

Urinary Water-Soluble Vitamins and Their Metabolite Contents as Nutritional Markers for Evaluating Vitamin Intakes in Young Japanese Women

Tsutomu FUKUWATARI and Katsumi SHIBATA

Department of Food Science and Nutrition, School of Human Cultures, The University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522–8533, Japan

(Received October 30, 2007)

Summary Little information is available to estimate water-soluble vitamin intakes from urinary vitamins and their metabolite contents as possible nutritional markers. Determination of the relationships between the oral dose and urinary excretion of water-soluble vitamins in human subjects contributes to finding valid nutrition markers of water-soluble vitamin intakes. Six female Japanese college students were given a standard Japanese diet in the first week, the same diet with a synthesized water-soluble vitamin mixture as a diet with approximately onefold vitamin mixture based on Dietary Reference Intakes (DRIs) for Japanese in the second week, with a threefold vitamin mixture in the third week, and a sixfold mixture in the fourth week. Water-soluble vitamins and their metabolites were measured in the 24-h urine collected each week. All urinary vitamins and their metabolite levels except vitamin B₁₂ increased linearly in a dose-dependent manner, and highly correlated with vitamin intake ($r=0.959$ for vitamin B₁, $r=0.927$ for vitamin B₂, $r=0.965$ for vitamin B₆, $r=0.957$ for niacin, $r=0.934$ for pantothenic acid, $r=0.907$ for folic acid, $r=0.962$ for biotin, and $r=0.952$ for vitamin C). These results suggest that measuring urinary water-soluble vitamins and their metabolite levels can be used as good nutritional markers for assessing vitamin intakes.

Key Words biomarker, human, urine, vitamin

A nutritional marker can be an indicator of nutritional status with respect to intake or metabolism of dietary constituents. Nutritional markers can be designated into one or more of three categories. 1) a means of validation of dietary instruments, 2) surrogate indicators of dietary intakes, or 3) integrated measures of nutritional status for a nutrient (1). Nutritional markers may be interpreted more broadly as a biological consequence of dietary intake or dietary patterns, and contribute to setting recommendations, tolerable levels and guidelines. Recent validation studies have developed the urinary compounds as nutritional markers to estimate nutrient intakes. For example, 24-h urinary nitrogen has been established as a marker for protein intake (2), the same as urinary potassium for energy and potassium intake (3), and urinary sugars for sugar intake (4).

Water-soluble vitamins are absorbed from the digestive tract after ingestion, stored in the liver, delivered to peripheral sites and then excreted to urine. Urinary water-soluble vitamins or their metabolites decrease markedly as vitamin status declines, and they are affected by recent dietary intake. Urinary excretion of water-soluble vitamins such as thiamin, riboflavin and niacin has been used for setting Dietary Reference Intakes (DRIs) in the USA and Japan (5, 6). However, only a single study investigated urinary vitamins as a possible marker for intake. Individuals' 30-d means of

thiamin intake are highly correlated with their mean 24-h urine thiamin levels under strictly controlled conditions, showing 24-h urinary thiamin as a useful marker for thiamin intake under strictly controlled conditions (7). Although pharmacological doses of water-soluble vitamin intake such as vitamin B₂ (8), nicotinamide (9) and biotin (10) dramatically increase urinary vitamin levels, few studies have investigated the relationship between several oral doses and dietary intake and urinary excretion of vitamin C, to the best of our knowledge (11, 12).

To determine whether urinary levels of water-soluble vitamins and their metabolites can be used as possible markers for estimating their intakes, six female Japanese college students were given a standard Japanese diet with or without a 1-, 3- and 6-fold vitamin mixture based on Dietary Reference Intakes (DRIs) for Japanese. The 24-h urinary excretion of water-soluble vitamins and their metabolites was measured, and the relationships between vitamin oral dose and urinary excretion were determined. This is the first report clearly to show that 24-h urinary vitamins and their metabolite levels were correlated to their intakes, and can be used as nutritional markers for their intakes.

SUBJECTS AND METHODS

Subjects. Six healthy female Japanese college students participated in the present experiment. They did not have regular use of medications or dietary supple-

E-mail: fukkie@shc.usp.ac.jp

Table 1. The composition of the diets.

