Relationships of Nutrient Intake and Lifestyle-Related Factors to Serum Folate and Plasma Homocysteine Concentrations in 30–69 Year-Old Japanese

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Summary We studied non-hospitalized 30–69 y-old Japanese subjects to ascertain the influences of a 677C-T methylene-tetrahydrofolate reductase (MTHFR) genotype, nutritional intake and lifestyle-related factors on plasma homocysteine (Hcys) and serum folate concentrations. Hcys was higher and serum folate was lower in males than in females (p<0.01). The Hcys concentration was higher in the VV group than in the AA and AV groups for both males and females. However, a relatively low serum folate concentration of 18±7nmol/L was found in the entire male group as compared with 22±10nmol/L in all females. In the female subjects, serum folate concentrations differed among MTHFR genotypes, being lowest in the VV group. In all male subjects, log folate intake per 1,000 kcal was a significant positive predictor of log serum folate concentration (p<0.01), while in females the log vitamin C intake per standard body weight was a significant positive variable (p<0.001) predicting the log serum folate concentration. Smokers had significantly lower serum folate concentrations, regardless of dietary folate intake. High folate and vitamin C consumptions, appears to be beneficial to normal and heterozygous MTHF genotype subjects for maintaining serum folate concentrations. Even a 400 µg daily intake of folate might be less than what is needed, especially for homozygous MTHFR subjects and smokers, to maintain an adequate serum folate concentration.

Key Words Folate, homocysteine, vitamin C, smoking, methylenetetra-hydrofolate reductase genotype

A large number of epidemiological association studies have identified moderate hyperhomocysteinemia as an independent risk factor for stroke, peripheral vascular disease, and myocardial infarction (1–5). The plasma homocysteine (Hcys) concentration increases with excessive methionine intake, deficiencies of several B vitamins (3, 6–8), medications affecting Hcys metabolism (9), and genetic disorders involving impaired Hcys metabolism (6, 10, 11). Numerous reports, concerning the effects of folic acid, B6, and B12 supplementation, have suggested adequate intakes of these vitamins to be necessary (12–15). In addition, several investigations of the relationship between habitual nutrient intake and serum folate concentrations have been conducted (8, 16–18), the results of which have mainly supported those of the supplementation studies. Recently, a lack of association was reported between plasma Hcys and angiographically confirmed coronary artery disease in the era of fortification of cereal grain with folic acid in the USA (19). That study did, however, show that the dietary approach effectively increases serum folate and decreases Hcys and protects against coronary artery disease.

On the other hand, individuals who are homozygous for the methylenetetra-hydrofolate reductase (MTHFR) 677C-T mutation, which specifies an alanine to valine substitution, are considered to be at risk for elevated plasma Hcys as compared to individuals with the normal or heterozygous genotype (3, 10, 11, 20, 21). This is because reduced MTHFR activity results in a lower serum folate concentration. Thus, the MTHFR homozygous subject is considered to be at high risk for developing atherosclerosis (22, 23), making strategies aimed at reducing the risk highly desirable.

However, there have been few reports examining relationships among plasma Hcys, folate concentration...
and dietary folate intake in the Japanese population. Research on the optimal dietary folate consumption for Japanese, and better preventive measures against atherosclerosis, are thus needed.

Therefore, we studied the effects of difference in MTHFR genotype, nutritional intake and lifestyle-related factors on serum folate and plasma homocysteine concentrations in the non-hospitalized Japanese population.

**SUBJECTS AND METHODS**

**Subjects.** The entire 30–69y old population of Kisei-cho, a rural fishing and farming village in Mie Prefecture, Japan, was invited to undergo a general health examination and participate in this study. Out of a potential 1,128 male and 1,360 female subjects, 270 (23.9%) and 340 (25.0%), respectively, agreed to participate. The study was conducted each December from 1997 through 2002.

