The Urinary Excretory Ratio of Nicotinamide Catabolites Was Associated with the Conversion Ratio of Tryptophan to Nicotinamide in Growing Rats Fed a Niacin-Free 20% Casein Diet

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Weaning rats were fed a niacin-free 20% casein diet. Twenty-four-h urine samples were collected, and nicotinamide and its catabolites were measured. A correlation was found between the urinary excretory ratio of nicotinamide catabolites (N1-methyl-2-pyridone-5-carboxamide + N1-methyl-4-pyridone-3-carboxamide)/N1-methylnicotinamide and the tryptophan-nicotinamide conversion ratio during growing period of the rats. This indicates the possibility that the conversion ratio can be deduced from the excretory ratio.

Key words: N1-methylnicotinamide; N1-methyl-2-pyridone-5-carboxamide; N1-methyl-4-pyridone-3-carboxamide; tryptophan-nicotinamide conversion ratio

The vitamin Nam is biosynthesized from the essential amino acid Trp in mammalian liver, including the human liver.1,2) The metabolism of nicotinic acid, Nam, and Trp in mammals is given in reference 3. It is said that the pathway Trp to Nam plays a critical role in preventing Nam deficiency pellagra in humans, because protein malnutrition frequently causes pellagra.4) In order to calculate the conversion ratio of Trp to Nam, animals and humans must eat a special diet that configures a preformed niacin-free refined diet for several days.5,6) This means that calculating the conversion ratio is very difficult.

Shibata6) had found that the conversion ratio of Trp to Nam is affected by age, and the excretory ratio of (2-Py + 4-Py)/MNA is too, but the conversion ratio could not be calculated in the experiment because the diet of rats contained a pre-formed niacin-free refined diet for several days.5,6) This means that the excretory ratio of (2-Py + 4-Py)/MNA can be used as a surrogate biomarker of the conversion ratio of Trp to Nam during the growing period of rats. As a first step, we investigated the relationship between the excretory ratio and the conversion using 24-h urine samples. The urinary excretory ratio of Nam catabolites was associated with the conversion ratio of Trp to Nam in growing rats fed a niacin-free 20% casein diet. We report these results in detail here.

The care and treatment of the experimental animals confirmed to The University of Shiga Prefecture Guidelines for the Ethical Treatment of Laboratory Animals. The room temperature was maintained at about 22 °C and about 60% humidity and a 12 h/12 h light/dark cycle (06:00–18:00/18:00–06:00) was imposed.

Male 3-week-old Wistar rats purchased from CLEA Japan (Tokyo) were placed immediately in individual CL-301 metabolism cages purchased from CLEA Japan, and were fed freely with a conventional purified diet consisting of 20% vitamin-free milk casein, 0.2% t-methionine, 46.9% gelatinized cornstarch, 23.4% sucrose, 5% corn oil, 3.5% AIN-93-G mineral mixture,7) and a 1% AIN-93 vitamin mixture containing choline bitartrate, but without niacin, for 30 d.

Twenty-four h urine samples were collected from 9:00 to next 9:00 for days 7, 16, 23, and 30 of the experiment in amber bottles containing 1 mL of 1 mol/L HCl, and were stored at −20 °C until needed. The urine contents of Nam, 2-Py, and 4-Py were measured simultaneously by the HPLC method of Shibata et al.8) The urine content of MNA was also measured by this method.9) The conversion ratio was calculated by comparing the Trp intake during urine collection with the sum of urinary excretion of Nam, MNA, 2-Py, and 4-Py.10)

Pearson correlation coefficients were calculated to determine the association between the conversion ratio of Trp to Nam and the urinary excretory ratio of (2-Py + 4-Py)/MNA. The calculation was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

The weaning rats had free access to the niacin-free 20% casein diet for 30 d. The changes in food intake and in growth during the experiment were normal. Figure 1 shows the urinary excretion of Nam, MNA, 2-Py, and 4-Py. These compounds increased with age. The conversion ratio of Trp to Nam increased with age, as shown in Fig. 2A, and the excretory ratio of (2-Py + 4-Py)/MNA also increased with age as shown in Fig. 2B.

