Effect of Dietary Soy Protein Isolate Level on the Ratio of *N*¹-Methyl-2-pyridone-5-carboxamide plus *N*¹-Methyl-4-pyridone-3-carboxamide to *N*¹-Methylnicotinamide Excretion in Rats

Katsumi SHIBATA and Hiroko MATSUO

Department of Food Science and Nutrition, Teikoku Women's University,
Moriguchi, Osaka 570, Japan

Received October 17, 1988

A 10%, 20% or 40% soy protein isolate (SPI) diet containing sufficient niacin was fed to rats for 9 days and urine was collected for the last 2 days. The ratio of *N*¹-methyl-2-pyridone-5-carboxamide (2-py) plus *N*¹-methyl-4-pyridone-3-carboxamide (4-py) to *N*¹-methylnicotinamide (MNA) excretion increased with increasing in the dietary SPI level; this ratio in the groups with the 10%, 20% and 40% SPI diets was 0.56 ± 0.08 , 1.04 ± 0.34 and 10.33 ± 1.60 , respectively. The great increase in the ratio of 2-py plus 4-py to MNA excretion on changing the 20% SPI diet to the 40% SPI diet was attributed to the great decrease in MNA excretion in the group with the 40% SPI diet compared with the group with the 20% SPI diet. From these results and our previous results [Agric. Biol. Chem., 52, 1765 (1988)], it is suggested that the 2-py + 4-py/MNA excretion ratio can be used for assessment of the protein nutrition.

We have hypothesized^{1,2,12)} that the ratio of *N*¹-methyl-2-pyridone-5-carboxamide (2-py) plus *N*¹-methyl-4-pyridone-3-carboxamide (4-py) to *N*¹-methylnicotinamide (MNA) excretion is a more useful index for assessing the protein nutrition than as one form assessing the niacin nutrition, because this excretion ratio increases with increasing casein levels¹⁾ and decreases on the administration of a 5-times concentration of niacin compared with the normal level.²⁾ It is known that tryptophan-niacin metabolism is affected by the amino acid composition of a diet,^{3~5)} so we further investigated whether there are similar findings or not when soy protein isolate (SPI) was used as the dietary protein source instead of casein.

Materials and Methods

Chemicals. Kynurenic acid (KA) and MNA chloride were purchased from Tokyo Kasei Kogyo Co., Ltd. 5-Hydroxyindole-3-acetic acid (5-HIAA) was purchased from Aldrich Chemical Co. 2-Py and 4-py were synthesized by the methods of Pullman and Colowick,⁶⁾ and

Shibata *et al.*,⁷⁾ respectively. SPI was a gift from Fuji Oil Co., Ltd. The vitamin and mineral mixtures were obtained from the Oriental Yeast Kogyo Co., Ltd. All other chemicals used were of the highest purity available from commercial sources.

Animal and diets. Rats of the Wistar strain (five weeks old) were purchased from Clea Japan Inc. The rats were immediately placed individually in rat metabolic cages (CT-10; obtained from Clea Japan Inc.) and fed *ad libitum* a 20% casein diet¹⁾ for 10 days. Then, the experimental diets (Table I) were fed *ad libitum* for a further 9 days, and the rats were killed by decapitation at around 9:00

Table I. THE COMPOSITIONS OF THE EXPERIMENTAL DIETS

	10% SPI	20% SPI	40% SPI
SPI	10	20	40
Sucrose	26.3	23.0	16.3
α -Cornstarch	52.7	46.0	32.7
Corn oil	5	5	5
Mineral mixture ^a (Oriental's ration)	5	5	5
Vitamin mixture ^a (Oriental's ration)	1	1	1

^a See ref. 1.

a.m. on the 10th day. The livers were removed, 5-volumes of cold 50 mM potassium phosphate buffer, pH 7.0, was immediately added, and then the livers were homogenized with a Teflon-glass homogenizer. The resulting homogenates were used as enzyme sources. Urine was collected in tinfoil-wrapped amber bottles containing 1 ml of 1 M HCl and 1 ml of 1% L-cysteine for the last 2 days and stored at -25°C . However, measurement of 5-HIAA was carried out immediately. The room temperature was kept at $22 \pm 2^{\circ}\text{C}$ and the humidity was about 60%. The lighting-schedule was 06:00–18:00 (light) and 18:00–06:00 (dark). Body weight and food intake measurements were performed at around 9:00 a.m.

