Relationship between Homocysteine, Folate, Vitamin B12 and Physical Performance in the Institutionalized Elderly

Misora Ao1, Nao Inuiya1, Junko Ohta2, Satoshi Kurose1, Yasusei Abe3, Naho Niki4, Shino Inoue5, Shizuo Tanaka6, Takashi Miyawaki1 and Kiyoshi Tanaka1,2

1Department of Food and Nutrition, Kyoto Women's University, Kyoto 605-8501, Japan
2Faculty of Nutrition, Kobe Gakuin University, Hyogo 651-2180, Japan
3Department of Health Science, Graduate School of Medicine, Kansai Medical University, Osaka 573-1010, Japan
4Nursing Care Home, Life in Kyoto, Kyoto 615-8256, Japan
5Nursing Care Home, Airanomori Ujigokasho, Kyoto 611-0011, Japan
6Nursing Care Center, Care House Ajisai, Kyoto 619-1154, Japan
7Nursing Care Center, Care House Yamabuki, Kyoto 611-0021, Japan
8Nursing Care Home, Villa Joyo, Kyoto 610-0114, Japan

(Received December 21, 2017)

Summary
Hyperhomocysteinemia causes various diseases including cardiovascular disease, osteoporotic fracture and dementia. Although there have been reports that hyperhomocysteinemia decreases physical performance, findings are inconsistent on the association of homocysteine, folate, vitamin B12 and physical performance. Considering that lower physical performance increases the risk of fall and fracture in the elderly, the effect of nutritional status on physical function must be clarified. This is a cross-sectional study conducted from April 2015 to November 2016. Eighty-six residents and users in five care facilities were evaluated for their blood homocysteine, folate and vitamin B12 concentrations and indices for physical performance; lower limb muscle strength, handgrip strength and gait speed. Analyses of physical performance were done in women only, considering the high proportion of women in the study population and the muscular gender difference. In the third tertile of plasma homocysteine concentration, handgrip strength was significantly lower than in the first tertile \((p=0.027)\). In the first tertile of serum folate concentration, handgrip strength was significantly lower than in the third tertile \((p=0.002)\). Although not statistically significant, lower limb muscle strength in the third tertile of folate was higher than in the first \((p=0.061)\) and second \((p=0.057)\) tertile. In the multiple regression analysis, however, only serum folate concentration was a significant contributor except for age. In subjects with their serum folate and vitamin B12 concentrations both exceeding the median, lower limb muscle strength was higher. Low serum folate concentration is a risk factor for lower physical performance independent of homocysteine in elderly women.

Key Words
folate, vitamin B12, homocysteine, physical performance, elderly

Homocysteine (Hcy) is an intermediate in the methionine cycle. Methionine is metabolized to S-adenosylmethionine, S-adenosyl-Hcy, then to Hcy.

Hcy is metabolized to methionine with vitamin B12 and folate as cofactors, or to cysteine vitamin B6-dependently. Therefore, blood Hcy concentration is increased by the deficiency of these vitamins (hyperhomocysteinemia) (1). Hyperhomocysteinemia has been known to be a risk factor for cardiovascular disease, which is independent of dyslipidemia (2).

Vitamin deficiency causes classical deficiency diseases with typical phenotypic changes. For example, vitamin B12 deficiency causes beriberi, and vitamin D deficiency leads to rickets (3, 4). In vitamin insufficiency, which is milder than deficiency, such classical diseases do not occur, but the risk for various diseases is increased. For example, it is well known that vitamin D insufficiency is a serious risk factor for osteoporotic fracture (5). Similarly, vitamin B12 deficiency causes pernicious anemia and folate deficiency leads to megaloblastic anemia or neurological disorders. Moreover, insufficiency of these vitamins causes hyperhomocysteinemia, and increases the risk for cardiovascular diseases (6–8).

Recently, hyperhomocysteinemia has also been reported to be a risk for various other diseases including osteoporotic fracture and dementia (9, 10). Since Japan has been becoming a super-aging society, such a relationship would be important both clinically and socially (11).

