

⟨Original Article⟩

## Lower serum levels of folate and vitamin B12 in Japanese childbearing aged women in comparison with that of the United States levels

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**Summary** This study assessed the internationally standardized serum concentrations of folate and vitamin B12 (VB12) in Japanese women of childbearing age not consuming fortified foods, and compared them with those in United States. Serum folate and VB12 concentrations were determined by the Access Immunoassay System, in which observed values were standardized by the World Health Organization (WHO) International Standard 03/178. A total of 125 women voluntarily (aged 18-26 y) participated in this study, and the central 95 percentile of serum folate concentrations was 12.6-51.4 nmol/L (median 24.6 nmol/L) and that of serum VB12 was 229-779 pmol/L (median 415 pmol/L). Although none of the women were determined to have folate and VB12 deficiencies, the central 95 percentile for folate and median concentration were significantly lower than those reported in U.S. NHANES 2011-2012 (18.9-96.5 nmol/L, median 43.5 nmol/L: both men and women of all age groups). The central 95 percentile of serum VB12 in Japanese women was the same as that of U.S. NHANES 1999-2004 (women of all age groups). Because newborns with neural tube defects are now increasing in Japan, Japanese women of childbearing age should take much more folate so as to increase their serum folate level to that of the U.S. population.

**Key words:** Folic acid, Vitamin B12, Standardization, Neural tube defects, Vitamin deficiency

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## 1. Introduction

Folate is utilized to make red blood cells and the chemical components of the nervous system, and also has the role of DNA methylation. Vitamin B12 (VB12) is necessary for the synthesis of DNA during cell division. The metabolic interrelationship between folate and VB12 is well-known. Both folate and VB12 are required for the single-carbon transfer metabolism. Folate deficiency causes megaloblastic anemia, arteriosclerosis, vascular disease, and cancer. Also, numerous observational studies have suggested an association between folate deficiency and depression and dementia in the elderly, as well as indicated a decreasing risk of impaired growth and neural tube defects (NTDs) in infants<sup>1</sup>. For pregnant women to decrease the risk of having infants with NTDs, all cereal grain products such as bread and flour were fortified with folic acid at a level of 140 mg/100 g in the U.S. and Canada since 1996<sup>2</sup>.

In the National Health and Nutrition Examination Survey (NHANES) in the U.S.<sup>3</sup>, the central 95 percentile of serum folate concentration during 1988-1994 was reported as 3.9-52.6 nmol/L (median 12.9 nmol/L) in women of all age groups (4 years of age and older), and that of VB12 during 1991-1994 was 145-804 pmol/L (median 345 pmol/L). After fortification (1999-2004), the central 95 percentile of serum folate and VB12 in the same age group increased to 10.9-84.5 nmol/L (median 30.8 nmol/L) and 149-930 pmol/L (median 356 pmol/L), respectively.

In contrast to the U.S. and Canadian population, it is supposed that serum levels of folate and VB12 are different from those reported in NHANES, because the Japanese population do not eat foods fortified with folic acid. Unfortunately previous reports for Japanese women of childbearing age could not be compared with NHANES, because Japanese values had not been adjusted to international standardization. Therefore, in this study, we assessed internationally standardized concentrations of folate and VB12 in Japanese women, and considered whether there are any differences in the serum

levels between the Japanese and U.S. population reported in 1999-2004 and the latest NHANES results<sup>3,4</sup>.

## 2. Materials and methods

### 2.1. Study participants

Blood specimens collected from 125 self-reported healthy women aged 18-26 y (median 20 y) were subjected to analyses of complete blood counts (CBCs), serum albumin, serum iron and total iron-binding capacity (TIBC), plasma total homocysteine (tHcy), serum folate and VB12. Subjects with low serum albumin levels and severe iron deficiency anemia were not present in our volunteers (Table 1). All volunteers provided a 3-day dietary history prior to examination to calculate amounts of dietary intakes of folate and VB12 together with the energy intake, and to verify that they were free of habitual smoking, drinking or dietary supplements. None of them were pregnant. Written informed consent was obtained from all volunteers, and our study was in compliance with the rules for human studies at Showa Women's University (Ethics Committee approval number: 06-05) and Toho University Ohashi Medical Center (Ethics Committee approval number: 08-12).

