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Urinary Excretion Levels of Water-Soluble Vitamins in Pregnant and Lactating Women in Japan

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Summary Recent studies have shown that the urinary excretion levels of water-soluble vitamins can be used as biomarkers for the nutritional status of these vitamins. To determine changes in the urinary excretion levels of water-soluble vitamins during pregnant and lactating stages, we surveyed and compared levels of nine water-soluble vitamins in control (non-pregnant and non-lactating women), pregnant and lactating women. Control women (n=37), women in the 2nd (16–27 wk, n=24) and 3rd trimester of pregnancy (over 28 wk, n=32), and early- (0–5 mo, n=54) and late-stage lactating (6–11 mo, n=49) women took part in the survey. The mean age of subjects was ~30 y, and mean height was ~160 cm. A single 24-h urine sample was collected 1 d after the completion of a validated, self-administered comprehensive diet history questionnaire to measure water-soluble vitamins or metabolites. The average intake of each water-soluble vitamin was ≈ the estimated average requirement value and adequate intake for the Japanese Dietary Reference Intakes in all life stages, except for vitamin B_6 and folate intakes during pregnancy. No change was observed in the urinary excretion levels of vitamin B_2, vitamin B_6, vitamin B_{12}, biotin or vitamin C among stages. Urine nicotinamide and folate levels were higher in pregnant women than in control women. Urine excretion level of vitamin B_1 decreased during lactation and that of pantothenic acid decreased during pregnancy and lactation. These results provide valuable information for setting the Dietary Reference Intakes of water-soluble vitamins for pregnant and lactating women.

Key Words water-soluble vitamin, pregnant women, lactating women, urine, nutrient intake

Dietary habits of pregnant and lactating women have a critical impact on the health of future infants. For this reason, the Japanese Dietary Reference Intakes regarding the amounts of addition of water-soluble vitamins have been set (*1*). The additional intake amounts for pregnant and lactating women were calculated based on data concerning the changes in the metabolic characteristics of vitamins in pregnant women and the loss of vitamins as a result of lactation. However, there is little information about whether nutritional statuses of water-soluble-vitamins in Japanese pregnant and lactating women are good or not. Therefore, we examined the nutritional status of pregnant and lactating women and speculated about the validity of the additional amount of some water-soluble vitamins in the dietary reference intakes for pregnant and lactating women.

A potential approach for determining the validity for the additional amounts is based on the observation that a water-soluble vitamin or its catabolite(s) can be detected in the urine (*2–11*). Using this approach, the urinary excretion of a water-soluble vitamin or its catabolite(s) occurs when the dietary intake exceeds an individual necessary amount.

MATERIALS AND METHODS

Subjects. Japanese pregnant (16–40 wk gestation) or lactating women (0–11 mo postpartum) were recruited from a parenting circle in The University of Shiga Prefecture between April 2010 and February 2012. Japanese married non-pregnant, non-lactating women also were recruited as controls through the parenting circle to match the mean ages and heights of the pregnant and lactating women. The purpose and protocol of this study was explained to all participants before joining the study, and written informed consent was obtained from each participant. We excluded participants diagnosed with a cold or influenza, and those who had taken multi-vitamin supplements at least once during the previous month. In addition, we excluded participants whose 24-h urine collection was considered incomplete: a collection time outside the 22–26-h range, urine volume <250 mL, or extremely low or high energy intake (<500 or >4,000 kcal/d) (*12*). Twenty-four of the 2nd trimester pregnant (16–27 wk gestation) and 32 of the

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3rd trimester pregnant (after 28 wk gestation) women, 54 (0–5 mo) and 49 (6–11 mo) of the lactating women, and 37 non-pregnant, non-lactating women (used as control women) were found to be eligible. This study was reviewed and approved by the Ethical Committee of The University of Shiga Prefecture.

Diet history assessment. Dietary habits during the preceding month were assessed using a previously validated, self-administered comprehensive diet history questionnaire (DHQ) (*13–15*). All answered DHQs, as well as a lifestyle questionnaires, were checked at least twice for completeness. When necessary, forms were reviewed with the subject to ensure the clarity of answers. The DHQ is a 16-page, structured questionnaire that consists of the following seven sections: general dietary behavior; major cooking methods; consumption frequency and semi-quantitative portion size of 122 selected food and non-alcoholic beverage items; dietary supplements; consumption frequency and semi-quantitative portion size of 19 cereals usually consumed as staple foods (rice, bread and noodles) and miso (fermented soybean paste) soup; and open-ended items for foods consumed regularly (more than once a week) but not appearing in the DHQ. Items and portion sizes were derived primarily from data in the National Nutrition Survey of Japan and several recipe books for Japanese dishes (*15*).

