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

Katsumi SHIBATA¹, Tsutomu FUKUWATARI¹, Mari OHTA¹, Hidemi OKAMOTO¹, Toshiaki WATANABE², Tooru FUKUI³, Mamoru NISHIMURA⁴, Masayuki TOTANI⁵, Mieko KIMURA⁶, Nobuko OHISHI⁷, Mieko NAKASHIMA⁷, Fumio WATANABE⁸, Emi MIYAMOTO⁸, Shigeru SHIGEOKA⁹, Tooru TAKEDA⁹, Megumi MURAKAMI⁹, Hiroshi IHARA¹⁰ and Naotaka HASHIZUME¹⁰

¹ School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522–8533, Japan

² School of Humanities for Environmental Policy and Technology, University of Hyogo, Himeji, Hyogo 670–0092, Japan

³ Byotai Seiri Laboratory, Tokyo 173–0025, Japan

⁴ Division of Human Nutrition, The Incorporated Administrative Agency of Health and Nutrition, Tokyo 162–8636, Japan

⁵ Graduate School of Life Science, Showa Women's University, Tokyo 154–8533, Japan

⁶ Takeda Research Institute of Life Science and Preventive Medicine, Kyoto 600–8813, Japan

⁷ Institute of Applied Biochemistry, Mitake, Gifu 505–0116, Japan

⁸ Department of Health Science, Kochi Women's University, Kochi 780–8515, Japan

⁹ Faculty of Agriculture, Kinki University, Nara 631–8505, Japan

¹⁰ Department of Laboratory Medicine, Ohashi Hospital, Toho University School of Medicine, Tokyo 153–8515, Japan

(Received January 31, 2005)

Summary We investigated the levels of water-soluble vitamins except for vitamin B₆ in the blood and urine of Japanese college male ($n=10$) and female ($n=10$) students. They consumed for 7 d a semi-purified diet based on Japanese Dietary Reference Intakes to assess the Recommended Dietary Allowances (RDA) for water-soluble vitamins and to present some new normal values for blood and urine levels of water-soluble vitamins in Japanese. The blood and the 24-h urine samples were collected on the last day of the experiment and measured. The values of total vitamin B₁ in whole blood, total vitamin B₂ in whole blood, total cyanocobalamin in serum, total nicotinamide in whole blood, total pantothenic acid in whole blood, total folates in serum, total biotin in serum, and ascorbic acid in plasma were 104 ± 17 pmol/mL (mean $\pm$ SD), 216 ± 25 pmol/mL, 0.34 ± 0.05 pmol/mL, 59.1 ± 5.0 nmol/mL, 2.45 ± 0.37 nmol/mL, 15.6 ± 4.6 pmol/mL, 8.3 ± 0.5 pmol/mL, and 62 ± 10 nmol/mL, respectively, in males, and 90 ± 23 , 234 ± 18 , 0.67 ± 0.20 , 61.9 ± 6.0 , 2.48 ± 0.30 , 30.2 ± 8.6 , 8.4 ± 0.3 , and 67 ± 14 , respectively, in females. There was a significant difference in the values of cyanocobalamin and total folates between men and women. The urinary excretion of vitamin B₁, vitamin B₂, cyanocobalamin, sum of the catabolic metabolites of nicotinamide, pantothenic acid, folates, biotin, and ascorbic acid were 665 ± 114 nmol/d, 562 ± 325 nmol/d, 93 ± 31 pmol/d, 84 ± 26 μ mol/d, 9.3 ± 2.3 μ mol/d, 19.4 ± 2.8 nmol/d, 83 ± 18 pmol/d, and 148 ± 51 μ mol/d, respectively, in males, and 495 ± 212 , 580 ± 146 , 145 ± 49 , 83 ± 19 , 16.9 ± 1.3 , 22.7 ± 2.7 , 83 ± 23 , and 140 ± 51 , respectively, in females. There was a significant difference in the urinary excretion of cyanocobalamin, pantothenic acid and total folates between men and women. These values will be useful for the nutritional assessment of water-soluble vitamins for Japanese, although the examination period was short. In future, an experiment with various age groups and re-evaluation by repeated experiments will provide more accurate values.

Key Words water-soluble vitamins, blood vitamin concentrations, DRIs (Dietary Reference Intakes), humans, Japanese

In Japan, revision of the “Recommended Dietary Allowance (RDA)” has been done every 5 y since 1970 by the Ministry of Health, Labor, and Welfare. During these 3 decades, the life span of Japanese has extended and retained the first position in the world. This distinction may be attributed to the fact that the nutritional guidance and health promotion programs are excellent

in Japan. In particular, in June 1999, “The 6th revision of the Japanese RDA—Dietary Reference Intakes (DRIs)—” (1) was laid down by the Ministry of Health, Labor, and Welfare. In this revision, the dietary reference intakes of all of the water-soluble vitamins (vitamin B₁, vitamin B₂, vitamin B₆, vitamin B₁₂, niacin, pantothenic acid, folic acid, biotin, and vitamin C) were first established. However, many RDAs were determined based on reports from foreign countries. So, we mea-

sured the values for the blood and urine of Japanese adult male and female subjects, who ingested a diet prescribed by “The 6th revision of Japanese RDA—DRIs—” (1) to assess the validity for the DRIs of water-soluble vitamins.

In clinical examination, blood values are used for assessment of vitamins, but in a nutritional examination, those values would not be suitable sometimes. As a general rule, the concentrations of nutrients in the body are well controlled in healthy subjects. We think that the concentrations of water-soluble vitamins in the blood are kept almost constant in healthy adult subjects but that the urinary amounts are relatively varied, because the excess water-soluble vitamins are excreted into the urine. In other words, the blood concentrations of water-soluble vitamins would be kept constant when the subjects have ingested a more than adequate amount of nutrients, whereas the urinary excretion of water-soluble vitamins would vary, because the requirements of nutrients are different among subjects. Therefore, the measurement of urinary excretion of water-soluble vitamins should be the most reliable index for nutritional status of water-soluble vitamins. So, we investigated the water-soluble vitamin contents in the urine when subjects were given a diet based on the recommended Japanese DRIs. As far as we know, this study is the first of its kind. The values listed in the present paper will be useful as indices for evaluating the nutritional status of water-soluble vitamins.

MATERIALS AND METHODS

Subjects. Healthy Japanese college students, consisting of 10 males and 10 females, participated in the present experiment. Their ages, body weights and heights are shown in Table 1. Prior to the experiment, they had physical checkups, and their hematological and blood biochemical analyses showed normal values. This study was reviewed and approved by The Ethical Committee of The Incorporated Administrative Agency of Health and Nutrition.

