

# Chapter 3

## Nutritional Aspects of Tryptophan Metabolism

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**Abstract** Niacin is biosynthesized from L-tryptophan (Trp). There is a substantial amount of Trp in the body which can be efficiently converted to niacin especially during critical situations. Therefore, it is very important to know the factors affecting the conversion ratio of Trp to niacin. In animal experiments, the conversion ratios decreased with increasing dietary protein levels. Feeding of diets containing unsaturated fatty acids increased the conversion ratio, while feeding of diets with saturated fatty acids did not. In the effects of carbohydrate, the conversion ratio was higher in diets containing starch than in diets containing sucrose. Vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, or vitamin B<sub>6</sub> deficiency affects the metabolism of Trp to niacin. Mineral deficiency also affects the metabolism of Trp to niacin. With regard to hormones, thyroxin increased the Trp to niacin conversion ratio, while estrone, progesterone, and adrenaline decreased. Certain chemicals can also impact niacin synthesis; alloxane and streptozotocin decreased the conversion ratio, while pyrazinamide, phthalates, valproic acid, and clofibrate increased the ratio. Moderate food restriction greatly decreased the conversion. In human experiments, the urinary excretion ratio of anthranilic acid formation from Trp was calculated to be approximately 0.06 % against Trp intake, kynurenic acid 0.13 %, xanthurenic acid 0.11 %, 3-hydroxyanthranilic acid 0.13 %, and quinolinic acid 0.34 % in basal metabolic level. Supplementing healthy women with up to 5.0 g/day of Trp had no adverse effects. Urinary excretion of 3-hydroxykynurenine is a good surrogate biomarker for excess Trp ingestion.

**Keywords** Tryptophan • Niacin • Urine • Nicotinamide • N<sup>1</sup>-methylnicotinamide • N<sup>1</sup>-methyl-2-pyridone-5-carboxamide • N<sup>1</sup>-methyl-4-pyridone-3-carboxamide • Quinolinic acid

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## List of Abbreviations

ACMS	2-Amino-3-carboxymuconate-6-semialdehyde
ACMSD	2-Amino-3-carboxymuconate-6-semialdehyde decarboxylase
AnA	Anthranilic acid
3-HA	3-Hydroxyanthranilic acid
3-HK	3-Hydroxykynurenine
IDO	Indoleamine 2,3-dioxygenase
KA	Kynurenic acid
KYN	Kynurenine
MNA	<i>N</i> <sup>1</sup> -methylnicotinamide
Nam	Nicotinamide
NiA	Nicotinic acid
2-Py	<i>N</i> <sup>1</sup> -methyl-2-pyridone-5-carboxamide
4-Py	<i>N</i> <sup>1</sup> -methyl-4-pyridone-3-carboxamide
PiA	Picolinic acid
QA	Quinolinic acid
TDO	Tryptophan 2,3-dioxygenase
Trp	L-Tryptophan
XA	Xanthurenic acid

## Introduction

The vitamin niacin is biosynthesized from the essential amino acid the L-tryptophan (Trp) (Fig. 3.1), and one defines it as the conversion ratio of Trp to niacin. Over 20 % of enzymes need pyridine nucleotide coenzymes. When the metabolic rate is accelerated, the need of pyridine nucleotide coenzymes concomitantly increase. Such a case, the conversion ratio of Trp to niacin would be increased. Therefore, it is very important to know what affects the conversion ratio of TRP to niacin.

It has been generally accepted in the nutrition field that in the normal state, 60 mg of Trp has equal activity to 1 mg of nicotinamide (Nam) in humans based on several human studies [1–4]. During pregnancy, there is an observed increase in the TRP to niacin conversion ratio [5].

## Analyses

### *Metabolites*

TRP [6] and its metabolites, including kynurenine (KYN) [7], kynurenic acid (KA) [8], anthranilic acid (AnA) [9], 3-hydroxykynurenine (3-HK) [9], xanthurenic acid (XA) [10], 3-hydroxyanthranilic acid (3-HA) [10], 2-oxoadipic acid (2-OAA) [11],