Analyses. The content of Nam, 2-py and 4-py were simultaneously measured by the high-performance liquid chromatographic (HPLC) method of Shibata *et al.*⁷⁾ The MNA content in urine was determined by the HPLC method of Shibata.⁸⁾ The KA and 5-HIAA contents in urine were determined by the HPLC methods of Shibata⁹⁾ and Shibata *et al.*,¹⁰⁾ respectively. The methods used for measuring the activities of Nam methyltransferase and MNA oxidase were described in refs. 11 and 12, respectively.

Statistical analysis. The significance of differences was evaluated by means of Bartlett's test and Duncan's new multiple range test.¹³⁾

Results

Body weight gain, food intake and food efficiency ratio

The gain in body weight, food intake and the food efficiency ratio were higher in the

groups with the 20% and 40% SPI diets than in the group with the 10% SPI diet, as shown in Table II. The intake of nicotinic acid was also higher in the groups with the 20% and 40% SPI diets than in the group with the 10% SPI diet (Table II). The intake of tryptophan increased with the increasing SPI levels (Table II).

Urinary excretion of Nam, MNA, 2-py and 4-py, and the ratio of 2-py plus 4-py to MNA excretion

The urinary excretion of Nam was almost the same in the groups with the 10%, 20% and 40% SPI diets, as shown in Table III. The urinary excretion of MNA in the group with the 20% SPI diet was slightly higher than that in the group with the 10% SPI diet, but the MNA excretion was much lower in the group with the 40% SPI diet than in the groups with the 10% and 20% SPI diets (Table III). The urinary excretion of 2-py was slightly higher in the group with the 20% SPI diet than in the groups with the 10% and 40% SPI diets (Table III). The urinary excretion of 4-py showed the tendency to increase with increases in the SPI level (Table III). The sum of the urinary excretion of Nam, MNA, 2-py and 4-py was higher in the group with the 20% SPI diet than in the groups with the 10% and 40% SPI diets (Table III). The ratio of 2-py plus 4-py to

Table II. EFFECTS OF THE DIETARY SPI LEVEL ON THE GAIN IN BODY WEIGHT, FOOD INTAKE AND THE FOOD EFFICIENCY RATIO (FER)

	10% SPI	20% SPI	40% SPI
Gain in body weight (g/9 days)	14.5 ± 5.5^a	58.4 ± 7.9^b	59.2 ± 6.7^b
Food intake (g/9 days)	127.1 ± 12.4^a	150.7 ± 5.2^b	143.3 ± 5.8^b
FER	0.112 ± 0.035^a	0.371 ± 0.047^b	0.413 ± 0.039^b
Tryptophan intake* (mg/9 days)	172.8 ± 16.9^a	420.4 ± 24.3^b	779.0 ± 31.1^c
Nicotinic acid intake** (mg/9 days)	7.6 ± 0.7^a	9.0 ± 0.3^b	8.6 ± 0.3^b

Values are means $\pm$ S.D. for 5 rats; values having different superscript letters in the same row are statistically significantly different at $p < 0.05$.

* This value was calculated from data showing that the tryptophan content of SPI is 1.5 g per 100 g protein ("Standard Tables of Food Composition in Japan, Amino Acid Composition of Foods [revised edition]", Resources Council, Science and Technology Agency, Japan, The Printing Bureau, The Minister of Finance, Japan, 1986, pp. 122–123) and the protein content of SPI is 90.6%.