### 2.2. Analyses

CBCs were measured by a Sysmex XE-2100 hematology analyzer (Sysmex Corporation, Hyogo, Japan). Serum albumin concentrations were determined by modified bromocresol purple method. Serum iron and TIBC were determined by Nitroso-PSAP method. Plasma tHcy concentrations were measured by the high-performance liquid chromatography (HPLC)<sup>5</sup>. Serum concentrations of folate and VB12 were measured by the Access Immunoassay System (Beckman Coulter, Inc., Brea, CA, USA) according to the instructions of the automated method and the manufacturer's reagents. Its observed values were standardized by measuring for accuracy to the WHO International Standard 03/178<sup>6</sup>. Assigned folate values of WHO 03/178 were certified by the reference measurement

Table 1 Static and dynamic variables of the volunteers in nutritional assessment.

	This study	NHNS in Japan, 2013	
		20 - 29	30 -39
Age (y)	22.2±5.9 (18 - 45: 20.0)	20 - 29	30 -39
BMI (kg/m <sup>2</sup> )	< 18.5 (n= 19, 15.2%)	21.5%*	17.6%
	18.5 - 25 (n= 99, 79.2%)	67.8%	69.1%
	> 25 (n= 7, 5.6%)	10.7%	13.3%
Energy intake (kcal/d)	1792±373 (918 - 2604: 1774)	1628±530 (1612)	1665±463 (1658)
Folate intake (µg/d)	260±107 (112 - 734: 230)	217±98 (198)	233±116 (221)
VB12 intake (µg/d)	5.3±4.9 (0.5 - 19.0: 3.4)	4.6±5.3 (2.8)	4.8±5.6 (2.8)
Serum albumin (g/dL)	4.8±0.3 (4.1 - 5.2: 4.9)	4.7±0.4	4.5±0.3
Serum Iron (µg/dL)	102.8±41.2 (22 - 205: 102.0)	70.8±31.5**	71.9±37.1**
TIBC (µg/dL)	358.1±49.6 (265 - 496: 351.5)	360.2±55.0	362.2±55.5
RBC (10 <sup>4</sup> /µL)	442.1±28.5 (363 - 495: 443.0)	449.2±34.8	440.6±32.3
Hemoglobin (g/dL)	13.1±15.7 (10.4 - 495: 13.2)	13.0±1.0	12.7±1.2
Hematocrit (%)	41.0±2.5 (35.0 - 47.4: 41.0)	40.6±3.0	40.0±3.3
MCV (fL)	92.9±5.2 (79 - 102: 93.5)	90.6±5.1	90.9±6.2
MCHC (%)	31.9±1.2 (29.1 - 34.7: 32.0)	31.9±1.0	31.9±1.3

All values are mean±SD (range: median).

\*Significantly higher than this study.

\*\*Significantly lower than this study.

procedure (LC-MS/MS). Their serum VB12 values were standardized by the radio-protein binding assay (Quantaphase II: Bio-Rad Laboratories, Hercules, CA, USA)<sup>7</sup>. Here, the serum folate concentration is the sum of folate vitamers (i.e., 5-methyltetrahydro-folic acid, 5-formyltetrahydrofolic acid, and folic acid), and the serum VB12 concentration is the sum of VB12 vitamers (i.e., methylcobalamin, adenosyl-cobalamin, hydroxocobalamin, and cyanocobalamin).

Currently, the concentrations suggesting folate and VB12 deficiencies defined by World Health Organization (WHO) are < 10 nmol/L and < 150 pmol/L, respectively<sup>8</sup>. Hyperhomocysteinemia was defined as > 10.4 µmol/L of tHcy<sup>9</sup>. Macrocytosis was defined as MCV greater than 100 fL. Plasma tHcy is a sensitive marker of the folate status, and MCV is a marker of macrocytosis.

### 2.3. Statistical analysis

Data are presented as mean±SD or median. Serum concentrations of folate and VB12 in subjects took these vitamins from diets above or below Dietary Reference Intakes for Japanese (DRIs) were compared using the Mann-Whitney U-test. Prevalence of hyperhomocysteinemia in these subjects was compared using the z-test. The statistical significance was defined as  $p < 0.05$ .