Diet and experimental design. All subjects were housed in the same facility for 9 d. The experimental design is shown in Fig. 1. The 24-h urine samples were

collected from the second urinary excretion on day 7 to the first one on day 8. After the volumes of the urine samples had been measured, the collected urine samples were immediately treated as described under “Analyses” to avoid destruction of water-soluble vitamins, and then stored at -20°C until needed. The blood was taken from a cubital vein at 08:30 on day 8 before breakfast, and treated immediately to avoid destruction of water-soluble vitamins, and stored at -20°C until needed.

The daily schedule was partly restricted: The lights

Table 1. Characteristics of the subjects.

Subjects	Age (y)	Height (cm)	Body weight (kg)	BMI
Male 1	19	160.2	58.50	22.79
Male 2	19	173.0	56.05	18.73
Male 3	21	176.1	70.40	22.70
Male 4	21	170.1	61.00	21.08
Male 5	21	170.5	60.95	20.97
Male 6	19	180.5	55.55	17.05
Male 7	20	166.0	57.10	20.72
Male 8	23	170.0	56.80	19.65
Male 9	20	182.0	58.70	17.72
Male 10	21	183.6	79.00	23.43
Mean	20.4	173.2	61.40	20.48
SD	1.3	7.4	7.52	2.18
SEM	0.4	2.4	2.38	0.69
Female 1	21	161.8	52.55	20.27
Female 2	22	164.8	60.90	22.42
Female 3	20	159.9	57.40	22.45
Female 4	21	162.5	51.10	19.35
Female 5	21	166.4	52.70	19.03
Female 6	21	167.7	56.50	20.09
Female 7	21	163.4	51.90	19.44
Female 8	20	158.3	51.00	20.35
Female 9	20	170.9	56.05	19.19
Female 10	20	163.6	51.35	19.19
Mean	20.7	163.9	54.15	20.08
SD	0.7	3.7	3.37	1.28
SEM	0.2	1.2	1.07	0.41

Fig. 1. Scheme of the experimental design.

Table 2. Composition of the male diet.

	(g/d)	Remarks
Wheat flour (Soft flour), first grade	315	Protein: $315 \text{ g} \times 0.08 = 25.2 \text{ g}$. Tryptophan content was 1.1%, so 277 mg of tryptophan were supplied. Carbohydrate: $315 \text{ g} \times 0.759 = 239 \text{ g}$ Lipid: $315 \text{ g} \times 0.017 = 5.4 \text{ g}$
Gluten	56	The gluten contained 81.6% protein, so the net protein amount was 45.7 g. Tryptophan content was 1.1%, so 503 mg of tryptophan were supplied. Total protein = 70.9 g Total tryptophan = 780 mg
Cornstarch	118	
Sucrose	30	Total carbohydrate = 387.1 g
Fats		Total fat = 50.3 g
Soybean oil	7.0	
Rapeseed oil	21.0	
Coconut oil	8.5	
Lard	8.4	
Dietary fiber		Soluble dietary fiber used, "Fibersol" was obtained from Matsutani Chemical Industry Co., Ltd. (Osaka, Japan), and insoluble dietary fiber used, Ramie powder, was obtained from Tosco Co., Ltd. (Tokyo, Japan).
Soluble	0.8	
Insoluble	14.3	
Mineral mixtures	14.6	The composition is shown below.
Total amount	592.5	Total energy = ca. 2,300 kcal = 9,614 kJ

For breakfast and supper, 177.75 g of the above powder mixture was added to 110 mL of water, and mixed well, and was baked for 9 min at 250°C. The weight of the baked meal was ca. 214 g. The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 237.0 g of the above mixture was added to 150 mL of water, and mixed well, and was baked for 10 min at 250°C. The weight of the baked meal was ca. 285 g. The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects.

Composition of the mineral mixture: 1,200 mg of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 1,049 mg of CaCO_3 , 2,124 mg of KH_2PO_4 , 3,558 mg of KHCO_3 , 2,594 mg of $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 49.8 mg of $\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$, 17.6 mg of $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 22.9 mg of ZnCl_2 , 7.1 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.2 mg of KI, and 4,000 mg of NaCl.

Composition of the vitamin mixture: 4.0 mg (2,000 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 4.47 mg of *dl*- α -tocopherol (7 mg was supplied from oils), 24 μg of phyloquinone (41 μg was supplied from oils), 1.2 mg of thiamin-HCl, 1.2 mg of riboflavin, 2.0 mg of pyridoxine-HCl, 2.4 μg of cyanocobalamin, 4.2 mg of nicotinamide (13.0 mg of nicotinamide was supplied from 780 mg of tryptophan in protein), 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, 100 mg of ascorbic acid, made up to 1 g with sucrose.

were turned off at 22:00 in order to promote sleep and the subjects got up at 06:00. The breakfast time was 08:00–09:00, lunch 12:30–13:10, and dinner 18:30–19:00. They consumed a semi-purified diet based on Japanese DRIs during experiment. The composition and the amount of the semi-purified diet are shown in Table 2 for males and in Table 3 for females. The experiment was carried out from March, 1st through March, 8th, 2002 for females, and from August 27th through September 3rd, 2002 for males.

Chemicals. Wheat flour (soft flour, first grade) was obtained from Nisshin Flour Milling Inc. (Tokyo). Wheat gluten, raw cornstarch, soybean oil, 13 kinds of vitamins (see Tables 2 and 3), and minerals (see Tables 2 and 3) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rapeseed oil was purchased from Ajinomoto Co. Ltd. (Tokyo, Japan). Coconut oil and lard were both obtained from Clea Japan (Tokyo, Japan). "Fibersol," used as a soluble dietary fiber, was obtained from Matsutani Chemical Industries (Osaka, Japan) and "Ramie powder," used as an insoluble dietary fiber, was from Tosco (Tokyo, Japan).

Thiamin hydrochloride ($\text{C}_{12}\text{H}_{17}\text{ClN}_4\text{OS} \cdot \text{HCl} = 337.27$),

riboflavin ($\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_6 = 376.37$), cyanocobalamin ($\text{C}_{63}\text{H}_{88}\text{CoN}_{14}\text{O}_{14}\text{P} = 1355.40$), nicotinamide (Nam; $\text{C}_6\text{H}_6\text{N}_2\text{O} = 122.13$), calcium pantothenate ($\text{C}_{18}\text{H}_{32}\text{N}_2\text{O}_{10} \cdot \text{Ca} = 476.54$), folic acid ($\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6 = 441.40$), D(+)-biotin ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3\text{S} = 244.31$), and L(+)-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6 = 176.13$) were purchased from Wako Pure Chemical Industries. N^1 -Methylnicotinamide (MNA) chloride ($\text{C}_7\text{H}_9\text{N}_2\text{O} \cdot \text{HCl} = 159.61$) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1 -Methyl-2-pyridone-5-carboxamide (2-Py, $\text{C}_7\text{H}_8\text{N}_2\text{O}_2 = 152.15$) and N^1 -methyl-4-pyridone-3-carboxamide (4-Py, $\text{C}_7\text{H}_8\text{N}_2\text{O}_2 = 152.15$) were synthesized by the methods of Pullman and Colowick (2) and Shibata et al. (3), respectively.

