〈Original Article〉

Dietary folate intake and serum folate status in Japanese women of childbearing age

Hiroshi Ihara[1], Toshiaki Watanabe[2], Yoshikazu Aoki[3], Yoichi Nagamura[4], Masayuki Totani[5] and Naotaka Hashizume[6]

Summary This study assessed the folate intake, serum folate concentrations and plasma total homocysteine (tHcy) concentrations in Japanese women of childbearing age. A total of 58 women voluntarily participated in this study. Precise dietary intake for 3 consecutive days was determined by their own dietary history. Serum folate concentrations were determined by automated competitive protein-binding assay and plasma tHcy by HPLC. The mean folate intake was 262±111 μg/d, and the mean concentrations in the serum were 5.4±2.1 ng/mL. The serum folate concentration was low in 12.1% of the subjects (＜3.0 ng/mL) and was marginal in 58.6% (3.0-5.9 ng/mL). The mean plasma tHcy concentration was 6.0±2.2 nmol/mL, and one subject evidenced hyperhomocysteinemia (≧11 nmol/mL). We noted positive relationships between folate intake and the folate concentrations in the serum. In addition, the plasma tHcy concentrations were negatively associated with the serum folate concentrations. In conclusion, the folate status of Japanese women of childbearing age was marginally deficient, with inadequate concentrations of serum folate, largely due to insufficient folate intake. Daily intake of folate exceeding 349 μg/d was recommended, the level at which intake folate deficiency (serum folate, ＜3.0 ng/mL) disappeared.

Key words: Folate status, Folic acid, Estimated Average Requirement (EAR), Folate intake, Homocysteine

1. Introduction

Folate performs major functions in a variety of physiological processes, including DNA synthesis, cell division, and amino acid interconversions[1]. The major known result of folate deficiency is megaloblastic anemia, and maternal folate deficiency may induce neural tube defects at birth[2]. In addition, a lack of sufficient dietary intake of folate has recently been reported to increase the risk of vascular disease in the elderly[3]. In 2005 Japan's Ministry of Health, Labour and Welfare established the Estimated Average

[1]Department of Laboratory Medicine, Toho University Ohashi Medical Center, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
[2]Department of Dietary Environment Analysis, School of Human Science and Environment, Himeji Institute of Technology, University of Hyogo, Himeji, Japan
[3]MECOM Co., LTD., Kanagawa, Japan
[4]Fujita Health University, School of Health Sciences, Aichi, Japan
[5]Graduate School of Human Life Science, Showa Women's University, Tokyo, Japan
[6]Department of Health and Nutrition, Wayo Women's University, Chiba, Japan

Recieved for Publication February 3, 2009
Accepted for Publication Maech 3, 2009

Requirement (EAR) of folate as 200 μ g/d for pregnant women[4], while the EAR in the same age group in Canada and the U.S. was 320 μ g/d for adult women and 520 μ g/d for pregnant women[5]. In the U.S., bread and grains are fortified with folate, however, Japanese women should take folate exclusively from food. Therefore, this study was designed to assess the folate status of women with childbearing potential by evaluating folate intake and determining serum folate concentration as indices.

2. Materials and methods

1. Subjects

Fasting specimens of venous blood collected from 58 healthy young female university students were subjected to analyses of complete blood counts (CBCs), reticulocyte, serum iron, serum ferritin, plasma total homocysteine (tHcy) and serum folate. All the volunteers provided a 3-day dietary history prior to examination to verify that they were free of habitual smoking, drinking or supplements. None of them were pregnant. In this study, written informed consent was obtained from all volunteers, and the protocol was approved by the Protection of Human Subjects Committee of Showa Women's University.

