Conversion Ratio of Tryptophan to Niacin in Japanese Women Fed a Purified Diet Conforming to the Japanese Dietary Reference Intakes

Tsutomu FUKUWATARI, Mari OHTA, Naoko KIMURA, Ryuzo SASAKI and Katsumi SHIBATA

Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, 2500 Hassakacho, Hikone, 522-8533 Japan
(Received February 3, 2004)

Summary In order to establish the human requirements of niacin, it is first important to know how much tryptophan is converted to niacin in the human body. In general, 60 mg of tryptophan is equivalent to 1 mg of niacin, whereas the conversion ratio of tryptophan to niacin is yet to be confirmed. The aim of this study was to know the conversion ratio of tryptophan to niacin in Japanese females fed a purified diet, which followed the Japanese Dietary Reference Intakes. Ten young Japanese females were housed in the same facility and given the same daily living activity schedule for 7 d. The composition of their purified diet was conformed to the Dietary Reference Intakes in Japan. The diet was niacin free. In order to investigate the conversion ratio, daily urinary outputs were collected. Tryptophan-niacin metabolites in the urine were measured and the conversion ratio of tryptophan to niacin calculated. The conversion ratio was calculated by comparing the dietary intake of tryptophan and the sum of the niacin catabolites such as N1-methylnicotinamide, N1-methyl-2-pyridone-5-carboxamide, and N1-methyl-4-pyridone-3-carboxamide, which were derived only from the dietary intake of tryptophan. The ratio was calculated as 1.5 ± 0.1 (mean ± SE for 10 women; in molar basis) on the last day of the experiment. It was calculated that if the excretory percentage of niacin metabolites in the urine were 60% of the tryptophan ingested, the conversion factor would be a value of 67, meaning that is 67 mg of tryptophan is equal to 1 mg of niacin.

Key Words conversion ratio, conversion factor, tryptophan-niacin, N1-methylnicotinamide, female human subjects

Over 80 years have passed since it was remarked that tryptophan could cure pellagra; it was first suggested by Goldberger and Wheeler in 1920 (1). In 1945, Krehl et al. (2) revealed that a pronounced growth-retarding feature of rats fed a diet containing corn was prevented by the supplements of suitable amounts of tryptophan. Furthermore, they revealed that 1 mg of nicotinic acid in 100 g of diet could be replaced by 50 mg of tryptophan. In 1947, Perlzweig et al. (3) reported that the ingestion of tryptophan by both infants and adults led to a prompt and marked increase in urinary excretions of the nicotinamide metabolite N1-methylnicotinamide (MNA). The first clear evidence that tryptophan could cure pellagra was reported by Goldsmith et al. (4) in 1952.

Estimations of how much tryptophan is convertible to nicotinamide are very important in deciding the niacin requirements of humans. In an experiment in the USA in 1956, Horwitt et al. (5) showed that approximately 60 mg of tryptophan in men is equivalent to 1 mg of niacin. This was calculated through comparisons of the level of MNA excretion with different levels of niacin and tryptophan intake. In their report, it was noted that the variation was quite large whilst group conversion rate averages varied between 46 to 1 and 86 to 1, depending on the length of the depletion and supplementation periods.

Increased rates of excretion of certain metabolites during human pregnancy has been reported by several investigators; for example, Wertz et al. (6) reported that during late pregnancy the amount of tryptophan equivalent to 1 mg of niacin varied from 7 to 30 mg (mean 18 mg). Goldsmith et al. (7) reported that an average of 3.3% of administered tryptophan was converted to niacin, or expressed in another way, they revealed that 55.8 mg of tryptophan was equivalent to 1 mg of niacin by comparing the excretion of niacin metabolites MNA and N1-methyl-2-pyridone-5-carboxamide (2-Py) after administration of tryptophan. In their report, it was also noted that the conversion ratio varied considerably among individual subjects: from 35 to 86 mg of tryptophan was found to be equivalent to 1 mg of niacin. These reports indicate high levels of variation in conversion ratio values. It has been suggested that it is not reasonable to assume the existence of an inflexible relationship between tryptophan and niacin consumption, which could be maintained through genetic and dietary variations (8). On the other hand, Horwitt also expounded that it was, however, important to elucidate some estimation of the relationships between tryp-
For breakfast and supper, 139 g of the above powder mixture was added to 91 mL of water, mixed well, and baked for 9 min at 250°C. The weight of the baked meal was 175 g. The meal and 0.3 g of the vitamin mixture (the composition is shown below) were supplied to the subjects. For lunch, 185.5 g of the above mixture was added to 122 mL of water, mixed well, and which was baked for 10 min at 250°C. The weight of the baked meal was 233 g. The meal and 0.4 g of the vitamin mixture (the composition is shown below) were supplied to the subjects.