	Diet 1	Diet 2	Average	RDA ³
Energy (kcal)	1,708	1,618	1,663	1,750
Protein (g)	68.5	61.5	65	50
Fat (g)	50.8	45.1	48.0	40–50
Carbohydrates (g)	236	237	237	—
Water-soluble vitamins ¹				
Vitamin B ₁ (mg as thiamin)	0.59	0.46	0.53 (2.0 μ mol)	0.74
Vitamin B ₂ (mg as riboflavin)	0.92	0.82	0.87 (2.3 μ mol)	1.05
Vitamin B ₆ (mg as pyridoxine)	1.24	0.86	1.05 (6.2 μ mol)	1.15
Vitamin B ₁₂ (μ g as cyanocobalamin)	7.4	11.3	2.4 (1.77 nmol)	2.4
Niacin equivalent ² (mg)	30.4	24.8	27.6 (226 μ mol)	10.2
Pantothenic acid (mg)	9.3	9.3	9.3 (42 μ mol)	5
Folates (μ g as pteroyl monoglutamic acid)	230	282	256 (0.58 μ mol)	200
Biotin (μ g)	67	53	60 (246 nmol)	30
Vitamin C (mg as L-ascorbic acid)	118	112	115 (0.65 mmol)	100

¹ Water-soluble vitamins except for vitamin B₁₂ are measured. Other nutrients are calculated by using the Standard Tables of Food Composition in Japan (15).

² The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (on a weight basis) of tryptophan taken was converted into niacin in the body.

³ The Recommended Dietary Allowance (RDA) for vitamin B₁ is 0.42 mg/1,000 kcal as thiamin, vitamin B₂ is 0.60 mg/1,000 kcal, vitamin B₆ is 0.023 mg/g protein, niacin is 5.8 mg NE/1,000 kcal, folic acid is 240 μ g/d and vitamin C is 100 mg/d for Japanese adults, and the Adequate Intake for pantothenic acid is 5 mg/d and biotin is 45 μ g/d for Japanese adult women (6).

The subjects consumed Diet 1 on days 1 and 3 each week, and Diet 2 on days 2 and 4.

ments, or habitual alcohol or cigarette consumption. Their age, body weight, height and body mass index (mean \pm SD) are 21.0 \pm 0.0 y old, 161.7 \pm 1.7 cm, 51.2 \pm 2.8 kg and 19.6 \pm 1.2, respectively. This study was reviewed and approved by The Ethical Committee of the National Institute of Health and Nutrition (Tokyo, Japan).

Chemicals. Thiamin hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinamide, calcium pantothenate, pteroylmonoglutamic acid (folic acid), D(+)-biotin, L(+)-ascorbic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-Pyridoxic acid (4-PIC) was manufactured by ICN Pharmaceuticals (Costa Mesa, CA, USA) and obtained through Wako Pure Chemical Industries. N¹-Methylnicotinamide (MNA) chloride was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). N¹-Methyl-2-pyridone-5-carboxamide (2-Py) and N¹-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized (13, 14). All the other chemicals used were of the highest purity available from commercial sources.

Diet. Two kinds of meals were given to the subjects. Diet 1 consisted of bread, margarine, ham, tomato, jelly and milk as breakfast; rice, miso-soup, Hamburg steak, cabbage, boiled spinach and Japanese tea as lunch; and

rice, raw skipjack, laver, pan-fried vegetables and Japanese tea as dinner. Diet 2 consisted of bread, margarine, ham, tomato, jelly and milk as breakfast; rice, miso-soup, broiled chicken, cabbage, simmered hijiki and Japanese tea as lunch; and rice, raw scallop, laver, pan-fried vegetables and Japanese tea as dinner. The nutrient elements are shown in Table 1. The subjects consumed Diet 1 on days 4 and 6 in each week, and Diet 2 on days 5 and 7. Water-soluble vitamins, except for vitamin B₁₂, in the diets were measured by the procedures described in *Determination of vitamins and their metabolites in urine and diets*. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan (15).

Experimental design. The subjects took the diet freely on days 1 to 3, and took the diet shown in Table 2 on days 4 to 7 in each week. Approximately 1-, 3- and 6-fold of the synthesized water-soluble vitamin mixture as vitamin mixture A, B and C shown in Dietary Reference Intakes for Japanese, 2005, were made (Table 2) (6). The subjects did not take any vitamin mixture in the first week, and then took the vitamin mixture A in the second week, the vitamin mixture B in the third week, and the vitamin mixture C in the fourth week. One third of the dose was put into a small gelatinous cap-

Table 2. The vitamin contents in the vitamin mixtures for 3 capsules per day.