Estimates of dietary intake for 150 food and beverage items, energy and nutrients were calculated using an ad hoc computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan (*16*). Because biotin is not listed in the table, we did not calculate dietary biotin intake. Information on dietary supplements and data from open-ended questionnaire items were not used in the calculation. Detailed descriptions of the methods used to calculate dietary intake and the validity of the DHQ have been published (*13–15*).

24-h urine collection. A single 24-h urine sample was collected one day after the completion of the DHQ to measure water-soluble vitamins or metabolites. Subjects were instructed both in writing and orally on the methods of urine collection and the necessity of obtaining a complete 24-h urine collection. Subjects were requested to eat and drink normally during the collection and to follow their usual pattern of activity. Subjects were then provided with a bag, three or four 1 L plastic bottles (containing no additives) and ten 400 mL cups. A recording sheet also was provided. In the morning, subjects were asked to discard the first specimen and to record the time (usually 06:00–09:00 h) on the sheet (the start of the collection period). Subjects were asked to collect all specimens by the time of the start of the collection period the following morning. When some specimens were missed, subjects were asked to record the estimated volume of missing urine and the time. The following morning, subjects were asked to collect the last specimen at the time when the first specimen was discarded the previous morning, and record the time on the collection sheet (the end of the collection period). The collection sheet was reviewed by the research staff when the samples were returned, and any missing information was obtained from the subjects. The height of urine in each bottle was measured and later converted into volume using an empirical formula based on repeated measurements of volume in identical bottles. All urine from the 24-h collection period was then combined and mixed thoroughly by vigorous stirring, and urinary aliquots taken and used for determination of vitamins and metabolites.

Chemicals. Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl; MW=337.27), riboflavin ($C_{17}H_{20}N_4O_6$=376.37), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$=1,355.40), nicotinamide ($C_6H_6N_2O$=122.13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca=476.54), folic acid (pteroylmonoglutamic acid, $C_{19}H_{19}N_7O_6$=441.40) and D(+)-biotin ($C_{10}H_{16}N_2O_3S$=244.31) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-Pyridoxic acid (4-PIC, $C_8H_9NO_4$=183.16) was made by ICN Pharmaceuticals (Costa Mesa, CA, USA) and obtained through Wako.

N^1-Methylnicotinamide (MNA) chloride ($C_7H_9N_2O$-HCl=159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$=152.15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$=152.15) were synthesized by the methods of Pullman and Colowick (*17*) and Shibata et al. (*18*), respectively. All other chemicals used were of the highest purity available from commercial sources.

Determination of vitamins and metabolites in urine. For analysis of thiamin, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of thiamin was determined by the HPLC-post labeled fluorescence method (*19*). For analysis of riboflavin, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of riboflavin was determined by the HPLC method (*20*). For analysis of 4-PIC, a metabolite of pyridoxal, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of 4-PIC was determined by the HPLC method (*21*).

For analysis for vitamin B_{12}, acetate buffer and potassium cyanide were added to urine, and vitamin B_{12} in urine was converted to cyanocobalamin by autoclave (*22*). Urinary content of cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmannii*, ATCC 7830 (*22*). For analysis of MNA, 2-Py and 4-Py, nicotinamide metabolites, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of 2-Py, 4-Py and MNA was determined by the HPLC method (*18*, *23*). For analysis of pantothenic acid, a urine sample was injected directly into a HPLC (*24*).

For analysis of folate, 1 mL of 1 mol/L ascorbic acid was added to 9 mL of urine. Urinary content of folate was determined by the microbioassay method using *Lactobacillus rhamnosus*, ATCC 27773 (*25*). For analysis of ascorbic acid, 4 mL of 10% metaphosphate was added to 4 mL of urine. Urinary content of reduced and oxidized ascorbic acid, and 2,3-diketogulonic acid, was determined by the HPLC method (*26*).

Other outcomes. Body height was measured to the nearest 0.1 cm with the subjects standing without

Table 1. Basic characteristics of control, pregnant and lactating women.