## 3. Results

### 3.1. Nutritional assessment

In this nutritional assessment our subjects took the same amount of folate, VB12 and energy intakes based on the data from the annual report of the National Health and Nutrition Survey (NHNS) in Japan, 2013<sup>10</sup>. In the NHNS 2013 survey, women at the age of 20-29 y took 217±98 µg/d of folate, 4.6±5.3 µg/d of VB12, and 1,628±530 kcal/d of energy on average. The mean intakes of our subjects were 260±107 µg/d of folate, 5.3±4.9 µg/d of VB12, and 1,792±373 kcal/d of energy. However, 63 of 125 women (50.4%: Table 2) took folate below the reference in DRIs (< 240 µg/d)<sup>11</sup> and 36 women (28.8%) took VB12 less than DRIs (< 2.4 µg/d). In addition, in 80 women (64.0%) the intake amount of energy was less than the daily requirement (< 2,000 kcal/d). Intake amounts of folate and VB12 taking from the diet significantly correlated to daily intakes for calories, respectively (Fig. 1). Their body mass index (BMI) ranged from 16.8 to 29.2 kg/m<sup>2</sup> (median 20.4 kg/m<sup>2</sup>), and 19 women (15.2%) were evaluated to be underweight (BMI < 18.5 kg/m<sup>2</sup>). This percentage of underweight women was not different from that reported in Japan NHNS 2013 in which it was indicated as 21.5% for women in their 20's. No significant differences were observed in laboratory

Table 2 Classification of patients based on their folate intakes and concentrations of folate and total homocysteine (tHcy).

Folate intakes	< DRIs (n = 63)		> DRIs (n = 62)	
Serum folate (< median)	37		25	
(> median)	26		37	
Plasma tHcy (< 10.4 $\mu\text{mol/L}$ )	28*	26	25*	37
(> 10.4 $\mu\text{mol/L}$ )	9	0	0	0

All values are numbers of subjects.

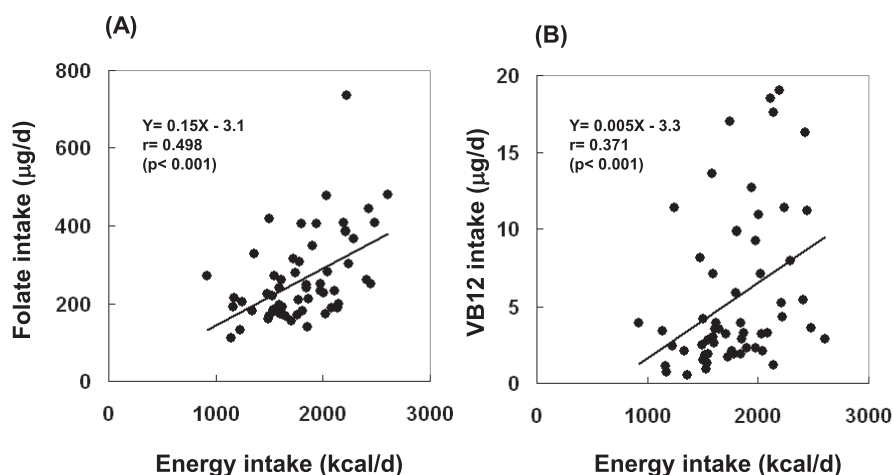


Fig. 1 Correlation between energy intake and folate (A) and VB12 (B) intake in 125 women of childbearing age.

values (Table 1) between our subjects and NHNS 2013, except for the serum iron levels.

### 3.2. Serum folate and VB12 concentrations

The serum folate concentration ranged from 12.7 to 69.3 nmol/L in 125 subjects. None of the women was determined to have a folate deficiency (< 10 nmol/L) based on blood data, despite that in half of women, the folate intake was lower than the reference values in DRIs. Although eating more vegetables, fruits, fried foods and especially drinking Japanese green tea are known to increase serum folate levels, serum folate concentrations were significantly but poorly correlated to amounts of folate intakes as shown in Fig. 2 (A) ( $r = 0.374$ ,  $p < 0.001$ ). The nutritional status in nearly one half of the subjects could not be correctly evaluated by food intake (highlighted dark in Table 2). However, serum folate concentrations in women taking folate above DRIs ( $29.9 \pm 12.6$  nmol/L) were significantly

higher than those taking folate below DRIs ( $24.3 \pm 7.1$  nmol/L). The central 95 percentile of serum folate concentrations were calculated after logarithmically transforming their concentrations, and thus defined as the antilog of [log mean  $\pm$  (2 log SD)] was 12.6-51.4 nmol/L (median 24.6 nmol/L).

