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Ⅲ. 研究成果の刊行物・別刷

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Effect of Nicotinamide Administration on the Tryptophan-Nicotinamide Pathway in Humans

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Abstract: The vitamin nicotinamide is synthesized in the liver from tryptophan, and distributed to non-hepatic tissues. Although it is generally accepted that 60 mg tryptophan is equivalent to 1 mg nicotinamide in humans, the conversion ratio of tryptophan to nicotinamide is changeable. To determine if *de novo* nicotinamide synthesis from tryptophan is influenced by nicotinamide intake itself, six young women consumed controlled diets containing 30.4 or 24.8 mg niacin-equivalent nicotinamide supplements with 0, 89, 310, or 562 μmol/day (0, 10.9, 37.8, or 68.6 mg/day, respectively), and urinary excretion of intermediates and metabolites of the tryptophan-nicotinamide pathway were measured. Urinary excretion of nicotinamide metabolites increased linearly in a dose-dependent manner. None of the intermediates, including anthranilic acid, kynurenic acid, xanthurenic acid, 3-hydroxyanthranilic acid, and quinolinic acid, changed at all, even when up to 562 μmol/day nicotinamide was given. That is, exogenous nicotinamide did not affect *de novo* nicotinamide synthesis. Therefore, when niacin equivalent is calculated, the intake of nicotinamide itself need not be considered as a factor that changes the tryptophan-nicotinamide conversion ratio.

Key words: Feedback inhibition, human, kynurenine pathway, NAD, nicotinamide, tryptophan, urine

Introduction

One of the general regulatory mechanisms in biosynthetic pathways is feedback inhibition by the end product. With regard to vitamins, mammals, including humans, can synthesize nicotinamide derivatives from the essential amino acid tryptophan. The conversion of tryptophan to nicotinamide is important for determining the niacin equivalent. The tryptophan-nicotinamide pathway exists in several tissues such as the liver, kidney, spleen, and brain, and the liver primarily contributes to the supply of nicotinamide to non-hepatic tissues [1]. Generally, 60 mg tryptophan is estimated to be equivalent to 1 mg nicotinamide in humans [2–10], although the conversion ratio of tryptophan to nicotinamide is altered by numerous factors such as vitamin deficiency [11, 12], high protein intake [13], hormones [14, 15], food restriction [16], exercise [17], and chemicals [18–21]. Since the amount of *de novo* synthesized nicotinamide derivatives from tryptophan is almost the same as intakes of nicotinamide and nicotinic acid in the Japanese population [22], using this conversion ratio to calculate niacin equivalent is important. Rat liver tryptophan 2,3-dioxygenase activity, which catalyzes the first irreversible step in the degradative me-

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tabolism of tryptophan to nicotinamide, is inhibited by pyridine nucleotide coenzymes in *in vitro* experiments [23]. We have previously reported that excess nicotinamide administration does not influence the upper part of the tryptophan-nicotinamide pathway in rats [24]. However, there are no reports on whether nicotinamide intake influences the tryptophan-nicotinamide pathway in humans. In the present study, we assessed the influence that administration of nicotinamide exerted on the production of an intermediary metabolite of the tryptophan-nicotinamide pathway.

Materials and Methods

Chemicals

Quinolinic acid (QA) and anthranilic acid (AnA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). N^1-methylnicotinamide (MNA) chloride, xanthurenic acid (XA), kynurenic acid (KA), and 3-hydroxyanthranilic acid (3-HA) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized by the methods of Pullman and Colowick [25] and Shibata *et al* [26]. Other chemicals used were of the highest purity available from commercial sources.

Subjects

Six healthy female Japanese college students who were not regularly using medication or dietary supplements, and who were not habitually consuming alcohol or cigarettes, participated in the experiment. Their age, body weight, height, and body mass index (BMI) are shown in Table I. This study was reviewed and approved by The Ethical Committee of the National Institute of Health and Nutrition (Tokyo, Japan).

Table I: Characteristics of the Subjects.