The composition of the mineral mixture: 1,100 mg of CaHPO₄·2H₂O, 860 mg of CaCO₃, 2,200 mg of KH₂PO₄, 3,500 mg of KHCO₃, 2,100 mg of MgCl₂·2H₂O, 60 mg of FeSO₄·7H₂O, 13 mg of MnSO₄·5H₂O, 19 mg of ZnCl₂, 6.3 mg of CuSO₄·5H₂O, 0.2 mg of KI, and 8,142 mg of NaCl.

The composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 µg of cholecaldiferol, 4.47 mg of dl-α-tocopherol (5 mg was supplied from oils), 13 µg of phylloquinone, 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.4 µg of cyanocobalamine, 5.5 mg of calcium pantothenate, 200 µg of pteroylmonoglutamic acid, 30 µg of D(+)-biotin, 100 mg of ascorbic acid, and to make up 1 g with sucrose.

### Table 1. The composition of the diets.

<table>
<thead>
<tr>
<th></th>
<th>(g/d)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free milk casein</td>
<td>39.5</td>
<td>The casein contains 87.5% protein, so the net protein amount is 34.6 g. Tryptophan content is 1.3%, so 449.3 mg of tryptophan is supplied.</td>
</tr>
<tr>
<td>Gluten</td>
<td>25.0</td>
<td>The gluten contains 81.6% protein, so the net protein amount is 20.4 g. Tryptophan content is 1.1%, so 224.4 mg of tryptophan is supplied.</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>274</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Fats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.1</td>
<td>S : M : P = 3 : 4 : 3</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>13.8</td>
<td>n -6 : n -3 = 4 : 1</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Lard</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Dietary fiber</td>
<td></td>
<td>The soluble dietary fiber used was “Fibersol” obtained from Matsutani Chemical Industry Co., Ltd. (Osaka, Japan), and the insoluble dietary fiber used was ramie powder obtained from Tosco Co., Ltd. (Tokyo, Japan).</td>
</tr>
<tr>
<td>Soluble</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Insoluble</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>18.0</td>
<td>The composition is shown below.</td>
</tr>
<tr>
<td>Total amount</td>
<td>463.5</td>
<td></td>
</tr>
</tbody>
</table>

For breakfast and supper, 139 g of the above powder mixture was added to 91 mL of water, mixed well, and baked for 9 min at 250°C. The weight of the baked meal was 175 g. The meal and 0.3 g of the vitamin mixture (the composition is shown below) were supplied to the subjects. For lunch, 185.5 g of the above mixture was added to 122 mL of water, mixed well, and which was baked for 10 min at 250°C. The weight of the baked meal was 233 g. The meal and 0.4 g of the vitamin mixture (the composition is shown below) were supplied to the subjects.

The composition of the mineral mixture: 1,100 mg of CaHPO₄·2H₂O, 860 mg of CaCO₃, 2,200 mg of KH₂PO₄, 3,500 mg of KHCO₃, 2,100 mg of MgCl₂·2H₂O, 60 mg of FeSO₄·7H₂O, 13 mg of MnSO₄·5H₂O, 19 mg of ZnCl₂, 6.3 mg of CuSO₄·5H₂O, 0.2 mg of KI, and 8,142 mg of NaCl.

The composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 µg of cholecaldiferol, 4.47 mg of dl-α-tocopherol (5 mg was supplied from oils), 13 µg of phylloquinone, 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.4 µg of cyanocobalamine, 5.5 mg of calcium pantothenate, 200 µg of pteroylmonoglutamic acid, 30 µg of D(+)-biotin, 100 mg of ascorbic acid, and to make up 1 g with sucrose.

### SUBJ ECTS AND METHODS

#### Subjects

Ten Japanese female college subjects aged 20.9 ± 0.7 y old (mean ± SE), 161.8 ± 1.4 cm tall, weighing 55.7 ± 1.7 kg at the beginning of the experiment, and 55.6 ± 1.5 kg at the end of the experiment were selected. This study was reviewed and approved by The Ethical Committee of National Institute of Health and Nutrition in Japan.