All other chemicals used were of the highest purity available from commercial sources.

Analyses

Blood and urine.

Vitamin B₁ (thiamin): The concentrations of total vitamin B₁ in whole blood and urine were measured by the HPLC-post labeled fluorescence method of Kimura et al. (4).

Vitamin B₂ (riboflavin): The concentration of total vitamin B₂ in whole blood was determined by the

Table 3. Composition of the female diet.

	(g/d)	Remarks
Wheat flour (Soft flour), first grade	315	Protein: $315 \text{ g} \times 0.08 = 25.2 \text{ g}$. Tryptophan content was 1.1%, so 277 mg of tryptophan were supplied. Carbohydrate: $315 \text{ g} \times 0.759 = 239 \text{ g}$ Lipid: $315 \text{ g} \times 0.017 = 5.4 \text{ g}$
Gluten	37.3	The gluten contained 81.6% protein, so the net protein amount was 29.8 g. Tryptophan content was 1.1%, so 328 mg of tryptophan was supplied. Total protein = 55 g Total tryptophan = 605 mg
Cornstarch	33	
Sucrose	20	Total carbohydrate = 292 g
Fats		Total fat = 40 g
Soybean oil	4.8	
Rapeseed oil	16.8	
Coconut oil	7.1	
Lard	5.9	
Dietary fiber		Insoluble dietary fiber used, Ramie powder, was obtained from Tosco Co., Ltd. (Tokyo, Japan).
Soluble	0	
Insoluble	10.1	
Mineral mixtures	12.0	The composition is shown below.
Total amount	462.0	Total energy = ca. 1,800 kcal = 7,524 kJ

For breakfast and supper, 139 g of the above powder mixture was added to 91 mL of water, and mixed well, and was baked for 9 min at 250°C. The weight of the baked meal was ca. 175 g. The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 185 g of the above mixture was added to 122 mL of water, and mixed well, and was baked for 10 min at 250°C. The weight of the baked meal was ca. 233 g. The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects.

Composition of the mineral mixture: 1,100 mg of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 900 mg of CaCO_3 , 2,200 mg of KH_2PO_4 , 3,500 mg of KHCO_3 , 2,083 mg of $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 60 mg of $\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$, 12.2 mg of $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 19 mg of ZnCl_2 , 6.3 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.2 mg of KI, and 2,120 mg of NaCl.

Composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 µg of cholecalciferol, 4.47 mg of *dl*- α -tocopherol (5 mg were supplied from oils), 24 µg of phylloquinone (31 µg was supplied from oils), 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.8 mg of nicotinamide (10.0 mg of nicotinamide was supplied from 605 mg of tryptophan in protein), 2.4 µg of cyanocobalamin, 5.5 mg of calcium pantothenate, 200 µg of pteroylmonoglutamic acid, 30 µg of D(+)-biotin, 100 mg of ascorbic acid, made up to 1 g with sucrose.

HPLC-lumiflavin method of Ohkawa et al. (5) modified slightly. Urinary concentration of riboflavin was analyzed according to the method of Ohkawa et al. (6).

Vitamin B₁₂ (cyanocobalamin): Serum vitamin B₁₂ concentration was determined by using a fully automated chemiluminescence analyzer (ACS 180; Beckman Coulter, Inc.) according to the manufacturer's instructions. The serum, prepared in the usual way, was directly applied to the analyzer.

Vitamin B₁₂ concentration in urine was assayed by the microbiological method with *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830 (7).

Niacin: The total nicotinamide content in whole blood was measured by the method of Shibata et al. (8).

The quantities of Nam, 2-Py and 4-Py in urine were measured simultaneously by the HPLC method of Shibata et al. (3). The content of MNA was measured by the method of Shibata (9).

Pantothenic acid: The heat-extracted supernatant of whole blood was treated with a mixture of 2 enzymes (phosphatase and amidase) for decomposing bound pantothenic acid such as CoA, phosphopantetheine, and pantetheine to form free pantothenic acid (10). The

enzyme-treated materials were directly used for measuring total pantothenic acid by using *Lactobacillus plantarum* ATCC 8014 (11). Total pantothenic acid means the sum of free pantothenic acid, and that derived from pantetheine, phosphopantetheine, CoA, and acyl CoA including acetyl CoA.

The content of free pantothenic acid in urine was directly measured by using *Lactobacillus plantarum* ATCC 8014 (11).

Folates: Serum concentrations of folates were determined by an automated method based on the competitive protein-binding assay using the ACS at the Clinical Laboratory of Mitsubishi BCL in Kobe. Herbert (12) suggested that there was no significant loss of serum folate activity after storage at -20°C for up to 106 d. We also found that the solution of folic acid was stable for at least 3 wk in the freezer at -20°C. Therefore, ascorbic acid was not added prior to storage of serum and urine samples in this study.

The concentrations of urinary folates were determined by an automated method based on the competitive protein-binding assay using the ACS as mentioned above.

Biotin: Biotin levels in the serum were microbiologically quantified with *Lactobacillus plantarum* ATCC 8014 according to the agar plate assay developed by Fukui et al. (13).

The content of free biotin in urine was directly measured by using *Lactobacillus plantarum* ATCC 8014.

Vitamin C: Total ascorbic acid (vitamin C) was determined by an HPLC-UV method according to Fujiwara et al. (14).

Vitamin C in urine was determined by the 2,4-dinitrophenylhydrazine (DNPH) method according to Shigeoka et al. (15).

Statistical analysis. For the statistical evaluation, the significance of the differences in the mean concentrations of water-soluble vitamins in blood and urine between two sexes was tested by using Student's paired, two-tailed *t*-test and non-parametric Mann-Whitney *U* test. Differences of $p < 0.05$ were considered to be statistically significant. InStat software (version 2.00; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

RESULTS AND DISCUSSION

Vitamin B₁

The content of total vitamin B₁ (free thiamin + thiamin monophosphate: TMP + thiamin diphosphate: TDP + thiamin triphosphate: TTP) in whole blood is shown in Table 4. Vitamin B₁ concentrations in males were 104 ± 17 pmol/mL (range 81–122) and in females 90 ± 23 pmol/mL (range 38–122) on the final day of the study. No significant difference between males and females was observed. However, a wide distribution range in blood vitamin B₁ concentrations of females was found; in particular, very low vitamin B₁ concen-

trations (38 pmol/mL) could be found. On the first day of the study, blood vitamin B₁ concentrations in males were 82 ± 10 pmol/mL (range 74–106) and in females 88 ± 19 pmol/mL (range 48–112).