Bone is formed through calcium phosphate deposition onto the protein matrix composed mainly of collagen. It has been reported that hyperhomocysteinemia dis-
rupts the collagen-cross links and impairs bone strength (12, 13). Furthermore, previous papers have reported the relationship between Hcy and muscle strength (14–18). It is possible that hyperhomocysteinemia increases the fracture risk through two mechanisms: impaired bone strength and decreased muscle strength. However, recent data are not necessarily consistent. In some papers, insufficiencies of vitamin B₁₂ or folate were reported to cause hyperhomocysteinemia, which in turn, leads to low physical performance. In contrast, these vitamins were related to low physical performance independently of Hcy in other reports. There have been only a few reports on the relationship among Hcy, folate, vitamin B₁₂ and physical performance in Japanese elderly. Therefore in this paper, we have studied such a relationship in the institutionalized elderly.

**MATERIALS AND METHODS**

**Subjects and study design.** This is a cross-sectional study conducted from April 2015 to November 2016. The participants are 86 residents and users in five care facilities. Written consent to participate in this study was obtained from each subject after explanation of the objective and protocol of this study. The study protocol was approved by the Ethical Committee of Kyoto Women’s University (Ethics Approval number: 28-9), and the study was performed complying with the Declaration of Helsinki.

Subjects were either ambulatory or users of a wheelchair, but not bedridden. Subjects were excluded if they were taking vitamin B₁₂, folate, or vitamin B₆. Since food fortification of folate or vitamin B₁₂ is not done in Japan, the possibility for the undeclared use of supplementation was considered to be possible in subjects with extremely high concentrations for these vitamins. Participants whose serum vitamin B₁₂ or folate concentrations were above average plus 2SD, 1,162.8 pmol/L for vitamin B₁₂, and 37.5 nmol/L for folate, were excluded (19). In addition, participants whose plasma Hcy concentration exceeded average plus 2SD, 30.9 μmol/L were excluded. A total of 77 subjects (65 women, 12 men) were included in this analysis. Since there is a marked gender difference in muscle strength and female subjects constitute most participants in the current study, analyses for muscle strength were done in female subjects only.

**Biochemical measurements.** Non-fasting blood was drawn by venipuncture. After centrifugation, serum or plasma was stored at −70°C until measurement. Serum concentrations of vitamin B₁₂ and folate were measured by chemiluminescent enzyme immunoassay (CLEIA). Plasma Hcy concentration was measured by HPLC. Serum albumin concentration was measured by dye-binding (BCG) methods and serum total cholesterol concentration was measured by enzymatic (cholesterol oxidase) methods. Estimated glomerular filtration rate (eGFR; mL/min/1.73 m²) was calculated as 194 × creatinine⁻¹.₉₀₄ × age⁻₀.₂₈⁷ for men and 194 × creatinine⁻¹.₉₀₄ × age⁻₀.₂₈⁷ × 0.₇₃₉ for women (20).

**Statistical analyses.** Statistical analyses were done using SPSS version 22 (IBM Japan, Tokyo). Spearman’s correlation was used to examine the relationship between plasma Hcy and serum folate and vitamin B₁₂ concentrations. The difference between two independent groups was analyzed by the unpaired t test. Comparisons of subjects’ characteristics among tertiles of Hcy or folate were carried out using one-way ANOVA followed by Tukey’s multiple comparison test. The homogeneity of the variance was analyzed by the Levene test. Data were analyzed by the Kruskal-Wallis test when the variances were unequal. Multiple regression analysis was employed to assess the contributing factors for muscle strength. The significance level of the associations was set at p<0.05.

**RESULTS**

Background profiles of the study subjects are shown in Table 1. They were aged 83.5 ± 7 years on average with no gender difference. Their eGFR was almost the same as the reference value of 60 mL/min/1.73 m², and there was no gender difference (https://www.jsn.or.jp/guide/line/pdf/CKD_evidence2013/all.pdf). Their BMI and serum concentrations of albumin and total cholesterol were within the reference range, suggesting that these subjects were not generally malnourished. Although the men had higher height and weight than the women, there was no difference in BMI. Mean lower limb muscle strength was lower than that in general Japanese in
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Comparisons of physical performance among tertiles of serum vitamin B12 concentrations and plasma Hcy concentration. Post hoc test showed a significant difference between the first and third tertile in handgrip strength ($p=0.002$). Although not statistically significant, lower limb muscle strength in the third tertile was higher than in the first ($p=0.061$) and second ($p=0.057$) tertile. There was no significant association between serum folate concentration and gait speed. Nor were there any significant differences in the indices for physical performance between the tertiles of serum vitamin B12 concentrations.