	Control	2nd trimester pregnant women (16–27 wk)	3rd trimester pregnant women (over 28 wk)	Lactating women (0–5 mo)	Lactating women (6–11 mo)
n	37	24	32	54	49
Age (y)	32.4±3.7	31.2±3.5	31.7±3.9	32.1±3.7	31.6±3.0
Height (cm)	160±5	159±5	160±5	160±5	159±5
Body weight (kg)	51.7±6.6^{a}	53.6±4.4^{a}	59.1±7.8^{b}	53.7±6.8^{a}	50.4±6.1^{a}
BMI (kg/m^2)	20.2±2.1^{a}	21.3±1.8^{a}	23.1±2.4^{b}	20.9±2.3^{a}	19.9±1.9^{a}
Energy intake (kcal/d)	1,684±355^{a}	1,812±338^{ab}	1,765±388^{ab}	1,967±439^{b}	1,904±383^{ab}
Protein intake (%energy)	13.5±1.6	13.1±2.0	13.7±1.7	13.6±1.3	13.6±1.4
Fat intake (%energy)	30.5±5.6	29.4±6.6	28.8±4.2	28.6±4.3	27.5±4.4
Carbohydrate intake (%energy)	56.0±6.1	57.5±6.8	57.4±4.9	57.8±4.8	58.9±5.2

Values are means±SD. The means in a row without a common superscripted letter differ, $p<0.05$, determined by one-way ANOVA followed by Tukey-Kramer multiple comparison tests.

Table 2. Water-soluble vitamin intake in control, pregnant and lactating women.

	Control	2nd trimester pregnant women (16–27 wk)	3rd trimester pregnant women (over 28 wk)	Lactating women (0–5 mo)	Lactating women (6–11 mo)
n	37	24	32	54	49
Vitamin B_1 (mg/d)	0.69±0.19^{a}	0.76±0.20^{ab}	0.75±0.21^{ab}	0.86±0.28^{b}	0.80±0.21^{ab}
Vitamin B_2 (mg/d)	1.2±0.4^{a}	1.3±0.6^{ab}	1.4±0.5^{ab}	1.5±0.5^{b}	1.4±0.4^{ab}
Vitamin B_6 (mg/d)	0.87±0.27^{a}	1.00±0.32^{ab}	0.97±0.28^{ab}	1.11±0.32^{b}	1.06±0.28^{b}
Vitamin B_{12} (μg/d)	4.9±2.0^{a}	5.8±3.1^{ab}	6.0±3.2^{ab}	6.5±2.9^{b}	6.2±2.2^{ab}
Niacin (mgNE/d)	24±6^{a}	25±7^{ab}	25±6^{ac}	29±7^{b}	28± 6^{bc}
PaA (mg/d)	5.0±1.5^{a}	5.7±2.1^{ab}	5.7±1.7^{ab}	6.2±1.6^{b}	6.0±1.5^{b}
Folate (μg/d)	256±93	286±111	282±111	301±100	302±107
Vitamin C (mg/d)	79±30	104±54	98±47	102±53	93±27
Vitamin B_1 (mg/1,000 kcal)	0.41±0.07	0.42±0.09	0.42±0.07	0.43±0.07	0.42±0.08
Vitamin B_2 (mg/1,000 kcal)	0.71±0.17	0.70±0.24	0.74±0.17	0.72±0.13	0.71±0.12
Vitamin B_6 (mg/1,000 kcal)	0.51±0.12	0.56±0.14	0.55±0.12	0.55±0.11	0.56±0.12
Vitamin B_{12} (μg/1,000 kcal)	2.9±1.0	3.0±1.1	3.2±1.1	3.2±1.0	3.3±1.1
Niacin (mgNE/1,000 kcal)	14±2	14±2	14±2	15±2	15±2
PaA (mg/1,000 kcal)	3.0±0.5	3.1±0.8	3.2±0.6	3.1±0.5	3.1±0.5
Folate (μg/1,000 kcal)	152±44	154±47	156±47	151±31	159±49
Vitamin C (mg/1,000 kcal)	47±16	54±22	52±18	50±16	50±13

Values are means±SD. The means in a row without a common superscripted letter differ, $p<0.05$, determined by one-way ANOVA followed by Tukey-Kramer multiple comparison tests.

wearing shoes. Body weight in light, indoor clothes was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as body weight (kg) divided by the square of body height (m). Current smoking status was self-reported in the lifestyle questionnaire, whereas current dietary supplement use was assessed in the DHQ.

Statistical analyses. The statistical significance was determined by one-way ANOVA followed by Tukey-Kramer multiple comparison tests and $p<0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Subject characteristics are shown in Table 1. The mean age of the sample was ~30 y old, and mean height was ~160 cm. Energy intake was increased by pregnancy and lactation compared to non-pregnant, non-lactating period, but no difference among non-pregnant, non-lactating women, pregnant women and late-stage lactating women was observed; the only statistically significantly higher value occurred in the early-stage lactating women (0–5 mo). Energy balance was the same in all experimental and control subjects.

