intake level was used in this study. This finding is not consistent with reports by other researchers. Korslund et al. (25) and Kies and Fox (9) reported the improvement of N balance by methionine supplementation of textured vegetable protein at an intake level of 4 g N/day, but not at an intake level of 8 g N/day. Zezulka and Calloway (10) observed that methionine supplementation of SPI improved the N balance at an intake level of 3.0 g N/day of SPI. Young et al. (14) studied the effect of methionine supplementation on SPI more extensively at two nitrogen intake levels of SPI with graded supplementary levels of methionine. They observed improvement of the N balance when 0.6 or 1.1% methionine was added to SPI but no effect when 1.6% methionine was added to 0.5 g soy protein/kg/day. These results showed that methionine had a supplementary effect on SPI only in subjects given a low-protein diet with an adequate concentration of added methionine.

In the present study, nitrogen intake was inadequate and the level of L-methionine supplementation was 1.0%. Some investigators (26) reported a high content of S-containing amino acids in SPI (Fujipro R) of 175 mg/g N compared with those reported by others, 125 mg/g N (1) or 138 mg/g N (27). On supplementation of 1.0% methionine, the content of S-containing amino acids in the diet nearly approximates the suggested pattern for adults proposed by FAO/WHO (1973) (28). However, the nitrogen balance was not always improved by this diet, and varied in different subjects. The result indicates that there are significant variations in the responses of different subjects, and that S-containing amino acids in SPI are sufficient to meet the suggested pattern (1973) for some subjects while not for others. Kishi et al. (29) and Koishi et al. (30) reported no improvement in nitrogen balance by supplementing SPI (Fujipro R) with either 1.0 or 1.5% methionine at an intake level of 90 mg N/kg in adult male subjects, while others (31) reported an improvement in 4 of 5 subjects.

We conclude from the present results and the above discussion that the isolated soy protein tested was of good quality, and that its utilization by adult women was not consistently improved by supplementation with L-methionine under the conditions of the present experiment.

We are grateful to Fuji Oil Co., Osaka, Japan, for supporting this study.

REFERENCES

1) Yamaguchi, M., Iwaya, M., and Miyazaki, M. (1980): Limiting amino acids of soy J. Nutr. Sci. Vitaminol.
METHIONINE SUPPLEMENTATION OF SPI 129

protein isolate and their supplementary effects. *Nutr. Sci. Soy Protein (Japan)*, 1, 10–15.

2) Horii, M. (1980): Nutritional efficiency of rice protein. *J. Jpn. Soc. Food Nutr.*, 33, 127–136.

3) Sakamoto, K., and Fukuzawa, H. (1973): Effect of fortification of meatless meat with amino acid (Part 1): Effect of fortification with lysine and methionine. *J. Jpn. Soc. Food Nutr.*, 26, 177–183.

4) Inoue, G., Kishi, K., and Yagi, I. (1980): Quality of soy protein isolate and the effect of sulfur amino acid supplementation on the protein utilization in growing rats. *Nutr. Sci. Soy protein (Japan)*, 1, 6–9.

5) Bressani, R. (1975): Nutritional contribution of soy protein to food systems. *J. Am. Oil Chem. Soc.*, 52, 254A–262A.

6) Yamaguchi, M., Iwaya, M., and Miyazaki, M. (1981): Limiting amino acids of soy protein isolate and their supplementary effects (Part 2). *Nutr. Sci. Soy Protein (Japan)*, 2, 77–81.

7) Young, V. R., Scrimshaw, N. S., Torun, B., and Viteri, F. (1979): Soy bean protein in human nutrition: An overview. *J. Am. Oil Chem. Soc.*, 56, 110–120.

8) Vemury, M. K. D., Kies, C., and Fox, H. M. (1976): Comparative protein values of several vegetable protein products fed at equal nitrogen levels to human adults. *J. Food Sci.*, 41, 1086–1091.

9) Kies, C., and Fox, H. M. (1971): Comparison of the protein nutritional value of TVP, methionine riched TVP and beef at two levels of intake for human adults. *J. Food Sci.*, 36, 841–845.

10) Zezulka, A. Y., and Calloway, D. H. (1976): Nitrogen retention in men fed varying levels of amino acids from soy protein with or without added l-methionine. *J. Nutr.*, 106, 212–221.