The reference value of erythrocyte vitamin B₁ (70–90 pmol/mL) was proposed by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (USA and Canada) (16). However, the reference value in whole blood has not yet been proposed. In Japan, the content of vitamin B₁ in whole blood is frequently used. Kimura et al. (17) reported that the total vitamin B₁ concentration of farm village inhabitants was 109 ± 10 pmol/mL and that of fishing village inhabitants was 119 ± 19 pmol/mL in a survey of rural areas in 1982. And after these subjects were supplied with vitamin B₁ (1.5 mg/d) in daily foods for 1 wk, the total vitamin B₁ concentration of farm village inhabitants was 137 ± 7 pmol/mL and that of fishing village inhabitants was 157 ± 16 pmol/mL. The total vitamin B₁ concentration in whole blood of young men (29.4 ± 4.5 y old) was 140 ± 36 pmol/mL ($n=10$) and that of young women (23.5 ± 3.1 y old), 117 ± 21 pmol/mL ($n=10$), which was reported by Itokawa et al. (18). Takeda et al. (19) reported that the total vitamin B₁ concentration in whole blood of Japanese adult males and females was 119 ± 33 pmol/mL ($n=524$) and 104 ± 27 pmol/mL ($n=345$), respectively. In the survey study of other countries by Kimura et al. (20), the total vitamin B₁ concentration in the whole blood of Chinese adult males and females living in urban areas was 164 ± 34 pmol/mL ($n=100$) and 159 ± 37 pmol/mL ($n=100$). In the survey for vitamin B₁ status of inhabitants in northeast rural Thailand by Kimura et al. (21), the total vitamin B₁ concentration in whole blood of

Table 4. Blood values of water-soluble vitamins for Japanese college students who consumed on a semi-purified diet based on the Japanese dietary reference intakes.

	Mean	SD	Minimum	Maximum	Median
Males ($n=10$)					
Total thiamin in whole blood (pmol/mL)	104	17	81	122	106
Total riboflavin in whole blood (pmol/mL)	216	25	175	259	211
Cyanocobalamin in serum (pmol/mL)	0.34	0.05	0.26	0.43	0.34
Total Nam in whole blood (nmol/mL)	59.1	5.0	52.8	69.9	60.0
Total pantothenic acid in whole blood (nmol/mL)	2.45	0.37	1.86	3.27	2.36
Folates in serum (pmol/mL)	15.6	4.6	9.9	23.1	15.0
Biotin in serum (pmol/mL)	8.3	0.5	7.8	9.0	8.2
Ascorbic acid in plasma (nmol/mL)	62	10	47	80	60
Females ($n=10$)					
Total thiamin in whole blood (pmol/mL)	90	23	38	122	91
Total riboflavin in whole blood (pmol/mL)	234	18	205	259	238
Cyanocobalamin in serum (pmol/mL)	0.67*	0.20	0.41	0.92	0.67
Total Nam in whole blood (nmol/mL)	61.9	6.0	55.4	75.4	61.0
Total pantothenic acid in whole blood (nmol/mL)	2.48	0.30	2.08	2.83	2.51
Folates in serum (pmol/mL)	30.2*	8.6	21.0	51.7	29.0
Biotin in serum (pmol/mL)	8.4	0.3	7.8	9.0	8.6
Ascorbic acid in plasma (nmol/mL)	67	14	47	100	61

* Significant difference from male value at $p < 0.05$, calculated by Student's *t* test.

Table 5. Urinary values of water-soluble vitamins for Japanese college students who consumed a semi-purified diet based on the Japanese dietary reference intakes.

	Mean	SD	Minimum	Maximum	Median
Males (<i>n</i> =10)					
Thiamin (nmol/d)	665	114	467	821	669
Riboflavin (nmol/d)	562	325	155	1,208	492
Cyanocobalamin (pmol/d)	93	31	69	170	85
Sum (MNA+2-Py;4-Py) (μ mol/d)	84	26	54	128	74
Pantothenic acid (μ mol/d)	9.3	2.3	6.2	12.2	10.2
Folates (nmol/d)	19.4	2.8	14.5	23.9	19.6
Biotin (pmol/d)	83	18	59	112	78
Ascorbic acid (μ mol/d)	148	51	87	231	134
Females (<i>n</i> =10)					
Thiamin (nmol/d)	495*	212	286	988	458
Riboflavin (nmol/d)	580	146	366	849	569
Cyanocobalamin (pmol/d)	145*	49	94	252	112
Sum (MNA+2-Py;4-Py) (μ mol/d)	83	19	53	111	81
Pantothenic acid (μ mol/d)	16.9*	1.3	14.8	18.6	17.3
Folates (nmol/d)	22.7*	2.7	20.4	29.0	22.3
Biotin (pmol/d)	83	23	54	120	78
Ascorbic acid (μ mol/d)	140	51	89	257	131

* Significant difference from male value at $p < 0.05$, calculated by Student's *t* test.

males and females working on farms was 74 ± 37 pmol/mL ($n=59$) and 76 ± 46 pmol/mL ($n=47$), and the total vitamin B₁ concentration in the whole blood of males and females working in factories was 96 ± 34 pmol/mL ($n=279$) and 105 ± 27 pmol/mL ($n=21$), respectively.

The urinary excretion of free vitamin B₁ in males and females is shown in Table 5. A significant difference between sexes was observed. The reference value of urinary excretion of vitamin B₁ (133–333 nmol/d) has been proposed by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (USA and Canada) (16). All of the present values were over this reference value. So, the RDA of vitamin B₁ in the 6th revision was evaluated as good in adult men and women.

Murata et al. (22) reported that vitamin B₁ levels of 24 h-urine samples collected from 13 women in summer and winter were 545 ± 402 nmol/d and 522 ± 445 nmol/d, respectively. Itokawa et al. (23) reported that the urinary excretion of vitamin B₁ in adult men (21–24 y old) was 472 ± 339 nmol/d ($n=21$). Therefore, the present values were almost the same as the previously reported ones (22, 23).

Based on the results of a thiamin deficiency study, Nishio et al. (24) concluded that the urinary excretion of vitamin B₁ would not be a useful index of thiamin depletion because the urinary excretion decreased so quickly before the clinical deficiency was revealed. But the use of the urinary values helps to assess the vitamin B₁ nutritional status, because the urinary excretion reflects the immediate vitamin B₁ intake.