** This value was calculated from data showing that these diets contain 6 mg of nicotinic acid per 100 g diet.

Table III. EFFECTS OF THE DIETARY SPI LEVEL ON THE URINARY EXCRETION OF NAM AND ITS METABOLITES, AND THE RATIO OF 2-Py + 4-Py TO MNA EXCRETION

	10% SPI	20% SPI	40% SPI
Nam (nmol/daily urine)	461 ± 280	554 ± 381	390 ± 163
MNA (nmol/daily urine)	5565 ± 1182 ^a	7173 ± 1550 ^a	892 ± 336 ^b
2-Py (nmol/daily urine)	441 ± 62 ^a	927 ± 105 ^b	648 ± 154 ^a
4-Py (nmol/daily urine)	2617 ± 222 ^a	6180 ± 1072 ^b	7343 ± 546 ^b
Nam + MNA + 2-Py + 4-Py (nmol/daily urine)	9084 ± 1610 ^a	14835 ± 1476 ^b	9163 ± 822 ^a
2-Py + 4-Py/MNA	0.56 ± 0.08 ^a	1.04 ± 0.34 ^b	10.33 ± 1.60 ^c

Values are means ± S.D. for 5 rats; values having different superscript letters in the same row are statistically significantly different at $p < 0.05$.

Table IV. EFFECTS OF THE DIETARY SPI LEVEL ON THE URINARY EXCRETION OF KA AND 5-HIAA

	10% SPI	20% SPI	40% SPI
KA (nmol/daily urine)	527 ± 30 ^a	1127 ± 225 ^b	1918 ± 184 ^c
5-HIAA (nmol/daily urine)	341 ± 45	364 ± 129	382 ± 59

Values are means ± S.D. for 5 rats; values having different superscript letters in the same row are statistically significantly different at $p < 0.05$.

Table V. EFFECTS OF THE DIETARY SPI LEVEL ON THE ACTIVITIES OF NAM METHYLTRANSFERASE AND MNA OXIDASE IN LIVER

	10% SPI	20% SPI	40% SPI
Nam methyltransferase (nmol/hr/g liver)	439 ± 52 ^a	450 ± 32 ^a	644 ± 103 ^b
MNA oxidase (nmol/hr/g liver)			
2-Py formation	36 ± 13 ^a	43 ± 4 ^a	1148 ± 156 ^b
4-Py formation	65 ± 33 ^a	378 ± 24 ^b	2986 ± 679 ^c

Values are means ± S.D. for 5 rats; values having different superscript letters are statistically significantly different at $p < 0.05$.

MNA excretion increased with the increasing SPI levels, in particular, this excretion ratio increased greatly when the 20% SPI diet was changed to the 40% SPI diet (Table III).

Urinary excretion of KA and 5-HIAA

The urinary excretion of KA increased with the increasing SPI levels, as shown in Table IV. The urinary excretion of 5-HIAA remained constant, regardless of the SPI level (Table IV).

Activities of Nam methyltransferase and MNA oxidase in liver

The activity of Nam methyltransferase was

higher in the group with the 40% SPI diet than in the groups with the 10% and 20% SPI diets, as shown in Table V. The enzyme activity of 2-py formation slightly increased when the 10% SPI diet was changed to the 20% SPI diet, and when the 20% SPI diet was changed to the 40% SPI diet, the activity of 2-py formation greatly increased (Table V). The enzyme activity of 4-py formation increased with the increasing SPI levels (Table V).