	V. mix. A	V. mix. B	V. mix. C
Thiamin	0.56 mg/d (2.1 $\mu\text{mol/d}$)	1.78 mg/d (6.7 $\mu\text{mol/d}$)	3.89 mg/d (14.7 $\mu\text{mol/d}$)
Riboflavin	0.92 mg/d (2.4 $\mu\text{mol/d}$)	2.95 mg/d (7.8 $\mu\text{mol/d}$)	5.74 mg/d (15.3 $\mu\text{mol/d}$)
Pyridoxine	0.96 mg/d (5.7 $\mu\text{mol/d}$)	3.21 mg/d (19.0 $\mu\text{mol/d}$)	6.61 mg/d (39.1 $\mu\text{mol/d}$)
Nicotinamide	9.2 mg/d (75 $\mu\text{mol/d}$)	36.4 mg/d (298 $\mu\text{mol/d}$)	67.4 mg/d (552 $\mu\text{mol/d}$)
Pantothenic acid	4.8 mg/d (22 $\mu\text{mol/d}$)	15.0 mg/d (68 $\mu\text{mol/d}$)	31.4 mg/d (143 $\mu\text{mol/d}$)
Pteroylmonoglutamic acid	205 $\mu\text{g/d}$ (0.46 $\mu\text{mol/d}$)	530 $\mu\text{g/d}$ (1.20 $\mu\text{mol/d}$)	1,340 $\mu\text{g/d}$ (3.04 $\mu\text{mol/d}$)
Biotin	26 $\mu\text{g/d}$ (107 nmol/d)	84 $\mu\text{g/d}$ (344 nmol/d)	182 $\mu\text{g/d}$ (746 nmol/d)
L-Ascorbic acid	98 mg/d (0.56 mmol/d)	296 mg/d (1.68 mmol/d)	600 mg/d (3.41 mmol/d)

sule, and the capsule was administered three times daily after breakfast, lunch and dinner. The 24-h urine samples were collected from the second urinary sample on the last day to the first sample on the next day in each week. The urine sample volumes were measured, and the samples were immediately treated as described below, to avoid destruction of water-soluble vitamins and their metabolites, and then stored at -20°C until needed.

Determination of vitamins and their metabolites in urine and diets. For analysis of urinary thiamin, riboflavin, 4-PIC, MNA, 2-Py and 4-Py, 1 mL of 1 mol/L HCl was added to 9 mL urine. For analysis of urinary pantothenic acid and biotin, urine samples were not treated. For analysis of urinary folic acid, 1 mL of 1 mol/L-ascorbic acid was added to 9 mL urine. For analysis of urinary ascorbic acid, 4 mL of 10% metaphosphate was added to 4 mL urine. Urinary thiamin was determined by the HPLC-post labeled fluorescence method (16). Urinary riboflavin was determined by the HPLC method (17). Urinary 4-PIC was determined by the HPLC method (18). Urinary 2-Py, 4-Py and MNA, nicotinamide metabolites, were determined by the HPLC method (13, 19). Urinary pantothenic acid was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (20). Urinary folic acid was determined by the microbioassay method using *Lactobacillus casei* ATCC 2733 (21). Urinary biotin was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (22). Urinary reduced and oxidized ascorbic acid, and 2,3-diketogluconic acid, were determined by the HPLC method (23).

For analysis of water-soluble vitamins in the diets, Diet 1 and 2 were homogenized in water. Vitamin B₁ as sum of thiamin, TMP, TDP and TTP in the diets was determined by the HPLC-post labeled fluorescence method (16). Riboflavin, FMN and FAD in the diets were converted to lumiflavin by photolysis, and then determined by the HPLC method (17). Vitamin B₆ vitamers in the diets was converted to pyridoxine by autoclave

under acidic condition, and total pyridoxine was determined by the microbioassay method using *Saccharomyces carlsbergensis* strain 4228 ATCC 9080 (24). NAD and NADP in the diets were converted to nicotinamide by autoclave, and total nicotinamide was determined by the HPLC method (13). Bound pantothenic acid such as CoA and pantetheine in the diets was digested to free form by alkaline phosphatase and pigeon liver amidase, and total pantothenic acid was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (20). Foliates in the diets were digested to pteroylmonoglutamic acid by conjugase and protease, and pteroylmonoglutamic acid as total folic acid was determined by the microbioassay method using *Lactobacillus casei* ATCC 2733 (21). Bound biotin in the diet was converted to free form by autoclave under acidic conditions, and total biotin was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (22). Reduced and oxidized ascorbic acid, and 2,3-diketogluconic acid in the diets were determined by the HPLC method (23).

Statistical analysis. Linear regression analysis was carried out using a computer program, GraphPad Prism version 4.03 (GraphPad Software, Inc., San Diego, CA 92130, USA). Correlation coefficients were calculated using the method of Pearson product-moment correlation coefficient. The significance of the linear correlation coefficient was tested using Fisher's transformation test.