11) Wang, M.-F., Kishi, K., Takahashi, T., Komatsu, T., Ohnaka, M., and Inoue, G. (1983): Efficiency of utilization of soy protein isolate in Japanese young men. *J. Nutr. Sci. Vitaminol.*, 29, 201–216.

12) Scrimshaw, N. S., and Young, V. R. (1979): Soy protein and human nutrition, a review with new data, *in Soy Protein and Human Nutrition*, ed. by Wilcke, H. L., Hopkins, D. T., and Waggle, D. H., Academic Press, Inc., New York, pp. 121–148.

13) Kaneko, K., Inayama, T., and Koike, G. (1985): Utilization of soy protein isolate mixed with rice protein in Japanese women. *J. Nutr. Sci. Vitaminol.*, 31, 99–106.

14) Young, V. R., Puig, M., Queiroz, E., Scrimshaw, N. S., and Rand, W. M. (1984): Evaluation of the protein quality of an isolated soy protein in young men: relative nitrogen requirements and effect of methionine supplementation. *Am. J. Clin. Nutr.*, 39, 16–24.

15) Calloway, D. H., and Karzer, M. S. (1982): Menstrual cycle and protein requirements of women. *J. Nutr.*, 112, 356–366.

16) Koishi, H. (1962): A critical examination on the Folin’s method for determination of creatinine concentration in the urine. *Osaka City Med. J.*, 8, 1–15.

17) Weatherburn, M. W. (1967): Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.*, 39, 971–974.

18) Gornall, A. G., Bordawill, C. J., and Maxima, M. D. (1949): Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.*, 177, 751–766.

19) Doumas, B. T., Watson, W. A., and Biggs, H. G. (1971): Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta*, 31, 87–96.

Vol. 32, No. 1, 1986
20) Reitman, S., and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminase. *Am. J. Clin. Pathol.*, 28, 56–63.

21) Brozek, J., Grand, F., Anderson, J. T., and Kies, A. (1963): Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann. N. Y. Acad. Sci.*, 110, 113–140.

22) Kaneko, K., and Koike, G. (1983): Obligatory N loss and utilization of egg and rice mixed protein in young Japanese women. *J. Nutr. Sci. Vitaminol.*, 29, 455–466.

23) Kaneko, K., and Koike, G. (1985): Utilization and requirement of egg protein in Japanese women. *J. Nutr. Sci. Vitaminol.*, 31, 43–52.

24) Inoue, G., Fujita, Y., and Niiyama, Y. (1973): Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. *J. Nutr.*, 103, 1673–1687.

25) Korslund, M., Kies, C., and Fox, H. M. (1973): Comparison of the protein nutritional value of TVP, methionine-enriched TVP and beef for adolescent boys. *J. Food Sci.*, 38, 637–638.

26) Kiriyama, S., Kasai, T., Chiji, H., Harayama, K., and Nishino, K. (1984): Differential inducibility of threonine imbalance of L-methionine and oligo-L-methionine supplemented to a 8% casein or 10% soy protein isolate diet in rats. *Nutr. Sci. Soy Protein (Japan)*, 5, 31–37.

27) Arai, S. (1983): Nutritional properties of an oligopeptide mixture prepared from soy protein isolate by enzymatic modification. *Nutr. Sci. Soy Protein (Japan)*, 4, 22–25.

28) Report of a Joint FAO/WHO Ad Hoc Expert Committee (1973): Energy and protein requirements, Wld. Hlth./Org. Rep. Ser. No. 522.

29) Kishi, K., Maekawa, M., Yamamoto, S., Shizuka, F., and Inoue, G. (1984): Effect of methionine supplementation to soy protein isolate on short-term nitrogen balance in adult men. *Nutr. Sci. Soy Protein (Japan)*, 5, 88–93.

30) Koishi, H., Okuda, T., and Miyoshi, H. (1984): Nutritive effects of L-methionine supplement to the soy protein isolate in human body. *Nutr. Sci. Soy Protein (Japan)*, 5, 99–103.

31) Takahashi, T., and Yamada, T. (1984): Effect of supplementing methionine to soy protein isolate on the protein utilization in male adult humans. *Nutr. Sci. Soy Protein (Japan)*, 5, 94–98.