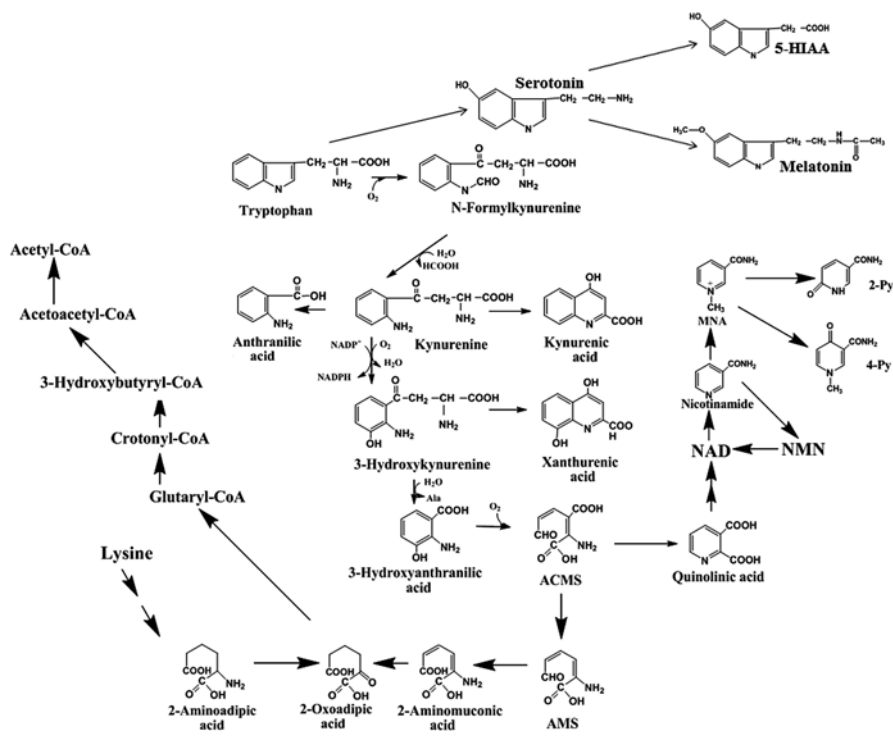


Fig. 3.1 Major metabolic pathway of Trp

quinolinic acid (QA) [12], *N*<sup>1</sup>-methylnicotinamide (MNA) [13], Nam [14], *N*<sup>1</sup>-methyl-2-pyridone-5-carboxamide (2-Py) [14], and *N*<sup>1</sup>-methyl-4-pyridone-3-carboxamide (4-Py) [14], were measured as described.

## Enzymes

Trp 2,3-dioxygenase (TDO) [EC 1.13.11.11] [15], kynureninase [EC 3.7.1.3] [15], kynurenine aminotransferase [EC 2.6.1.7] [15], 3-hydroxyanthranilic acid dioxygenase [EC 1.13.11.6] [15], 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase (ACMSD) [EC 4.1.1.45] [16], and quinolinic acid phosphoribosyltransferase (QPRT) [EC 2.4.2.19] [17] were measured as described.

## Conversion Ratio

The conversion ratio was calculated from the following equation:

$$\text{Conversion ratio} = \frac{(\text{urinary excretion of Nam} + 2 - \text{Py} + 4 - \text{Py}, \text{ mol / day})}{(\text{Trp intake}, \text{ mol / day})} \times 100$$

## Rat Experiment

### Effects of Dietary Protein Levels

The conversion ratio decreased with increasing dietary protein levels (Table 3.1). The activity of ACMSD increased with increasing dietary protein levels [18], while those of the other enzymes did not. The increased ACMSD activity means that ACMS, which is synthesized from Trp, is metabolized into acetyl-CoA but not into niacin. Therefore, the low conversion ratio in the group of high-protein diet might be attributed to the increase in the activity of liver ACMSD.

It is known that niacin itself is not necessary in rats when Trp is given in adequate amounts, because rats can biosynthesize niacin from Trp [19]. The rats fed the 20 % and 40 % casein diets did not require niacin for optimum growth, but the rats fed the 70 % casein diet needed it [20]. This phenomenon was attributed to the supposition that liver ACMSD activity increased according with the dietary casein levels. The conversion ratio in rats fed on the 70 % casein diet became extremely low, and then the rats needed niacin.