RESULTS

Vitamin B₁

The urinary excretion of thiamin in the first week was $0.288 \pm 0.074 \mu\text{mol/d}$ to 0.53 mg/d ($2.0 \mu\text{mol/d}$) of thiamin intake (mean \pm SD, $n=6$), and the level increased linearly until the fourth week taking 4.42 mg/d ($22.4 \mu\text{mol/d}$) (Fig. 1A). The correlation between urinary and oral thiamin was significantly high ($y=0.281x-0.514$, $r=0.959$; $p<0.0001$). The urinary recovery of thiamin (mean \pm SD, $n=6$) was

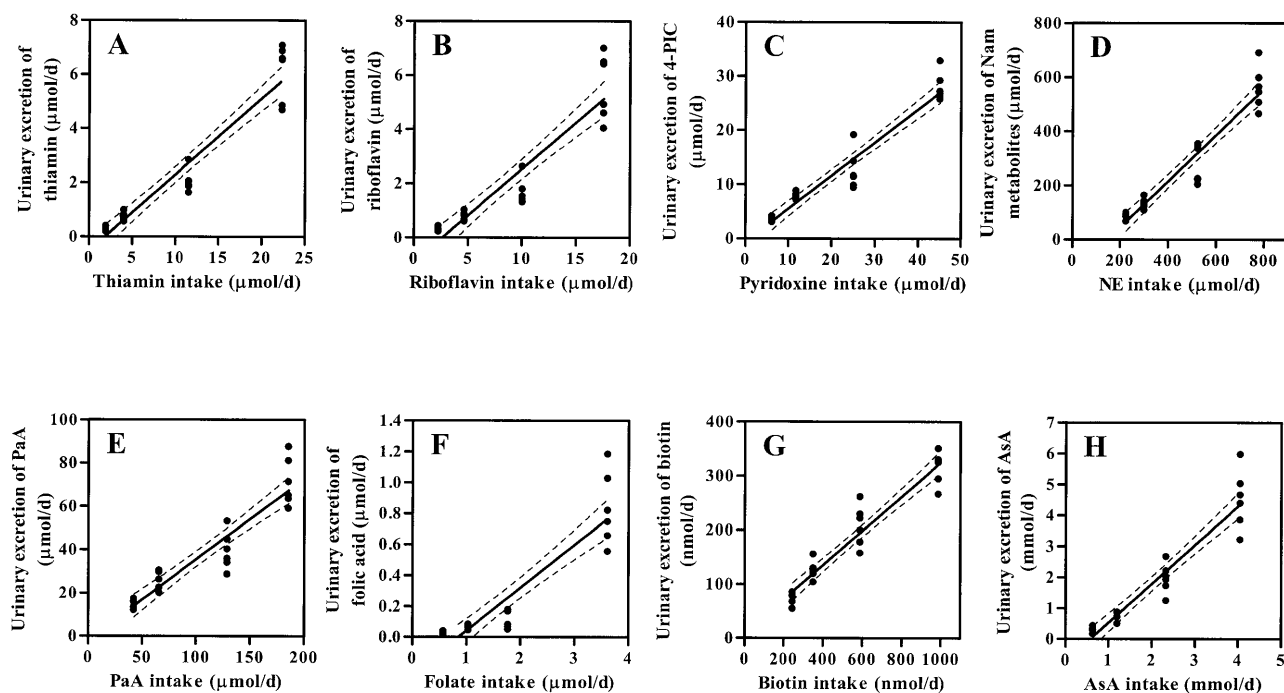


Fig. 1. Regression and 95% CI of oral dose and urinary excretion of vitamin B₁ (A), vitamin B₂ (B), vitamin B₆ (C), niacin (D), pantothenic acid (E), folate (F), biotin (G) and vitamin C (H). Values are individual points of six subjects in each dose. 4-PIC signifies 4-pyridoxic acid, a catabolite of vitamin B₆ vitamers, and the Nam metabolites signify the total amount of nicotinamide metabolites, MNA, 2-Py and 4-Py.

14.4±3.7, 19.0±4.3, 17.6±3.6 and 27.2±4.7% in the first, second, third and fourth week, respectively.

Vitamin B₂

The urinary excretion of riboflavin in the first week was 0.283±0.073 μmol/d to 0.87 mg/d (2.3 μmol/d) of riboflavin intake, and the level increased linearly until the fourth week taking 6.61 mg/d (17.6 μmol/d) (Fig. 1B). The correlation between urinary and oral riboflavin was significantly high ($y=0.342x-0.901$, $r=0.926$; $p<0.0001$). The urinary recovery of riboflavin was 12.3±3.2, 16.1±3.5, 16.4±5.0 and 31.6±6.9% in the first, second, third and fourth week, respectively.