2. Analyses

CBCs and reticulocyte were measured by a Sysmex XE-2100 hematology analyzer. Serum iron and TIBC were determined by a Hitachi 7170 automated analyzer. Serum ferritin concentration was measured by a BCS 600 automated analyzer. Plasma tHcy was measured by the HPLC[6]. Serum folate concentration was determined by Immulite 2000, DPC, Los Angeles, CA, USA, which gave a value close to that of a candidate reference method, i.e., LC/MS/MS[7]. A number of different forms of folate are present in blood, although the predominant folate vitamer in serum is 5-methyltetrahydrofolic acid. Therefore, serum folate concentration determined by the Immulite was total folate (i.e., the sum of 5-methyltetrahydrofolic acid, 5-formyltetrahydrofolic acid and folic acid). The serum folate concentration of 3.0 ng/mL was determined to be equivalent to 6.8 nmol/L using the conventional conversion factor of 2.266[7]. Although red cell folate is a better index of body stores than the serum folate, the current routine procedure for determining the folate status is serum folate concentration. In this study, we used the following guidelines[8] for the interpretation of serum folate concentration: deficiency (＜3.0 ng/mL), marginal deficiency (3.0-5.9 ng/mL) and normal (≧6.0 ng/mL). Although for hyperhomocysteinemia, the upper limit of normal plasma tHcy has been suggested to be 12 nmol/mL in the world literature[8], we used ＜11 nmol/mL for Japanese women[9].

3. Statistical data analysis

Data differences were analyzed by the Mann-

Table 1 Age, anthropometrical measurements, and hematological indices

Age (y)	20.6±1.6 (18-26)
Height (cm)	159±4.9 (148-169)
Body weight (kg)	51.4±6.4 (37-75)
Body mass index (BMI)	20.4±2.1 (16.8-28.6)
Red blood cell count (M/ μ L)	4.42±0.28 (3.63-4.90)
Blood, hemoglobin (g/dL)	13.1±1.0 (10.4-15.7)
Mean corpuscular volume (MCV, fL)	93±5 (79-102)
Reticulocyte count (‰)	12±3 (6-21)
Serum, iron (μ g/dL)	104±43 (220-205)
Serum, ferritin (ng/mL)	21±15 (2-74)
Energy intake (kcal/d)	1,792±375 (918-2,604)
Folate intake (μ g/d)	262±111 (112-734)

Values are means±SD (ranges).

Table 2 Biomarkers of folate status

	All subjects	Subjects classified by amounts of folate intake	
		< EAR	≧EAR
Serum, folate			
Deficiency (<3.0 ng/mL)	7 (12.1)	2 (9.5)	5 (13.5)
Marginal deficiency (3.0-5.9 ng/mL)	34 (58.6)	14 (66.7)	20 (54.1)
Normal (≧ 6.0 ng/mL)	17 (29.3)	5 (23.8)	12 (32.4)
Plasma, tHcy			
Normal (< 11 nmol/mL)	55 (94.8)	19 (90.5)	36 (97.3)
Hyperhomocysteinemia (≧ 11 nmol/mL)	3 (5.2)	2 (9.5)	1 (2.7)

Values are numbers (percentages).
tHcy, total homocysteine.

Whitney U test. Statistical significance was defined as *P*<0.05.

3. Results

1. Age, anthropometrical measurements, and hematological indices

The age of the subjects was 20.6±1.6 y. Their height and weight, 159±4.9 cm and 51.4±6.4 kg, respectively, were similar to those of JDRIs (Dietary Reference Intakes for Japanese, 2005) standards of 157.7 cm and 50.0 kg. Their body mass index (BMI) was 20.4±2.1 (Table 1). Among the 58 subjects, three evidenced marginal iron deficiency (Hb, <12 g/dL, iron <29 μg/dL, and ferritin, <10 ng/mL), but no subject was diagnosed with megaloblastic anemia.

2. Dietary intakes of energy and folate

Dietary intakes of the subjects' energy and folate are shown in Table 1. The intake of energy was 1,792±375 kcal/d, which was 87% of the Estimated Energy Requirement (EER). The folate intake was 262±111 μg/d, which was 131% of the EAR of folate for Japanese women, but 36% of the subjects consumed at levels below the EAR.