### Effects of Dietary Constant Protein Levels but Differing Trp Level

As it had been known that the amounts of Nam increase with increasing intake of Trp, our data [18, 20] conflict common sense for the conversion. The sum total urinary excretion of Nam and its metabolites did not increase as the level of dietary casein increased [21]. While the sum total urinary excretion of Nam and its catabolites

**Table 3.1** Effects of dietary protein levels on the conversion ratio of Trp to niacin and the activity of liver ACMSD

	20 % casein diet	40 % casein diet	70 % casein diet
Conversion ratio of Trp to niacin (%)	1.87 ± 0.27 <sup>c</sup>	1.01 ± 0.03 <sup>b</sup>	0.402 ± 0.03 <sup>a</sup>
ACMSD (μmol/h/g of liver)	1.67 ± 0.03 <sup>a</sup>	2.97 ± 0.33 <sup>a</sup>	11.67 ± 0.67 <sup>b</sup>

Statistical significance was determined by analysis of variance followed by Tukey's multiple comparison tests. Values are mean ± SE for five rats. Mean values in a row with uncommon superscript letter differs,  $p < 0.05$

in the groups fed the 10 % casein-30 % gelatin, 20 % casein-20 % gelatin, and 40 % casein diets were about 5, 7, and 11  $\mu\text{mol/day}$ , respectively, values were significantly different from each other [22]. Therefore, it was found that only when the total protein intake was constant the sum total urinary excretion increases with increasing intake of Trp.

### *Effects of Composition of Amino Acids*

The conversion ratio in the group fed with a preformed niacin-free, 9 % casein-sucrose diet was 3.5 %. The ratio was decreased to 0.2 % by the addition of 2 % glycine, 0.078 % L-threonine, and 0.2 % L-cystine and restored to 3.2 % by the further addition of 0.1 % Trp [23]. The same phenomena were observed in other types of Trp-limiting diets [24]. These results clearly prove that the conversion ratio was lowest when the rats were fed with the Trp-limiting diets. Therefore, we think that the pellagrigenic factor of corn is simply due to a low content of Trp, but the adverse effect is due to a low conversion ratio of Trp to niacin.

The conversion ratio was significantly lower in the groups fed with the 3 % L-leucine-, L-valine-, or L-isoleucine-added diet than in the group fed with the control diet [25]. Namely, the inhibition of the conversion was observed not only by the addition of L-leucine but also by the addition of L-valine or L-isoleucine. The ratio in the group fed with an amino acid diet-simulating rice protein was around 2.5 %. The ratio was decreased by the addition of the limiting amino acids, except for Trp, and increased by the addition of all the limiting amino acids, including Trp [26]. The composition of amino acids proved to greatly affect the conversion ratio.

### *Effects of Dietary Fat*

Feeding of diets containing unsaturated fatty acids increased the conversion ratio, while diets containing saturated fatty acids did not affect it (Table 3.2). The increased conversion ratio in the group fed with unsaturated fatty acids might be attributed to the decreased activity of ACMSD because it is known that feeding of diets containing unsaturated fatty acids inhibits the ACMSD activity but that feeding diets containing saturated fatty acids does not [27].

**Table 3.2** Effects of dietary fat level and the kind of dietary fat on the conversion ratio of Trp to niacin

	20 % casein + 0 % fat diet	20 % casein + 20 % corn oil diet	20 % casein + 20 % soybean diet	20 % casein + 20 % lard diet
Conversion ratio of Trp to niacin (%)	2.27 $\pm$ 0.20 <sup>a</sup>	4.40 $\pm$ 0.57 <sup>b</sup>	4.47 $\pm$ 0.58 <sup>b</sup>	2.66 $\pm$ 0.38 <sup>a</sup>

Statistical significance was determined by analysis of variance followed by Tukey's multiple comparison tests. Values are mean  $\pm$  SE for five rats. Mean values in a row with uncommon superscript letter differs,  $p < 0.05$

**Table 3.3** Effect of dietary carbohydrate on the conversion ratio of tryptophan to niacin

	Starch diet	Sucrose diet
Conversion ratio of Trp to niacin(%)	1.83±0.13	0.80±0.12*

Statistical significance was determined by Student's *t*-test. Values are mean±SE for five rats

\**p*<0.05

### *Effects of Dietary Carbohydrates*

The conversion ratio was higher in diets containing starch than in diets containing sucrose (Table 3.3) [18]. This phenomenon also might be due to the change of the activity of ACMSD.

### *Effects of Dietary Vitamins*

#### **Vitamin B<sub>1</sub>-Free Diet**

We clarified the following two phenomena: (1) severe food restriction gave an increase of the conversion ratio compared to free access control group [28, 29] and (2) the conversion ratio also increased by vitamin B<sub>1</sub> deficiency compared to free access control group [30]. So, another study was set up to clarify whether or not a true reason about an increase in the conversion ratio is vitamin B<sub>1</sub> deficiency or severe food restriction [31].