Subjects were excluded if all necessary data had not been obtained, or if they were being treated with medications affecting the plasma Hcys or folate concentration, such as anti-hyperlipemic, anti diabetes mellitus, anti inflammatory, antiepileptic, antiulcer, antacid, thiazide diuretic and anti-rheumatoid arthritis drugs. Thus, a total of 147 males and 293 females served as the subjects of this study. The subjects’ jobs included construction (24.8%), clerical (21.8%), fishing (17.3%), farming (12.8%), factory labor (8.3%) and other (2.3%) for males, 0.3% of whom were unemployed. For women, jobs were housewife (47.9%), clerk (28.3%), factory laborer (16.8%), farmer (3.5%) and others (3.5%). The aims of the study were fully explained to all participants, and written informed consent was obtained from all subjects before enrollment. The study protocol was approved by the Human Ethics Committee of the Saitama Social Insurance Hospital.

**Biochemical and physical examinations.** Fasting blood samples, collected by clean venipuncture, were allowed to clot at room temperature for 2–4 h and then centrifuged at 1,000×g for 10 min at room temperature. Plasma samples were obtained by adding sodium fluoride to fasting blood samples for measuring glucose and Hcys. Serum and plasma thus separated were transferred into 1.5 mL tubes. Serum total protein, uric acid, creatinine, total and HDL-cholesterol concentrations, and lower serum total cholesterol, diastolic blood pressure, serum triglycerides, uric acid, and creatinine concentrations, and lower serum total protein, total and HDL-cholesterol concentrations.

In Table 1. Compared with females, males had higher diastolic blood pressure, serum triglycerides, uric acid, and creatinine concentrations, and lower serum total protein, total and HDL-cholesterol concentrations.

The respective polymorphism frequencies of MTHFR genotypes AA, AV and VV were 51.7, 38.8 and 9.5% in males, and 50.0, 38.8 and 11.2% in females. This genotype distribution was compatible with the Hardy-Weinberg equilibrium. Plasma Hcys and serum folate concentrations (BMI in kg/m^2) were calculated using body weight and height measurements.

**Genotyping.** Blood samples were drawn into tubes with EDTA-2Na, and DNA for genotyping was extracted from theuffy coat using a commercially available DNA preparation kit (Gene Trapping by Liquid Extraction, TaKaRa Biochemicals). Polymerase chain reaction amplification of genomic DNA samples was performed using specific oligonucleotide primers in a TaKaRa Taq™ (TaKaRa Biochemicals). The primers used generated a 198-base pair (bp) fragment. The MTHFR genotype determination was accomplished using a modification of the polymerase chain reaction and Hinfl restriction enzyme digestion procedure (22). If the mutation was present, the Hinfl restriction enzyme digested the 198-bp fragment into 175- and 23-bp fragments that were identified by gel electrophoresis. The MTHFR genotypes Ala/Ala, Ala/Val and Val/Val are shown as AA, AV and VV, respectively.

**Diet survey.** Dietary records were kept for 2 d before the serum was obtained. Dietary intake was confirmed by an interview with a dietician. Nutrient intake was calculated using Excel-Eiyokun software (Kenpakusha Co. Ltd., Tokyo) based on the table of nutrient contents published by the Japanese Science and Technology Agency (5th edition). Questionnaires were used to determine habitual smoking or smoking experience, periods of smoking cessation, frequency and number of cigarettes. Type and frequency according to liquor, supplement and health food intakes were asked.

**Statistical analysis.** An Apple computer with Statview J 5.0 software (SAS Institute Inc., USA) was used for statistical analysis. Data are presented as means±SD. Means were compared using the Mann-Whitney U test. Frequency analysis was performed with the chisquare test for independence. Spearman correlation coefficients were used to analyze the relationships of serum folate concentration to nutrient intakes. Normality of distribution was assessed be applying the Kolmogorov-Smirnov test, and multiple regression analyses were performed using natural logarithms of the serum folate concentration, and intakes of protein, fat, carbohydrates and vitamin C per standard body weight (BMI=22). As B vitamins play important roles in energy metabolism, log B vitamin intakes per 1,000 kcal were used as explanatory variables for log serum folate concentrations. A value of p<0.05 was considered statistically significant.

**RESULTS**

Subject characteristics, and clinical data are shown in Table 1. Compared with females, males had higher diastolic blood pressure, serum triglycerides, uric acid, and creatinine concentrations, and lower serum total protein, total and HDL-cholesterol concentrations.