Subjects	Age (Yr)	Height (cm)	Body weight (kg)	BMI
Woman 1	21	161.0	50.0	19.3
Woman 2	21	161.0	52.5	20.3
Woman 3	21	162.0	46.0	17.5
Woman 4	21	160.7	53.0	20.5
Woman 5	21	160.5	53.0	20.6
Woman 6	21	165.0	52.5	19.3
Mean	21.0	161.7	51.2	19.6
SD	0.0	1.7	2.8	1.2

Diet

Two kinds of meals were given to the subjects. The nutrient elements in each meal are shown in Tables II and III. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan (5th edition) [27].

Experimental design

The outline of the experiment is shown in Figure 1. The subjects spent their time as usual except for diets, on the following schedule: meal-times (breakfast 07:00–07:30 h, lunch 12:00–12:30 h, and dinner 18:00–18:30 h), wake-up time (06:30 h), and bed time (23:00 h). The experimental period was about 4 weeks. Only the diets shown in Tables II and III were given to the subjects during the first week. The subjects were given the diets with 89 μmol/day (10.9 mg/day) nicotinamide in the second week, 310 μmol/day (37.8 mg/day) in the third week, and 562 μmol/day (68.6 mg/day) in the fourth week. Nicotinamide was divided into a ratio of 3:4:3 and administered daily after breakfast, lunch, and dinner. The 24-hour urine samples were collected from the second urinary sample on the fourth day to the first urinary sample after 06:30 h (wake-up time) on the fifth day in each week. The urine sample volumes were measured, and 1 mL of 1 mol/L HCl was added to 9 mL urine samples to stabilize the metabolites in the tryptophan-nicotinamide pathway. The acidified urine samples were stored at –20° C until needed.

Analyses

Urinary MNA was measured using the high-performance liquid chromatography (HPLC) method of Shibata [28]. The 2-Py and 4-Py contents in the urine were simultaneously measured using the HPLC [26]. The contents of KA [29], XA [30], 3-HA [30], AnA [31], and QA [32] in the urine were measured using HPLC.

Results

Relationship between nicotinamide intake and urinary excretion of nicotinamide metabolites

The increased administration of nicotinamide led to a significant increase in the urinary excretion of nicotinamide metabolites, which are the sum of MNA, 2-Py, and 4-Py (Figure 2). The relationship between nicotinamide intake and urinary excretion was linear. This result suggests that exogenous nicotinamide is well absorbed into the body,

Table II: The Composition of Diet 1.

	Breakfast	Lunch	Dinner	Total
Energy (kcal)	402	689	617	1784
Protein (g)	19.5	23.8	25.2	68.6
Fat (g)	15.7	25.5	9.6	50.8
Carbohydrates (g)	46.0	85.8	104.4	248.8
Fat-soluble vitamins				
Vitamin A (μg)	150	309	419	878
Vitamin D (μg)	1	0	2	3
Vitamin E (mg)	1.1	2.1	2.4	5.6
Vitamin K (μg)	8	204	98	311
Water-soluble vitamins[1]				
Vitamin B_1 (mg as thiamin)	0.35	0.17	0.07	0.59
Vitamin B_2 (mg as riboflavin)	0.47	0.20	0.25	0.92
Vitamin B_6 (mg as pyridoxine)	0.20	0.36	0.68	1.24
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.5	6.2	7.4
Niacin equivalent[2] (mg)	3.5	8.4	18.5	30.4
Pantothenic acid (mg)	1.97	4.21	3.14	9.32
Folic acid (μg as pteroyl monoglutamic acid)	52	134	44	230
Biotin (μg)	21	20	26	67
Vitamin C (mg as L-ascorbic acid)	34	34	50	118
Minerals				
Na (mg)	794	1175	850	2845
K (mg)	592	601	625	1993
Ca (mg)	249	142	85	479
Mg (mg)	47	71	74	192
P (mg)	380	293	317	1071
Fe (mg)	0.8	3.4	2.6	6.7
Zn (mg)	1.8	3.7	2.5	8.0
Cu (mg)	0.15	0.44	0.43	1.02

[1] Water-soluble vitamins except for vitamin B_{12} were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan, fifth revised edition -2000-, Resources Council, Science and Technology Agency, Japan.
[2] The niacin equivalent intake was calculated as follows: The average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

and excess amount of nicotinamide is catabolized to MNA and its further metabolites 2-Py and 4-Py.