#### **Vitamin B<sub>2</sub>-Free Diet**

In riboflavin-deficient rats, the activities of 2-Py-forming MNA oxidase and 4-Py-forming MNA oxidase were not detected in vitro [32]. These results show that the two MNA oxidases are FAD-dependent enzymes. On the contrary, the Nam methyltransferase activity was higher in riboflavin-deficient rats than in control rats. Therefore, the resulting excretion ratio of (2-Py + 4-Py/MNA) was greatly lower in the riboflavin-deficient rats than in the control rats.

#### **Vitamin B<sub>6</sub>-Free Diet**

The urinary excretion of KA decreased while that of XA increased drastically in the vitamin B<sub>6</sub>-free diet. The conversion ratio was lower in the vitamin B<sub>6</sub>-free diet than in the vitamin B<sub>6</sub>-containing diet [33].

## ***Effects of Hormones***

### **Thyroxine**

It has been reported that thyroxine increased the activity of kynurenine aminotransferase and reduced the activity of kynurenine 3-monooxygenase, with a resulting increase in the reaction of kynurenine to KA [34]. Traditionally, these changes were taken to mean that thyroxine suppressed the conversion ratio; however, the effect of this hormone on the conversion ratio has not been actually demonstrated. We, therefore, investigated the effect on the conversion of feeding to rats, a preformed niacin-free, 20 % casein diet containing 0.002 % thyroxine [26]. The ratio was statistically higher in hyperthyroid rats than in the control rats. Furthermore, the present data suggests that ACMSD played a critical role in this conversion.

### **Sex Hormones**

It is known that deaths attributable to pellagra, which is considered to be a disease caused by the disturbance of Trp metabolism, have been approximately twofold higher in women than in men [35]. Administration of ovarian hormones significantly decreased the conversion ratio [30, 36]. Feeding a diet containing testosterone had no effect on any parameter [30].

### **Adrenaline**

The administration of glycemia-affecting chemicals such as alloxane [15], streptozotocin [37], and 6-aminonicotinamide [38] decreases the conversion ratio. Adrenaline is also known to increase the glucose level. The conversion ratio was reduced to half by the intraperitoneal injection of adrenalin [39].

### **Prednisolone**

The urinary excretion of Nam, MNA, 2-Py, and 4-Py was each higher in the prednisolone group than in the physiological saline group after 1 day; however, each excretion was lower in the prednisolone group than in the saline group after 3, 4, 5, and 6 days [40]. On around the 7th day, the urinary excretion in the prednisolone group was restored to the initial values. The initial rapid increase of prednisolone decrease in the urinary excretion of Nam and its metabolites by an injection of prednisolone is considered to be attributable to a respective elevation of the liver TDO level and the liver ACMSD level.

## Chemicals Substances

Table 3.4 summarizes the effects of some chemical substances on the metabolism of Trp to niacin.

### Food Restriction

The conversion ratio was lower in both restricted groups than in the ad libitum-fed control group during the experimental period (control group,  $1.37 \pm 0.24$  %; 80 %-restricted group,  $0.20 \pm 0.04$  %; 65 %-restricted group,  $0.15 \pm 0.02$  %). control vs. restricted groups,  $p < 0.01$ ) [29]. Food restriction, even at mild levels, suppressed the conversion ratio compared to the ad libitum-fed control group.