Vitamin B₆

The urinary excretion of 4-PIC, a metabolite of vitamin B₆, in the first week was 3.44±0.41 μmol/d to 1.05 mg/d (6.2 μmol/d) of pyridoxine intake, and the level increased linearly until the fourth week taking 7.66 mg/d (45.2 μmol/d) (Fig. 1C). The correlation between urinary 4-PIC and oral pyridoxine was significantly high ($y=0.611x-0.59$, $r=0.966$; $p<0.0001$). The urinary recovery of 4-PIC was 55.4±6.6, 65.1±5.5, 49.9±14.3 and 61.9±5.9% in the first, second, third and fourth week, respectively.

Niacin

The urinary excretion of nicotinamide metabolites in the first week was 85.6±10.8 μmol/d to 27.6 mg niacin equivalents (NE)/d (226 μmol/d) of niacin intake, and the level increased linearly until the fourth week taking 95.0 mg NE/d (779 μmol/d) (Fig. 1D). The correlation between urinary nicotinamide metabolites and oral niacin was significantly high ($y=0.852x-125.9$, $r=0.957$; $p<0.0001$). The urinary recovery of nicotin-

amide metabolites was 37.9±4.8, 43.6±6.2, 53.4±13.6 and 71.9±10.1% in the first, second, third and fourth week, respectively.

Pantothenic acid

The urinary excretion of pantothenic acid in the first week was 14.6±2.0 μmol/d to 9.3 mg/d (42 μmol/d) of pantothenic acid intake, and the level increased linearly until the fourth week taking 40.7 mg/d (186 μmol/d) (Fig. 1E). The correlation between urinary and oral pantothenic acid was significantly high ($y=0.378x-1.6$, $r=0.951$; $p<0.0001$). The urinary recovery of pantothenic acid was 34.4±4.8, 39.1±6.1, 30.5±6.7 and 38.4±5.9% in the first, second, third and fourth week, respectively.

Folate

The urinary excretion of folic acid in the first week was 0.022±0.009 μmol/d to 256 μg/d (0.58 μmol/d) of folate intake, and the level increased linearly until the fourth week taking 1.60 mg/d (3.62 μmol/d) (Fig. 1F). The correlation between urinary folic acid and oral folate was significantly high ($y=0.277x-0.235$, $r=0.907$; $p<0.0001$). The urinary recovery of folic acid was 3.8±1.5, 5.1±1.5, 5.5±3.3 and 22.9±6.5% in the first, second, third and fourth week, respectively.

Biotin

The urinary excretion of biotin in the first week was 74.5±12.0 nmol/d to 60 μg/d (246 nmol/d) of biotin intake, and the level increased linearly until the fourth week taking 242 μg/d (990 nmol/d) (Fig. 1G). The correlation between urinary and oral biotin was significantly high ($y=0.316x+8.2$, $r=0.962$; $p<0.0001$). The urinary recovery of biotin was 30.3±4.9, 35.6±4.8, 35.1±6.4 and 31.8±3.0% in the first, second,

third and fourth week, respectively.

Vitamin C

The urinary excretion of ascorbic acid and 2,3-diketogluconic acid in the first week was 0.29 ± 0.08 mmol/d to 115 mg/d (0.65 mmol/d) of ascorbic acid intake, and the level increased linearly until the fourth week taking 715 mg/d (4.06 mmol/d) (Fig. 1H). The correlation between urinary ascorbic acid and 2,3-diketogluconic acid and oral ascorbic acid was significantly high ($y = 1.26x - 0.73$, $r = 0.952$; $p < 0.0001$). The urinary recovery of ascorbic acid and 2,3-diketogluconic acid was 45.2 ± 12.6 , 57.3 ± 9.6 , 83.6 ± 20.4 and $111.2 \pm 23.5\%$ in the first, second, third and fourth week, respectively.

DISCUSSION

To investigate the relationship between oral dose and urinary excretion of water-soluble vitamins and their metabolites, young Japanese women were administered a diet with or without varying amounts of the vitamins for 1 wk. Amount of the nutrients including water-soluble vitamins in the diets were close to RDA in DRIs (5, 6) and previous dietary assessment in free-living Japanese young women (25). The concentrations of all eight water-soluble vitamins and their metabolites in 24-h urine samples increased linearly in a dose-dependent manner, and strongly correlated with their intakes. These findings show that water-soluble vitamins and their metabolite levels in 24-h urine reflect the vitamin intakes under strictly controlled conditions, and suggest that vitamin intakes can be estimated from 24-h urinary vitamins and their metabolite contents.

