Plasma Total Homocysteine, Folate, and Vitamin B₁₂ Status in Korean Adults

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Summary Elevated plasma total homocysteine (tHcy) levels have been established as a risk factor for occlusive cardiovascular disease. Also known is that plasma folate and vitamin B₁₂ influence homocysteine metabolism as cosubstrate and cofactor, respectively. However, not much information is available describing plasma tHcy levels and their relationship to plasma folate and vitamin B₁₂ status in Koreans. We measured the plasma levels of tHcy, folate, and vitamin B₁₂ in 195 adults (99 males, 96 females; 23–72 y old in the lower middle class). The mean plasma tHcy levels of males, 11.18±3.88 μmol/L, was significantly higher (p<0.001) than that of females, 9.20±2.65 μmol/L. The distribution of tHcy levels of males showed a wide range, 3–50 μmol/L, with a long tail toward higher values. Thus the incidence of hyperhomocysteinemia (≥15 μmol/L) in males, 10.1%, was significantly higher (<0.02) than the 2.1% in females. As age increased, plasma tHcy levels tended to be higher in females. Therefore, sex differences in plasma tHcy levels disappeared in subjects over fifty. On the other hand, both plasma folate (6.47±3.06 vs 7.96±3.55 ng/mL, p<0.01) and vitamin B₁₂ levels (537.0±222.0 vs. 664.1±309.8 ng/mL, p<0.01) were significantly lower in males than in females. A plasma folate deficiency (<3.0 ng/mL) was found in 6.1% of males and 2.1% of females. And a vitamin B₁₂ deficiency (<150 pg/mL) was detected in 2.0% and 1.0%, respectively. Plasma tHcy levels were related with inversely plasma concentrations of folate (r=-0.37249, p<0.001) as well as vitamin B₁₂ (r=-0.22560, p<0.01) in both sexes. Plasma levels of tHcy and the prevalence of hyperhomocysteinemia in Korean adults are similar to findings in the West. Our results indicate that male adults may be in worse condition for cardiovascular disease (CVD) than females. And improving folate and vitamin B₁₂ status may reduce plasma tHcy level, which may be more important in males.

Key Words homocysteine, folate, vitamin B₁₂, Korean, age-sex difference

In recent years, there has been increasing interest in the metabolism of homocysteine because clinical and epidemiological observations have shown that elevated plasma total homocysteine (tHcy) levels are related to the pathogenesis of atherosclerosis (1–5). The prevalence of cardiovascular disease (CVD) has increased in Korea. Cardiovascular disease has become the leading cause of death among Koreans, and especially, mortality from ischemic heart disease in 1999 increased 77.9% compared with what it was in 1990 (6).

It has been well established that folate and vitamin B₁₂ deficiencies may result in hyperhomocysteinemia (≥15 μmol/L). Plasma concentrations of folate and vitamin B₁₂ and, to a lesser extent, of vitamin B₆, are inversely related to plasma tHcy levels (7–9). Indeed, it has been suggested that about two-thirds of hyperhomocysteinemia cases are due to inadequate blood levels of one or more of these vitamin cofactors (8). Although interest in homocysteine metabolism is growing in Korea, there are relatively few studies of plasma tHcy levels in normal subjects. Moreover, not much information is available about plasma levels of the vitamins related to homocysteine metabolism in Korean adults. Some differences in plasma tHcy levels may exist among races and ethnic groups (10, 11).

Thus in this study we investigated plasma tHcy concentrations and the status of folate and vitamin B₁₂ status in Korean adults and assessed the differences by age and sex. We also analyzed the correlation between plasma tHcy levels and vitamin B₁₂, as well as folate status.

MATERIALS AND METHODS

Subjects. A total of 195 adults (male: 99, female: 96; 23–72 y old) who approved the purpose of this study participated in it. They did not knowingly have any diseases and were receiving no medical treatments.
As shown in Table 1, a third of them were educated for less than 12 years, and two-thirds had monthly incomes of less than $1,000. Most were classified in the low middle class and resided in a large city, Gwangju, Korea. It seemed that male and female subjects alike had normal plasma lipid profiles. Triacylglyceride concentrations of male subjects were significantly higher than those of females (p<0.001), but HDL-cholesterol levels were higher in females than in males (p<0.05).

This study was approved by the Korean Research Foundation and written informed consent was obtained from each subject.

Blood collection. Blood was collected in the fasting state by venipuncture in a tube containing EDTA, cooled on ice, and centrifuged at 3,000 g for 30 min. Plasma was divided into small vials and stored at -20°C until analysis.