**Table 3.4** Effects of some chemical substances on the metabolism of Trp to niacin

Chemicals	Effects
Alloxane [15]	A marked increase was observed in the activities of TDO, ACMSD, and Nam methyltransferase upon the injection of alloxane; on the other hand, the activities of kynureninase and NAD <sup>+</sup> synthetase were decreased by the injection of alloxane. These changes mean that the conversion ratio is lower in the alloxane diabetic rat than in normal rat
Streptozotocin [37]	Marked increases in the activities of ACMSD and Nam methyltransferase were observed in the streptozotocin-induced diabetic rats, but increased TDO activity was not observed
Pyrazinamide [41, 42]	The administration of pyrazinamide and pyrazinoic acid, antituberculosis drugs, significantly increased the metabolites, 3-HA and beyond, especially QA, Nam, MNA, 2-Py, and 4-Py, and therefore significantly increased the conversion ratio. However, no difference in the upper metabolites of the Trp to Nam pathway such as AnA, KA, and XA was apparent by the addition of pyrazinamide and pyrazinoic acid
Phthalates [7, 43]	Of the phthalate esters with different side chains tested, di(2-ethylhexyl) phthalate (DEHP) and its metabolite, mono(2-ethylhexyl)phthalate (MEHP), most strongly enhanced the production of QA and degradation products of Nam, while 3-HA was unchanged. This pattern of metabolic change led us to assume that these esters lowered ACMSD protein or its activity. Although DEHP could not be tested because of its low solubility, MEHP reversibly inhibited ACMSD from rat liver and mouse kidney, and also the recombinant human enzyme. Correlation between inhibition of ACMSD by phthalate esters with different side chains and urinary excretion of QA supports the notion that phthalate esters perturb Trp metabolism by inhibiting ACMSD
Valproic acid [44]	Valproic acid is a short-chained, branched fatty acid that is widely used in humans as an anticonvulsant and mood stabilizer. The conversion ratio was increased by feeding a diet containing valproic acid. Of the intermediates formed during the conversion of Trp to Nam, the Trp to 3-HA step was not affected by the administration of valproic acid, while such metabolites beyond QA as Nam and its catabolites were significantly increased
Clofibrate [45]	Clofibrate enhanced the conversion ratio without any side effects and supports again the claim that the activity of ACMSD exerts a critical influence on the conversion ratio



## ***Loading***

### **Tryptophan**

Young rats were fed on a 20 % casein diet with 0, 0.5, 1.0, 2.0, or 5.0 % added Trp for 30 days [46]. The apparent toxicity and growth retardation was observed in the 5.0 % Trp-added group. Metabolites of the Trp-Nam pathway such as AnA, KA, XA, 3-HA, and QA in urine increased in a dose-dependent manner. Of these metabolites, urine KA progressively increased, and urine AnA dramatically increased in the 2.0 and 5.0 % Trp-added groups. The urine ratio of AnA/KA is a useful index to monitor an excessive Trp intake.

### **Quinolinic Acid**

Dietary QA had no adverse effects up to 35 mg/100 g of diet. Dietary QA is approximately 1/9 as active as niacin in growing rats [47].

### **Picolinic Acid**

Picolinic acid (PiA) is an endogenous metabolite of Trp that has been reported to possess a wide range of physiological actions. Toxicity of PiA was higher compared with analogs such as nicotinic acid and QA. Feeding an ordinary diet containing 0.05 % and 0.1 % PiA did not elicit decreased intake of food or loss in body weight. PiA did not affect the *in vitro* liver activities of QPRT and ACMSD, a Zn-dependent enzyme. PiA administration did not affect Trp metabolites such as AnA, KA, and XA. However, QA and subsequent metabolites such as Nam and its catabolites were increased by administration of a diet containing 0.05 % PiA but not by a 0.1 % PiA diet. These results suggest that the *in vivo* activity of ACMSD is controlled by Zn level [48].

## **Human Experiments**

### ***Basal Metabolic Fate***

Fate of dietary Trp in humans was investigated [49]. The urinary excretion amounts of Trp, kynurenine, AnA, KA, 3-HK, XA, 3-HA, and QA were about 40, 20, 4, 1, 10, 4, 3, 5, and 20  $\mu\text{mol/day}$ , respectively. In this experiment, Trp was ingested at levels of 674 mg/day or 3300  $\mu\text{mol/day}$ . The percentage of AnA formation from Trp was calculated to be approximately 0.06 %, KA 0.13 %, XA 0.11 %, 3-HA 0.13 %, and QA 0.34 %.

## ***Tryptophan Loading***

Healthy Japanese women were randomly assigned to receive placebo (0 g/day) or 1.0, 2.0, 3.0, 4.0, or 5.0 g/day of Trp for 21 days each with a 5-week washout period between trials [50]. Food intake, body weight, general biomarkers in blood and urine, and amino acid composition in blood and urine were not affected by any dose of Trp. Administration of up to 5.0 g/day Trp had no effect on a profile of mood states category measurement. The urinary excretion of NAM and its catabolites increased in proportion to the ingested amounts of Trp, indicating that participants could normally metabolize this amino acid. The urinary excretion of Trp metabolites, including kynurenine, AnA, KA, 3-HK, 3-HK, and QA, all of which are intermediates of the Trp–kynurenine–QA pathway, was in proportion to Trp loading. The response of 3-HK was the most characteristic of these Trp metabolites. This finding suggests that the urinary excretion of 3-HK is a good surrogate biomarker for excess Trp ingestion.