The respective polymorphism frequencies of MTHFR genotypes AA, AV and VV were 51.7, 38.8 and 9.5% in males, and 50.0, 38.8 and 11.2% in females. This genotype distribution was compatible with the Hardy-Weinberg equilibrium. Plasma Hcys and serum folate concentrations
### Table 1. Subject characteristics and clinical data.

|                      | Males       | Females     |
|----------------------|-------------|-------------|
| Number               | 147         | 294         |
| Age                  | 54.4±11.1   | 60.7±8.7    |
| Height (cm)          | 165.2±6.2   | 153.0±3.9** |
| Weight (kg)          | 64.6±9.9    | 54.7±6.1**  |
| Body mass index      | 23.6±3.1    | 23.4±3.0    |
| Systolic blood pressure (mmHg) | 131±18 | 129±18     |
| Diastolic blood pressure (mmHg) | 83±11 | 81±11*     |
| Total protein (g/L)  | 74±4        | 76±4*       |
| Total cholesterol (mmol/L) | 5.69±0.89 | 5.88±0.93* |
| HDL-cholesterol (mmol/L) | 1.49±0.39 | 1.62±0.39** |
| LDL-cholesterol (mmol/L) | 3.54±0.87 | 3.68±0.85  |
| Triglycerides (mmol/L) | 1.48±1.17 | 1.15±0.68** |
| Glucose (mmol/L)      | 6.1±1.6     | 5.8±1.5     |
| Uric acid (µmol/L)    | 358±83      | 270±58**    |
| Creatinine (µmol/L)   | 91.4±13.9   | 71.3±9.9**  |

Values are means±SD. Significant difference between all male and female subjects at *p<0.05 and **p<0.01.

### Table 2. Plasma homocysteine and serum folate concentration by MTHFR genotype and gender.

|                      | Male | Female |
|----------------------|------|--------|
| Number               | 147  | 294    |
| Homocysteine (µmol/L) | 11.1±3.2 | 9.0±2.1** |
| Folate (nmol/L)      | 18±7 | 17±6   |

Values are means±SD. Significant difference between male and female subjects at **p<0.01.

### Table 3. Daily nutritional intakes by MTHFR genotype groups and gender.

|                      | Male | Female |
|----------------------|------|--------|
| Number               | 147  | 294    |
| Energy (kcal/kg)§    | 41.2±10.1 | 37.2±7.9** |
| Protein (g/kg)       | 1.5±0.4 | 1.5±0.4 |
| Lipids (g/kg)        | 1.0±0.4 | 1.0±0.4 |
| Carbohydrate (g/kg)  | 6.0±1.8 | 5.5±1.3** |
| Thiamin (mg/1,000 kcal) | 0.49±0.58 | 0.57±0.66 |
| Riboflavin (mg/1,000 kcal) | 0.66±0.37 | 0.78±0.49** |
| Niacin (mg/1,000 kcal) | 9.2±3.4   | 9.9±4.1   |
| Folate (µg/1,000 kcal) | 162±56   | 216±87** |
| Vitamin B6 (mg/1,000 kcal) | 0.75±0.55 | 0.82±0.66 |
| Vitamin B12 (µg/1,000 kcal) | 5.64±4.03 | 5.53±4.95 |
| Vitamin C (mg/kg)    | 2.5±2.5 | 3.6±3.7** |

Values are means±SD. Significant difference between all male and female subjects at **p<0.01.

<sup>§</sup> kg: divided by standard body weight.
Table 4. Regression coefficients of nutrients affecting serum to serum folate concentration as determined by multiple regression analysis.

| Nutrient | Intercept | Energy (kcal/kg) | Carbohydrates (g/kg) | Protein (g/kg) | Riboflavin (mg/kg) | Folate (mg/kg) | Vitamin B6 (mg/kg) | Vitamin B12 (mg/kg) | Vitamin C (mg/kg) | R² | adj. R² | P-value |
|----------|-----------|------------------|----------------------|---------------|-------------------|----------------|-------------------|-------------------|-----------------|-----|---------|---------|
| Females  | 0.580     | 0.879            | 0.736                | -0.060        | -0.914            | -0.133         | -0.467            | -0.787            | -0.388          | 0.659 | <0.001  |<0.001          |
|          | 0.060     | 0.101            | 0.17                 | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.294     | 0.094            | 0.039                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.578     | 0.294            | 0.618                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.580     | 0.879            | 0.736                | -0.060        | -0.914            | -0.133         | -0.467            | -0.787            | -0.388          | 0.659 | <0.001  |<0.001          |
|          | 0.060     | 0.101            | 0.17                 | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.294     | 0.094            | 0.039                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.578     | 0.294            | 0.618                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.580     | 0.879            | 0.736                | -0.060        | -0.914            | -0.133         | -0.467            | -0.787            | -0.388          | 0.659 | <0.001  |<0.001          |
|          | 0.060     | 0.101            | 0.17                 | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.294     | 0.094            | 0.039                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |
|          | 0.578     | 0.294            | 0.618                | -0.133        | -0.219            | -0.013         | -0.19             | -0.229            | -0.110          | 0.741 | >0.10   | >0.10   |