Vitamin B₂

The total vitamin B₂ content expressed in whole blood is shown in Table 4. The values were almost the

same between the 2 groups. Hiraoka (25) reported that the mean value and the reference interval were 225 pmol/mL and 154–311 pmol/mL, respectively, for the blood riboflavin levels of healthy female students aged 21–22 y whose intake of vitamin B₂ was above the RDA. The value obtained in the present study coincides with their data. There is a paper stating that over 180 pmol/mL of vitamin B₂ in whole blood indicates a well-nourished individual (16). In the present experiment, only one person was below the value. Therefore, the RDA of vitamin B₂ in the 6th revision would be evaluated as good.

The vitamin B₂ content in urine is shown in Table 5. The values were almost the same between the male and female students. Roughead and McCormick (26) reported that the amount of urinary riboflavin determined by the HPLC procedure exceeded 319 nmol/d under conditions of riboflavin intake of ca. 1.5 mg/d in healthy residents of rural Georgia, USA. Two of our subjects were below the value of 319 nmol/d. Urinary riboflavin separated from other flavin derivatives including catabolites by HPLC is considered to be useful for estimation of the vitamin B₂ requirement. The average in our study was ca. 550 nmol/d and the range was 155 to 1,208 nmol/d.

Vitamin B₁₂

The concentration of vitamin B₁₂ in serum reflects both vitamin B₁₂ intake and storage. The lower limit (cut-off value) of the serum vitamin B₁₂ concentration is considered to be approximately 0.12–0.18 pmol/mL for adults (16). The serum concentrations of vitamin B₁₂ found in the present study are shown in Table 4. These values are over the cut-off value, and so the RDA in the 6th revision was evaluated as good. But, the diet

used for the experiment contained a small amount of vitamin B₁₂. So, the accurate amount of vitamin B₁₂ consumed was 4.2 μ g/d. The mean values for males and females were 0.34 pmol/mL and 0.67 pmol/mL, respectively, and this difference was significant. Those values at day -1 were 0.34 ± 0.07 pmol/mL and 0.48 ± 0.14 pmol/mL, respectively. Therefore, daily intake of 2.4 μ g of cyanocobalamin for 7 d maintained the serum vitamin B₁₂ level in the male subjects, and increased it in the female subjects. Fernandes-Costa et al. (27) reported mean serum vitamin B₁₂ values for young men ($n=77$) and women ($n=82$) to be 0.477 and 0.604 pmol/mL, respectively. Similar results have been also obtained by other workers (28, 29). At the present time, we cannot explain why there is such a difference in the serum vitamin B₁₂ concentration between men and women and do not know whether there is any gender-based difference in vitamin B₁₂ requirements.

Amounts of urinary vitamin B₁₂ are shown in Table 5. The mean value in men and women was 93 pmol/d and 145 pmol/d, respectively, and this difference was significant.

Mollin and Ross (30) reported that the daily excretion of vitamin B₁₂ in urine ranged from 81.2 to 199.3 pmol/d in 6 normal subjects, but decreased significantly [to less than 36.9 pmol] in 7 patients with pernicious anemia.

Adams (31) demonstrated that the mean value of urinary vitamin B₁₂ was 121 pmol/d in non-vitamin B₁₂-deficient subjects and found a linear correlation between the serum vitamin B₁₂ level and daily loss of vitamin B₁₂ in the urine. From the present data, the urinary excretion of vitamin B₁₂ in Japanese would be ca. 100 pmol/d in men and 150 pmol/d in women.

The values for serum and urinary vitamin B₁₂ between men and women were significantly different, as stated above. However, the RDA of vitamin B₁₂ in the 6th revision is the same for men and women. This problem should be resolved in the future.

Niacin (total Nam)

The content of total Nam in whole blood is shown in Table 4. There was no difference in the value between sexes. The present mean value for men and women was ca. 60 μ mol/mL of whole blood. This value is about the same as was reported previously (32), in a study in which the subjects were female college students ($n=19$), who consumed self-selected foods. In a report published in 1950, Nose (33) reported that the content of total niacin in whole blood was 62.7 ± 5.3 nmol/mL ($n=10$). In 1953, Katsura and Sakakida (34) reported a mean value of 61 ± 5 nmol/mL for 10 men (24–29 y old) and 56 ± 6 nmol/mL for 10 women (19–50 y old). Therefore, the RDA of niacin equivalent intake in the 6th revision was evaluated as good.

In humans, nicotinamide itself is minimally excreted into urine, but its catabolic metabolites, MNA, 2-Py, and 4-Py are excreted. The sum of the urinary excretion of MNA+2-Py+4-Py for the male and female students is shown in Table 5. Shibata and Matsuo (35) reported a

value of ca. 100 μ mol/d, for female college students who consumed self-selected foods. Okamoto et al. (36) reported that when female college students consumed a diet containing 25 mg niacin equivalent/d, the urinary excretion of the sum was ca. 80 μ mol/d. The present and previous data (35, 36) indicate that the urinary excretion of the sum of MNA+2-Py+4-Py changes according to the niacin equivalent intake. In fact, Shibata and Matsuo (35) and Moyer et al. (37) revealed a high correlation coefficient between the sum and the niacin equivalent intake. Therefore, determination of the sum of MNA, 2-Py, and 4-Py would provide an excellent index for a niacin nutritional assessment. We would like to propose that adult humans (18–69 y olds) including men and women should ingest sufficient niacin equivalent to maintain this urinary sum at a level over 50 μ mol/d.

Pantothenic acid

The content of total pantothenic acid in whole blood is shown in Table 4. No difference in value was observed between sexes. The present mean value was ca. 2.5 nmol/mL of whole blood. Wittwer et al. (38) reported that a normal value for pantothenic acid in the whole blood ranged from 1.57 to 2.66 nmol/mL, and Song et al. (39) reported the concentration in adult women to be 2.40 ± 0.05 ($n=47$) nmol/mL. They also reported that the correlation between total pantothenic acid in blood and the intake of pantothenic acid was weak. As to the paper by Fry et al. (40), the total pantothenic acid did not decrease even when the subjects were fed a diet without pantothenic acid for 9 wk. Therefore, the total pantothenic acid in blood does not reflect the intake of pantothenic acid and should not be used for assessing the nutritional status of pantothenic acid.

The urinary content of pantothenic acid in the males and the females is shown in Table 5. The value was about 2 times higher in the females than in the males, and this difference was significant. There is clear evidence that the urinary excretion of pantothenic acid is a reliable index for assessing the nutritional status of pantothenic acid. For example, Fox and Linkswiler (41) reported that the correlation coefficient between dietary pantothenic acid intake and the urinary excretion of pantothenic acid was 0.805. Previously reported values (40, 41) for urinary pantothenic acid were ca. 10–20 μ mol/d in adults. So, we conclude the RDA of pantothenic acid in the 6th revision is good, and propose that the urinary excretion of pantothenic acid is a suitable index for the nutritional assessment of pantothenic acid. A value of 10 μ mol/d would be a suitable intake of pantothenic acid for maintaining health in males, whereas 15 μ mol/d would be appropriate for females.