In the present study, the correlations between urinary levels and their intakes for vitamin B₂ and folic acid were lower than those for other vitamins tested. The urinary riboflavin level linearly increased in a dose-dependent manner at 0.87 to 3.82 mg (2.3 to 10.1 μmol) vitamin B₂ intake, and then the level dramatically increased when the subjects took 6.61 mg (17.6 μmol) vitamin B₂. The urinary folic acid contents also showed a similar pattern to riboflavin: the contents linearly increased at 0.256 to 0.786 mg (0.58 to 1.78 μmol) folate intakes, and then dramatically increased at 1.60 mg (3.62 μmol) intake. The urinary vitamin levels may be affected by several factors such as absorption in the digestive tract, storage in the tissue, energy expenditure, tissue turnover and reabsorption in the kidney. However, no report has disclosed whether these factors change the urinary excretions of vitamins when humans take vitamins at the range used in the present study. Investigation of relationships for oral dose to urinary, blood and stored vitamin levels may explain what the dramatic increases in urinary riboflavin and folic acid mean.

We previously reported the levels of water-soluble vitamins and their metabolites in 24-h urine samples from young Japanese women consuming a semi-purified diet with a vitamin mixture for 7 d (26). The levels were 0.665 ± 0.114 μmol thiamin/d to 0.71 mg/d (2.7 μmol /d) thiamin intake; 0.580 ± 0.145 μmol ribo-

flavin/d to 1.0 mg/d (2.7 μmol /d) riboflavin intake; 83 ± 19 μmol nicotinamide metabolites/d to 12.8 mg/d (105 μmol /d) nicotinamide equivalent intake; 16.9 ± 1.3 μmol pantothenic acid/d to 5.0 mg/d (23 μmol /d) pantothenic acid intake; 22.7 ± 2.7 nmol folic acid/d to 200 μg /d (454 nmol/d) folic acid intake; 83 ± 23 nmol biotin/d to 30 μg /d (123 nmol/d) biotin intake; and 0.140 ± 0.051 nmol ascorbic acid/d to 100 mg/d (0.568 mmol/d) ascorbic acid intake (26). Intake of vitamin B₁, vitamin B₂ and folic acid was the same in the present and previous studies, and the urinary excretion of these vitamins in the present study was half or less than that in the previous study (26). Furthermore, urinary excretion of nicotinamide metabolites and pantothenic acid was the same in the present and previous studies, and the intake of niacin and pantothenic acid in the present study was twice that in the previous study (26). The form of vitamins differed between the two studies. The subjects obtained the vitamins from the diet in the first week in the present study, and they took a vitamin mixture in the previous one (26). As for niacin, most nicotinic acid in cereals binds to sugars, and bioavailability of this form is less than half that of free nicotinic acid (27). Pyridoxine-5'- β -D-glucoside (PN-glucoside) is a major naturally occurring form of vitamin B₆ in fruits (28), vegetables and cereal grains, and the bioavailability of PN-glucoside is $\sim 50\%$ relative to pyridoxine (29). Bioavailability of pantothenic acid in food is also half that of free pantothenic acid (30). Supplements of folic acid are nearly 100% bioavailable under fasting conditions (31), and a long-term controlled dietary study indicated that the bioavailability of folate in a typical mixed diet was no more than 50% relative to that in a formula diet (32). A recent study showed that bioavailability of food folate was 78% of that of folic acid according to an isotope (33). Most water-soluble vitamins, except vitamin C, bind to proteins or sugars in food, and the bioavailability of these forms is considered to be lower than that of the free forms (5).

The primary indicators selected to determine water-soluble vitamin sufficiency are the levels in urine, blood and/or serum. However, blood pantothenic acid and plasma biotin concentrations are not sensitive indicators of inadequate intake of these vitamins (34, 35). The present study shows the first evidence that urinary excretion of all eight water-soluble vitamins and their metabolites is highly correlated with vitamin intake when the subjects take a standard diet with or without 1-, 3- and 6-fold vitamins based on DRIs. The next step in this type of study is to determine the number of days reflecting vitamin and metabolite contents in 24-h urine samples, and to determine whether urinary vitamins and their metabolites in spot urine samples reflect their intakes in everyday life. We propose that estimating urinary 24-h water-soluble vitamin and their metabolite excretion is a good approach for assessing vitamin intakes in individuals. Furthermore, these results will contribute to determine dietary guidelines and recommendations.