Compared with females, males had higher Hcys concentrations and lower folate concentrations. The Hcys concentration was 11.1±3.2 (mean±SD) µmol/L in all male subjects, a value significantly higher than the 9.0±2.1 µmol/L in all female subjects (p<0.01), and the Hcys concentration was higher in the VV group than in the AA and AV groups for both males and females, the respective values being 13.5±4.8 µmol/L (p<0.05) and 10.3±2.9 µmol/L (p<0.01). However, a relatively low serum folate concentration of 18±7 nmol/L was found in the entire male group as compared with 22±10 nmol/L in all females. In the female subjects, serum folate concentrations differed among MTHFR genotypes, being lowest in the VV group (AA vs. VV p<0.01). The correlation between plasma log Hcys concentration and the serum log folate concentration was negative in all males (r=-0.301, p<0.001), AA (r=-0.225, p=0.051), AV (r=-0.383, p<0.01), and VV (r=-0.284, NS), and in all females (r=-0.169, p<0.01), AA (r=-0.204, p=0.05), AV (r=-0.052, NS) and VV (r=-0.397, p<0.05).

Dietary intakes, in terms of nutrient content, are shown in Table 3. Compared with females, males had higher intakes of energy per standard body weight (p<0.01) and of carbohydrates per standard body weight (p<0.01), and lower intakes of riboflavin per 1,000 kcal (p<0.01), of folate per 1,000 kcal (p<0.01) and of vitamin C per standard body weight (p<0.01). There were no differences in nutrient intake among MTHFR genotype groups, except for a higher vitamin C intake in the VV group than in the AA and AV groups in females (p<0.05).

The log serum folate concentration correlated positively with age in all males and all females (male: r=0.17, p=0.040, female: r=0.197, p=0.012). A multiple correlation coefficient table expressing log serum folate concentration by nutrient intake is presented as Table 4. In males, log folate intake was a positive variable independently predicting the log serum folate concentration in the AA (p=0.07), AV (p<0.05) and VV (p<0.05) groups. However, log folate intake was not a significant variable while log vitamin C intake was a positive variable independently predicting the log serum folate concentration in the female AA (p<0.01) and AV (p<0.01) groups. Almost the same results were obtained when using log vitamin B intakes per standard body weight as explanatory variables for serum folate concentrations (data are not shown).

Habitual smoking, alcohol consumption and vitamin B supplement and health food intakes are presented in Table 5. Forty-two percent of the males smoked habitually, while only 5.1% of the females were smokers (p<0.01). The percentage habitually consuming alcohol was also higher in males than in females (p<0.01). Vitamin B supplements and health foods consisted mostly of vitamins B1 and B2. No subject took supplemental folate, vitamin B6, or B12, which should affect serum folate concentrations. Plasma Hcys concentra-
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Intakes did not differ among groups stratified according to smoking habit, alcohol consumption or supplement and health food intakes. However, the serum folate concentration was lower in smokers than in non-smokers, for both males and females. Serum folate concentrations were $21 \pm 8 \text{ nmol/L}$ ($n=49$), $18 \pm 7 \text{ nmol/L}$ ($n=35$), and $17 \pm 6 \text{ nmol/L}$ ($n=61$) in male non-smokers, former smokers, and current smokers, and the respective values for females were $23 \pm 10 \text{ nmol/L}$ ($n=275$), $16 \pm 3 \text{ nmol/L}$ ($n=3$), and $16 \pm 5 \text{ nmol/L}$ ($n=15$) (smokers vs non-smokers: $p<0.05$). No significant differences in serum folate concentration were observed among the alcohol consumption or the supplement and health food intake groups.