Folates

The serum content of folates is presented in Table 4. The serum concentrations of folates were 15.6 ± 4.6 pmol/mL for males and 30.2 ± 8.6 pmol/mL for females. The large sex difference was observed with the females showing a significantly higher level. Those values at

day -1 were 19.6 ± 6.2 pmol/mL and 25.4 ± 5.8 pmol/mL, respectively. Therefore, daily intake of 200 μ g of pteroylmonoglutamic acid for 7 d maintained the serum level of folates in the male subjects, and increased it in the female subjects. No subjects with extremely low amounts of serum folates (6.8 pmol/mL) were found in either group.

Milne et al. (42) reported that the serum content of folates was 18.9 ± 6.1 pmol/mL in 40 male volunteers, aged 19 to 54 y, without underlying disease in the USA. The mean plasma concentration of folates was 23.3 ± 6.3 pmol/mL ($n=39$) and 23.3 ± 7.5 pmol/mL ($n=41$) in 80 free-living men and women aged 50–87 y in the USA (43). In 6 healthy Caucasian males (aged 22–31 y) the serum value was 18 pmol/mL (44). It was 17.1 ± 1.3 pmol/mL in 30 apparently healthy subjects (12 males and 18 females from 37 to 69 y of age) in Turkey (45). The Framingham Heart Study of 885 elderly people found the plasma folates concentrations to be greater for women (46): The level was 12.7 pmol/mL for men, which was significantly different from the 16.3 pmol/mL for women. Thus, a significant sexual difference was observed, which is in accordance with the present findings. On the other hand, Yukawa et al. (47) reported that in a folate-deficient group of the neurological patients, serum folates levels were significantly lower in females (5.0 ± 2.0 pmol/mL, $n=11$) than in males (6.6 ± 2.0 pmol/mL, $n=25$). A normal value for serum folates has not yet been proposed, but from these findings, the baseline values of folates in serum (or plasma) ranged from approximately 15 to 25 pmol/mL. There was also no difference of race among Japanese, Asians and Caucasians. From these findings, we conclude the RDA of folic acid in the 6th revision value of 200 μ g/d is good.

The urinary content of folates is shown in Table 5. The urinary concentrations of folate were 19.4 ± 2.8 nmol/d in the males and 22.7 ± 2.7 nmol/d in the females. A significant difference in the values of the two groups was observed. The urinary excretion of folates ranged from 15 to 30 nmol/d in the present study, which is closely in accord with the previously reported value (11.3–90.7 nmol/d) (48). This would be a normal value for Japanese subjects.

Biotin

The amount of biotin supplemented in the semi-purified diet in this study was 30 μ g/d, which followed the Japanese DRIs. As biotin is widely distributed in many foodstuffs, biotin is also contained in natural sources such as wheat gluten and raw cornstarch compounded as ingredients of the semi-purified diet. Therefore, the daily biotin intake was 142.7 μ g for young men and 118.2 μ g for young women.

Table 4 presents the serum biotin concentrations in both groups. The serum concentrations of biotin were 8.3 ± 0.5 pmol/mL in men and 8.4 ± 0.3 pmol/mL in women.

In a large Japanese population of 190 healthy adults (18 to 66 y, mean 34.4 y), Fukui et al. (13) found the mean total biotin level in their serum to be

10.9 ± 2.2 pmol/mL. From these findings, normal values for total biotin in serum were from 7.8 to 12.2 pmol/mL. Watanabe et al. (49) also demonstrated that the serum biotin level in 685 elderly people was 10.2 ± 7.2 pmol/mL, and that in 2,004 reference people, 9.4 ± 1.4 pmol/mL, and the mean and SD of biotin levels did not differ between men and women. The normal value for serum biotin in reference people was determined to range from 6.5 to 12.1 pmol/mL. So, the present data are within this range. However, the ingested biotin was over 30 μ g/d because the ingredients, raw cornstarch and gluten, contained a great deal of biotin. The evaluation of the present RDA (30 μ g/d) of biotin could not be precisely made in the present experiment.

The biotin content in the urine is shown in Table 5. The urinary concentrations of biotin were 83.0 ± 18.3 pmol/d in men and 83.2 ± 22.5 pmol/d in women. No sex difference was observed. These results are in accord with the findings of previous studies in the USA and UK: Sweetman et al. (50) reported that 600 adults, aged 20–50 y, excreted biotin at the rate of 24.6–204.7 pmol/d. Similar findings were also obtained by Jung et al. (51) and Bitsch et al. (52), who demonstrated 187.1 ± 44.6 pmol/d in 40 adults (45.7 ± 10.9 y) and 40.9 pmol/d in 28 adults, respectively. As the ingestion of biotin was over 30 μ g/d, the evaluation of the present RDA of biotin should be made carefully.

Vitamin C

The plasma level of vitamin C is shown in Table 4. No difference in the values of vitamin C was observed between sexes. The mean value of plasma vitamin C was ca. 64.5 nmol/mL. Levine et al. (53) reported a mean value of vitamin C in plasma of 62.0 ± 10.2 nmol/mL for 15 women. Kobata et al. (54) reported that when 7 female subjects (average age of 25 y) were given a diet containing 111.0 ± 33.0 mg/d of vitamin C, the plasma level of vitamin C remained constant throughout the experimental period, at 56.8 ± 10.8 nmol/mL. It was also reported that the mean value of vitamin C in plasma was 56.0 ± 4.0 nmol/mL for 7 men (20–26 y old), who were administered 50 mg of the vitamin twice daily in the fasting state, at least 2-h before breakfast and dinner (53, 54). Levine et al. (55) demonstrated that the plasma saturation of vitamin C occurred between 200 and 400 mg daily doses, whereas a 100 mg daily dose resulted in saturation of vitamin C in neutrophils, monocytes, and lymphocytes. Furthermore, they indicated that a 100 mg daily dose would be a suitable intake of vitamin C for maintaining the steady-state plasma concentration at a value corresponding to the RDA of vitamin C (56). Recently Ihara et al. (57) performed an experiment with 176 young Japanese women (19–26 y old) and reported that a vitamin C intake of ca. 99-mg daily is sufficient to produce a serum vitamin C concentration above the lower reference limit of 40 nmol/mL (7 mg/L). The data reported here clearly agree with the RDA of vitamin C for the Japanese population i.e., 100 mg daily (19). Healthy subjects require 100 mg daily to keep the plasma con-

centration at more than 40 nmol/mL.

