Dietary Folate Intake, Blood Folate Status, and Urinary Folate Catabolite Excretion in Korean Women of Childbearing Age

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Summary This study assessed folate intake, folate concentrations in plasma and erythrocytes, plasma total homocysteine (tHcy) concentrations, and urinary excretion of folate metabolites in Korean women of childbearing age. A total of 36 women voluntarily participated in this study. Precise dietary intake for 3 consecutive days was determined by weighing all foods consumed, and folate intake was calculated with a computer-aided dietary analysis system. Folate concentrations in plasma and erythrocytes were determined via microbiological methods and in plasma by HPLC. Urine excreted over the same period of time was collected and assayed for folate catabolites, para-aminobenzoylglutamate (pABG) and para-acetamidobenzoylglutamate (pAPABG) by reverse-phase HPLC after affinity chromatography. The mean folate intake was 206.9 ± 90.8 μg DFE/d, and the mean concentrations in plasma and erythrocytes were 10.5 ± 3.7 and 249.9 ± 77.8 nmol/mL, respectively. Erythrocyte folate concentration was low in 2.8% of the subjects (<140 ng/mL) and was marginal in 5.5% (140–156 ng/mL). The mean plasma tHcy concentration was 12.7 ± 0.2 nmol/mL, and 11% of the subjects evidenced hyperhomocysteinemia (>15 nmol/mL). The mean urinary excretion levels of pABG and pAPABG were 10.7 ± 3.8 and 89.1 ± 19.5 nmol/d, respectively. The means of folate reserve and folate turnover rate were 26.2 ± 11.6 and 10.5 ± 3.9, respectively. We noted positive relationships between folate intake and the folate concentrations in plasma and erythrocytes, as well as the urinary excretions of pAPABG and total folate catabolites. In addition, the erythrocytic folate concentrations were positively associated with the urinary excretions of pAPABG and total folate catabolites. In conclusion, the folate status of Korean women of childbearing age was marginally deficient with inadequate concentrations of erythrocyte folate and elevated plasma tHcy, largely due to insufficient folate intake. The marginally deficient folate status was confirmed by the low excretion of folate catabolites in urine.

Key Words folate status, folate catabolite, homocysteine, childbearing-aged women

Folate performs major functions in a variety of physiological processes, including DNA synthesis, cell division, and amino acid interconversions (1). Maternal folate deficiencies may induce neural tube defects (NTDs) (2), congenital defects including heart defects and orofacial clefts (3–5), other adverse pregnancy outcomes (6), and certain cancers (7). Adequate folate supplementation for women of childbearing age has been demonstrated to reduce the risk of NTDs (8–10). The fact that neural tubes close by the 28th day after conception (11) implies that peri-conception folate nutritional status is essential for the prevention of NTDs. However, an estimated 50% of pregnant women in the United Kingdom (12) and the United States (13) do not receive folate supplements, as well as over 40% in developing countries (14). Therefore, it is important to ensure adequate folate status in all women who are capable of becoming pregnant.

The Korean Nutrition Society initially introduced the Recommended Dietary Allowance of folate for Koreans in 1995 (15). Thereafter, in October 2005, the Estimated Average Requirement (EAR) of folate was established as 320 μg DFE/d for adult women, and 520 μg DFE/d for pregnant women (16). Previous studies of the folate status of Korean women with childbearing potential have shown that a considerable proportion of the women are marginally folate deficient (17–19) with insufficient folate intake levels (17–21). However, the majority of the previous studies relied solely on folate intake (17, 20), folate concentrations in plasma or erythrocytes (17, 19), and/or plasma total homocysteine (tHcy) concentrations (18). Minimal data is currently available regarding urinary folate catabolite excretion. Plasma folate concentration is a sensitive indicator of recent folate balance (22), erythrocyte folate concentration is generally considered to be an index of prolonged folate status (22), and elevated plasma tHcy concentration is thought to be a metabolic consequence of folate deficiency (23). Those are the

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common indices utilized for the evaluation of folate nutritional status. In addition, urinary folate catabolite excretion is considered to be another long-term indicator of the body folate pool size, which parallels functional measures of folate status (24). It slowly responds to changes in dietary folate intake, under the controlled research conditions of a metabolic protocol (25, 26).

Therefore, this study was designed to assess the folate status of women with childbearing potential by evaluating folate intake and determining plasma folate and tHcy concentrations as short-term indices, and erythrocyte folate concentration and urinary excretion of folate catabolites as long-term indices.