Physical examination and laboratory analysis. Weights and heights were measured and body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Plasma concentrations of total cholesterol, triacylglyceride, and high-density lipoprotein (HDL)-cholesterol, were analyzed by enzymatic methods using commercial kits (Eiken Co., Japan). Low-density lipoprotein (LDL)-cholesterol concentrations were calculated with Friedewald’s equation (12).

The plasma levels of tHcy were determined by using high-pressure liquid chromatography according to the modified methods of Araki et al. (13), Refsum et al. (14), and Ubbink et al. (15). The thyol adducts were separated by use of an isocratic system with a 0.1 μ acetate acid/acetate buffer (pH 5.5, containing 3% methanol) as a mobile phase and detected by fluorescence (excitation at 385 nm, emission at 515 nm). The flow rate was 0.7 mL/min and cysteamine was used as an internal standard. Plasma concentrations of folate were measured by a microbiological assay using the *Lactobacillus casei* (L. casei ATCC 7469). Plasma vitamin B₁₂ levels were analyzed with a radiobinding assay (Chiron Diagnostics Corporation, Norwood, MA, USA).

**RESULTS**

**Distributions of plasma tHcy, folate, and vitamin B₁₂ concentrations**

The frequencies, cumulative frequencies, and percentile distributions of plasma tHcy, folate, and vitamin B₁₂ levels are shown in Fig. 1 and Table 2. Plasma tHcy levels were widely distributed (3–51 μmol/L) with median values of 10.33 μmol/L in males and 9.03 μmol/L in females. Unlike the tHcy levels, the distributions of plasma concentrations of the two vitamins presented more skewed shapes toward higher concentrations in females. The 5th percentile of plasma folate values of both sexes was below the cutoff point, but that of females was not. Those of plasma vitamin B₁₂ values of both sexes were above the cutoff point.
Plasma total homocysteine (μmol/L)

Plasma folate (ng/mL)

Plasma vitamin B₁₂ (pg/mL)

Fig. 1. Frequency (upper) and cumulative frequency (lower) distributions of plasma total homocysteine, folate, and vitamin B₁₂ concentrations for 195 subjects (male 99, female 96).

Table 2. Percentile distributions of plasma total homocysteine, folate, and vitamin B₁₂ concentrations of the subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subject population</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total homocysteine</td>
<td>Male (n=99)</td>
<td>6.83</td>
<td>7.74</td>
<td>9.24</td>
<td>10.33</td>
<td>12.26</td>
<td>14.98</td>
<td>19.84</td>
</tr>
<tr>
<td>(μmol/L)</td>
<td>Female (n=96)</td>
<td>5.92</td>
<td>6.63</td>
<td>7.64</td>
<td>9.03</td>
<td>10.26</td>
<td>11.45</td>
<td>12.46</td>
</tr>
<tr>
<td>Plasma folate</td>
<td>Male (n=99)</td>
<td>2.85</td>
<td>3.30</td>
<td>4.37</td>
<td>5.98</td>
<td>7.72</td>
<td>10.45</td>
<td>13.51</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>Female (n=96)</td>
<td>3.42</td>
<td>4.21</td>
<td>5.28</td>
<td>7.51</td>
<td>9.28</td>
<td>13.35</td>
<td>14.37</td>
</tr>
<tr>
<td>Plasma vitamin B₁₂</td>
<td>Male (n=99)</td>
<td>192.4</td>
<td>282.9</td>
<td>371.6</td>
<td>507.5</td>
<td>659.0</td>
<td>850.4</td>
<td>975.7</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td>Female (n=96)</td>
<td>264.0</td>
<td>365.6</td>
<td>465.2</td>
<td>585.3</td>
<td>791.1</td>
<td>1,077.6</td>
<td>1,345.8</td>
</tr>
</tbody>
</table>