Figure 3 shows the relation found between the conversion ratio of Trp to Nam and the urinary excretory ratio of Nam catabolites. The Pearson coefficient value

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Abbreviations: Trp, tryptophan; Nam, nicotinamide; MNA, N1-methylnicotinamide; 2-Py, N1-methyl-2-pyridone-5-carboxamide; 4-Py, N1-methyl-4-pyridone-3-carboxamide
was 0.90, and \( p \) was 0.03. This correlation is significant.

A very strong correlation was found between the urinary excretory ratio of Nam catabolites (2-Py + 4-Py)/MNA in the 24-h urine samples and the Trp-Nam conversion during the growing period of the rats.

Pellagra results from a diet deficient in Nam and/or Trp. This disease is considered a public health problem in many maize-consuming African and Asian countries, especially populations facing to emergency and conflict.\(^{11-15}\)

Krehl et al.\(^ {16}\) found that Trp could completely counteract the growth retardation caused by corn grits diet in rats. The conversion ratio of Trp to Nam is not constant; it is affected by age,\(^ {5}\) various nutritional factors,\(^ {10,17-25}\) hormones,\(^ {26-28}\) and chemicals.\(^ {29-31}\)

Therefore, it is important in preventing a pellagra outbreak to know the conversion ratio of Trp to Nam under the conditions, but it is not possible to know this in case of emergency and conflict.

As for the biomarkers of pellagra, it is known that the blood NAD level does not reflect Nam nutritional status in pellagra patients,\(^ {32}\) and that the Nam itself does not appear in the urine even in healthy people.\(^ {5}\) On the contrary, urinary excretion of Nam catabolites such as

![Fig. 1. Effects of Age on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C), and 4-Py (D). Symbols mean represent ± SEM for six rats.](image1)

![Fig. 2. Effects of Age on the Conversion Ratio of Trp to Nam (A) and the Urinary Excretory Ratio of (2-Py + 4-Py)/MNA (B). Symbols mean represent ± SEM for six rats.](image2)
Fig. 3. Relation between the Conversion Ratio of Trp to Nam and the Urinary Excretory Ratio of Nam Catabolites.

Symbols mean represent ± SEM for six rats. The Pearson coefficient value was 0.90, and p was 0.03. The correlation is significant.

MNA, 2-Py, and 4-Py, and the excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples, are generally used as a laboratory test. Shibata and co-workers (10,17–22,33) found that the urinary excretory ratio of (2-Py + 4-Py)/MNA primarily reflected protein nutritional status, not Nam nutritional status, because the excretory ratio was decreased by the administration of an extremely large amount of Nam (13), and MNA (33) in rats. In addition, Shibata et al. (36) reported that the administration of 150 mg/d of Nam did not affect the excretory ratio in humans. Thus, increases in the excretory ratio do not bring improved Nam nutritional status. Shibata (33) proposed that the Nam catabolite excretory ratio reflects protein nutritional status.

Collection of a 24-h urine sample and feeding of a niacin-free refined diet are very hard to achieve in emergency and conflict situations. (2-Py + 4-Py)/MNA can be measured by using a spot urine sample instead of a 24-h urine sample. Therefore, it appears to be possible that the conversion ratio of Trp to Nam can be deduced by a spot urine sample instead of using a 24-h urine sample.

It is necessary to examine whether the same result is obtained when weaning rats are fed a diet containing other proteins or different concentrations of dietary proteins. In addition, it is also necessary to examine diurnal variations in the urinary excretory ratio of (2-Py + 4-Py)/MNA, even though the collection of spot urine samples from rats is difficult.

In the future, we plan to study the relation between the conversion ratio of Trp to Nam in 24-h urine samples and the urinary excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples the growing period of humans.

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