Relationship between nicotinamide intake and urinary excretion of the intermediates in the tryptophan-nicotinamide pathway

If nicotinamide inhibits the tryptophan-nicotinamide pathway in the liver, formation of one or more of the intermediates such as AnA, KA, XA, 3-HA, and QA should be reduced. Thus, the effects of nicotinamide administration on the urinary excretion of the intermediates were investigated, regardless of whether or not nicotinamide inhibits *de novo* nicotinamide synthesis. As shown in Figure 3, urinary excretion of AnA (Fig. 3A), KA (Fig. 3B), XA (Fig. 3C), 3-HA (Fig. 3D), and QA (Fig. 3E) was little affected by the increased intake of nicotinamide. In other words, the tryptophan-nicotinamide pathway is not affected, even when taking a large amount of nicotinamide.

Discussion

Humans can synthesize the vitamin nicotinamide from tryptophan in the liver, and the resultant nicotinamide is distributed to non-hepatic tissues. The purpose of the synthetic pathway in the liver is not the supply of NAD^+ but the supply of nicotinamide for non-hepatic tissues. We have clarified that the conversion pathway of nicotinamide from tryptophan is affected by various nutrients [11–13, 16], hormones [14, 15], exercise [17], and drugs [18–21] based on data concerning the urinary excretion of metabolic intermediates in the tryptophan-nicotinamide pathway. Furthermore, we have clarified that excessive nicotinamide administration does not influence the upper part of tryptophan-nicotinamide pathway in rats [24]. However, we still did not know whether nicotinamide itself affects this conversion pathway in humans. The present study was carried out to answer this question because the value of the conversion ratio of tryptophan to nicotinamide is important in deciding the niacin requirement.

Table III: The Composition of Diet 2.

	Breakfast	Lunch	Dinner	Total
Energy (kcal)	463	549	606	1693
Protein (g)	19.6	21.4	20.5	61.5
Fat (g)	22.3	12.8	10.0	45.0
Carbohydrates (g)	46.1	85.6	105.5	249.8
Fat-soluble vitamins				
Vitamin A (μg)	294	144	444	882
Vitamin D (μg)	1	0	0	1
Vitamin E (mg)	2.7	0.6	2.9	6.2
Vitamin K (μg)	12	98	100	210
Water-soluble vitamins[1]				
Vitamin B_1 (mg as thiamin)	0.35	0.09	0.02	0.46
Vitamin B_2 (mg as riboflavin)	0.47	0.18	0.17	0.81
Vitamin B_6 (mg as pyridoxine)	0.20	0.35	0.31	0.86
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.3	10.3	11.3
Niacin equivalent[2] (mg)	7.0	8.1	9.7	24.8
Pantothenic acid (mg)	1.97	3.73	3.55	9.25
Folic acid (μg as pteroyl monoglutamic acid)	52	125	105	282
Biotin (μg)	21	12	20	53
Vitamin C (mg as L-ascorbic acid)	34	25	53	112
Minerals				
Na (mg)	833	1237	1080	3177
K (mg)	594	851	615	2235
Ca (mg)	250	173	96	523
Mg (mg)	47	113	96	257
P (mg)	381	253	317	1032
Fe (mg)	0.8	6.2	3.2	10.2
Zn (mg)	1.9	2.8	4.2	8.9
Cu (mg)	0.15	0.33	0.47	0.95

[1] Water-soluble vitamins except for vitamin B_{12} were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan, fifth revised edition -2000-, Resources Council, Science and Technology Agency, Japan.
[2] The niacin equivalent intake was calculated as follows: The average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