3. Serum folate concentration and plasma tHcy concentration

The serum folate concentration was 5.4±2.1 ng/mL (range, 2.6-12.9 ng/mL) and the plasma tHcy concentration was 6.0±2.2 nmol/mL (range, 3.4-15.6 nmol/mL). The numbers (and percentages) of the subjects with folate deficiency, as determined by the concentrations of serum folate and plasma tHcy, are presented in Table 2. Seven subjects (12.1%) were determined to have a folate deficiency (<3.0 ng/mL), as estimated by their serum folate concentration, and 34 subjects (58.6%) were diagnosed with marginal folate deficiency (3.0-5.9 ng/mL). No significant differences were observed in the percentage of deficiency as determined by serum folate among subjects with folate levels lower than EAR or not. Three subjects evidenced hyperhomocysteinemia (≧ 11 nmol/mL). Plasma tHcy concentration in the folate-deficient subjects (9.4±3.6 nmol/mL) was significantly higher (P<0.001) than either those in subjects with marginal folate deficiency (5.6±1.4 nmol/mL) or subjects with normal folate levels (5.3±1.3 nmol/mL).

4. Correlation among folate status indexes

As shown in Fig. 1, a positive linear relationship was observed between folate intake and serum folate concentration (r= 0.401, P<0.001). Plasma tHcy concentration was negatively correlated with serum folate concentration (r= -0.432, P<0.001: Fig. 2). Although it is not shown in a figure, folate intake was also positively correlated with the intake of energy (r= 0.498, P<0.001).

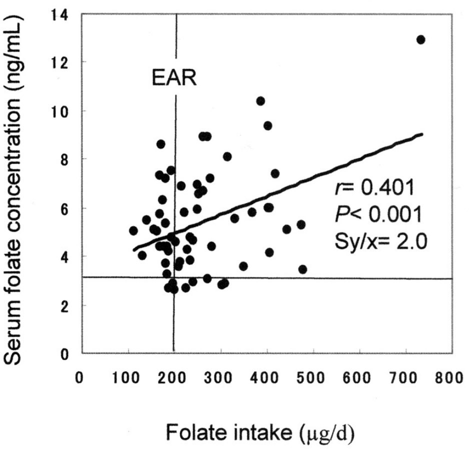

Fig. 1 Relationship of daily amounts of folate intake to serum folate concentrations.
EAR: Estimated Average Requirement.

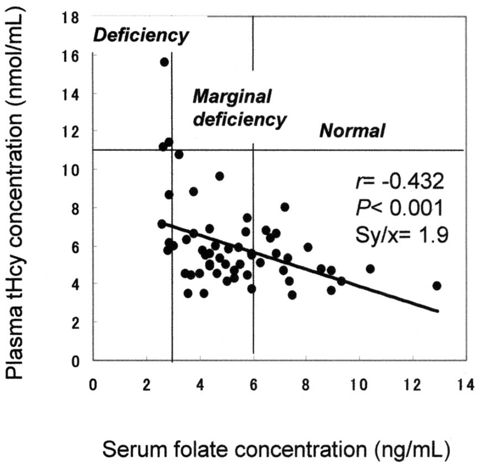

Fig. 2 Negative correlation between plasma levels of tHcy and serum folate concentrations.
tHcy: total homocysteine.

4. Discussion

In this study, Japanese women of childbearing age were found to be consuming insufficient quantities of folate, largely due to their diets with low folate density and insufficient energy intake. Consequently, several of our subjects evidenced inadequate folate states. Our subjects should increase folate intake in their diets and supplements, as serum folate concentration was positively correlated to the amount of folate intake. As shown in Fig. 1, when daily intake of folate exceeded 349 μg/d, the folate deficiency (serum folate, <3.0 ng/mL) decreased. In our previous report[10], successive dietary supplements of folic acid could change serum folate levels at 3 and 7 days after the start of administration.