Differences in plasma tHcy, folate, and vitamin B₁₂ levels by sex and age

The plasma levels of tHcy, folate, and vitamin B₁₂ by sex and age are shown in Table 3. The mean of plasma tHcy levels were significantly higher in male subjects than in female subjects (p<0.001). Plasma tHcy values did not differ among the various age categories in males; however, those of females tended to be higher as age increased. In age categories below 50, the females had significantly higher tHcy levels (from p<0.05 to p<0.01), but sex differences disappeared in subjects over 50. Female subjects had significantly higher means of both plasma folate and vitamin B₁₂ concentrations than male subjects did (p<0.01). In the corre-
Table 3. Plasma total homocysteine, folate, and vitamin B₁₂ concentrations of the subjects sex and age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age (y)</th>
<th>Male (n=99)</th>
<th>Female (n=96)</th>
<th>p-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>±SD</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td>Plasma total homocysteine</td>
<td>20-29</td>
<td>11.54±3.16NS³</td>
<td>8.91±1.07ab³</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>11.22±4.54b</td>
<td>8.72±1.93b</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>11.07±4.20b</td>
<td>8.45±1.94b</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>10.20±3.00b</td>
<td>9.43±1.66ab</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
<td>11.94±3.68a</td>
<td>10.66±5.36a</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>11.18±3.88</td>
<td>9.20±2.65</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4. Status of plasma total homocysteine, plasma folate, and plasma vitamin B₁₂ of the subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Status</th>
<th>Male (n=99)</th>
<th>Female (n=96)</th>
<th>Chi-square²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (&lt;15 μmol/L)</td>
<td>89 (89.9)¹</td>
<td>94 (97.9)</td>
<td>p=0.02</td>
</tr>
<tr>
<td></td>
<td>High (≥15 μmol/L)</td>
<td>10 (10.1)</td>
<td>2 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Plasma folate</td>
<td>Deficiency (&lt;3.0 ng/mL)</td>
<td>6 (6.1)</td>
<td>2 (2.1)</td>
<td>p=0.089</td>
</tr>
<tr>
<td></td>
<td>Borderline (3.0-5.9 ng/mL)</td>
<td>44 (44.4)</td>
<td>33 (34.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal (≥6.0 ng/mL)</td>
<td>49 (49.5)</td>
<td>61 (63.5)</td>
<td></td>
</tr>
<tr>
<td>Plasma vitamin B₁₂</td>
<td>Deficiency (&lt;150 pg/mL)</td>
<td>2 (2.0)</td>
<td>1 (1.0)</td>
<td>p=0.579</td>
</tr>
<tr>
<td></td>
<td>Normal (≥150 pg/mL)</td>
<td>97 (98.0)</td>
<td>95 (99.9)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means±standard deviations.
2 Different superscripts in a row indicate significant differences between male and female subjects after Student’s t-test.
3 p-values in a column indicate significant differences among age categories after ANOVA with Duncan’s multiple range test.

Prevalence of hyperhomocysteinemia and the status of folate and vitamin B₁₂

The prevalence of hyperhomocysteinemia (≥15 μmol/L) and the status of folate and vitamin B₁₂ are shown in Table 4. The incidence of hyperhomocysteinemia showed a sex difference (p<0.02): 10 of 99 males (10.1%) and 2 of 96 females (2.1%) had hyperhomocysteinemia. A plasma folate deficiency (<3.0 ng/mL) was found in 6.1% of males and in 2.1% of females: however, 44.4% of males and 34.4% of females had a borderline deficiency (3.0-5.9 ng/mL). However, a sex difference was not found in plasma folate status. Only 2 of male subjects (2.0%) and one of females (1.0%) had a deficiency (<150 pg/mL) of plasma vitamin B₁₂, and...
Correlation of plasma tHcy to plasma folate and vitamin B₁₂

The correlation between the levels of plasma tHcy and the concentrations of folate and vitamin B₁₂ are shown in Fig. 2. Plasma tHcy levels had a significant negative correlation with plasma folate concentrations both in males ($r = -0.3117, p < 0.001$) and in females ($r = -0.2217, p < 0.05$). On the other hand, a negative relationship ($r = -0.2771, p < 0.05$) was found between plasma tHcy levels and plasma vitamin B₁₂ concentrations only in males, but it was not evident in females. The relationship between tHcy and folate ($R^2 = 0.088, p < 0.001$) was stronger than that between tHcy and vitamin B₁₂ ($R^2 = 0.034, p < 0.01$).

DISCUSSION

This study was conducted to describe the distribution of plasma tHcy, folate and vitamin B₁₂ concentrations in Korean adults and to assess relationships among them. Our results showed that the plasma tHcy levels, especially in male subjects, were widely distributed and showed a skewed shape, with a long tail toward higher values, as found in Western subjects (16, 17). We assumed that the prevalence of hyperhomocysteinemia in Korea might be lower than in the West because several reports have shown racial and ethnic differences in plasma tHcy levels (10, 11). The diets of Koreans were quite likely to be centered around more grains and vegetables and less fat than the diets of Westerners. Our as-
sumption, however, was not confirmed. The mean plasma tHcy level for our subjects tended to be slightly higher compared with the values in the U.S. NHANES report (11) and the values from subjects who consumed no vitamin supplements in the Hordaland Homocysteine Study for Norway (16). In comparison to the reports previously published in Korea, our mean value is quite similar to the 10.8 μmol/L of Han et al. (18) and the 10.7 μmol/L of Jang et al. (19). However, other studies have reported lower plasma tHcy levels than our values: 6.4 μmol/L of Lee et al. (20) and 5.9 μmol/L of Kim et al. (21). It is difficult to directly compare the five values of Koreans, including ours, because in each study, the number of subjects was very limited and their ages and sexes were different. There is no representative data of plasma tHcy of Koreans.