Nicotinamide administration led to a significant increase in the urinary excretion of the nicotinamide metabolites MNA, 2-Py, and 4-Py in a dose-dependent manner (Figure 2). This result means that the exogenous nicotinamide was equally well absorbed and utilized in the body. We did not measure the contents of nicotinamide and NAD in the blood, because the objective of the experiment was simply to know whether or not large amounts of exogenous nicotinamide affect the tryptophan-nicotinamide pathway. For this purpose, the most appropriate method is to measure the metabolites excreted into the urine, as reported previously [11–21, 24]. Therefore, six young women were recruited for our study, and the influence that nicotinamide administration exerted on the production of intermediates in the tryptophan-quinolinic acid pathway, which is in the upper part of the tryptophan-nicotinamide pathway, was examined to measure the urinary excretion of the metabolites. The present data clearly show that excessive nicotinamide intake does not influence the tryptophan-quinolinic acid metabolism. This result means that we can calculate the niacin equivalent regardless of the intake of nicotinamide.

The tryptophan-nicotinamide metabolic pathway is divided into two parts: one involves tryptophan-quinolinic acid metabolism and the other quinolinic acid-nicotinamide metabolism. Cho-Chung *et al* [23] have reported that rat liver tryptophan 2,3-dioxygenase activity, which catalyzes the first irreversible step in the degradative metabolism of tryptophan to nicotinamide, is inhibited by pyridine nucleotide coenzymes in *in vitro* experiments. However, the present study shows that excessive nicotinamide administration does not decrease the formation of intermediates from tryptophan (Figure 3). NAD^+ synthesis from nicotinamide is well regulated by NAD^+ through inhibiting the activity of nicotinamide phosphoribosyltransferase [33], which catalyzes the formation of nicotinamide mononucleotide from nicotinamide and 5-phosphoribosyl-1-pyrophosphate. Therefore, *in vivo*, even when excessive nicotinamide is given, the liver pyridine nucleotide concentrations do not increase, since the cel-