Intakes of water-soluble vitamins are shown in Table 2. The limitation of the present study is that nutrient intakes were calculated by DHQ (*13–15*) but not by a weight food record method (*27*). Generally speaking, a reported weak point of several food survey questionnaires is pointed out that portion sizes differ from indi-

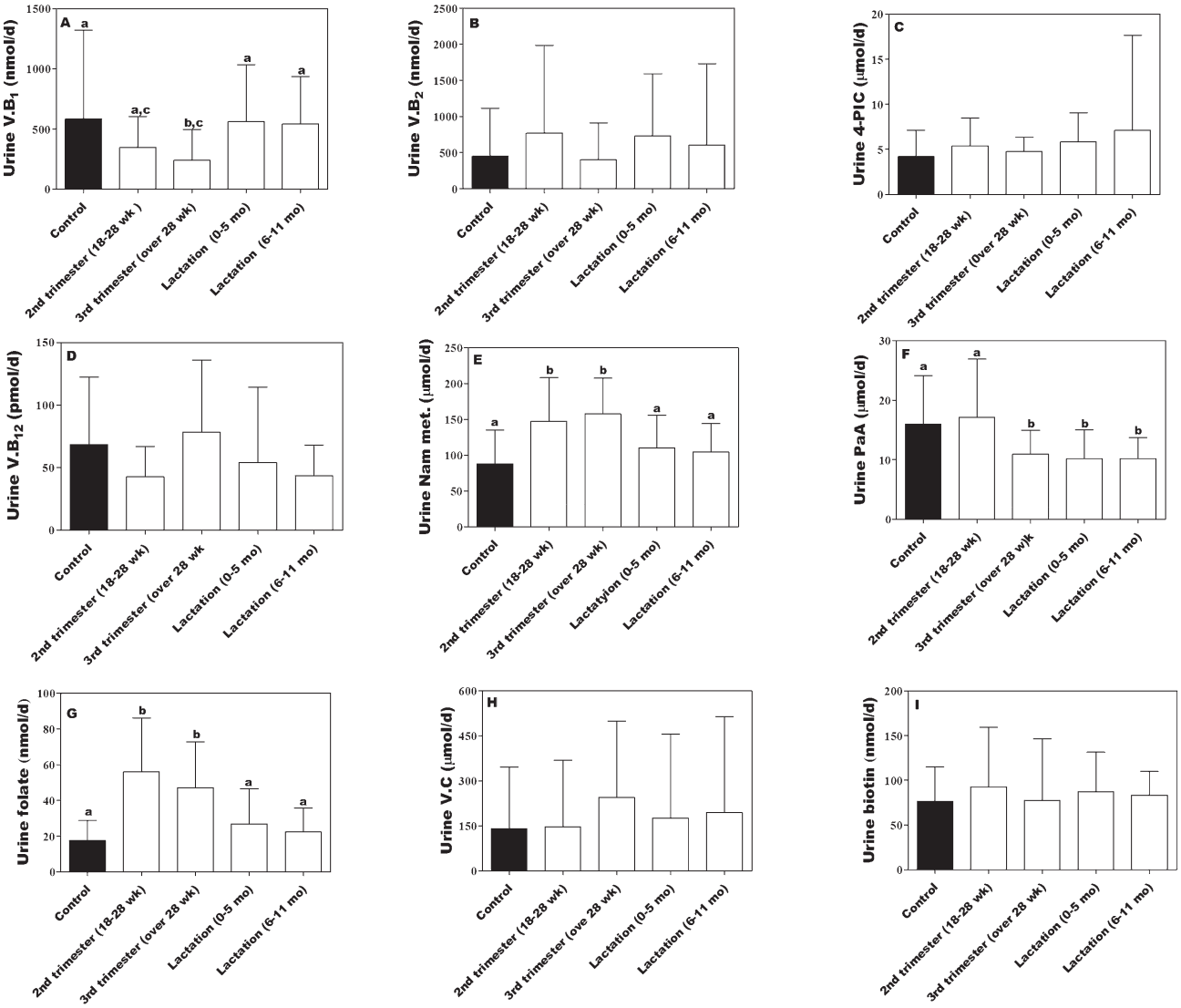

Fig. 1. Urinary excretion levels of water-soluble vitamins in control (non-pregnant, non-lactating women), pregnant and lactating women. (A) vitamin B_1; (B) vitamin B_2; (C) 4-PIC, a catabolite of vitamin B_6; (D) vitamin B_{12}; (E) sum of Nam and its catabolites; (F) pantothenic acid; (G) folate; (H) vitamin C; (I) biotin. Numbers of the subjects in control women, women in the 2nd trimester of pregnancy (16–28 wk), the 3rd trimester of pregnancy (over 28 wk), lactating women (0–5 mo), and lactating women (6–11 mo) are 37, 24, 32, 54, and 49, respectively. Values are means±SD. The columns without a common superscripted letter differ, $p<0.05$, determined by one-way ANOVA followed by Tukey-Kramer multiple comparison tests.