However, there was a large Sy/x (2.0 ng/mL) around the regression line in Fig. 1. For example, although several subjects took 210-225 μg/d of folate, their serum folate concentration still varied from 2.7 to 6.9 ng/mL. Estimates of intake from a 3-day dietary history were not as accurate, but these differences could arise as a result of polymorphism in MTHFR (methylenetetrahydrofolate reductase) enzyme activity. Serum folate levels were somewhat more affected by genotypes of MTHFR than folate intake levels[11]. Genotyping of every volunteer will be necessary for selection of volunteers in any future study.

In addition, one of the important findings from our subjects was that no clinical symptoms of folate deficiency were found among seven subjects, despite the fact that their serum folate concentrations were below 3 ng/mL. Furthermore, except for three (11.1, 11.4, and 15.6 nmol/mL), the plasma tHcy concentrations of the remaining four subjects did not exceed 11 nmol/mL (Fig. 2). Elevated levels of homocysteine in the blood were reported to be associated with atherosclerosis as well as an increased risk of heart attacks, strokes, blood clot formation, and possibly Alzheimer's disease. This phenomenon calls for further investigation.

References

1) Edited by CA. Burtis and Ashwood ER: DB. McCormick, Greene HL: Vitamins. Tietz textbook of clinical chemistry, 2nd ed. 1275-1316. WB Saunders Company, USA (1994)
2) Thame G, Guerra-Shinohara EM and Moron AF: Serum folate by two methods in pregnant women carrying fetuses with neural tube defects. Clin. Chem., 48: 1094-1095, 2002
3) Boushey CJ, Beresford SA, Omenn GS and Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. probable benefits of increasing folic acid intakes. JAMA, 274:

1049-1057, 1995

4) Ministry of Health, Labour, and Welfare, Japan: Dietary reference intakes for Japanese, 2005 [Jpn]. 92-95, Daiichi-Shuppan, Japan (2005)

5) National Research Council: Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. 196-305. National Academy Press, USA (2000)

6) Araki A and Sako Y: Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J. Chromatogr., 422: 43-52, 1987

7) Thorpe SJ, Heath A, Blackmore S, Lee A, Hamilton M, O'broin S, Nelson BC and Pfeiffer C: International standard for serum vitamin B_{12} and serum folate: international collaborative study to evaluate a batch of lyophilised serum for B_{12} and folate content. Clin. Chem. Lab. Med., 45: 380-386, 2007

8) Sauberlich HE: Folate (folic acid, pteroylmonoglutamic acid, folacin). Laboratory tests for the assessment of nutritional status, 2nd ed. 103-134. CRC Press, Washington, D.C., (1999)

9) Moriyama Y, Kawamura M, Iso H, Kimura H, Yamamoto Y, Okamura T, Abe K and Yorimitsu K: Effects of lifestyle factors on plasma level of homocysteine and α-tocopherol in young women [Jpn]. Rep. Pub. Hlth. Kochi, 50: 47-52, 2004

10) Shibata K, Fukuwatari T, Ohta M, Okamoto H, Watanabe T, Fukui T, Nishimuta M, Totani M, Kimura M, Ohishi N, Nakashima M, Watanabe F, Miyamoto E, Shigeoka S, Takeda T, Murakami M, Ihara H and Hashizume N: Values of water-soluble vitamins in blood and urine of Japanese young men and women consuming a semi-purified diet based on the Japanese Dietary Reference Intakes. J. Nutr. Sci. Vitaminol., 51: 319-328, 2005

11) Hiraoka M: Folate intake, serum folate, serum total homocysteine levels and methylenetetrahydrofolate reductase C6771 polymorphism in young Japanese women. J. Nutr. Sci. Vitaminol., 50: 238-245, 2004