Table 5. Habitual smoking, alcohol consumption, and supplement or health food use by gender.

|                      | Males | Females |
|----------------------|-------|---------|
| Number               | 147   | 294     |
| Smoking status       |       |         |
| Never                | 33.8b | 93.9    |
| Former smoker        | 24.1  | 1.0     |
| Current smoker       |       |         |
| <10/d                | 24.1  | 5.1     |
| 10-20/d              | 6.9   | 0.0     |
| 21/d<                | 11.0  | 0.0     |
| Alcohol consumption  |       |         |
| None                 | 32.4  | 73.0    |
| 1-3 times/mo         | 6.9   | 8.3     |
| 1-4 times/wk         | 11.7  | 6.9     |
| 5-6 times/wk         | 11.0  | 7.6     |
| Daily                | 37.9  | 4.2     |
| Supplement and/or health food use | 28.1 | 29.3 |

*Values are percentages.
Significant difference between all male and female subjects at * * $p<0.01$.

Disguised according to serum folate concentration versus folate intake and smoking status (non-smoker vs. former and current smoker) are shown in Fig. 1. Folate intake was separated according to mean values of 162 $\mu$g/1,000 kcal for males and 216 $\mu$g/1,000 kcal for females. In all males, high folate intake and being a non-smoker were associated with the highest serum folate concentration of $24 \pm 9 \text{ nmol/L}$, while the lowest value of $16 \pm 6 \text{ nmol/L}$ ($p<0.01$) was seen in those with low folate intake who smoked. In all females, high folate intake and being a non-smoker were associated with a higher serum folate concentration, $25 \pm 12 \text{ nmol/L}$, than in the other groups. The value was lowest, $16 \pm 4 \text{ nmol/L}$ ($p<0.01$), in those women with low folate intake who smoked. The prevalence of MTHFR genotypes did not differ among subgroups stratified by smoking and folate intake.

**DISCUSSION**

The plasma Hcys concentration correlated negatively with the serum folate concentration in all males and in all females, as reported previously (8, 13-15). In regards to the relations between the serum folate concentration and dietary nutrient intakes, the significant probability and correlation values for these associations were higher in males than in females. The log serum folate concentration showed a weak positive correlation with age in all males and all females, and mean age did not differ significantly between males and females. Thus, age was not an important reason for the difference in serum folate concentrations between males and females in this study. Males had lower folate and ascorbic acid intakes and a higher energy intake than females, suggesting the level of folate intake per 1,000 kcal to be insufficient in males. We speculate that the differences in nutrient intake between genders contributed to the lower serum folate concentration in males.

On the other hand, the higher folate intake in females resulted in that not being a significant correlation.
between the serum folate concentration and folate intake on multiple regression analysis. Among our female subjects, mean dietary folate intake was 216 μg/1,000 kcal, i.e. 406 μg/d. In contrast, in our male subjects, 162 μg/1,000 kcal of dietary folate intake, i.e. 399 μg/d, almost the same total daily consumption as in our female subjects, was apparently inadequate to maintain the serum folate concentration at the same level as in females. Four hundred micrograms per day has been indicated to be adequate for maintaining the serum folate level, and serves as the basis for determining the recommended dietary allowance for folate intake in the US and European countries (24). The United States Food and Drug Administration issued a regulation requiring that all flour products, including rice, pasta, cornmeal, etc. be fortified with 140 μg of folic acid per 100 g to increase folic acid intake in order to decrease the incidence of neural tube defects (25). This strategy increased serum folate concentrations and contributed to lowering plasma Hcy as a risk factor for coronary artery disease (19). However, the Japanese recommended dietary allowance for folate intake is only 200 μg/d (26). Our results suggest that folate consumption at a level higher than that currently recommended for Japanese may be warranted for our subjects.

Low serum and red blood cell concentrations of folate, were reported in smokers (27). McDonald et al. reported both serum folate and red blood cell folate to be lower in pregnant women who smoked than in pregnant women who did not smoke, and suggested that smoking might accelerate folate metabolism or cause changes in redox status (28). We observed significantly lower serum folate concentrations in smokers, independent of dietary folate intake, in both males and females. Notably, male smokers whose folate intake was lower than 162 μg/1,000 kcal showed the lowest serum folate level, lower than even that of the male VV genotype group. The higher prevalence of smoking might be one of the reasons for the low serum folate concentration in our male subjects. Further studies with large numbers of subjects are necessary to clarify the relations of serum folate concentration with both smoking duration and number of cigarettes.