## ***Nicotinamide Loading***

To determine if *de novo* Nam synthesis from Trp is influenced by Nam intake itself, young women consumed controlled diets containing 0, 10.9, 37.8, or 68.6 mg/day respectively, and urinary excretion of Trp metabolites were measured [51]. None of the intermediates, including AnA, KA, XA, 3-HA, and QA, changed at all. That is, exogenous Nam did not affect *de novo* Nam synthesis.

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## **References**

1. Horwitt MK, Harvey C, Rothwell W, Cutler J, Haffron D. Tryptophan-niacin relationships in man studies with diets deficient in riboflavin and niacin, together with observations on the excretion of nitrogen and niacin metabolites. *J Nutr.* 1956;60(1 Suppl):1–43.
2. Goldsmith GA, Miller ON, Unglaub WG. Efficiency of tryptophan as a niacin precursor in man. *J Nutr.* 1961;73(2):172–6.
3. Nakagawa I, Takahashi T, Sasaki A, Kajimoto M, Suzuki T. Efficiency of conversion of tryptophan to niacin in humans. *J Nutr.* 1973;103(8):1195–9.
4. Fukuwatari T, Ohta M, Kimtjra N, Sasaki R, Shibata K. Conversion ratio of tryptophan to niacin in Japanese women fed a purified diet conforming to the Japanese dietary reference intakes. *J Nutr Sci Vitaminol.* 2004;50(6):385–91.

5. Wertz AW, Lojkin ME, Bouchard BS, Derby MB. Tryptophan-niacin relationships in pregnancy. *J Nutr.* 1958;64(3):339–53.
6. Shibata K, Onodera M, Aihara S. High-performance liquid chromatographic measurement of tryptophan in blood, tissues, urine, and foodstuffs with electrochemical and fluorometric detections. *Agric Biol Chem.* 1991;55(6):1475–81.
7. Fukuwatari T, Ohsaki S, Fukuoka S, Sasaki R, Shibata K. Phthalate esters enhance quinolinate production by inhibiting alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase (ACMSD), a key enzyme of the tryptophan pathway. *Toxicol Sci.* 2004;81(2):302–8. doi:[10.1093/toxsci/kfh204](https://doi.org/10.1093/toxsci/kfh204).
8. Shibata K. Fluorimetric micro-determination of kynurenic acid, an endogenous blocker of neurotoxicity, by high-performance liquid chromatography. *J Chromatogr.* 1988;430(2):376–80.
9. Shibata K, Onodera M. Measurement of 3-hydroxyanthranilic acid and anthranilic acid in urine by high-performance liquid chromatography (analytical chemistry). *Agric Biol Chem.* 1991;55(1):143–8.
10. Shibata K, Onodera M. Simultaneous high-performance liquid chromatographic measurement of xanthurenic acid and 3-hydroxyanthranilic acid in urine. *Biosci Biotechnol Biochem.* 1992;56(6):974.
11. Shibata K, Yasui M, Sano M, Fukuwatari T. Fluorometric determination of 2-oxoadipic acid, a common metabolite of tryptophan and lysine, by high-performance liquid chromatography with pre-chemical derivatization. *Biosci Biotechnol Biochem.* 2011;75(1):185–7. doi:[10.1271/bbb.100723](https://doi.org/10.1271/bbb.100723).