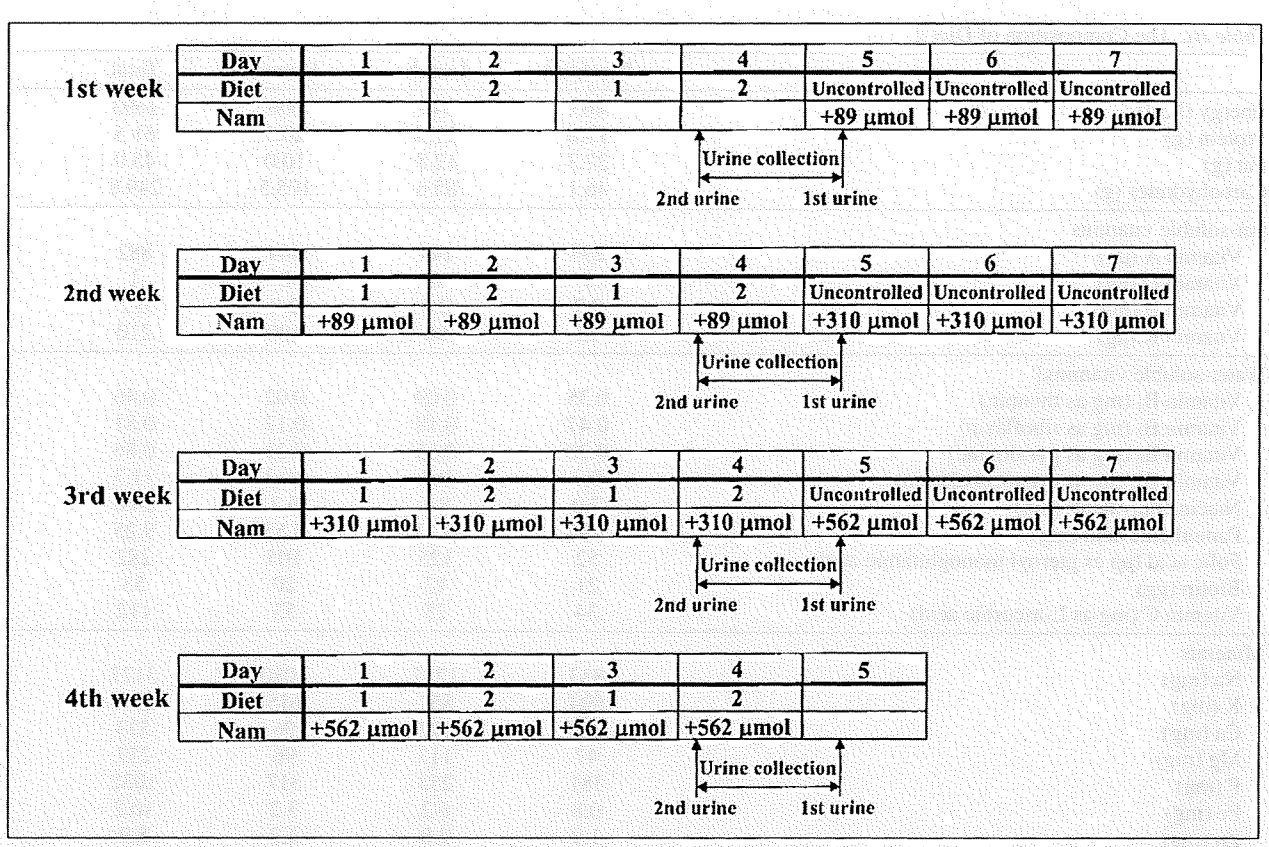

Figure 1: Scheme of the experimental design. Subjects were given the diet and nicotinamide (Nam) as indicated. The compositions of diets 1 and 2 are shown in Tables II and III, respectively. Nicotinamide was divided into a ratio of 3:4:3 and administered daily after breakfast, lunch, and dinner. The 24-hour urine samples were collected from the second urinary sample on the fourth day to the first sample on the fifth day in each week. The urine sample volumes were measured, and 1 mL of 1 mol/L HCl was added to 9 mL urine samples to stabilize the metabolites in the tryptophan-nicotinamide pathway. The acidified urine samples were stored at –20° C until needed.

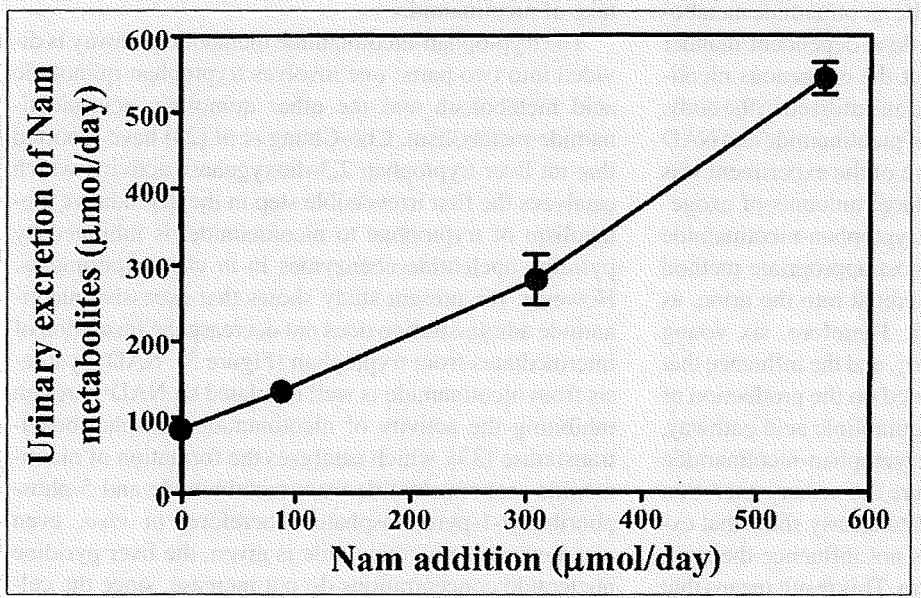

Figure 2: Effect of nicotinamide (Nam) administration on urinary excretion of its metabolites. Values are presented as the mean ± SEM for six subjects. Nam metabolites signify the total amount of nicotinamide metabolites MNA, 2-Py, and 4-Py. The experimental conditions were as outlined in Figure 1 and in the Materials and Methods section.

