

Note

Increased Conversion Ratio of Tryptophan to Niacin in Severe Food Restriction

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The effect of food restriction on the conversion ratio of tryptophan to niacin was investigated, because it is known that the conversion ratio is influenced by nutritional factors. A 20% casein diet was fed to rats *ad libitum* (control), 1/2 the food of the control, 1/4 the food of the control, or starved for 9 days, and urine samples were collected to measure the urinary excretion of such tryptophan metabolites as kynurenic acid, xanthurenic acid, and nicotinamide. The conversion ratio in the 1/2, 1/4, or starving group increased at day 1 of the experiment, but returned to the original value from day 2. Only in the starving group did the conversion ratio extremely increase from day 6 to day 9, being about 5-times higher than that of the original value on day 9. The possible mechanism by which the conversion ratio increased during food restriction is discussed.

Key words: tryptophan metabolism; nicotinamide; energy restriction; niacin; rat

Dietary Trp can be converted to niacin in mammals, including humans. It has been assumed that the efficiency of the conversion ratio of Trp to niacin is constant.^{1–4} However, we have indicated that this is not so and that it is influenced by nutritional factors.^{5,6} A deficiency of micro-nutrients, especially water-soluble vitamins, is prone to appear when food intake is limited, because they cannot be stored in the body. However, there is one exception pattern, which is niacin. In this present work, we investigated the effect of food restriction on the conversion ratio of Trp to niacin.

2-Py and 4-Py were synthesized by the methods of Pullman and Colowick⁷ and of Shibata *et al.*,⁸ respectively. The mineral and vitamin mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan), all other chemicals used being of the highest purity available from commercial sources.

The animal room temperature was maintained at around 22°C and about 60% humidity, and a 12-hr light/12-hr dark cycle was operated. Body weight and food intake were measured daily at around 09:00 a.m., and food and water were renewed daily.

Male rats of the Wistar strain (7 weeks old) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). To accustom the rats to these conditions, they were initially fed *ad libitum* for 10 days with an NiA-

free 20% casein diet.⁵ They were then divided into four groups at 09:00 a.m. (the initial day is designated as day 0), and the control group was fed *ad libitum* for 9 days with the same diet, the second group was fed the half amount of the control group, the third group was fed a quarter of the amount of the control group, and the fourth group was completely starved. The 24-h urine samples (09:00 a.m.–09:00 a.m.) from –1 at 09:00 a.m. to day 9 at 09:00 a.m. were collected daily in amber bottles with 1 ml of 1 M HCl and stored at –25°C until needed. The rats were killed by decapitation on day 9 at around 09:00 a.m. after the last urine samples from day 8 at 09:00 a.m. to day 9 at 09:00 a.m. had been collected. The liver of each animal was removed, and a portion (approximately 1 g) was immediately homogenized with a Teflon-glass homogenizer in five volumes of a cold 50 mM KH₂PO₄-K₂HPO₄ buffer (pH 7.0). These homogenates were used as enzyme sources for measuring Trp oxygenase,¹⁰ kynureninase the reaction was done without adding exogenous pyridoxal phosphate,¹⁰ NMN adenylyltransferase,¹³ NAD⁺ synthetase,¹³ Nam methyltransferase,¹⁴ 2-Py-forming MNA oxidase,¹⁴ and 4-Py-forming MNA oxidase.¹⁴ One part of each homogenate was centrifuged at 105,000 × *g* for 20 min to measure the activities of 3-HA oxygenase¹⁰ and AC-MSDase.¹² Another portion of the liver (approximately 1 g) was also immediately homogenized with a Teflon-glass homogenizer in ten volumes of cold 0.25 M sucrose. These homogenates were used as enzyme sources for measuring the activity of kynurenine aminotransferase (the each was done without adding exogenous pyridoxal phosphate), and one part of each homogenate was prepared as in the literature for measuring the activity of kynurenine 3-hydroxylase.⁹

The contents of Nam, 2-Py and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata *et al.*,⁸ while the content of MNA in the urine was measured by the HPLC method of Shibata.¹⁵ The contents of KA¹⁶ and XA¹⁷ in the urine were measured by HPLC methods. To calculate the conversion ratios of Trp to niacin {(Nam + MNA + 2-Py + 4-Py) (mol/day) × 100/available Trp (mol/day)},¹⁸ Trp to KA, and Trp to XA, the available Trp was calculated according to the following rule: one gram gain of body weight retained 7.12 μmol of Trp in the body and one gram loss of body weight liberated 7.12 μmol of available Trp.¹⁹ In the present experiment, one gram of food gave 11.05