**MATERIALS AND METHODS**

**Subjects.** The subjects for this study were recruited from among women aged 15 to 45 y living in Gwangju (a large city) and Sunchon (a small city), Korea. Smokers and users of oral contraceptive agents were excluded from participation in this study. A total of 36 healthy women voluntarily participated. The objectives and procedures of this study were explained to each of the subjects, and all subjects provided written informed consent. The research protocols were approved by the Research Ethics Committee of the Chonnam National University Hospital.

**Dietary folate consumption assay.** Daily dietary intake was determined for 3 consecutive days, using a weighed food record method: The subjects were instructed as to how to weigh and record their meals using a dietary scale (±1 g) provided to them. The intakes of energy, protein, and folate were calculated with a computer-aided dietary analysis system (CAN-Pro version 2.0, The Korean Nutrition Society).

**Blood sampling and analytical methods.** Venous blood samples from the fasting subjects were collected once, on the day after the 3-d dietary survey. The blood samples were employed for a variety of laboratory measurements. Hemoglobin concentration and hematocrit were determined with a hematocrit analyzer (Coulter STKS; Beckman Coulter, Fullerton, CA). A diluted sample of 100 μL of whole blood with 900 μL of potassium phosphate buffer (1% Na-ascorbate, pH 6.3) was stored at −20°C until analysis for erythrocyte folate.

Plasma was obtained by centrifuging whole blood immediately after collection at 3,000 rpm for 15 min; it was then stored at −20°C for folate and tHcy analyses. Folate concentrations in the plasma and erythrocytes were determined via a microbiological assay (27), using Lactobacillus casei (L. casei ATCC 7469; ATCC, Manassas, VA) and a microplate reader (EL 800; Bio-Tec Instruments Inc., USA).

The whole blood and plasma samples were kept at 37°C for 10 min prior to determining folate concentrations. Each 50 μL of plasma or 20 μL of whole blood was loaded into a 96-well microplate, to which 0.1 M phosphate buffer was added (1% Na-ascorbate, pH 6.3), followed by stepwise dilution. The microplate was incubated with 150 μL of L. casei solution for 18 h at 37°C, using folic acid solution as a standard. The absorbance of folic acid was measured with a microplate reader (EL800, Bio-Tec Instruments, Inc.) at 490 nm.

Plasma tHcy concentrations were determined by HPLC (model 501; Waters, Milford, MA) using the method described by Araki and Sako (28). Each 100 μL of plasma was mixed with 30 μL of 10% cold TBP (tributylphosphine) solution (Sigma Chemical Co., St. Louis, MO), vigorously shaken, and incubated for 30 min at 4°C for reduction. Thereafter, 270 μL of cold 10% TCA (trichloroacetic acid) solution was added, mixed vigorously, and centrifuged for 5 min at 4,000 rpm at 4°C to precipitate the proteins. One hundred microliters of the upper solution was carefully transferred to a new EP tube, and 20 μL of 1.55 M NaOH, 250 μL of 0.125 m borate buffer (pH 9.5, 4 mM EDTA), 250 μL of thiol-specific fluorogenic reagent, and 100 μL of cold 0.1% SBD-F (thiol-specific fluorogenic reagent) were added to the supernatant layer, mixed vigorously, and incubated with shaking at 60°C for 60 min. After cooling on ice and filtering through an HV filter (0.45 μL, Millipore), the thiols adducts were separated using an isocratic system with 0.1 M acetic acid/acetate buffer (pH 5.5, containing 3% methanol) as the mobile phase and detected via fluorescence (excitation at 385 nm and emission at 515 nm). The column used was a Prodigy ODS 2 (150 A, 150 mm×3.2 mm, 5 μm, Phenomenex, Torrance, USA). The flow rate was 0.7 mL/min and cysteamine (Sigma Chemical Co.) was used as an internal standard.

**Urine collection and analytical methods.** Twenty-four-hour urine was collected daily in a PET bottle containing 3 g of sodium ascorbate during the same 3 consecutive days as the dietary survey. The subjects were instructed to keep the urine refrigerated at all times during the collection period to protect against bacterial growth. After the total urine volume was recorded, the urine was thoroughly mixed, dispensed into 300 mL portions, and stored at −20°C.