The sum of urinary excretion of vitamin C in men and women is shown in Table 5. No sex difference was observed, and the mean value was ca. 144 μ mol/d. Levine et al. (53, 55) reported the effect of 30–1,250 mg doses daily on the urinary excretion of vitamin C. In their report (53, 55), no vitamin C was excreted in the urine of men or women until a 100-mg dose has been given orally or intravenously. With intravenous administration, the entire dose was excreted at the 500–1,250 mg doses. At an oral dose of 500 mg daily and higher, vitamin C excretion increased linearly. It was also reported that bioavailability or gastrointestinal absorption declined at intravenously delivered doses above 200 mg or at oral doses above 500 mg (53, 55). Our data presented here and the data reported previously indicate that a 100-mg daily dose would be a suitable intake of vitamin C to maintain the steady-state plasma concentration without affecting the urinary excretion.

CONCLUSION

We measured the values of water-soluble vitamins except for vitamin B₆ in the blood and urine of Japanese young men and women who consumed a diet based on the DRIs in the 6th revision of Recommended Dietary Allowance in the Japanese population (1). The RDAs of water-soluble vitamins except for vitamin B₁₂ and biotin were evaluated. All of the values of water-soluble vitamins indicated these RDAs to be good. So, we propose that the present values except for vitamin B₁₂ and biotin might be used as normal values for Japanese adult men and women. The requirement of vitamin B₁₂ and biotin were so low that we could not precisely control them in the present diet.

Acknowledgments

The Ministry of Health, Labor and Welfare supported this investigation.

REFERENCES

- 1) Ministry of Health, Labor and Welfare. 1999. Recommended Dietary Allowances in the Japanese Population, 6th Ed, Dietary Reference Intakes, p 112–114. Tokyo.
- 2) Pullman ME, Colowick SP. 1954. Preparation of 2- and 6-pyridones of N¹-methylnicotinamide. *J Biol Chem* **206**: 121–127.
- 3) 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-4-pyridone-3-carboxamide, by high-performance liquid chromatography. *J Chromatogr* **424**: 23–28.
- 4) Kimura M, Fujita T, Itokawa Y. 1982. Liquid chromatographic determination of the total thiamin content of blood. *Clin Chem* **28**: 29–31.
- 5) Ohkawa H, Ohishi N, Yagi K. 1982. A simple method for micro-determination of flavin in human serum and whole blood by high-performance liquid chromatography. *Biochem Int* **4**: 187–194.
- 6) Ohkawa H, Ohishi N, Yagi K. 1983. New metabolites of riboflavin appear in human urine. *J Biol Chem* **258**: 5623–5628.
- 7) Watanabe F, Abe K, Katsura H, Takenaka S, Mazumder ZH, Yamaji R, Ebara S, Fujita T, Tanimori S, Kirihata M, Nakano Y. 1998. Biological activity of hydroxo-vitamin B₁₂ degradation product formed during microwave heating. *J Agric Food Chem* **46**: 5177–5180.
- 8) Shibata K, Kawada T, Iwai K. 1987. High-performance liquid chromatographic determination of nicotinamide in rat tissue samples and blood after extraction with diethyl ether. *J Chromatogr* **422**: 257–262.
- 9) Shibata K. 1987. Ultramicro-determination of N¹-methylnicotinamide in urine by high-performance liquid chromatography. *Vitamins (Japan)* **61**: 599–604.
- 10) Masugi I. 1972. Pantothenic acid metabolism in young animals and childhood. *Vitamins (Japan)* **46**: 261–265.
- 11) Skeggs HR, Wright LD. 1944. The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. *J Biol Chem* **156**: 21–26.
- 12) Herbert V. 1962. Minimal daily adult folate requirement. *Arch Int Med* **110**: 649–653.
- 13) Fukui T, Inuma 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.
- 14) Fujiwara Y, Otsuka M, Ihara H, Ito S, Fujisaki M, Inomata M, Tomabechi K, Kodaka K, Igarashi O, Okuda K, Mino M, Chibata I, Hashizume N, Itokawa Y. 2001. Proposed standard for total ascorbic acid values in human plasma: HPLC procedure as a reference method. *J Jpn Soc Nutr Food Sci* **54**: 41–44.
- 15) Shigeoka S, Yokota A, Nakano Y, Kitaoka S. 1979. The effect of illumination on the L-ascorbic acid content in *Euglena gracilis* z. *Agric Biol Chem* **43**: 2053–2058.
- 16) Institute of Medicine, ed. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline. National Academy Press, Washington, DC.
- 17) Kimura M, Sayama N, Takashima M, Nakabayashi J, Itokawa Y, Tsunematu T. 1982. A field survey on nutritional status of vitamin B₁ in Shimane prefecture. *Vitamins (Japan)* **56**: 479–486.
- 18) Itokawa Y, Kimura M, Nishino K, Miyata S, Mino M, Tamai H. 1993. Thiamin and riboflavin status in elderly—Effect of single administration of a multivitamin preparation—. *Vitamins (Japan)* **67**: 675–679.
- 19) Takeda A, Suyama T, Suzuki T, Imanishi M, Takeda T, Takeda R, Kitamura R, Tamai H, Kimura M. 2002. Vitamin B₁ nutritional status assessed by blood vitamin B₁ value of middle aged Japanese men and women. *Vitamins (Japan)* **76**: 349–353.
- 20) Kimura M, Takeda A, Takeda R, Takeda T, Imanishi M, Suzuki T, Suyama T, Minakuchi Y, Kitamura R. 2001. Vitamin B₁ nutritional status and estimation assessed by blood vitamin B₁ value of middle aged Japanese men and women. *Jpn J Nutr Assess* **18**: 507–510.
- 21) Kimura M, Saito N, Itokawa Y. 1988. Thiamin status of inhabitants on North-East Thailand. *Trace Nutr Res* **4**: 163–170.
- 22) Murata K, Komuro H, Asano M. 1979. Urinary and blood thiamine levels in healthy women. *Vitamins (Japan)* **53**: 1–9.
- 23) Itokawa Y, Kimura M, Nishino K, Mino M, Kitagawa M, Matsuoka M, Otsuka H. 1991. Changes in blood concentrations and urinary excretion of thiamin and ribo-