In the same subjects, serum VB12 concentration ranged from 204 to 953 pmol/L. None of the women were determined to have a VB12 deficiency (< 150 pmol/L) based on blood data. No significant correlation was observed between serum VB12 concentrations and amounts of VB12 intakes ( $r = 0.039$ ,  $p > 0.05$ , Fig. 2 (B)). Serum VB12 concentrations were not different between subjects taking VB12 above DRIs ( $451 \pm 143$  pmol/L) and below DRIs ( $405 \pm 138$  pmol/L). Eating fish and dairy foods increased serum VB12 levels. The central 95 percentile of VB12 defined as the antilog of [log mean  $\pm$  (2 log SD)] was 223-779 pmol/L (median 415 pmol/L).

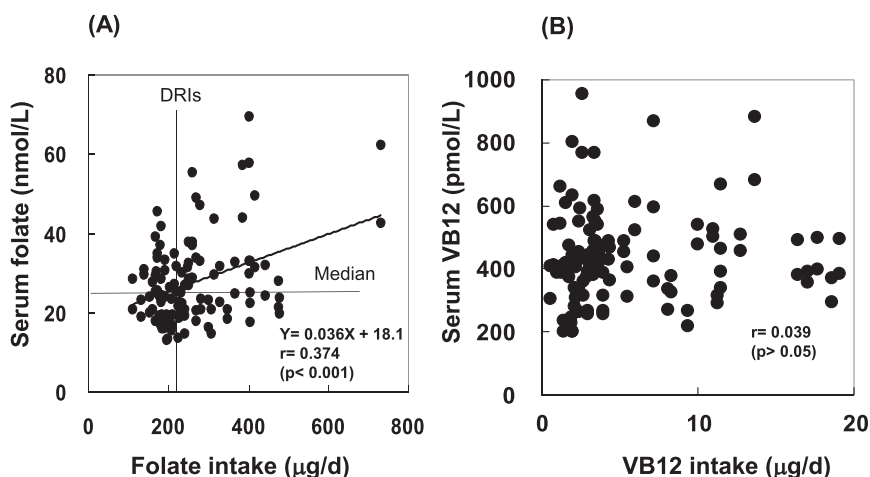


Fig. 2 Correlation between intakes of folate (A) and VB12 (B) and their serum concentrations in 125 women of childbearing age.

### 3.3. Relation to tHcy and MCV

Serum concentrations of folate and VB12 were compared with plasma tHcy concentrations (Fig. 3) and MCV (no data in Fig.). Plasma tHcy concentrations were negatively correlated to serum concentrations of folate ( $r = 0.373$ ,  $p < 0.05$ ) and VB12 ( $r = 0.249$ ,  $p < 0.05$ ). On the other hand, MCV was not correlated to serum concentrations of folate ( $r = 0.035$ ,  $p > 0.05$ ) and VB12 ( $r = 0.111$ ,  $p > 0.05$ ). In addition, BMI was poorly correlated with serum concentrations of folate ( $r = -0.203$ ,  $p < 0.05$ ) and VB12 ( $r = 0.032$ ,  $p > 0.05$ ). Nine and four women

represented elevated levels of tHcy ( $> 10.4 \mu\text{mol/L}$ ) and MCV ( $> 100 \text{ fL}$ ), respectively. All of the nine women with hyperhomocysteinemia ( $> 10.4 \mu\text{mol/L}$ ) ingested folate below DRIs (Table 2), but ingested VB12 above DRIs. The elevation of MCV in this four subjects was negligibly small at 101, 101, 102 and 102 fL.

## 4. Discussion

Although both folate and VB12 were water-soluble vitamins, their amounts stored in the body