Abbreviations: NiA, nicotinic acid; Nam, nicotinamide; NMN, nicotinamide mononucleotide; Trp, L-tryptophan; MNA, N¹-methyl-nicotinamide; 2-Py, N¹-methyl-2-pyridone-5-carboxamide; 4-Py, N¹-methyl-4-pyridone-3-carboxamide; KA, kynurenic acid; XA, xanthurenic acid; 3-HA, 3-hydroxyanthranilic acid; ACMSDase, aminocarboxymuconate-semialdehyde decarboxylase.

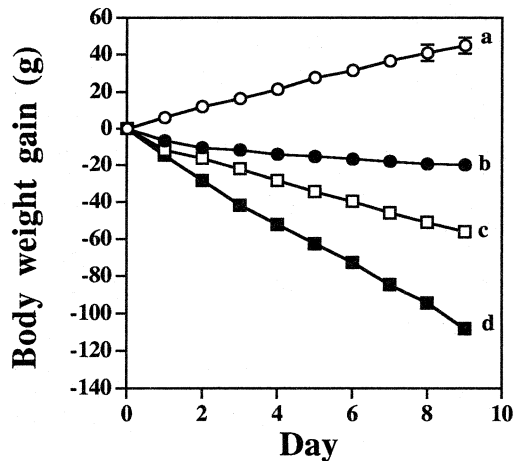


Fig. 1. Effect of Food Restriction on the Body Weight Gain.

○, *ad libitum* feeding; ●, 1/2 feeding; □, 1/4 feeding; ■, starving.

Each point is the mean \pm SEM for five rats; values with different superscript letters on the last day of the experiment are statistically different at $p < 0.01$, as determined by Duncan's new multiple-range test.

μ mol of available Trp. For example, in the *ad libitum* group, the food intake per day was 18.62 g, and the body weight gain per day was 6 g, which gave 163 μ mol of available Trp; $\{(18.62 \times 11.05) - (6 \times 7.12)\} = 163$. In the 1/2 group, the food intake was 8.54 g and the body weight loss per day was -2.46 g, which gave 112 μ mol of available Trp; $\{(8.54 \times 11.05) - (-2.46 \times 7.12)\} = 112$. In the starving group, the food intake was nil and the body weight loss was -11.69 g, which gave 83 μ mol of available Trp; $\{(0 \times 11.05) - (-11.69 \times 7.12)\} = 83$.

The body weights decreased by the dependent on the extent of the energy restriction as shown in Fig. 1.

The daily urinary excretion of the sum (Nam + MNA + 2-Py + 4-Py) is shown in Fig. 2(A), and the daily change in the conversion ratio of Trp to niacin is shown in Fig. 2(B). At the beginning of the experiment, on day 1, the conversion ratio increased more in the restricted-feed groups than in the control, which was dependent on the extent of food restriction. After that, the increased conversion ratio returned to the original value in each case. This phenomenon might be the result of increased excretion of corticosteroid, which has been shown to increase in acute starvation,^{20,21)} and Trp oxygenase activity is known to increase in corticosteroid administration^{21,22)} and prednisolone.²³⁾ Only in the starving group the conversion ratio extremely increase from day 6 to day 9, being about 5-times higher than that of the control group and the original value (Fig. 2). To clarify why the conversion ratio increased that much, the effect of food restriction on the enzyme activities involved in the conversion of Trp to niacin was investigated. As shown in Table I, the activity of Trp oxygenase was much higher in the starving group than in the other three groups. A similar result has been reported by Satyanarayana and Rao.²⁴⁾ However, the activities of NMN adenylyltransferase and NAD⁺ synthetase were lower in the 1/4 and starving groups than in the control and 1/2 groups. As the conversion ratio of the 1/4