Concentrations of the urinary folate catabolites, pABG and ApABG, were determined by reverse-phase HPLC (29) after affinity chromatography (30). The urine samples were adjusted to neutral pH and filtered through Whatman number 1 filter paper. The filtered urine samples were applied to 2 mL FBP-Affigel columns containing immobilized folate-binding protein to remove the intact folate. After affinity chromatography, the 20 mL urine samples were adjusted to a final concentration of 0.1 M HCl and applied to glass columns (200 mm×15 mm) filled to a height of 5 cm with a slurry of Dowex 50 W (50×8-400, Sigma Chemical Co.) cation exchange resin, equilibrated with 0.1 M HCl. ApABG was obtained in the fraction eluted with 50 mL of 0.1 M HCl effluent, which was pooled and retained. A second portion of the 0.1 M HCl eluate (50 mL) was applied to the column, and the pABG fraction was obtained in the eluate (100 mL) with 0.6 M HCl. The fraction containing ApABG was acidified to 0.2 M HCl and heated for 60 min at 100°C to deacetylate ApABG. After deacetylation, the fraction was adjusted to 0.1 M HCl and reapplied to the re-equil-
ibrated ion exchange columns. After washing with 100 mL of 0.1 M HCl, pABG converted from ApABG was eluted and collected in 100 mL of 0.6 M HCl. The two pABG fractions were kept separate throughout the process and derivatized. One milliliter of sodium nitrite solution (1%) and 1 mL of 5.0 M HCl were added to each of the pABG fractions, and the reaction carried out at room temperature. After 5 min, 1 mL of ammonium sulfamate solution (50 g/L) was added. Then, after 5 min, 1 mL of N-(1-naphthyl)-ethylenediamine solution (10 g/L) was added to couple pABG. Both fractions were maintained overnight at room temperature to allow the complete formation of the purple-colored azo-N-(1-naphthyl)-ethylenediamine derivative of pABG (azo-pABG). Each fraction was applied to a preactivated (5 mL of methanol wash followed by 5 mL H2O) C18 Sep Pak cartridge (Waters). After washing with 10 mL of 0.05 M HCl, the azo-pABG was eluted in 4 mL of 100% methanol. The solution was evaporated until dry at 40°C under a nitrogen stream. The residue was reconstituted in 250 µL of H2O and added to 25 µL of 5 M HCl and 25 µL of zinc powder suspension (1 g/3 mL H2O). After 15 min of mixing, the mixture was transferred to a 1-mL Eppendorf tube and centrifuged for 10 min. The supernatant from the centrifuged mixture was removed, filtered, and stored at -20°C until HPLC analysis.

The quantity of pABG was determined by reverse-phase HPLC with an octadecylsilica column (Ultramex C18, 5-μm particle size, 4.6 mm×250 mm; Phenomenex). The HPLC injection volume was 100 µL and the flow rate was 1.0 mL/min. The isocratic mobile phase (pH 4.0) was composed of acetonitrile (2%) and 0.1 M formic acid (98%). A UV absorption detector (Dionex AD 20) was used for monitoring at 280 nm. The quantification of pABG (representing excreted pABG and ApABG) was estimated from a standard curve using commercial pABG (Sigma Chemical Co.).

Statistical analysis. All statistical analyses were conducted using SPSS, version 10.0 (SPSS Inc., Chicago, IL). The data are expressed as the means±SD for the subjects. Pearson’s correlation coefficients were utilized to describe the relationships among the folate status indices. A p-value of <0.05 was considered to be significant. Since the data for weight, folate intake, dietary folate density, plasma tfHcy concentration, folate concentrations of plasma and erythrocytes, and urinary excretions of pABG and ApABG did not evidence standard distributions, those values were transposed into natural log form prior to statistical analysis.

RESULTS

Age, anthropometrical measurements, and hematological indices

The age of the subjects was 23.8±5.8 y. The height and weight of the subjects, 160.4±5.3 kg and 54.9±8.1, respectively were similar to those of the KDRIs (Dietary Reference Intakes for Koreans) standard (160 cm and 56.3 kg, respectively). The Hb concentration and Hct of the subjects were 12.6±1.6 g/dL and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Energy, protein, and folate intakes.</th>
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</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1,779±50.0 (560-3,013.8)</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>64.0±20.6 (33.2-116.8)</td>
</tr>
<tr>
<td>Folate (µg DFE/d)</td>
<td>206.9±90.8 (85.2-584.5)</td>
</tr>
</tbody>
</table>

Values are means±SD (ranges).

Fig. 1. Distribution of dietary folate intakes. EAR, estimated average requirement.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Concentrations of plasma and erythrocyte folate and plasma total homocysteine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma folate (ng/mL)</td>
<td>10.5±3.7 (5.8-22.8)</td>
</tr>
<tr>
<td>Erythrocyte folate (ng/mL)</td>
<td>249.9±77.8 (138.8-463.0)</td>
</tr>
<tr>
<td>Plasma tfHcy (nmol/mL)</td>
<td>12.7±0.2 (6.8-28.0)</td>
</tr>
</tbody>
</table>

Values are means±SD (ranges).

tHcy, total homocysteine.