#### Diet and experimental design

All subjects were housed in the same facility for 9 d (March 3rd to March 11th, 2003). The experiment started at 06:00 on March 4th and ended at 06:00 on March 11th. Day 1 started at 06:00 on March 4th and ended at 06:00 on March 5th. The last experimental day, day 7, started at 06:00 on May 10th and ended at 06:00 on May 11th. Urine samples from the 24 h periods (06:00–06:00) were collected daily. The collected urine was immediately acidified through the addition of conc. HCl to make the final concentration 0.1 mol/L, the urine volume was measured, and the samples stored at −20°C until needed. Blood was taken from a venous vein of the forearm and tested immediately for the total quantities of nicotinamide (13), NAD (14), and NADP (15), respectively.

The daily schedule was partly restricted: lights were turned off at 22:00 and turned on at 06:00; breakfast
Conversion Ratio of Tryptophan to Niacin was from 08:00-09:00, lunch from 12:30-13:10, and dinner from 18:30-19:00. The composition of the semi-purified diet and the amounts are shown in Table 1. The total energy was approximately 1,800 kcal/d, the total protein was approximately 55 g/d, and the total fat energy ratio was 25%. The diet contained 674 mg of tryptophan per day (approximately 3.3 mmol; 0.674/204 = 3.3 × 10⁻³ mol), this was calculated by assuming that the protein content of the casein and gluten obtained from Wako Pure Chemical Industries (Osaka, Japan) were 87.5% and 81.6%, respectively whilst the tryptophan contents of the casein and gluten were 1.3% and 1.1%, respectively. The time the subjects were awake and asleep were not monitored. Body weight and height were measured daily before breakfast. The subjects attended lectures throughout days 1 to 7 from 09:00-12:10 and 13:10-18:00.

Chemicals. Vitamin-free milk casein, gluten, cornstarch, sucrose, vitamins, quinolinic acid (QA) and anthranilic acid (AnA) were purchased from Wako Pure Chemical Industries. Kynurenine sulfate, MNA chloride, xanthurenic acid (XA), kynurenic acid (KA), 3-hydroxyanthranilic acid (3-HA) were obtained from Tokyo Kasai Kogyo (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick (16) and Shibata et al. (17). Other chemicals used were of the highest purity available from commercial sources.

Analyses. Urinary MNA was measured using the HPLC method of Shibata (18). The 2-Py and 4-Py contents of the urine were simultaneously measured using the HPLC method of Shibata et al. (17). The contents of KA (19), XA (20), 3-HA (20), AnA (21), and QA (22) in the urine were measured using the HPLC methods. The total nicotinamide (free nicotinamide + NAD + NADP) content in the whole blood was measured using the HPLC method of Shibata et al. (17). The NAD (NAD⁺ + NADH) (13) and NADP (NADP⁺ + NADPH) (14) contents were measured using respective enzyme cycling methods.

RESULTS

Niacin content of the blood
When humans are well nourished, the functioning of the body is kept constant. The total nicotinamide content of human whole blood is controlled at levels of 50–80 nmol/mL, NAD 25–40 nmol/mL, and NADP 8–15 nmol/mL (23). In this experiment, the total amounts of nicotinamide, NAD, and NADP were within the normal ranges (Table 2).

Urinary excretions of the upper metabolites of tryptophan-niacin metabolism

Table 2. Blood levels of total nicotinamide, NAD, and NADP in Japanese young women fed on the purified diet for 7 d.

<table>
<thead>
<tr>
<th>Value (nmol/mL of whole blood)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nicotinamide</td>
<td>68.4 ± 2.2</td>
</tr>
<tr>
<td>(NAD + NADP + free nicotinamide)</td>
<td>35.3 ± 1.4</td>
</tr>
<tr>
<td>NAD (NAD⁺ + NADH)</td>
<td>9.8 ± 0.3</td>
</tr>
<tr>
<td>NADP (NADP⁺ + NADPH)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects.

![Fig. 1. Mean daily changes in the urinary excretions of AnA (A), KA (B), XA (C), 3-HA (D), and QA (E) in Japanese females fed a semi-purified diet. Each point was mean ± SE for 10 subjects. They were fed a daily diet containing 674 mg of tryptophan and 0 mg of niacin.](image)
Fig. 2. Metabolic pathway of tryptophan to niacin. (1) Tryptophan 2,3-dioxygenase, (2) formylase, (3) kynurenine 3-hydroxylase, (4) 3-hydroxykynureninase, (5) kynurenine aminotransferase, (6) 3-hydroxyanthranilic acid oxygenase, (7) aminocarboxymuconate semialdehyde decarboxylase, (8) non-enzymatic, (9) quinolinic acid phosphoribosyltransferase, (10) NMN (or NAMN) adenyllytransferase, (11) NAD synthase, (12) NAD kinase, (13) nicotinamide phosphoribosyltransferase, (14) NAD glycohydrolase, (15) nicotinamide methyltransferase, (16) MNA oxidase (2-Py-forming), (17) MNA oxidase (4-Py-forming). ACMS: α-amino-β-carboxymuconate-semialdehyde, AMS: α-aminomuconate-semialdehyde.
Conversion Ratio of Tryptophan to Niacin