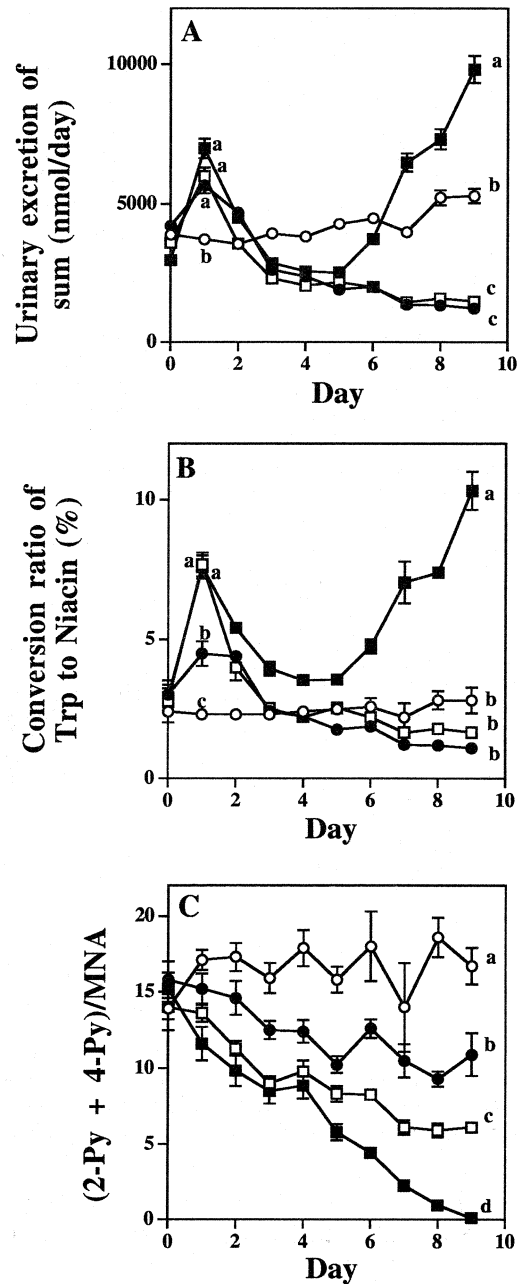


Fig. 2. Effect of Food Restriction on the Urinary Excretion of the Sum (A), the Conversion Ratio of Trp to Niacin (B), and the Urinary Excretion Ratio of (2-Py + 4-Py)/MNA (C).

○, *ad libitum* feeding; ●, 1/2 feeding; □, 1/4 feeding; ■, starving.

Each point is the mean \pm SEM for five rats; values with different superscript letters on the last day of the experiment are statistically different at $p < 0.01$, as determined by Duncan's new multiple-range test.

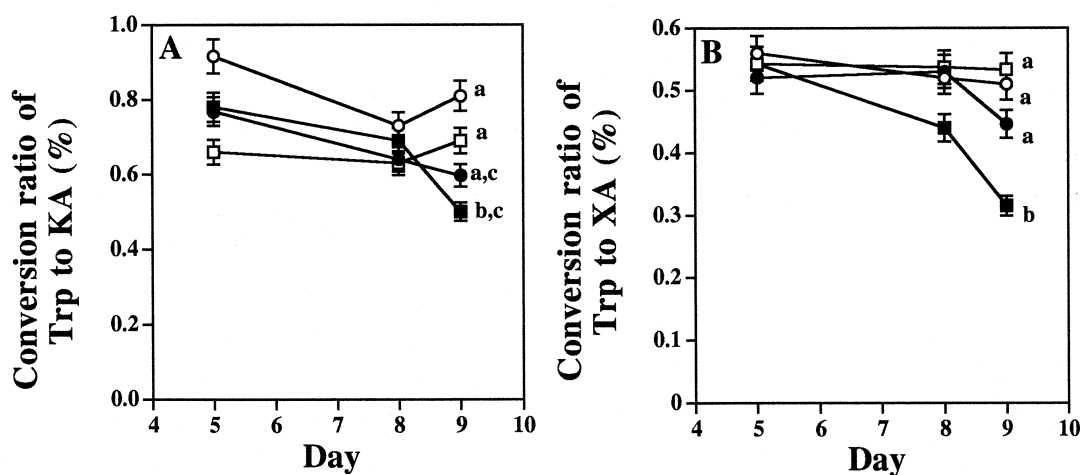
Sum = Nam + MNA + 2-Py + 4-Py.

group was no higher than that of the control, the changes in these enzyme activities would not had influenced the conversion ratio under food restriction. It is known that the activity of ACMSDase plays a crucial role in the conversion of Trp to niacin.^{5,6)} In the present experiment, its activity was not influenced by severe food restriction. Accordingly, we consider that the activity of ACMSDase was affected by nutritional changes

Table I. The Effects of Dietary Restriction on the Enzyme Activities Involved in Trp to Niacin Metabolism.