<table>
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<tr>
<th>Table 3</th>
<th>Direct and indirect biomarkers of folate status.</th>
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</thead>
<tbody>
<tr>
<td>Plasma folate</td>
<td>Normal (≥6.0 ng/mL)</td>
</tr>
<tr>
<td>Borderline (3.0-5.9 ng/mL)</td>
<td>2.8 (1)</td>
</tr>
<tr>
<td>Deficiency (&lt;3.0 ng/mL)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>RBC folate</td>
<td>Normal (≥157 ng/mL)</td>
</tr>
<tr>
<td>Borderline (140-157 ng/mL)</td>
<td>5.5 (2)</td>
</tr>
<tr>
<td>Deficiency (&lt;140 ng/mL)</td>
<td>2.8 (1)</td>
</tr>
<tr>
<td>Plasma tfHcy</td>
<td>Normal (&lt;15 nmol/mL)</td>
</tr>
<tr>
<td>Hyperhomocysteinemia (≥15 nmol/mL)</td>
<td>11.1 (4)</td>
</tr>
</tbody>
</table>

Values are percentages (numbers).
RBC, red blood cell; tfHcy, total homocysteine.

37.1±3.8%, respectively. Among the 36 subjects, 19.4% (7 subjects) evidenced marginal iron deficiency (Hb <12 g/dL and Hct <36%), and 5.6% (2 subjects) had Hb concentrations of below 9 g/dL.

Dietary intakes of energy, protein, and folate

Dietary intakes of energy, protein, and folate of the subjects are shown in Table 1. The intake of energy was 1,779±503.0 kcal/d, which was 85% of the Estimated Energy Requirement (EER). The protein intake, 64.0±20.6 g/d, was 1.8 times the EER of protein. The folate intake was 206.9±90.8 µg DFE/d, which was
65% of the EAR of folate for Koreans. As is shown in
Fig. 1, the majority (83.3%) of the subjects consumed
folate at levels below the EAR.

Plasma and erythrocyte folate concentrations and plasma tHcy concentration

Concentrations of plasma and erythrocyte folate and plasma tHcy of the subjects are presented in Table 2. The percentages of the subjects with folate deficiency, as determined by the concentrations of plasma and erythrocyte folate and plasma tHcy, are presented in Table 3. The plasma and erythrocyte folate concentrations were 10.5 ± 3.7 and 249.9 ± 77.8 ng/mL, respectively. The plasma tHcy concentration was 12.7 ± 0.2 nmol/mL. No subject was determined to be folate-deficient (<3.0 ng/mL), as estimated by plasma folate concentrations, and only 1 subject was diagnosed with marginal folate-deficiency (3.0–5.9 ng/mL). However, as determined by the erythrocyte folate concentrations, 2.8% (1 subject) of the subjects were folate-deficient (<140 ng/mL) and 5.5% (2 subjects) were marginally folate-deficient (140–156 ng/mL). Furthermore, 11% (4 subjects) evidenced hyperhomocysteinemia (>15 nmol/mL).

Table 4. Urinary excretion of folate catabolites.

| pABG (nmol/d) | 10.7 ± 3.8 (5.1–21.1) |
| ApABG (nmol/d) | 89.1 ± 19.5 (48.6–134.3) |
| pABG+ApABG (nmol/d) | 99.7 ± 20.7 (58.9–141.5) |

Values are means ± SD (ranges).
pABG, para-aminobenzoyleglutamate; ApABG, para-acet-
amiobenzoyleglutamate.

Correlations among folate status indexes

As shown in Fig. 2, positive linear relationships were observed between folate intake and plasma folate concentration (r = 0.341, p < 0.05), erythrocyte folate concentration (r = 0.330, p < 0.05), and urinary excretion of ApABG (r = 0.329, p < 0.05); but not between folate intake and pABG excretion (r = 0.135, p = 0.432). Although it is not shown in Fig. 1, total urinary excretion of folate catabolites showed a positive correlation with folate intake (r = 0.335, p < 0.05). Positive linear relations were also observed (Fig. 3) between plasma folate concentration and urinary excretion of pABG (r = 0.389, p < 0.05), but not ApABG (r = 0.293, p = 0.083) and between erythrocyte folate concentration and urinary excretion of ApABG (r = 0.513, p < 0.01), but not pABG (r = 0.227, p = 0.183). Total urinary excretion of folate catabolites was positively correlated with both plasma and erythrocyte folate concentrations (r = 0.347, p < 0.05 and r = 0.526, p < 0.01, respectively, data not shown). We also noted a negative relationship between the concentrations of erythrocyte folate and plasma tHcy (r = -0.332, p < 0.05).