Some vitamin-vitamin interactions are well known for accumulating homocystein by a folate, vitamin B₆ or vitamin B₁₂ deficiency, and requiring vitamin B₂ and vitamin B₆ for conversion of nicotinamide from tryptophan (36). These vitamin-vitamin interactions can be seen in some vitamin deficiencies, and little is known about how administrations of large amounts of water soluble vitamins affect other vitamins' metabolism. However, 1 g of ascorbic acid administration for 7 d does not alter plasma pyridoxal 5'-phosphate level or urinary excretion of 4-PIC (37). We previously reported that 150 mg (1.22 mmol) of nicotinamide administration increased nicotinamide metabolites approximately 800 μ mol in 24 h urine (9). Chronic administration of a multivitamin supplement containing 150 mg of nicotinamide (1.22 mmol/d), 5.45 mg of fursulthiamin hydrochloride (12.5 μ mol/d), 3.5 mg of riboflavin (9.3 μ mol/d), 4.5 mg of pyridoxine hydrochloride (22 μ mol/d), 6.5 μ g of cyanocobalamin (4.8 nmol/d), 15 mg of calcium pantothenate (63 μ mol/d as pantothenic acid) and 125 mg of ascorbic acid (0.71 mmol/d) increased nicotinamide metabolites approximately 700 μ mol in 24 h urine, showing that these doses of vitamin intake did not affect nicotinamide metabolism (38). Intestinal cells transport biotin, pantothenic acid and lipoate via a sodium-dependent multivitamin transporter (SMVT), and biotin uptake is inhibited by pantothenic acid at a micromolar range in vitro (39). This SMVT system is the major biotin uptake system in the intestinal cells, and physiological (nanomolar) concentrations of pantothenic acid have no effect on the biotin uptake in vitro (40). These reports and the present results that urinary excretions of biotin and pantothenic acid linearly or more increased with administration of vitamins mixtures suggest that biotin and pantothenic acid do not inhibit their absorption in the present study. Moreover, urinary excretions of other vitamins or their metabolites increased linearly in a dose-dependent manner, suggesting no major effect on water soluble vitamin metabolism or absorption because of vitamin administration.

Acknowledgments

This investigation is part of the results in "Studies on the requirement of water-soluble vitamins in Japanese (principal investigator, Katsumi Shibata)", which was supported by the Ministry of Health, Labor and Welfare.

REFERENCES

- 1) Potischman N, Freudenheim JL. 2003. Biomarkers of nutritional exposure and nutritional status: an overview. *J Nutr* **133**: 873S–874S.
- 2) Bingham SA. 2003. Urine nitrogen as a biomarker for the validation of dietary protein intake. *J Nutr* **133**: 921S–924S.
- 3) Tasevska N, Runswick SA, Bingham SA. 2006. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. *J Nutr* **136**: 1334–1340.
- 4) Tasevska N, Runswick SA, McTaggart A, Bingham SA. 2005. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev* **14**: 1287–1294.
- 5) Food and Nutrition Board, Institute of Medicine. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline. National Academy Press, Washington DC.
- 6) The Ministry of Health, Labour, and Welfare. 2004. Dietary Reference Intakes for Japanese, 2005. Tokyo (in Japanese).
- 7) Tasevska N, Runswick SA, McTaggart A, Bingham SA. 2007. Twenty-four-hour urinary thiamine as a biomarker for the assessment of thiamine intake. *Eur J Clin Nutr* DOI: 10.1038/sj.ejcn.1602829.
- 8) Zemleni J, Galloway JR, McCormick DB. 1996. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* **63**: 54–66.
- 9) Shibata K, Matsuo H. 1990. Changes in blood NAD and NADP levels, and the urinary excretion of nicotinamide and its metabolism in women student after nicotinamide administration. *Vitamins* **64**: 301–306.
- 10) Zemleni J, Mock DM. 1999. Bioavailability of biotin given orally to humans in pharmacologic doses. *Am J Clin Nutr* **69**: 504–508.
- 11) Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JE, King J, Cantilena LR. 1996. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* **93**: 3704–3709.
- 12) Levine M, Wang Y, Padayatty SJ, Morrow J. 2001. A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci USA* **98**: 9842–9846.
- 13) Pullman ME, Colowick SP. 1954. Preparation of 2- and 6-pyridones of N¹-methylnicotinamide. *J Biol Chem* **206**: 121–127.
- 14) Shibata K, Kawada T, Iwai K. 1988. Simultaneous micro-determination of nicotinamide and its major metabolites, N¹-methyl-2-pyridone-5-carboxamide and N¹-methyl-3-pyridone-4-carboxamide, by high-performance liquid chromatography. *J Chromatogr* **424**: 23–28.
- 15) Resources Council, Science and Technology Agency. 2000. Standard Tables of Food Composition in Japan, Fifth revised edition —2000—. Tokyo (in Japanese).
- 16) Fukuwatari T, Suzuura C, Sasaki R, Shibata K. 2004. Action site of bisphenol A as metabolic disruptor lies in the tryptophan-nicotinamide conversion pathway. *J Food Hyg Soc Japan* **45**: 231–238.
- 17) Ohkawa H, Ohishi N, Yagi K. 1983. New metabolites of riboflavin appear in human urine. *J Biol Chem* **258**: 5623–5628.
- 18) Gregory JF 3rd, Kirk JR. 1979. Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. *Am J Clin Nutr* **32**: 879–883.
- 19) Shibata K. 1987. Ultramicro-determination of N¹-methylnicotinamide in urine by high-performance liquid chromatography. *Vitamins* **61**: 599–604.
- 20) Skeggs HR, Wright LD. 1944. The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. *J Biol Chem* **156**: 21–26.
- 21) Aiso K, Tamura T. 1998. Trienzyme treatment for food folate analysis. Optimal pH and incubation time for α -