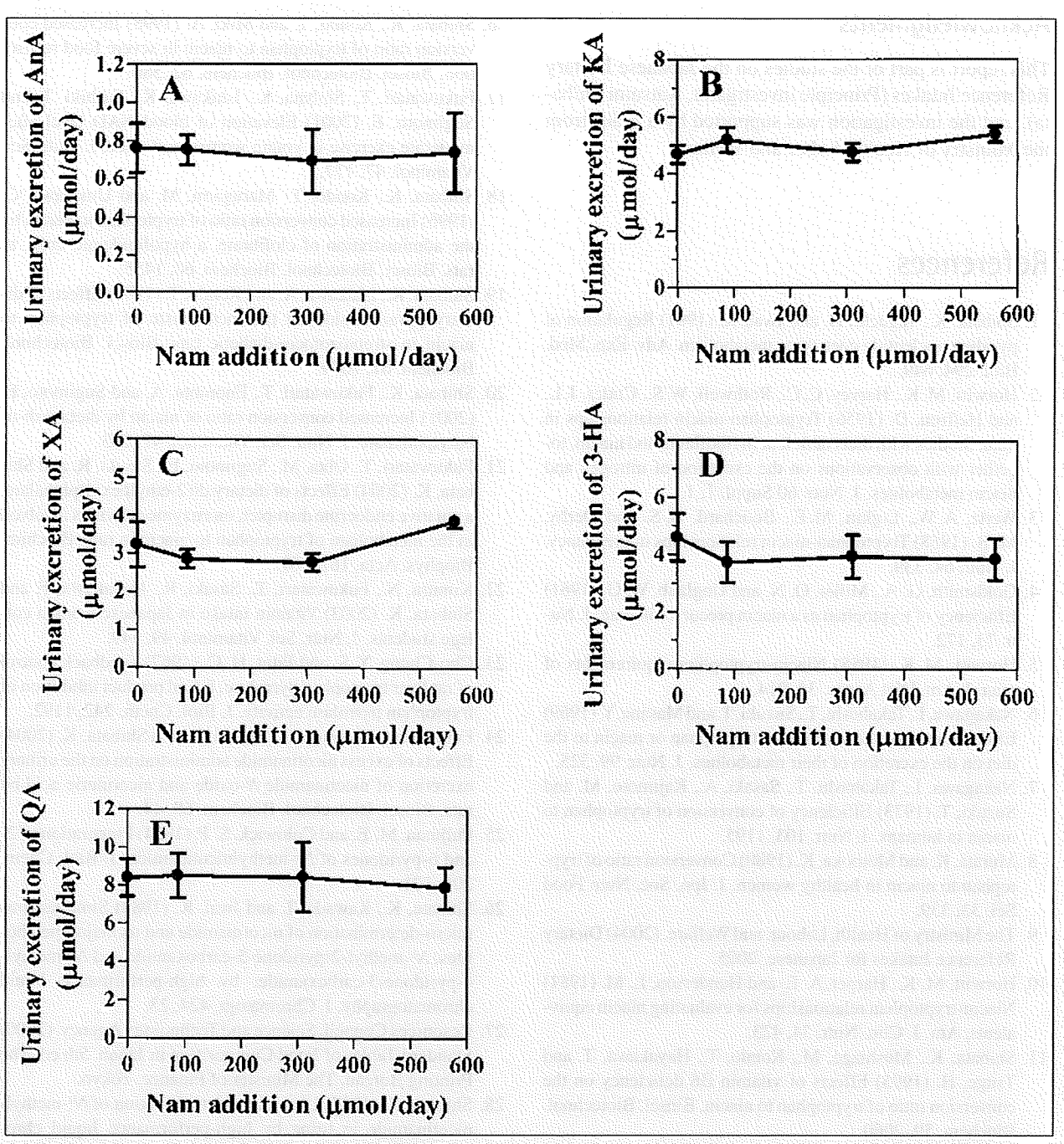

Figure 3: Effect of nicotinamide (Nam) administration on urinary excretion of anthranilic acid (A), kynurenic acid (B), xanthurenic acid (C), 3-hydroxyanthranilic acid (D), and quinolinic acid (E).
Values are presented as the mean ± SEM for six subjects. The experimental conditions were as outlined in Figure 1 and in the Materials and Methods section.

lular pyridine nucleotide coenzyme concentrations are well controlled, as mentioned above.