12. Mawatari K, Oshida K, Iinuma F, Watanabe M. Determination of quinolinic acid by liquid chromatography with fluorimetric detection. *Adv Exp Med Biol.* 1996;398:697–701.
13. Shibata K. Ultramicro-determination of N<sup>1</sup>-methylnicotinamide in urine by high-performance liquid chromatography. *Vitamins.* 1987;61:599–604.
14. Shibata K, Kawada T, Iwai K. Simultaneous micro-determination of nicotinamide and its major metabolites, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide, by high-performance liquid chromatography. *J Chromatogr.* 1988;424(1):23–8.
15. Shibata K. Tryptophan-niacin metabolism in alloxan diabetic rats and partial prevention of alloxan diabetes by nicotinamide (biological chemistry). *Agric Biol Chem.* 1987;51(3):811–16.
16. Ichiyama A, Nakamura S, Kawai H, Honjo T, Nishizuka Y, Hayaishi O, et al. Studies on the metabolism of the benzene ring of tryptophan in mammalian tissues. II. Enzymic formation of alpha-aminomuconic acid from 3-hydroxyanthranilic acid. *J Biol Chem.* 1965;240:740–9.
17. Shibata K, Fukuwatari T, Sugimoto E. Reversed-phase high-performance liquid chromatography of nicotinic acid mononucleotide for measurement of quinolinate phosphoribosyltransferase. *J Chromatogr B Biomed Sci Appl.* 2000;749(2):281–5.
18. Shibata K. Effects of protein-amino acids, lipid, and carbohydrate on the conversion ratio of tryptophan to niacin. *Vitamins (in Japanese).* 1996;70:369–82.
19. Krehl WA, Teplý LJ, Elvehjem CA. Corn as an etiological factor in the production of a nicotinic acid deficiency in the rat. *Science.* 1945;101(2620):283. doi:[10.1126/science.101.2620.283](https://doi.org/10.1126/science.101.2620.283).
20. Kimura N, Fukuwatari T, Sasaki R, Shibata K. The necessity of niacin in rats fed on a high protein diet. *Biosci Biotechnol Biochem.* 2005;69(2):273–9. doi:[10.1271/bbb.69.273](https://doi.org/10.1271/bbb.69.273).
21. Shibata K, Nomamoto R, Iwai K. Effect of dietary protein levels on the urinary excretion of nicotinamide and its metabolites in rats (Food & Nutrition). *Agric Biol Chem.* 1988;52(7):1765–9.
22. Shibata K, Matsuo H. Effect of dietary tryptophan levels on the urinary excretion of nicotinamide and its metabolites in rats fed a niacin-free diet or a constant total protein level. *J Nutr.* 1990;120(10):1191–7.
23. Shibata K. Conversion ratio of tryptophan to niacin in rats fed with a nicotinic acid-free, tryptophan-limiting diet. *Biosci Biotechnol Biochem.* 1995;59(4):715–16.
24. Shibata K, Shimada H, Kondo T. Effects of feeding tryptophan-limiting diets on the conversion ratio of tryptophan to niacin in rats. *Biosci Biotechnol Biochem.* 1996;60(10):1660–6. doi:[10.1271/bbb.60.1660](https://doi.org/10.1271/bbb.60.1660).