- flavin during oral administration of multivitamin tablets to healthy adults for 44 weeks. *Vitamins (Japan)* **65**: 535–544.
- 24) Nishio M, Fujiwara M, Kitamura M, Nakata S. 1948. Studies on experimental thiamine deficiency in man and thiamine requirement. *Vitamins (Japan)* **1**: 256–257.
 - 25) Hiraoka M. 2001. Nutritional status of vitamin A, E, C, B₁, B₂, B₆, nicotinic acid, B₁₂, folate, and β -carotene in young women. *J Nutr Sci Vitaminol* **47**: 20–27.
 - 26) Roughead ZK, McCormick DB. 1991. Urinary riboflavin and its metabolites: effects of riboflavin supplementation in healthy residents of rural Georgia (USA). *Eur J Clin Nutr* **45**: 299–307.
 - 27) Fernandes-Costa F, van Tonder S, Metz J. 1985. A sex difference in serum cobalamin and transcobalamin levels. *Am J Clin Nutr* **41**: 784–786.
 - 28) Low-Beer TS, McCarthy CF, Austad WI, Brzechwa-Ajdukiewicz A, Read AE. 1968. Serum vitamin B₁₂ levels and vitamin B₁₂ binding capacity in pregnant and non-pregnant Europeans and West Indians. *Br Med J* **4**: 160–161.
 - 29) Metz J, Hart D, Harpending HC. 1971. Iron, folate, and vitamin B₁₂ nutrition in a hunter-gatherer people: A study of the Kung Bushmen. *Am J Clin Nutr* **24**: 229–242.
 - 30) Mollin DL, Ross GIM. 1952. The vitamin B₁₂ concentrations of serum and urine of normal and of patients with megaloblastic anaemias and other diseases. *J Clin Pathol* **5**: 129–139.
 - 31) Adams JF. 1970. Correlation of serum and urine vitamin B₁₂. *Br Med J* **1**: 138–139.
 - 32) Shibata K, Matsuo H. 1989. Levels of NAD, NADP and their related compounds in human blood. *Vitamins (Japan)* **63**: 569–572.
 - 33) Nose Y. 1950. On the nicotinic acid content in blood and urine of healthy body. *Vitamins (Japan)* **3**: 16–17.
 - 34) Katsura E, Sakakida H. 1953. Supplement of knowledge on the method for estimating nicotinic acid and its normal blood level. *Vitamins (Japan)* **6**: 904–908.
 - 35) Shibata K, Matsuo H. 1989. Correlation between niacin equivalent intake and urinary excretion of its metabolites, N¹-methylnicotinamide, N¹-methyl-2-pyridone-5-carboxamide, and N¹-methyl-4-pyridone-3-carboxamide, in humans consuming a self-selected food. *Am J Clin Nutr* **50**: 114–119.
 - 36) Okamoto H, Ishikawa A, Yoshitake Y, Kodama N, Nishimuta M, Fukuwatari T, Shibata K. 2003. Diurnal variations in human urinary excretion of nicotinamide catabolites: effects of stress on the metabolism of nicotinamide. *Am J Clin Nutr* **77**: 406–410.
 - 37) Moyer EZ, Goldsmith GA, Miller ON, Miller J. 1963. Metabolic patterns in preadolescent children. Intake of niacin and tryptophan and excretion of niacin or tryptophan metabolites. *J Nutr* **79**: 423–430.
 - 38) Wittwer CT, Schweitzer C, Pearson J, Song WO, Windham CT, Wyse BW, Hansen RG. 1989. Enzymes for liberation of pantothenic acid in blood: Use of plasma pantotheinase. *Am J Clin Nutr* **50**: 1072–1078.
 - 39) Song WO, Wyse BW, Hansen RG. 1985. Pantothenic acid status of pregnant and lactating women. *J Am Diet Assoc* **85**: 192–198.
 - 40) Fry PC, Fox HM, Tao HG. 1976. Metabolic response to a pantothenic acid deficient diet in humans. *J Nutr Sci Vitaminol* **22**: 339–346.
 - 41) Fox HM, Linkswiler H. 1961. Pantothenic acid excretion on three levels of intake. *J Nutr* **75**: 451–454.
 - 42) Milne DB, Johnson LK, Mahalko JR, Sandstead HH. 1983. Folate status of adult males living in metabolic unit: Possible relationships with iron nutriture. *Am J Clin Nutr* **37**: 768–773.
 - 43) McKay DL, Perrone G, Rasmussen H, Dalla G, Blumberg JB. 2000. Multivitamin/mineral supplementation improves plasma B-vitamin status and homocysteine concentration in healthy older adults consuming a folate-fortified diet. *J Nutr* **130**: 3090–3096.
 - 44) Von der Porten AE, Gregory JF III, Toth JP, Cerda J, Curry SH, Bailey LB. 1992. In vivo folate kinetics during chronic supplementation of human subjects with deuterium-labeled folic acid. *J Nutr* **122**: 1293–1299.
 - 45) Turgan N, Boydak B, Habif S, Apakkan S, Ozmen D, Mutaf I, Bayindir O. 1999. Plasma homocysteine levels in acute coronary syndrome. *Jpn Heart J* **40**: 729–736.
 - 46) Tucker KL, Selhub J, Wilson PW, Rosenberg IH. 1996. Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr* **126**: 3025–3031.
 - 47) Yukawa M, Naka H, Murata Y, Katayama S, Kohriyama T, Mimori Y, Nakamura S. 2001. Folic acid-responsive neurological diseases in Japan. *J Nutr Sci Vitaminol* **47**: 181–187.
 - 48) Tamura T, Stokstad EL. 1973. The availability of food folate in man. *Br J Haematol* **25**: 513–532.
 - 49) Watanabe T, Yasumura S, Shibata H, Fukui T. 1998. Biotin status and its correlation with other biochemical parameters in the elderly people of Japan. *J Am Coll Nutr* **17**: 48–53.
 - 50) Sweetman L, Surh L, Baker H, Peterson RM, Nyhan WL. 1981. Clinical and metabolic abnormalities in a boy with dietary deficiency of biotin. *Pediatrics* **68**: 553–558.
 - 51) Jung U, Helbich-Endermann M, Bitsch R, Schneider S, Stein G. 1998. Are patients with chronic renal failure (CRF) deficient in biotin and is regular biotin supplementation required? *Z Ernahrungswiss* **37**: 363–367.
 - 52) Bitsch R, Salz I, Hotzel D. 1989. Studies on bioavailability of oral biotin doses for humans. *Int J Vitam Nutr Res* **59**: 65–71.
 - 53) Levine E, 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.
 - 54) Kobata T, Inoue K, Ishii K, Higuchi M. 1998. Vitamin status in young women with different physical activity levels. *Vitamins (Japan)* **72**: 363–371.
 - 55) Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, 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.
 - 56) Institute of Medicine, ed. 2000. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. In: Vitamin C, p 95–185. National Academy Press, Washington, DC.
 - 57) Ihara H, Shino Y, Hashizume N. 2004. Recommended dietary allowance for vitamin C in the United States is also applicable to a population of young Japanese women. *J Clin Lab Anal* **18**: 305–308.