Those homozygous for the MTHFR genotype are well recognized as being at risk for a lower serum folate concentration, as compared to individuals with the normal or heterozygous genotype, because of their reduced MTHFR activity (29, 30). MTHFR homozygous genotype subjects require higher folate intake than do individuals with the normal or heterozygous genotype to achieve similar serum folate and to lower Hcy concentrations. Fortunately, those individuals are responsive to folate intervention (31, 32). Among our female subjects the serum folate concentration was lower in the MTHFR VV genotype than in the AA and AV genotype groups, though in males no difference was observed among genotype groups. The effect of smoking on serum folate concentration appeared to be strong because the prevalence of smoking was higher in the AA (63.2%) and AV (73.7%) groups than in the VV group (50.0%). These results suggest the gene-environmental interaction between MTHFR activity and smoking effects to have an important impact on serum folate levels. Numerous reports have indicated that smoking is a strong risk factor for atherogenic diseases based on the risk of oxidation and endothelial function injury (33). The lower serum folate concentrations in smokers should be considered an additional reason for smoking cessation aimed at preventing atherosclerosis.

The serum folate concentration is recognized to depend not only on dietary folate intake, but also on energy metabolism, the effect being increased absorption with ascorbic acid in the digestive tract. Furthermore, riboflavin, in the form of flavin adenine dinucleotide, is a cofactor not only for glucose and fatty acid metabolism but also for MTHFR. Riboflavin deficiency has been associated with reduced forms of folate, including tetrahydrofolate and 10-formyltetrahydrofolate (7, 30).

In females, there was no association between folate intake and serum folate concentration. However, ascorbic acid intake appeared to be a significant independent variable determining for serum folate concentration in all females, and in the AA and AV genotype groups. In addition to folate, ascorbic acid in gastric juice contributes to salvaging of labile inactive 5-methyl-5,6-H2PteGlu into active 5-methyl-H4PteGlu which contributes to the maintenance of folate absorption (34). Broekmans et al. reported increased intake of mixed fruits and vegetables to increase serum folate and vitamin C concentrations, and decrease plasma Hcy concentrations in Dutch volunteers (18). Adequate vitamin C consumption might contribute to increasing serum folate concentrations, especially in the normal and heterozygous MTHFR groups with adequate folate consumption among our female subjects. We should also consider the bioavailability of folates from different sources, the effects of eating patterns, methods of cooking and the condition of the digestive tract in order to assess dietary folate consumption. Further studies on folate, in terms of dietary consumption in food, i.e., focusing on nutrient combinations, are needed.

We observed no effects of supplement use on serum folate concentrations despite supplement and health food users accounting for approximately 30% of our subjects. This was because no subject used supplements containing folate, vitamin B6, or B12, which would affect serum folate concentrations. However, numerous reports on the effects of folic acid supplementation as a means of increasing serum folate concentrations have been published (12–15, 35). Folic acid containing dietary supplements use was reported the most important predictor of blood folate concentrations when taken in the previous 24 h (36). In the present study, the subjects used supplements and health foods with many types and doses of vitamins. We did not take into consideration either the volume or the duration of the supplements and health foods used, and could not determine the intake time before blood collection. Further
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studies, focusing on these issues and the effects of supplement use on serum folate concentrations, are needed as supplement use is currently increasing in Japan.

In conclusion, the serum folate concentration was significantly lower in males than in females, and also lower in the homozygous MTHFR subjects than in the other two groups. Even a 400 μg daily intake of folate might be less than what is needed, especially for homozygous MTHFR subjects and smokers, to maintain an adequate serum folate concentration. In addition, to maintain an optimal serum folate concentration, relatively high dietary folate and vitamin C consumption appears to be beneficial to normal and heterozygous MTHFR genotype subjects. Further studies are necessary to clarify the requirements and optimal food consumption and intakes of nutrients for folate metabolism in homozygous MTHFR subjects.

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