25. Shibata K, Taniguchi I, Onodera M. Effect of adding branched-chain amino acids to a nicotinic acid-free, low-protein diet on the conversion ratio of tryptophan to nicotinamide in rats. *Biosci Biotechnol Biochem.* 1994;58(5):970–1.
26. Shibata K, Toda S. Effects of thyroxine on the conversion ratio of tryptophan to nicotinamide in rats. *Biosci Biotechnol Biochem.* 1994;58(10):1757–62.
27. Shibata K, Onodera M. Changes in the conversion rate of tryptophan-nicotinamide according to dietary fat and protein levels. *Biosci Biotechnol Biochem.* 1992;56(7):1104–8.
28. Shibata K, Kondo T, Miki A. Increased conversion ratio of tryptophan to niacin in severe food restriction. *Biosci Biotechnol Biochem.* 1998;62(3):580–3. doi:10.1271/bbb.62.580.
29. Shibata K, Nakata C, Fukuwatari T. Moderate food restriction suppresses the conversion of L-tryptophan to nicotinamide in weaning rats. *Biosci Biotechnol Biochem.* 2014;78(3):478–81. doi:10.1080/09168451.2014.890025.
30. Shibata K, Toda S. Effects of sex hormones on the metabolism of tryptophan to niacin and to serotonin in male rats. *Biosci Biotechnol Biochem.* 1997;61(7):1200–2. doi:10.1271/bbb.61.1200.
31. Shibata K, Kobayashi R, Fukuwatari T. Vitamin B<sub>1</sub> deficiency inhibits the increased conversion of tryptophan to nicotinamide in severe food-restricted rats. *Biosci Biotechnol Biochem.* 2014;1–6. doi:10.1080/09168451.2014.962473.
32. Shibata K. The catabolism of nicotinamide in riboflavin-deficient rats. *Vitamins (in Japanese).* 1990;64:589–95.
33. Shibata K, Mushiage M, Kondo T, Hayakawa T, Tsuge H. Effects of vitamin B<sub>6</sub> deficiency on the conversion ratio of tryptophan to niacin. *Biosci Biotechnol Biochem.* 1995;59(11):2060–3. doi:10.1271/bbb.59.2060.
34. Okamoto H, Okada F, Hayaishi O. Kynurenine metabolism in hyperthyroidism. A biochemical basis for the low NAD(P) level in hyperthyroid rat liver. *J Biol Chem.* 1971;246(24):7759–63.
35. Miller DF. Pellagra deaths in the United States. *Am J Clin Nutr.* 1978;31(4):558–9.
36. Shibata K, Kondo T. Effect of progesterone and estrone on the conversion of tryptophan to nicotinamide in rats. *Biosci Biotechnol Biochem.* 1993;57:1890–3.
37. Shibata K. Tryptophan-NAD metabolism in streptozotocin diabetic rats. *Agric Biol Chem.* 1988;52:1993–8.
38. Shibata K, Ogawa A, Taniguchi I. Effects of dietary 6-aminonicotinamide, an antagonist of nicotinamide, on the metabolism of tryptophan to nicotinamide in rats. *Biosci Biotechnol Biochem.* 1994;58(4):727–33.
39. Shibata K. Effects of adrenalin on the conversion ratio of tryptophan to niacin in rats. *Biosci Biotechnol Biochem.* 1995;59(11):2127–9.
40. Shibata K. Effect of prednisolone on the urinary excretion of nicotinamide and its metabolites in rats fed with a niacin-free diet. *Agric Biol Chem.* 1990;54(5):1195–200.
41. Shibata K. Effects of pyrazinamide on tryptophan-niacin conversion in rats (Biological Chemistry). *Agric Biol Chem.* 1990;54(9):2463–4.
42. Fukuwatari T, Sugimoto E, Shibata K. Growth-promoting activity of pyrazinoic acid, a putative active compound of antituberculosis drug pyrazinamide, in niacin-deficient rats through the inhibition of ACMSD activity. *Biosci Biotechnol Biochem.* 2002;66(7):1435–41. doi:10.1271/bbb.66.1435.
43. Shibata K, Fukuwatari T, Enomoto A, Sugimoto E. Increased conversion ratio of tryptophan to niacin by dietary di-n-butylphthalate. *J Nutr Sci Vitaminol.* 2001;47(3):263–6.
44. Shibata K, Kondo R, Sano M, and Fukuwatari T. Dietary valproate increases the conversion of tryptophan to nicotinamide in rats. *Biosci Biotechnol Biochem.* 2013;77(2):295–300.
45. Shibata K, Kondo T, Marugami M, Umezawa C. Increased conversion ratio of tryptophan to niacin by the administration of clofibrate, a hypolipidemic drug, to rats. *Biosci Biotechnol Biochem.* 1996;60(9):1455–9. doi:10.1271/bbb.60.1455.
46. Okuno A, Fukuwatari T, Shibata K. Urinary excretory ratio of anthranilic acid/kynurenic acid as an index of the tolerable amount of tryptophan. *Biosci Biotechnol Biochem.* 2008;72(7):1667–72. doi:10.1271/bbb.70630.

47. Shibata K, Tanaka K, Murata K. Efficiency of exogenous quinolinic acid as niacin in rats (Food & Nutrition). *Agric Biol Chem.* 1986;50(8):2025–32.
48. Shibata K, Fukuwartari T. Large amounts of picolinic acid are lethal but small amounts increase the conversion of tryptophan-nicotinamine in rats. *J Nutr Sci Vitaminol.* 2014;60:335–40.
49. Hiratsuka C, Fukuwatari T, Shibata K. Fate of dietary tryptophan in young Japanese women. *Int J Tryptophan Res.* 2012;5:33–47. doi:[10.4137/IJTR.S10497](https://doi.org/10.4137/IJTR.S10497).
50. Hiratsuka C, Fukuwatari T, Sano M, Saito K, Sasaki S, Shibata K. Supplementing healthy women with up to 5.0 g/d of L-tryptophan has no adverse effects. *J Nutr.* 2013;143(6):859–66. doi: [10.3945/jn.112.173823](https://doi.org/10.3945/jn.112.173823).
51. Fukuwatari T, Shibata K. Effect of nicotinamide administration on the tryptophan-nicotinamide pathway in humans. *Int J Vitam Nutr Res.* 2007;77(4):255–62.