- amylase and protease treatment. *J Nutr Sci Vitaminol* **44**: 361–370.
- 22) Fukui T, Iinuma K, Oizumi J, Izumi Y. 1994. Agar plate method using *Lactobacillus plantarum* for biotin determination in serum and urine. *J Nutr Sci Vitaminol* **40**: 491–498.
 - 23) Kishida K, Nishimoto Y, Kojo S. 1992. Specific determination of ascorbic acid with chemical derivatization and high-performance liquid chromatography. *Anal Chem* **64**: 1505–1507.
 - 24) Association of Official Analytical Chemists. 2000. Official Methods of Analysis, 17th ed. AOAC Inc, Arlington, VA.
 - 25) Kimura N, Fukuwatari T, Sasaki R, Hayakawa F, Shibata K. 2003. Vitamin intake in Japanese women college students. *J Nutr Sci Vitaminol* **49**: 149–155.
 - 26) Shibata K, Fukuwatari T, Ohta M, Okamoto H, Watanabe T, Fukui T, Nishimuta M, Totani M, Kimura M, Ohishi N, Nakashima M, Watanabe F, Miyamoto E, Shigeoka S, Takeda T, Murakami M, Ihara H, Hashizume N. 2005. Values of water-soluble vitamins in blood and urine of Japanese young men and women consuming a semi-purified diet based on the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol* **51**: 319–328.
 - 27) Carter EG, Carpenter KJ. 1982. The bioavailability for humans of bound niacin from wheat bran. *Am J Clin Nutr* **36**: 855–861.
 - 28) Gregory JF 3rd, Sartain DB. 1991. Improved chromatographic determination of free and glycosylated forms of vitamin B-6 in foods. *J Agric Food Chem* **39**: 899–905.
 - 29) Nakano H, McMahan LG, Gregory JF 3rd. 1997. Pyridoxine-5'- β -D-glucoside exhibits incomplete bioavailability as a source of vitamin B-6 and partially inhibits the utilization of co-ingested pyridoxine in humans. *J Nutr* **127**: 1508–1513.
 - 30) Tarr JB, Tamura T, Stokstad ELR. 1981. Availability of vitamin B₆ and pantothenate in an average American diet in man. *Am J Clin Nutr* **34**: 1328–1337.
 - 31) Gregory JF 3rd. 1997. Bioavailability of folate. *Eur J Clin Nutr* **51**: S54–S59.
 - 32) Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. 1987. Folate requirement and metabolism in nonpregnant women. *Am J Clin Nutr* **46**: 1016–1028.
 - 33) Winkels RM, Brouwer IA, Siebelink E, Katan MB, Verhoef P. 2007. Bioavailability of food folates is 80% of that of folic acid. *Am J Clin Nutr* **85**: 465–473.
 - 34) Eissenstat BR, Wyse BW, Hansen RG. 1986. Pantothenic acid status of adolescents. *Am J Clin Nutr* **44**: 931–937.
 - 35) Mock NI, Malik MI, Stumbo PJ, Bishop WP, Mock DM. 1997. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased biotin status in experimental biotin deficiency. *Am J Clin Nutr* **65**: 951–958.
 - 36) McCormick DB. 1989. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* **69**: 1170–1198.
 - 37) Shultz TD, Leklem JE. 1982. Effect of high dose ascorbic acid on vitamin B₆ metabolism. *Am J Clin Nutr* **35**: 1400–1407.
 - 38) Shibata K, Onodera M, Shimada S, Yasuda K. 1992. Changes in nicotinamide metabolism and its bioavailability during long-term administration of a multivitamin preparation. *Vitamins* **66**: 309–314.
 - 39) Chatterjee NS, Kumar CK, Ortiz A, Rubin SA, Said HM. 1999. Molecular mechanism of the intestinal biotin transport process. *Am J Physiol* **277**: C605–C613.
 - 40) Balamurugan K, Ortiz A, Said HM. 2003. Biotin uptake by human intestinal and liver epithelial cells: roles of the SMVT system. *Am J Physiol* **285**: G73–G77.