Fig. 3. Mean daily changes in the urinary excretions of MNA (A), 2-Py (B), and 4-Py (C) in Japanese females fed a semi-purified diet. Each point is the mean±SE for 10 subjects. They were fed a daily diet containing 674 mg of tryptophan and 0 mg of niacin.

Fig. 4. Daily changes in the conversion ratios (A) and the conversion factors (B) of tryptophan to niacin observed in Japanese females fed a semi-purified diet. Each point is the mean±SE for 10 subjects. They were fed a daily diet containing 674 mg of tryptophan and 0 mg of niacin.

XA, 3-HA, and QA collected during the experiment. As is shown in Fig. 2, these metabolites come only from the tryptophan. Each upper metabolite found in the urine remained at a relatively constant level during the experiment, although the subjects ate freely in the pre-experimental period. These findings may indicate that the subjects ate approximately similar amounts of tryptophan during the pre-experiment days. In this experiment, tryptophan was ingested at levels of 674 mg/d or 3,300 μmol/d. On the last day, day 7, the percentage of AnA formation from tryptophan was calculated to be approximately 0.06%, KA 0.13%, XA 0.11%, 3-HA 0.13%, and QA 0.34%.

Urinary excretions of the lower metabolites of tryptophan-niacin metabolism

Figure 3 shows the urinary excretions of MNA, 2-Py, and 4-Py collected during the experiment. The lower metabolites gradually decreased until day 4, but remained more constant from day 4 to day 7. In this experiment, these metabolites came only from the ingested tryptophan because no niacin was contained in the controlled diet fed to the subjects. However, the higher values of the sum (MNA+2-Py+4-Py) in the early days, days 1, 2, and 3, may suggest the effect of ingested niacin during the pre-experiment days.

Conversion ratio of and conversion factor for tryptophan to niacin

Figure 4A shows the conversion ratio of tryptophan to niacin during the experiment. The conversion ratio gradually decreased until day 4 after which they remained at a constant level. This data may indicate that the niacin stored during the pre-experiment days may have become exhausted over the final 3 d. Therefore, the data from days 1, 2, and 3 may not reflect the true conversion ratio of tryptophan to niacin. The sum of the urinary excretion of MNA, 2-Py, and 4-Py on the last day, day 7, was 48.2±3.9 μmol/d (mean±SE for 10 data). The conversion ratio, which was calculated using the following equation: \( \text{Conversion ratio} = \frac{(\text{MNA}+2\text{-Py}+4\text{-Py})}{(\text{tryptophan intake} \times 3,300 \text{ μmol/d}) \times 100} \), was 1.5±0.1%. Urinary excretions of nicotinamide were very small, <300 nmol/d, which is around the limit for detection with the methods used (17). Therefore, the nicotinamide values were not included when calculating the conversion ratio.

Generally, in nutritional fields, the amount of tryptophan equivalent to 1 mg of niacin is used. Thus, the conversion factor for tryptophan to niacin was calculated using the following equation: \( \frac{1}{\text{conversion ratio} \times 10^{-2} \times (1/0.6) \times (122/204)} \), where 0.6 stands for the percentage of urinary excretions after nicotinamide ingestion. Because approximately 60% of the
Table 3. Urinary excretions of tryptophan metabolites in a Japanese male (51 y old) fed on a purified diet followed by dietary reference intakes in Japan.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>AnA (μmol/d)</td>
<td>1.3</td>
<td>1.1</td>
<td>1.4</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>KA (μmol/d)</td>
<td>5.6</td>
<td>6.0</td>
<td>5.4</td>
<td>7.1</td>
<td>6.5</td>
</tr>
<tr>
<td>XA (μmol/d)</td>
<td>2.8</td>
<td>3.0</td>
<td>3.4</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>3-HA (μmol/d)</td>
<td>4.4</td>
<td>3.5</td>
<td>4.1</td>
<td>5.1</td>
<td>4.4</td>
</tr>
<tr>
<td>QA (μmol/d)</td>
<td>25.5</td>
<td>18.5</td>
<td>21.7</td>
<td>21.0</td>
<td>19.6</td>
</tr>
<tr>
<td>MNA (μmol/d)</td>
<td>12.9</td>
<td>22.7</td>
<td>14.0</td>
<td>32.3</td>
<td>22.6</td>
</tr>
<tr>
<td>2-Py (μmol/d)</td>
<td>68.5</td>
<td>54.8</td>
<td>48.8</td>
<td>49.2</td>
<td>48.0</td>
</tr>
<tr>
<td>4-Py (μmol/d)</td>
<td>9.5</td>
<td>8.6</td>
<td>7.1</td>
<td>7.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Sum=MNA+2-Py+4-Py (μmol/d)</td>
<td>90.9</td>
<td>86.2</td>
<td>69.9</td>
<td>89.1</td>
<td>77.6</td>
</tr>
<tr>
<td>Conversion ratio (%)</td>
<td>2.3</td>
<td>2.2</td>
<td>1.8</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Conversion factor ²</td>
<td>42.8</td>
<td>45.1</td>
<td>55.6</td>
<td>43.6</td>
<td>50.1</td>
</tr>
</tbody>
</table>