Discussion

In our previous studies,^{1,2,12)} when casein was used as the dietary protein source, the

urinary excretion of Nam and 2-py did not change, that of MNA decreased and that of 4-py increased with increasing dietary casein levels, and so that the total urinary excretion of Nam, MNA, 2-py and 4-py remained constant, regardless of the casein level.¹⁾ Furthermore, we have reported that when the concentration of niacin was raised 5-times compared with the normal level, the ratio of 2-py plus 4-py to MNA excretion decreased.²⁾ Therefore, we presented the hypothesis that the ratio of 2-py plus 4-py to MNA excretion is a more useful index for assessing the protein nutrition than as one for assessing the niacin nutrition,²⁾ although this excretion ratio is recommended as an index for assessing the niacin nutrition.¹⁴⁾ However, these findings are not consistent with the established theory; the urinary excretion of MNA, 2-py and 4-py increases with increasing niacin-equivalent intake [niacin (mg) + 1/60 tryptophan (mg)]. It was reported that tryptophan-niacin metabolism is affected by the amino acid composition of diet.^{3~5)} In the 1940's, several investigators^{15~18)} obtained similar results; extra casein in the rat diet leads to either no increase in the excretion of MNA or to an increase of less than that which would be expected if free tryptophan was administered in an amount equivalent to that present in the casein. Accordingly, it is possible that these unexpected findings reported previously^{1,2)} are due to the specific amino acid composition of casein. So, we further investigated the effect of the dietary SPI level on the urinary excretion of Nam, MNA, 2-py and 4-py, and the ratio of 2-py plus 4-py to MNA excretion. When the SPI level in the diet was changed, the following results were obtained: [1] the urinary excretion of Nam changed little, regardless of the SPI level; [2] the urinary excretion of MNA decreased greatly in the group with the 40% SPI diet compared with in the groups with the 10% and 20% SPI diets; [3] the urinary excretion of 4-py increased with increasing SPI levels; [4] the total urinary excretion of Nam, MNA, 2-py and 4-py was almost the same in the groups with the 10% and 40% SPI diets, but that in

the group with the 20% SPI diet was higher than that in the groups with the 10% and 40% SPI diets; [5] the ratio of 2-py plus 4-py to MNA excretion increased with increasing SPI levels; [6] the urinary excretion of KA increased with increasing SPI levels; [7] the urinary excretion of 5-HIAA remained constant regardless of the SPI level; [8] the activity of Nam methyltransferase increased in the group with the 40% SPI diet compared with the groups with the 10% and 20% SPI diets; [9] the enzyme activity of 2-py formation increased greatly when the 10% and 20% SPI diets were changed to the 40% SPI diet; and [10] the enzyme activity of 4-py formation increased with increasing SPI levels. The above findings were similar to the findings when the casein level in the diet was increased.^{1,2,12)} The ratio of 2-py plus 4-py to MNA excretion was almost the same in the groups with the 10% SPI diet and the 10% casein diet (0.56 ± 0.08 vs. 0.70 ± 0.04) or in the groups with the 40% SPI diet and the 40% casein diet (10.33 ± 1.60 vs. 10.79 ± 0.84), but this ratio was much higher in the group with the 20% casein diet (7.83 ± 1.06) than in the group with the 20% SPI diet (1.04 ± 0.34). This result shows that the ratio reflects the protein nutrition, because casein is superior to SPI as a protein source.

The flow to the serotonin pathway from tryptophan is constant, regardless of the protein intake. Although the flow to the kynurenine pathway from tryptophan increased with increasing SPI levels, the flow to the niacin pathway from tryptophan in the groups with the 10% and 40% SPI diets remained constant. This means that the strict control mechanism that keeps the NAD level normal must exist at the junction between the niacin and glutarate pathways. Aminocarboxymuconate-semialdehyde decarboxylase might be partly concerned with this control because this enzyme activity increases with increasing dietary casein levels.^{12,19,20)} In the group with the 20% SPI diet, the total urinary excretion of Nam, MNA, 2-py and 4-py was higher than that in the groups with the 10% and 40%