vidual to individual, so the validity of energy intake is low. The DHQ used in the present experiment (*13–15*) clearly describes portion sizes for major items of food to reduce the error of individual portion sizes. Nevertheless, a weak point of the DHQ is lower validity of energy intake compared to the weight food record method. Therefore, the values calculated per unit of energy have more validity compared with the values per day.

Energy adjusted water-soluble vitamin intakes in the pregnant women were consistent with a previous report investigating 997 pregnant Japanese women (*28*). Most of the intakes of water-soluble vitamins per day were higher for pregnant and lactating subjects compared to non-pregnant, non-lactating ones, but no difference was observed among non-pregnant, non-lactating women, pregnant women and late-stage lactating women, and the value was statistically significantly higher only in the early-stage lactating women (0–5 mo) compared to other groups. Because energy adjusted water-soluble vitamin intakes were the same for pregnant and lactating subjects, these higher values were the result of a higher of energy intake in early-stage lactation.

As described in Introduction, a potential approach for determining the validity for the additional amounts for pregnant and lactation women is based on the observation that a water-soluble vitamin or its catabolite(s) can be detected in the urine (*2–11*). Using this approach, the urinary excretion of a water-soluble vitamin or its catabolite(s) occurs when the dietary intake exceeds an individual requirement. In the present study, the values of the non-pregnant, non-lactating women were used as the control and as the reference values.

Figure 1 shows the urinary excretion amounts of water-soluble vitamins.

Vitamin B_1

The estimated average requirement (EAR) of vitamin B_1 in all stages is 0.45 mg/1,000 kcal (*1*). As shown in Table 2, the average intakes in all life stages were slightly lower than the corresponding EARs.

The vitamin B_1 intakes per 1,000 kcal were almost the same in all life stages (Table 2). However, the average urinary excretion levels of vitamin B_1 had a tendency to be lower and even lower in subjects in the 2nd and 3rd trimester than in the controls and lactating women (Fig. 1A). Therefore, the additional amount is needed for pregnant women. How large an amount of vitamin B_1 should be settled on is the next problem. Anyway, as a point of nutritional guidance, increased intake of vitamin B_1 should be advised for pregnant women.

We could not find an effect of pregnancy or lactation on the urinary excretion levels of vitamin B_1 in humans in the literature. The present data show that pregnancy might affect the urinary excretion levels of vitamin B_1.

Vitamin B_2

The estimated average requirement (EAR) of vitamin B_2 in all stages is 0.50 mg/1,000 kcal (*1*). As shown in Table 2, the average intakes were higher than the corresponding EARs in all life stages.

Differences in the intakes of vitamin B_2 in terms of energy intake were not observed among life stages (Table 2) and the urinary excretion levels were also not altered in any life stages (Fig. 1B). As a point of nutritional guidance, vitamin B_2 intakes for Japanese women in all stages were adequate.

We could not find any previous reports regarding the effects of pregnancy and lactation on the urinary excretion levels of vitamin B_2 in humans. The present data show that pregnancy and lactation did not affect the urinary excretion levels of vitamin B_2.

Vitamin B_6

The EAR of vitamin B_6 for 30–49 y old control women is 1.0 mg/d, 1.7 mg/d, during the 2nd and 3rd trimesters, and 1.3 mg/d in lactating women (*1*). The estimated energy requirement (EER) for 30–49 y old non-pregnant, non-lactating women is 1,750 kcal/d, 2,000 kcal/d and 2,200 kcal/d during the 2nd and 3rd trimesters of pregnancy, and 2,100 kcal/d in lactating women (*1*). The EAR of vitamin B_6 per 1,000 kcal becomes 0.57 mg/1,000 kcal in non-pregnant, non-lactating women, 0.85 mg/1,000 kcal, 0.77 mg/1,000 kcal during the 2nd and 3rd trimesters of pregnancy, and 0.62 mg/1,000 kcal in lactating women. As shown in Table 2, the average intakes in all life stages were lower than the EAR/1,000 kcal. The intake in the pregnant state was extremely low compared with the EAR/1,000 kcal. Nevertheless, the urinary excretion of 4-PIC, a major catabolite of vitamin B_6, in the pregnant subjects was not lower compared with the control subjects (Fig. 1C).