	<i>Ad libitum</i> feeding	1/2 restriction	1/4 restriction	Starving
Liver weight (g)	12.6±0.5 ^a	7.2±0.3 ^b	5.1±0.2 ^c	2.2±0.2 ^d
Trp oxygenase	1.14±0.13 ^a	2.58±0.33 ^b	2.87±0.52 ^b	9.69±2.24 ^c
Kynureninase	1.81±0.08	1.87±0.15	1.79±0.06	1.73±0.12
Kynurenine aminotransferase	1.24±0.04 ^a	1.63±0.05 ^b	1.81±0.06 ^c	0.83±0.05 ^b
Kynurenine 3-hydroxylase	1.22±0.04 ^a	1.48±0.19 ^a	1.28±0.13 ^a	0.50±0.08 ^b
3-HA oxygenase	618±18 ^a	771±30 ^b	770±21 ^b	828±29 ^b
ACMSDase	1.23±0.25	1.81±0.25	2.02±0.27	2.00±0.3
MNM adenylyltransferase	6.85±0.37 ^a	6.44±0.53 ^a	3.06±0.18 ^b	3.51±0.65 ^b
NAD ⁺ synthetase	0.39±0.05 ^a	0.43±0.17 ^a	0.13±0.04 ^b	0.27±0.06 ^b
Nam methyltransferase	0.30±0.02 ^a	0.57±0.02 ^b	0.82±0.04 ^c	1.15±0.08 ^d
2-Py-forming MNA oxidase	0.80±0.08 ^a	0.81±0.07 ^a	0.76±0.08 ^a	0.44±0.01 ^b
4-Py-forming MNA oxidase	2.52±0.23 ^a	1.25±0.14 ^b	0.78±0.06 ^c	0.57±0.04 ^d

Each value is expressed as $\mu\text{mol/hr/g}$ of liver and means \pm SEM for five rats; values with different superscript letters in the same row are statistically different at $p < 0.01$, as determined by Duncan's new multiple-range test.

**Fig. 3.** Effect of Food Restriction on the Conversion Ratios of Trp to KA (A) and to XA (B).

○, *ad libitum* feeding; ●, 1/2 feeding; □, 1/4 feeding; ■, starving.

Each point is the mean \pm SEM for five rats; values with different superscript letters on the last day of the experiment are statistically different at $p < 0.01$, as determined by Duncan's new multiple-range test.

such as the composition of diet, while the activity of Trp oxygenase was affected by energy restriction. Furthermore, the effects of food restriction on the upper metabolites on the conversion pathway of Trp to niacin were investigated. The urinary excretion of KA and XA was prone to decrease in the starving group, when compared with the other three groups as shown in Fig. 3. This is consistent with the result that the activity of kynurenine aminotransferase, which catalyses both the reactions of kynurenine to KA and 3-hydroxykynurenine to XA, was lower in the starving group than in the other three groups (Table I). The activity of kynurenine 3-hydroxylase was also lower in the starving group than in the other three groups, that of 3-HA oxygenase was the reverse, and that of kynureninase was almost the same among the groups (Table I). The lower activity of kynurenine 3-hydroxylase generally means a lower conversion of kynurenine to 3-hydroxykynurenine, which leads to accelerating the side-reaction of kynurenine to KA. However, the activity of kynurenine aminotransferase was also lower under the starving group and, therefore, the reaction of kynurenine to 3-

hydroxykynurenine *in vivo* is not thought to have been reduced. In fact, the conversion ratio of Trp to KA was no higher in the starving group than in the other three groups (Fig. 3(A)).

We have proposed that the excretory ratio of (2-Py+4-Py)/MNA reflects nutritional status;²⁵⁾ a lower value means an impaired nutritional status. As shown in Fig. 2(C), the excretion ratio was dependent on the extent of food restriction. As to be expected, the activity of Nam methyltransferase was increased and that of 4-Py-forming MNA oxidase was also increased by energy restriction (Table I).

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