In conclusion, we have shown that excessive nicotinamide does not influence the conversion pathway of tryptophan to nicotinamide in humans. This result means that the conversion ratio of tryptophan to niacin is not changed with either more or less nicotinamide intake. Thus, the niacin equivalent intake is calculated as usual: niacin intake (mg) = nicotinamide (mg) + nicotinic acid (mg) + 1/60 tryptophan (mg).

Acknowledgments

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Effects of Excess Biotin Administration on the Growth and Urinary Excretion of Water-Soluble Vitamins in Young Rats

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To determine the effects of excess biotin administration on growth and water-soluble vitamin metabolism, weaning rats were fed on a 20% casein diet containing 0.00002% biotin, or same diet with 0.04, 0.08, 0.10, 0.20, 0.50, 0.80 or 1.0% added biotin for 28 days. More than 0.08% biotin administration decreased the food intake and body weight gain compared with the levels in control rats. An accumulation of biotin in such tissues as the liver, brain and kidney increased in a dose-dependent manner, and the both bound and free biotin contents in the liver also increased in a dose-dependent manner. An excess administration of biotin did not affect the urinary excretion of other water-soluble vitamins, suggesting no effect on the metabolism of other water-soluble vitamins. The results of the food intake and body weight gain indicated that the lowest observed adverse effect level for young rats was 79.2 mg/kg body weight/day, while the no observed adverse effect level was 38.4 mg/kg/day. These results suggested immediately setting a tolerable upper intake level for biotin.

Key words: no observed adverse effect level (NOAEL); lowest observed adverse effect level (LOAEL); tolerable upper intake level (UL); urine; blood

Biotin is a water-soluble vitamin classified among the B-group of vitamins. In humans, biotin serves as a coenzyme for four carboxylases: pyruvate, acetyl-CoA, propionyl-CoA, and β-methylcrotonyl-CoA.[1] These carboxylases have important roles in fatty acid synthesis, branched-chain amino acid catabolism, odd-chain fatty acid metabolism, and gluconeogenesis. Although dietary biotin deficiency has not been reported in humans, biotin deficiency has caused growth retardation, alopecia, dermatitis and neurological impairment in experimental animals and humans.[2] In addition, biotin is important in the normal reproductive performance and embryonic growth and development of mammals.[3–5]

Some people have recently been taking 1–10 mg/d of biotin as a medical treatment because biotin has been found to be correlated with certain diseases such as diabetes mellitus,[6,7] liver[8] and skin[9] disorders, neurological abnormality,[10] and epilepsy.[11]

Biotin is a heterocyclic compound, an imidazolidone ring joined to a tetrahydrothiophene ring. The latter possesses a valeric acid side chain. The structure is unique, and biotin is more toxic than would be expected if a repeated excess dosage is administered. Indeed, single or repeated doses of biotin (total doses of 50 and 100 mg/kg body weight by subcutaneous injection) given to rats resulted in irregularities of the estrus cycle[12,13] and fetal and placental resorption in pregnant rats,[13] accompanied by decreased uterine weight, reduced glycogen and protein in the uterus, and reduced protein in the liver. However, these studies cannot be regarded as conclusive for human dietary biotin uptake, because of the route of administration. The administration of oral biotin in doses up to 100 mg/day to patients with holocarboxylase synthetase and biotinidase deficiency has not resulted in adverse effects,[14] although the metabolic defect may prevent or mask toxicity. The Japanese Dietary Reference Intake recommendation presents no data on the tolerable upper intake level (UL) for biotin.[15] Biotin toxicity in healthy humans has not been studied, and performing such a study with the risk of an adverse effect would not be permitted. In the present study, we investigated the effects of excess orally administered biotin on the food intake, body weight gain, tissue weight and water-soluble vitamin metabolism in young rats.

Materials and Methods

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine were purchased from Wako Pure Chemical

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Abbreviations: 4-PIC, 4-pyridoxic acid; Nam, nicotinamide; PaA, pantothenic acid; UL, tolerable upper intake level; MNA, N^1-methylnicotinamide; 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide; NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level