Multiple regression analysis was performed to study the contributing factors to muscle strength. Independent variables included for analysis were age, skeletal muscle mass percentage, serum albumin, folate, vitamin B12 concentrations and plasma Hcy concentration. Age has negatively, and serum folate concentration has positively contributed to handgrip strength (adjusted $R^2=0.376; p<0.001$). Similar results were obtained for lower limb muscle strength (Table 5).

Analysis for an interaction between folate, vitamin B12 and physical performance in women

Subjects were categorized into four groups according to their serum folate and vitamin B12 concentrations: group 1 (both lower than median), group 2 (folate lower than, and vitamin B12 higher than median), group 3 (folate higher than, and vitamin B12 lower than median), group 4 (both higher than median). According to ANOVA, lower limb muscle strength was significantly different between these groups ($p=0.027$), and

#### Table 2. Correlation of Hcy with folate or vitamin B12.

| Correlation of Hcy with | $r$   | $p$ value |
|------------------------|-------|-----------|
| Folate                 | -0.435| <0.001    |
| Vitamin B12            | -0.388| <0.001    |

The $r$ value represents the Spearman’s correlation coefficient.

Based on tertiles of folate are shown in Table 4. According to ANOVA, plasma Hcy concentration, lower limb muscle strength and handgrip strength were significantly different between tertiles of serum folate. The Post hoc test showed a significant difference between the first and third tertile in handgrip strength ($p=0.002$). Although not statistically significant, lower limb muscle strength in the third tertile was higher than in the first ($p=0.061$) and second ($p=0.057$) tertile. There was no significant association between serum folate concentration and gait speed. Nor were there any significant differences in the indices for physical performance between the tertiles of serum vitamin B12 concentrations.

#### Table 1. Background profiles of the study subjects.

|                     | Total ($n=77$) | Men ($n=12$) | Women ($n=65$) | $p$ value |
|---------------------|---------------|--------------|----------------|-----------|
| Age (y)             | 83.5±7.4      | 81.0±8.8     | 84.0±7.1       | 0.207     |
| Body height (cm)    | 147.6±9.2     | 161.0±5.9    | 145.0±7.3      | <0.001    |
| Body weight (kg)    | 49.1±9.5      | 57.7±10.9    | 47.4±8.3       | 0.001     |
| BMI (kg/m²)         | 22.3±3.3      | 22.1±3.5     | 22.4±3.3       | 0.783     |
| Skeletal muscle mass (%) | 21.9±3.5   | 27.6±2.9     | 20.8±2.3       | <0.001    |
| Fat percentage (%)  | 33.4±6.6      | 23.7±9.0     | 35.2±4.2       | 0.002     |
| Serum albumin (g/dL)| 4.02±0.34     | 4.08±0.27    | 4.01±0.35      | 0.530     |
| Total cholesterol (mg/dL)| 190.1±34.4 | 186.8±36.8  | 190.7±34.2     | 0.723     |
| eGFR (mL/min/1.73 m²)| 60.7±14.7    | 59.5±11.4    | 61.0±15.3      | 0.747     |
| Plasma Hcy (μmol/L)| 13.9±4.7      | 16.3±6.5     | 13.4±4.2       | 0.168     |
| Serum folate (nmol/L)| 15.1±7.9    | 13.4±8.4     | 15.4±7.9       | 0.416     |
| Serum vitamin B12 (pmol/L)| 420.3±177.1| 367.9±152.0 | 429.9±180.7   | 0.268     |
| Lower limb muscle strength (N) | 188.3±93.6 | 260.2±98.3  | 172.3±85.5    | 0.003     |
| Lower limb muscle strength (kgf)| 0.39±0.18  | 0.46±0.16    | 0.38±0.18      | 0.154     |
| Handgrip strength (kg) | 15.6±6.8     | 25.8±5.2     | 13.7±5.1       | <0.001    |
| Gait speed (m/s)     | 1.1±0.4       | 1.2±0.4      | 1.1±0.3        | 0.341     |

Data are expressed as mean±SD. Comparison was made according to gender using $t$ test. Lower limb muscle strength, handgrip strength, and gait speed were measured in 66 (men 12, women 54), 76 (men 12, women 64), and 42 subjects (men 9, women 33), respectively.