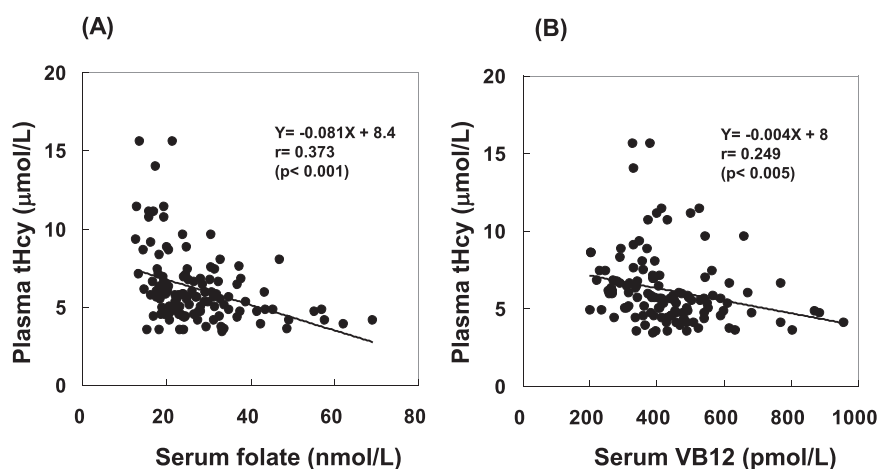


Fig. 3 Correlation between serum concentrations of folate (A) and VB12 (B) and plasma total homocysteine (tHcy) concentrations in 125 women of childbearing age.

are known as ca. 30 mg for folate (corresponding to amounts for 125 days in the Japanese DRIs) and 3 mg for VB12 (for more than 1,000 days of Japanese DRIs)<sup>11</sup>. This suggests that we easily become folate deficient by taking an insufficient amount of folate. However, it is considered that symptoms of VB12 deficiency do not appear because this vitamin accumulates at high concentration in the liver in the short period. In the U.S. and Canada, the fortification of cereal products with folic acid dramatically reduced infants with NTDs which were caused by maternal folate deficiency. On the other hand, in Japan, infants with NTDs still continue to increase<sup>12</sup>. Nowadays, the prevalence of NTDs was reported to be 4.7 and 5.1 per 10,000 live births in 2005 and 2012 in Japan, respectively. Japanese women are not actively using fortified foods, and as a result serum folate levels are lower than those in the U.S. and Canadian population. Therefore, we need think about how to raise awareness concerning these issues.

Our study provides a first-time analysis of internationally standardized serum concentrations of folate and VB12 in Japanese women of childbearing age, in which the central 95 percentile was 12.6-51.4 nmol/L (median 24.6 nmol/L) for folate and 223-779 pmol/L for VB12 (median 415 pmol/L). The latest NHANES 2011-2012 (both men and women of all age groups) presented the central 95 percentile determined by the reference measurement procedure (LC-MS/MS) as 18.9-96.5 nmol/L (median 43.5 nmol/L)<sup>4</sup>. When taking this study into consideration, the central 95 percentile and median observed in Japanese women would be lower than those in the U.S. population. Serum VB12 levels in Japanese women were similar to those from NHANES 1999-2004. However, we should be aware that serum folate levels in women of childbearing age in NHANES 2005-2006 (15-45 y) were observed to be 70% of that of other age groups<sup>13</sup>. Even after the consideration of this study, the median folate level of Japanese women of childbearing age is lower than that of U.S. women.

In addition to taking unfortified foods, the reason why the Japanese women have lower serum folate

levels would be the evidence of a high prevalence of genetic variation of methylenetetrahydrofolate reductase (MTHFR: EC 1.5.1.20) in the Japanese population. Among several variations in the genetic polymorphisms, C677>T mutation most strongly affects MTHFR activities. In the Japanese, the frequency of the homozygous TT genotype, heterozygous CT genotype, and homozygous CC genotype (wild type) was reported to be 14%, 42%, and 44% for men and 13%, 46%, and 41% for women, respectively in this order<sup>14</sup>. Consequently, serum folate levels were lowered in TT and CT genotypes as compared to the CC genotype<sup>15</sup>. Although further genetic studies are necessary for the discussion, women of childbearing age might require much higher serum folate levels than those reported above, to the levels of the U.S. or at least to our median levels. Furthermore, it is necessary to enlighten the public concerning the prevention of NTDs. Women of childbearing age, especially, should be ensured of their healthy eating habits through the health communication strategies taken by the academic institutions or the communities, as our study revealed that the amount of folate and VB12 taken from the diet will correlate to one's daily intake of calories.

In this study, we encountered no subjects with folate deficiency (<10 nmol/L) in Japanese women; however, the present finding would not be responsible for showing that Japanese women are safe from bearing infants with NTDs, since nine women represented hyperhomocysteinemia. On the contrary, hyperhomocysteinemia was not observed in subjects whose serum folate levels were higher than the median concentration (24.6 nmol/L). However, a total of 53 women (shown with an asterisk in Table 2) had normal homocysteine concentration despite their lower folate levels than median. In this study, we could not clarify why homocysteine had not accumulated in plasma.

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