The urinary excretion levels were not altered in any of the life stages (Fig. 1C). Therefore, the present EAR of vitamin B_6 in the pregnant stage is too high compared to the necessity for it. A further study is needed.

The EAR of vitamin B_6 for pregnant women is controversial because plasma PLP concentrations have been reported to decrease during pregnancy (*29–36*). Several investigators (*33–36*) proposed that 2–7 mg/d of vitamin B_6 should be supplemented during the 3rd trimester of pregnancy. However, we suggest that studies are required to evaluate fully the beneficial effects of maternal vitamin B_6 supplementation on infant conditions at birth.

Among the evaluation of the vitamin B_6 nutritional status, plasma PLP is considered the most relevant, but measurement of urinary 4-PIC, which is the major end product of vitamin B_6 metabolism, also is recommended (*37*, *38*). Increased elimination of 4-PIC means a surplus intake of vitamin B_6 above the necessary level of vitamin B_6. In our opinion, the present EAR for vitamin B_6 for pregnant and lactating women is higher than necessary. Further studies are needed to determine the EAR of vitamin B_6 for pregnant women.

Vitamin B_{12}

The EAR of vitamin B_{12} for 30–49 y old control women is 2.0 μg/d, 2.3 μg/d during the 2nd and 3rd trimesters, and 2.3 μg/d in lactating women (*1*). The EAR of vitamin B_{12} per 1,000 kcal becomes 1.1 μg/1,000 kcal in non-pregnant, non-lactating women, 1.2 μg/1,000 kcal, 1.0 μg/1,000 kcal during the 2nd and 3rd trimesters of pregnancy, and 1.1 μg/1,000 kcal in lactating women. As shown in Table 2, the average intakes during all life stages were higher than the corresponding EARs. The urinary excretion levels of vitamin B_{12} do not reflect the intake or the nutritional status of vitamin B_{12} (*6*). Vitamin B_{12} is different from other water-soluble vitamins with respect to its mechanism of absorption and main excretion route—the main excretion route is thought to be the bile (*39*). Available reports suggest that plasma concentrations of vitamin B_{12}, methylmalonic acid, and total homocysteine are effective biomarkers of a change in vitamin B_{12} intake (*40*). We report the urinary excretion levels as informative data (Fig. 1D). We could not find any previous reports on the effects of pregnancy and lactation on the urinary excretion levels of vitamin B_{12} in humans. Therefore, the present data are the first report. The present data show that pregnancy and lactation did not affect the urinary excretion levels of vitamin B_{12}.

Niacin

The EAR of niacin for 30–49 y old in all stages is 4.8 mg NE/1,000 kcal (*1*). As shown in Table 2, the average intakes in all life stages were triple the value of the EAR. When evaluating the nutritional status of niacin, the urinary excretion levels of nicotinamide catabolites such as MNA, 2-Py, and 4-Py traditionally were used as potential indices of body stores (*41–45*). The higher excretion amounts in pregnancy stages are attributed to the increased conversion of Trp→Nam during pregnancy (*46*), but not increased intake of tryptophan and niacin. The nutritional status of niacin for Japanese women is very good (Fig. 1E). The present data also support no need for supplementation of niacin dur-

ing pregnancy (*1*).

Pantothenic acid

The adequate intake (AI) of pantothenic acid for 30–49 y old non-pregnant, non-lactating women is 5 mg/d, 6 mg/d during the 2nd and 3rd trimesters of pregnancy, and 6 mg/d in lactating women (*1*). The AI of pantothenic acid per 1,000 kcal becomes 2.9 mg/1,000 kcal in non-pregnant, non-lactating women, 3.0 mg/1,000 kcal, 2.7 mg/1,000 kcal during the 2nd and 3rd trimesters of pregnancy, and 2.9 mg/1,000 kcal in lactating women. As shown in Table 2, the average intakes in all life stages were almost the same as the corresponding modified AI.

Song et al. (*47*) reported that the urinary excretion levels of pantothenic acid in pregnant women during the 3rd trimester of pregnancy were not different from those of non-pregnant, non-lactating women. The significant positive correlation between dietary vitamin intake and urinary excretion levels has been reported previously in the literature (*48*–*50*). In the present experiment, the urinary excretion levels were lower in the 3rd trimester and lactating women than in control and 2nd trimester women (Fig. 1F). This finding suggests that the necessity of pantothenic acid might be higher during the 3rd trimester of pregnancy and lactation than during either the 2nd trimester women or for non-pregnant, non-lactating women. The requirement for pantothenic acid in the 3rd trimester women and lactating women might be higher than the requirement for non-pregnant, non-lactating women. Because urinary excretion levels of pantothenic acid are closely related to dietary intake of the vitamin, urinary excretion levels have been used as an indicator of the nutritional status of pantothenic acid (*47*, *48*). Urinary excretion levels of pantothenic acid may provide a more reliable indicator of pantothenic acid status than blood pantothenic acid levels. Therefore, there is a possibility that an EAR of pantothenic acid can be set based on the data regarding the urinary excretion levels of pantothenic acid.