SPI diets. It is possible that the induction of aminocarboxymuconate-semialdehyde decarboxylase activity occurred on administration of the 40% SPI diet, but not on administration of the 20% SPI diet. Accordingly, quinolinic acid and its metabolites are formed more in the group with the 20% SPI diet than in the group with the 40% SPI diet, although the intake of tryptophan was higher in the group with the 40% SPI diet than in the group with the 20% SPI diet. The activity of Nam methyltransferase was slightly higher in the group with the 40% SPI diet than in the groups with the 10% and 20% SPI diets, but the sum of the urinary excretion of methylated compounds, such as MNA, 2-py and 4-py, was higher in the group with the 20% SPI diet than in the groups with the 10% and 40% SPI diets. The activity of 2-py formation was about the same in the groups with the 10% and 20% SPI diets, but this activity was about 30-times higher in the group with the 40% SPI diet than in the groups with the 10% and 20% SPI diets. Nevertheless, the order of the urinary excretion of 2-py was "20% SPI diet" > "40% SPI diet" > "10% SPI diet". These differences between the increasing order of the activities of Nam methyltransferase and the 2-py forming enzyme, and that of the methylated compounds (MNA + 2-py + 4-py) and 2-py excretion mean that enzyme activities determined *in vitro* do not always directly reflect *in vivo* conditions. The two enzyme activities could be more affected by the substrate concentration than by the enzyme concentration. On the other hand, the activity of the 4-py forming enzyme and the urinary excretion of 4-py increased with increasing dietary SPI levels.

The present results and our previous results^{1,2,12)} suggest that the increased ratio of

2-py plus 4-py to MNA excretion probably reflects an improved protein nutrition. This is partly attributed to that the activity of the 4-py forming enzyme is induced on improvement of the protein nutrition. In order to confirm this hypothesis, further study is needed.

Acknowledgment. The authors express their sincere thanks to Fuji Oil Co., Ltd. for the supply of SPI.

References

- 1) K. Shibata, R. Nomamoto and K. Iwai, *Agric. Biol. Chem.*, **52**, 1765 (1988).
- 2) K. Shibata and H. Matsuo, *Agric. Biol. Chem.*, **52**, 2747 (1988).
- 3) O. J. Koeppe and L. M. Henderson, *J. Nutr.*, **55**, 23 (1955).
- 4) K. Murata and T. Kimura, *J. Nutr.*, **98**, 427 (1969).
- 5) K. Murata, *Eiyo to Shokuryo*, **22**, 353 (1969).
- 6) M. E. Pullman and S. P. Colowick, *J. Biol. Chem.*, **206**, 121 (1954).
- 7) K. Shibata, T. Kawada and K. Iwai, *J. Chromatogr.*, **424**, 23 (1988).
- 8) K. Shibata, *Vitamins (Japan)*, **61**, 599 (1987).
- 9) K. Shibata, *J. Chromatogr.*, **430**, 376 (1988).
- 10) K. Shibata, M. Onodera, T. Kawada and K. Iwai, *J. Chromatogr.*, **430**, 381 (1988).
- 11) K. Shibata, *Agric. Biol. Chem.*, **50**, 1489 (1986).
- 12) K. Shibata, H. Taguchi and K. Iwai, *Agric. Biol. Chem.*, **52**, 3165 (1988).
- 13) K. Wakabayashi, "Zikken Data no Seiri," (in Japanese), Baifukan Co., Ltd., Tokyo, 1984, p. 44.
- 14) R. A. Clemens and R. C. Brown, *Food Tech.*, **40** (Feb.), 71 (1986).
- 15) S. A. Singal, A. P. Briggs, V. P. Sydenstricker and J. M. Littlejohn, *J. Biol. Chem.*, **166**, 573 (1946).
- 16) G. H. Bell, B. T. Scheer and H. J. Deuel, Jr., *J. Nutr.*, **35**, 239 (1948).
- 17) J. M. Hundley, *J. Nutr.*, **34**, 253 (1947).
- 18) B. S. Schweigert and P. B. Pearson, *J. Biol. Chem.*, **172**, 485 (1948).
- 19) H. Sanada and M. Miyazaki, *J. Nutr. Sci. Vitaminol.*, **30**, 113 (1984).
- 20) T. Hayakawa and K. Iwai, *Agric. Biol. Chem.*, **49**, 2431 (1985).