In their eighties, probably because most of these subjects were not able to live independently (21). Correlation of blood Hcy, folate and vitamin B12 concentrations

Plasma Hcy concentration exhibited significant negative correlation with both serum folate ($r=-0.435, p<0.001$) and vitamin B12 ($r=-0.388, p=0.001$) (Table 2). Similar results were obtained even when individually analyzed for men and women.

Comparisons of physical performance among tertiles of plasma Hcy in women

Subjects’ characteristics and physical performance according to tertiles of Hcy are shown in Table 3. ANOVA has revealed significant differences in serum folate, serum vitamin B12 and handgrip strength between tertiles. There was a significant difference between the first and third tertile in handgrip strength ($p=0.027$) shown by the post hoc analysis, but no significant association was observed between tertiles of plasma Hcy concentration and lower limb muscle strength or gait speed.

Comparisons of physical performance among tertiles of folate in women

Subjects’ characteristics and physical performance based on tertiles of folate are shown in Table 4. According to ANOVA, plasma Hcy concentration, lower limb muscle strength and handgrip strength were significantly different between tertiles of serum folate. The Post hoc test showed a significant difference between the first and third tertile in handgrip strength ($p=0.002$). Although not statistically significant, lower limb muscle strength in the third tertile was higher than in the first ($p=0.061$) and second ($p=0.057$) tertile. There was no significant association between serum folate concentration and gait speed. Nor were there any significant differences in the indices for physical performance between the tertiles of serum vitamin B12 concentrations.

Analysis for an interaction between folate, vitamin B12 and physical performance in women

Subjects were categorized into four groups according to their serum folate and vitamin B12 concentrations: group 1 (both lower than median), group 2 (folate lower than, and vitamin B12 higher than median), group 3 (folate higher than, and vitamin B12 lower than median), group 4 (both higher than median). According to ANOVA, lower limb muscle strength was significantly different between these groups ($p=0.027$), and
there was a significant difference in lower limb muscle strength between group 1 and group 4 in the post hoc test \((p=0.017)\), (Fig. 1). There were no significant associations with handgrip strength and gait speed (data not shown).

**DISCUSSION**

There have been reports on the relationship among folate, vitamin B_{12} and Hcy and physical performance. Elevated Hcy has been reported to be as an independent risk factor for low physical performance. A cohort study
from the Netherlands has shown that high plasma Hcy is an independent risk factor for lower physical performance in older women (14). Another cross-sectional study from Singapore has shown that physical and functional decline was associated with elevated Hcy (15). Additionally, analysis of the baseline data of B-PROOF (B-Vitamins for the PRevention Of Osteoporotic Fractures) Study has shown that elevated plasma Hcy was associated with reduced physical performance and muscle strength in older women (16).

However, previous reports are not necessarily consistent on the relative involvement of each vitamin. Physical and functional decline was associated with elevated Hcy and low folate, but not with vitamin B12, in the Singapore study (15). In the B-PROOF study, elevated plasma Hcy was associated with reduced physical performance and muscle strength independently of vitamin B12 and folate, but there was no significant association between folate, vitamin B12 and physical activity (16). In the Dutch study, high plasma Hcy was an independent risk factor for lower physical performance although the association between vitamin B12 and physical performance was less clear (14). Unfortunately, serum folate concentration was not measured in their study. In another cross-sectional study, the frequency of sarcopenia and dynapenia were significantly higher in vitamin B12 deficiency (<400 pg/mL) (17).

Such inconsistency can be due to various reasons. First, folate fortification may obscure the contribution of folate (15). In the current study, serum folate concentration, but not vitamin B12, has significantly contributed the muscle strength. Cereals are not fortified with folate in Japan, which may be related to the results that folate was the only risk factor for low physical function in the present study. Indeed, the serum folate concentration was 15.1 ± 7.9 nmol/L in the current study, while it was 24.1 ± 12.6 nmol/L from the folate fortified region (15). Difference in the background profiles of the study subjects would also greatly influence the results. Both folate and vitamin B12 deficiency are common in older people, mainly due to malabsorption or low intake (25, 26). However, folate deficiency (serum folate concentration <6.8 nmol/L) was more common than vitamin B12 deficiency (serum vitamin B12 concentration <150 pmol/L) in our study. The mean age in the current study population was higher than that in the previous studies (14–16). Additionally, subjects in our study were institutionalized elderly, whereas study subjects in the previous studies were community-dwelling. These differences in subjects’ characteristics between previous reports and the current study may have influenced the results.