Our results showed definite differences by sex in plasma tHcy levels; in comparison with females, males had significantly higher values, as in the results of other studies (11, 16, 22). The magnitude of the difference showed 1.98 μmol/L, which is greater than the 1 μmol/L found by Nygard et al. (23). Several researchers have suggested that the sex difference in plasma tHcy levels may be mediated by sex hormones, especially estrogen (24). This explanation is supported by the reduction in plasma tHcy levels in women during pregnancy (25–27), which is a state characterized by a high level of circulating estrogens. There are other consistent reports that plasma tHcy levels are lower in premenopausal women compared with postmenopausal women (28, 29); estrogen replacement therapy in the latter resulted in lower plasma tHcy levels (30, 31). Thus, the steep age-related increase in plasma tHcy levels in the women in this study may be explained by the change in hormonal status. Jacques et al. (11) mentioned a significant (p<0.01) age-sex interaction, reflecting that plasma tHcy levels in females tend to diverge from those in males at younger ages and to converge with those in males at older ages. This sex difference disappeared in subjects over 50 in our study. In two recent studies, the significant relationship of plasma tHcy levels with sex and age disappeared after adjustments were made for serum creatinine levels (32, 33). Creatine-creatinine synthesis, a function of muscle mass, is the major source of homocysteine (7), which may explain why plasma tHcy levels are higher in men than in women from a new perspective. Furthermore, another possible explanation is related to the increase in homocysteine levels during renal failure (34, 35). Brattström et al. (29) insisted that higher homocysteine levels in the elderly might, therefore, be related to the decline in renal function with age. According to the evidence from Arnadottir et al. (36), the plasma levels of homocysteine increase with even mild renal insufficiency (a creatinine clearance of <60 ml/min). In our study, however, the increase in plasma tHcy levels related to age was not found in male subjects. Sex hormones, muscle mass, and renal function were not evaluated in our study, and it was, therefore, not possible to interpret the actual cause of the age-related increase in plasma tHcy in female subjects.

It has been well established that plasma tHcy levels are influenced by the status of folate and vitamin B₁₂ in circulation required in homocysteine metabolism as substrate and coenzyme. Thus, deficiencies in folate and/or vitamin B₁₂ may result in hyperhomocysteinemia (8, 9, 37–39). It has been suggested that about two-thirds of hyperhomocysteinemia cases are due to inadequate blood levels of one or more of these vitamin cofactors (8). In our results, male subjects had lower plasma levels of folate as well as vitamin B₁₂ than female subjects did. Chambers et al. (10) evaluated the reason for the differences in plasma tHcy levels between Europeans and Asians by comparing the concentrations of plasma vitamin B₁₂ and folate. Our results, which showed that plasma tHcy levels were negatively correlated with both plasma folate (r=-0.37249, p<0.001) and vitamin B₁₂ (r=-0.22560, p<0.01), confirmed previous reports (8, 40). Thus, lower levels of plasma folate and vitamin B₁₂, in male subjects may also contribute to the sex difference in plasma tHcy. The negative relationship of plasma tHcy to plasma folate was stronger than that to vitamin B₁₂ in our study. It can be explained biochemically because folate acts as a limiting factor for homocysteine metabolism (38, 41). Folate, functioning as a donor of methyl group in the remethylation reaction, is used up quantitatively. Vitamin B₁₂, however, serves as a coenzyme and thus is not used up during the reaction in which it is involved. Vitamin B₁₂ does not seem to play a key role and is usually present in sufficient amounts because of large stores in the body (41).

Although the inverse correlation between plasma levels of tHcy and folate was significant for male and female subjects alike, it tended to be stronger in males (r=-0.3117, p<0.001) than in females (r=-0.2217, p<0.05). The correlation between plasma concentrations of tHcy and vitamin B₁₂ was negatively significant only for male subjects (r=-0.2771, p<0.05). This result suggests that folate status is more critical than vitamin B₁₂ for maintaining normal plasma tHcy levels. It also implies that the nutritional status of folate may be more important in males than females in relation to homocysteine metabolism.

In conclusion, we showed that the plasma tHcy levels of Korean adults are similar to those of Western populations. There is a marked sex difference in plasma tHcy, folate, and vitamin B₁₂ concentrations; the males had higher plasma levels of tHcy but lower folate and vitamin B₁₂ than the females did, however, it is not apparent in subjects over 50. Plasma tHcy levels had a significant inverse correlation with both plasma folate and vitamin B₁₂ concentrations. These findings should support dietary recommendations to improve folate and vitamin B₁₂ status to reduce tHcy levels, especially for males.

Acknowledgment

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REFERENCES