Folate

The EAR of folate for 30–49 y old non-pregnant, non-lactating women is 200 μg/d, 400 μg/d during the 2nd and 3rd trimesters, and 280 μg/d in lactating women (*1*). The EAR of folate per 1,000 kcal becomes 115 μg/1,000 kcal in non-pregnant, non-lactating women, 200 μg/1,000 kcal, 180 μg/1,000 kcal during the 2nd and 3rd trimesters of pregnancy, and 130 μg/1,000 kcal in lactating women. As shown in Table 2, the average intakes in control women and lactating women (0–5 mo and 6–11 mo) were higher compared to the corresponding EAR/1,000 kcal, while the intakes during stages of pregnancy were lower than the EAR/1,000 kcal. No difference was observed in the folate intakes among life stages in terms of 1,000 kcal (Table 2). However, the urinary excretion levels were higher in the pregnant women than in the controls and lactators (Fig. 1E). As based on the present urine data, the EAR during pregnancy is much higher than the necessary amount.

In 1970, the U.S. Food and Nutrition Board (*51*) set the recommended folate intake for pregnant women at 400 μg/d; this was reduced to 270 μg/d in 1989 mainly because of data showing that this amount was typically ingested by healthy, folate-replete adults (*52*). The recommendation was again increased to 400 μg/d (200 μg from food folate and 100 μg from folic acid) in 1998 (*53*), after the bioavailability of food folate and folic acid (pteroylmonoglutamic acid, which has two-fold the bioavailability of food folate) was considered. The study on "folate status and requirement in pregnancy" was published in 1968 (*54*). In the report, based on serum folate levels in non-pregnant women, the authors calculated a dose of an additional 100 μg of folic acid (equivalent to 200 μg food folate) needed during pregnancy. A limitation of this report was that the additional amount was only one point, e.g., 100 μg of folic acid. Therefore, a smaller addition of folic acid could keep serum levels normal. In addition, Willoughby and Jewell (*55*) reported that the minimum dose of folate needed during late pregnancy, in addition to a dietary folate intake of 50 μg per day, was close to 300 μg/d.

Increased folate catabolism and urinary folate excretion have been reported (*56*, *57*), which also may contribute to increased folate needs in pregnancy. However, these findings are controversial. One group reported that excretion of folate catabolites late in pregnancy was higher than in the non-pregnant state (*55*, *56*). These catabolites are cleavage products of the C-9–N-10 bond of folate, including *p*-aminobenzoylglutamate and *p*-acetamidobenzoylglutamate. In contrast, another group did not find an increase in urinary catabolites during the 2nd trimester in women (*58*). In addition, the same group reported no differences in urinary excretion of labeled folates or catabolites between the pregnant and non-pregnant women with the use of stable-isotope-labeled folates (*59*). Additional studies are needed. In the present study, the urinary excretory levels of folate were measured by microbiological assay using *Lactobacillus rhamnosus*, ATCC 27773 (*25*), which can use many kinds of folate compounds such as pteroylmonoglutamic acid (PteGlu), dihydroPteGlu, tetrahydroPteGlu, 5-formyl-tetrahydroPteGlu, 10-formyl-tetrahydroPteGlu, 5,10-methylene-tetrahydroPteGlu, and 5-methyl-tetrahydroPteGlu as growth factors (*60*). Therefore, the urinary amount did not contain the catabolites of folates. The urine folate amounts were higher in pregnant subjects than in control women or lactating subjects (Fig 1G). Japanese normal subjects, including women and men, provided a semi-purified diet containing 200 μg of chemically defined pteroylmonoglutamic acid have been reported to excrete on average ~20 nmol of folate daily (*2*). Similarly, in the present study control women excreted ~20 nmol/d. O'Keefe et al. (*61*) reported that subjects fed diets providing 200, 300, or 400 μg/d of folate had a mean daily excretion of folate of 3.8, 5.5, and 21.2 nmol, respectively. In the present survey, control women consumed ~250 μg/d food folate and the resulting excretion levels were ~20 nmol/d. These data suggest that the bioavailability of food folate might be higher in Japanese food than in

Western food. The present data indicate that the additional folate is not need during pregnancy. But further studies are needed.