Several mechanisms have been suggested on the relationship between low folate and low physical performance. Wei has studied the folate status and its association with muscle strength and gait measures in diabetic patients in Singapore over 65 y old, and reported that folate concentration was significantly correlated with handgrip and leg strength which was corrected by BMI (18). Leg strength was positively correlated with gait measures and negatively correlated with a history of falls. He has argued that folate deficiency may negatively affect strength and gait measurement through mechanisms independent of elevated Hcy concentration, such as neurotransmitter synthesis, myelination, synthesis of DNA and protein, DNA methylation and epigenetic regulation. Although this study is from Singapore, a folate fortified region, he has suggested the possibility that inclusion of diabetic patients receiving rather high doses of vitamin B12 may have affected the results. de Lau et al. have reported that a higher plasma folate concentration is associated with better global cognitive function and better performance on tests of psychomotor speed regardless of Hcy concentration (27). In their study, brain imaging was also studied, and folate deficiency was associated with white matter lesion, but not with decreased hippocampal or amygdalar volume. Since white matter lesion and decreased hippocampal or amygdalar volume represent small vessel disease and early manifestation of Alzheimer’s disease, respectively, they have concluded that folate deficiency leads to impaired cognitive function through vascular mechanisms.

There also have been reports demonstrating unique mechanisms between high Hcy, low vitamin B12 and low physical performance, respectively, although such a significant association was not observed in the current study. McDermott et al. have reported the association of lower calf muscle characteristics with inflammatory markers such as D-dimer, and Hcy in patients with peripheral arterial disease (28). Elevated Hcy was associated with multiple domains of disability mediated in part by muscle strength and gait speed. Another basis was suggested for these findings. Hcy is metabolized to highly reactive homocysteine thiolactone by methionyl
transfer RNA synthetase. Then, homocysteine thiolactone reacts with lysine residues of proteins, a process called homocysteinyllation. The mechanism could be a possible factor of protein damage by Hcy (29, 30). An additional mechanism, the contribution of hyperhomocysteinemia to ischemic change to the brain, has been suggested based on the MRI (magnetic resonance imaging) finding of white matter hyperintensities (31). Vitamin B12 insufficiency may also be related to low physical performance independently through its association with neurological problems (32).

The lack of an obvious relationship between vitamin B12 and physical performance in our study does not preclude the involvement of vitamin B12. Compared to subjects with their folate and vitamin B12 status both lower than median (folate, 13.6 nmol/L; vitamin B12, 391.1 pmol/L), lower limb muscle strength was significantly higher in those with their vitamin statuses both higher than median, but not in those with only one of these vitamins. Therefore, even if folate more potently affects physical performance, vitamin B12 also seems to have some role in the current population. Despite controversies concerning the mechanisms underlying the relationship among Hcy, folate and vitamin B12 and physical performance, maintaining the status of these vitamins would be of importance from a practical point of view.

Our study has some limitations. First, although plasma Hcy is also influenced by vitamin B6 status, its serum concentration could not be measured. Since vitamin B6 measurement is not approved under Japanese Health Insurance, its measurement is not common in clinical practice. An additional problem is related to the technical one. Serum vitamin B12 or folate can be measured by immunoassay, and is easily available. However, serum vitamin B6 must be measured by HPLC (33). Second, folate status was evaluated only by its serum concentration. Folate concentration in the serum and blood cells reflects the short-term and long-term folate status, respectively. Third, we could not measure methylenalonic acid (MMA). MMA has the advantage that it is influenced by vitamin B12 status alone, but can be measured only by gas chromatography mass spectrometry, which is difficult to perform (32). Finally, physical performance was measured in a limited number of subjects.

In conclusion, lower serum folate concentration was associated with decreased physical performance in elderly women, and maintaining appropriate folate status is of pivotal importance in the elderly to avoid impaired physical function and the unfavorable consequences thereof.

Acknowledgments
This study was supported by JSPS KAKENHI Grant Number 16K00881.

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