Fig. 2. Correlations between folate intake and folate concentrations in the plasma and erythrocytes and between folate intake and urinary excretion of folate catabolites.
DISCUSSION

In this study, Korean 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 intakes. This might be the consequence of poor dietary habits in young women. Among Korean women with childbearing potential, 27% of the teens and 45% of those in their twenties were underweight (BMI < 20) and their dietary intakes of energy, folate, iron, and calcium did not meet the respective EARs (31). Consequently, several of our subjects evidenced inadequate folate status. Although no women were demonstrated to be folate-deficient (< 3.0 ng/mL), with the exception of one subject (2.8%) with marginal folate-deficiency (3.0–5.9 ng/mL) as estimated by the plasma folate concentration. 2.8% of the subjects were folate-deficient (<140 ng/mL) and 5.5% were marginally folate-deficient (140–156 ng/mL), as determined by erythrocyte folate concentration. In addition, 11% evidenced hyperhomocysteinemia (≥ 15 mmol/L). The marginal folate status of the subjects in this study might impair homocysteine remethylation (32). These results are consistent with previous studies performed in Korea, in which it has been reported that a significant proportion of women with childbearing potential are marginally folate-deficient (17–19).

In humans, the rate of whole-body folate turnover is very slow (33). However, it is considered that the presence of ApABG in urine is indicative of folate catabolism (34). In a study of elderly American women (24), the subjects excreted 115.0±12.7 nmol/d of total folate catabolites when they consumed their normal diets, but 86.1±26.7 nmol/d when they consumed folate-depleted diets. The level of total folate catabolite excretion in this study was found to be lower than that obtained from the elderly American women when they were consuming their usual diets, but higher than when they were folate-depleted (24). In the above study, Wolfe et al. demonstrated that folate catabolite excretion was significantly reduced in response to a low-folate diet, which may be reflective of a reduction in tissue folate catabolism, or in the folate turnover rate. Erythrocyte folate concentration is considered to be a principal indicator of adequate folate status, due to its correlation with liver folate, and thus with tissue stores (35). The urinary excretions of folate catabolites imply that the subjects in this study took insufficient folate and were marginally folate-deficient. The means of folate reserve and folate turnover rate of the subjects in this study were 26.2±11.6 and 10.5±3.9, respectively. In the current study, folate intake was positively associated with plasma folate concentration (r=0.341, p<0.05) and erythrocyte folate concentration (r=0.330, p<0.05). Moreover, it was also correlated with urinary excretion of ApABG (r=0.329, p<0.05) and total folate catabolites (r=0.335, p<0.01). The results confirmed that urinary excretion of folate catabolites is indicative of the rate of turnover of total body folate, and that the turnover rate is dependent on folate intake (24). In addition, the folate concentration in erythrocytes, but not plasma, was associated with urin-
nary excretion of ApABG ($r=0.513$, $p<0.01$) and the total folate catabolites ($r=0.526$, $p<0.001$), and urinary pABG excretion was positively correlated with the folate concentrations in plasma, but not in the erythrocytes. The results support that the cleavage product, ApABG, accounts for the bulk of whole-body folate turnover (36) and supports the notion that urinary excretion of pABG, which is a minor component of total excretion of the catabolites (25), is reflective of recent dietary folate intake (37). In this study, ApABG constituted >85% of total folate catabolites, as has also been reported by Gregory et al. (26). In addition, all these results verify that the urinary excretion of folate catabolites may be a reliable indicator of folate turnover.

Since folate fortification began in the United States in 1998, the US population has consistently evidenced significantly higher serum and RBC folate concentrations (38). In Canada, following the introduction of folic acid fortification, the folate status of women of childbearing age has clearly improved (39). In Chile, after the mandatory fortification of wheat flour with folic acid, a measure which was targeted toward a 400 μg increase in the daily folate consumption of women of childbearing age, serum and RBC folate concentrations in a representative population sample increased by 284% and 144%, respectively (40). Berry et al. (41) reported a reduction in NTDs with folic acid intake in China. All of the aforementioned studies have suggested that sufficient folate consumption may guarantee adequate folate status.

On the basis of the overall results in this study, the folate status of Korean women of childbearing age is marginally deficient, with inadequate erythrocyte folate concentrations and plasma tHcy concentrations, largely due to insufficient folate intake. The marginally folate-deficient status was confirmed by the low urinary excretion rate of folate catabolites.

Acknowledgments

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Folate Status of Korean Childbearing Women