The subject was fed on a diet containing 65 g of protein or 3,900 μmol of tryptophan per day (see Table 1).

1 The conversion ratio was calculated from the following equation: conversion ratio = sum (μmol/d)/daily tryptophan intake (3,900 μmol/d) × 100.

2 The conversion factor was calculated from the following assumption that the about 60% of the ingested nicotinamide was excreted as MNA, 2-Py, and 4-Py (20); conversion factor = 1/[(conversion ratio × 10^-2) × (1/0.6) × (122/204)]. The value 0.6 was used for the calculation of the conversion factor. Because approximately 60% of the ingested nicotinamide was excreted as MNA, 2-Py, and 4-Py (24). Molecular weight of nicotinamide is 122 and tryptophan 204.

The experiment was conducted on a male subject. Diet was kept in line with the Dietary Reference Intakes of Japan (12). The total amount of protein consumed was 65 g/d. The subject was a Japanese male, 51 y old, 176.8 cm tall, and weighing 69.0 kg. His data is shown in Table 3. Urinary excretions of AnA, KA, XA, 3-HA, and QA were within the ranges obtained in the female experiments. The conversion factor was not, however, so high compared with the female experiments.

**DISCUSSION**

Niacin coenzymes are needed for over 400 kinds of enzymes in mammals. This requirement is met mostly through the B-group vitamins. As for the precursors of the niacin coenzymes, there are three known nutrients, namely nicotinic acid, nicotinamide and tryptophan. Among these, nicotinic acid and nicotinamide are directly incorporated into the coenzymes NAD and NADP, while tryptophan must be converted into quinolinic acid before becoming incorporated into coenzymes. The tryptophan to quinolinic acid conversion pathway (see Fig. 2) is intricately regulated and affected by many nutritional (25, 26) and hormonal (27) factors. This is why the SD of the conversion factor is so high. It is important therefore, to know the relevant values when humans are fed whole nutrients following the Dietary Reference Intakes.

There are currently three papers concerned with tryptophan–niacin conversion (9–11) in Japanese subjects. In these reports, significant variations were observed. In the present experiment, the subjects were fed a purified diet, which followed the Japanese Dietary Reference Intakes. Under the present conditions, the conversion ratio, {([MNA+2-Py+4-Py] (μmol/d))/(tryptophan intake 3,300 μmol/d))} × 100, 1.5% was observed on the final day of the experiment. The conversion factor was 67, which means that 1 mg of nicotinamide is made from 67 mg of tryptophan in the bodies of young Japanese women when fed the aforementioned diet. Under the present ideal restricted conditions, the SD still was high at approximately 25%, indicating that any variances might be attributed to hereditary factors.

A parallel experiment was conducted on a male subject. His conversion factor of 50 (Table 3) was within the range obtained in the female experiment.

In conclusion, the observed conversion factor of 67, which indicates that 67 mg of tryptophan is equivalent to 1 mg of nicotinamide or 1 mg of nicotinic acid, would be appropriate to Japanese adult. However, it is necessary to also consider that this value has a high variation of 25%.

**Acknowledgments**

This investigation was supported by a Grant-in-Aid for Scientific Research, The Ministry of Health, Labor and Welfare. We express our sincere thanks to Miss. Hideko Wada, Mako Kuzuya, Kozue Mato, Shiori Sato, Chie Suzu-ura, Satoko Taniguchi, Chisato Takahashi, and Mariko Nakano for their technical assistance during the measurements of tryptophan metabolites.

**REFERENCES**