Vitamin C

The EAR of vitamin C for 30–49 y old control women is 85 mg/d, 95 mg/d, during the 2nd and 3rd trimesters, and 125 mg/d in lactating women (*1*). The EAR of vitamin C per 1,000 kcal becomes 49 mg/1,000 kcal in non-pregnant, non-lactating women, 48 mg/1,000 kcal, 43 mg/1,000 kcal during the 2nd and 3rd trimesters of pregnancy, and 60 mg/1,000 kcal in lactating women.

As shown in Table 2, the average intakes in non-pregnant, non-lactating women was almost the same as the EAR/1,000 kcal, that in pregnant women was higher compared to the corresponding EAR/1,000 kcal (54 vs. 48 during the 2nd trimester, 52 vs. 43 mg/1,000 kcal) during the 3rd trimester, while those in lactating women were lower compared to the corresponding EAR/1,000 kcal (50 vs. 60 during the early-stage of lactation, and 50 vs. 60 mg/1,000 kcal for subjects in the late-stage of lactation). The urinary excretion levels did not differ in any life stage (Fig. 1H). Japanese control subjects, including women and men, provided a semi-purified diet containing chemically defined ascorbic acid (100 mg/d) have been reported to excrete ~150 μmol of ascorbic acid daily (*2*). Levine et al. (*62*) reported that the threshold of urinary excretion levels of vitamin C was between 60 and 100 mg daily in healthy young women. In their report (*62*), subjects fed 100 mg of chemically defined vitamin C daily excreted ~250 μmol of vitamin C. Another study (*63*) reported that the urinary excretion levels of vitamin C were ~150 μmol in females fed a diet containing 100 mg of vitamin C. We could not find effects of pregnancy or lactation on the urinary excretion levels of vitamin C in humans. Therefore, the present data are the first report. The present data show that pregnancy and lactation did not affect the urinary excretion levels of vitamin C.

Biotin

The present DHQ analysis could not calculate the intake of biotin. No differences in the urinary excretion amounts were observed among the life stages (Fig. 1I). The AI of biotin for 30–49 y old non-pregnant, non-lactating women is 50 μg/d, 52 μg/d during the 2nd trimester and the 3rd trimester, and 55 μg/d in lactating women (*1*). Japanese normal subjects, including women and men, provided a semi-purified diet containing 30 μg of chemically defined D(+)-biotin have been reported to excrete ~80 nmol of biotin daily (*2*). In the present survey, the urinary excretion levels of biotin were ~80 nmol/d for all life stages (Fig. 1I). Various studies have investigated the urinary excretion of biotin as an index of biotin nutritional status (*64–69*). Mock et al. (*69*) reported that biotin is catabolized to bisnorbiotin and biotin sulfoxide in humans and that the bioassay organism grows equally well on the biotin and biotin metabolites present in urine. In the present experiment, *Lactobacillus plantarum* was used as the bioassay organism to assess biotin. Therefore, the urinary excretion levels contain bisnorbiotin and biotin sulfoxide as well as biotin. The urinary excretion levels were not altered during any life stage (Fig. 1I). However, Mock et al. (*66–68*) have reported data from three studies regarding the biotin status of pregnant women. They measured two compounds: 3-hydroxyisovaleric acid, which reflects decreased tissue activity of the biotin-dependent enzyme methylcrotonyl-CoA carboxylase, and biotin. Biotin status decreases during pregnancy because the urinary excretion of biotin decreases from early to late pregnancy and excretion of 3-hydroxyisovaleric acid increases (*66*). In the second study (*68*), conflicting findings of biotin status in both early and late pregnancy were reported: 3-Hydroxyisovaleriac acid excretion was shown to be increased compared with the control, suggesting decreased activity of a biotin-dependent enzyme caused by tissue biotin depletion, and in early pregnancy, urinary excretion of biotin was normal; in late pregnancy, excretion was increased, suggesting biotin status was not decreased. In the third study, Mock et al. (*68*) designed another study to reconcile the conflicting findings. They used pregnant women with abnormally increased 3-hydroxyisovaleric acid excretion, and urine samples were collected before and after 14 d of supplementation with 300 μg biotin/d. The results show that 3-hydroxyisovaleric acid excretion decreased in some of the pregnant women administered biotin, with the conclusion that a marginal biotin deficiency frequently occurs during pregnancy.

The present data show that pregnancy and lactation did not affect the urinary excretion levels of biotin.

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