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Relationship between Homocysteine, Folate, Vitamin B_{12} and Physical Performance in the Institutionalized Elderly

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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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12}, 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 B_{12} and folate as cofactors, or to cysteine vitamin B_6-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 B_1 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 B_{12} 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-

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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 B_{12} 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 B_{12} 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 B_{12}, folate, or vitamin B_6. Since food fortification of folate or vitamin B_{12} 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 B_{12} or folate concentrations were above average plus 2SD, 1,162.8 pmol/L for vitamin B_{12}, 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 B_{12} 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 m^2) was calculated as $194\times\text{creatinine}^{-1.094}\times\text{age}^{-0.287}$ for men and $194\times\text{creatinine}^{-1.094}\times\text{age}^{-0.287}\times 0.739$ for women (*20*).

Measurement of physical performance. As the lower limb muscle strength, quadriceps muscle strength was evaluated using Locomoscan™ (AlCare Co. Ltd., Tokyo, Japan) (*21*). Results of the right and left lower limb muscle strength (N) were averaged, divided by gravitational acceleration of 9.797302 (m/s^2) and each subject's body weight (kg), and expressed in kgf.

Handgrip strength was evaluated according to The Japan Fitness Test (http://www.mext.go.jp/component/a_menu/sports/detail/__icsFiles/afieldfile/2010/07/30/1295079_04.pdf). In brief, measurement was performed in an upright position using a hand dynamometer (T.K.K5401, Digital Grip Dynamometer, Takei Scientific Instruments, Niigata, Japan).

Gait speed (m/s) was calculated as walking distance (4 m) divided by the time in seconds between when the subjects began walking and when one of the participants' feet completely crossed the end line (*22*). These physical performance tests were carried out only in subjects for whom these tests were able to be done with safety. Therefore, of the 77 subjects, the measurements of lower limb muscle strength, handgrip strength, and gait speed were conducted in 66 (men 12, women 54), 76 (men 12, women 64), and 42 (men 9, women 33) subjects, respectively.

Measurement of anthropometric and body composition. Body weight and body composition were measured with bioelectrical impedance analysis (BIA) equipment (Karada Scan; Omron HealthCare, Co. Ltd., Kyoto, Japan). Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m) (*23*, *24*).

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 B_{12} 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 y on average with no gender difference. Their eGFR was almost the same as the reference value of 60 mL/min/1.73 m^2, and there was no gender difference (https://www.jsn.or.jp/guideline/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

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/m^2)	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 m^2)	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 B_{12} (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.
omparison 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.

Table 2. Correlation of Hcy with folate or vitamin B_{12}.

Correlation of Hcy with	r	p value
Folate	−0.435	<0.001
Vitamin B_{12}	−0.388	<0.001

The r value represents the Spearman's correlation coefficient.

their eighties, probably because most of these subjects were not able to live independently (*21*).

Correlation of blood Hcy, folate and vitamin B_{12} concentrations

Plasma Hcy concentration exhibited significant negative correlation with both serum folate (r=−0.435, p<0.001) and vitamin B_{12} (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 B_{12} 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 B_{12} concentrations.

Multiple regression analysis to assess determinants for physical performance in women

Then 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 B_{12} 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 B_{12} and physical performance in women

Subjects were categorized into four groups according to their serum folate and vitamin B_{12} concentrations: group 1 (both lower than median), group 2 (folate lower than, and vitamin B_{12} higher than median), group 3 (folate higher than, and vitamin B_{12} 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 3. Characteristics of the subjects according to tertiles of Hcy in women.

	n	Tertile 1	Tertile 2	Tertile 3	*p* value
Age (y)	65	81.7±6.6	83.6±6.7	87.0±7.4	0.053
Skeletal muscle mass (%)	60	20.5±1.9	21.4±2.8	20.4±2.1	0.312
Plasma Hcy (μmol/L)	65	9.5±1.2	12.8±0.9	18.7±3.2	<0.001[1]
Serum folate (nmol/L)	65	18.8±8.1	14.8±6.9	12.2±7.6	0.021
Serum vitamin B_{12} (pmol/L)	65	515.1±183.7	380.2±154.2	394.1±181.2	0.022
Lower limb muscle strength (kgf)	54	0.45±0.20	0.34±0.18	0.31±0.13	0.066
Handgrip strength (kg)	64	15.1±5.3	14.5±4.6	11.0±4.9	0.024[2]
Gait speed (m/s)	33	1.2±0.4	1.1±0.3	0.9±0.4	0.435

Data are expressed as mean±SD.
Comparison was carried out using one-way ANOVA followed by Tukey's multiple comparison test.
[1] Plasma Hcy concentration was analyzed by Kruskal-Wallis test.
[2] There was a significant difference between first and third tertile in handgrip strength ($p=0.027$) by post hoc analysis.

Table 4. Characteristics of the subjects according to tertiles of folate in women.

	n	Tertile 1	Tertile 2	Tertile 3	*p* value
Age (y)	65	87.2±5.8	83.3±7.4	82.1±7.2	0.064
Skeletal muscle mass (%)	60	21.1±2.4	20.3±2.3	21.2±2.3	0.394
Plasma Hcy (μmol/L)	65	15.8±4.6	12.5±3.0	12.5±4.4	0.017
Serum folate (nmol/L)	65	7.0±1.6	13.7±2.5	24.1±6.0	<0.001[1]
Serum vitamin B_{12} (pmol/L)	65	429.7±186.2	421.9±153.1	439.2±211.0	0.949
Lower limb muscle strength (kgf)	54	0.32±0.15	0.33±0.18	0.46±0.19	0.028[2]
Handgrip strength (kg)	64	10.8±4.9	13.6±4.1	16.2±5.3	0.003[3]
Gait speed (m/s)	33	1.1±0.3	1.3±0.5	1.0±0.3	0.187

Data are expressed as mean±SD.
Comparison was carried out using one-way ANOVA followed by Tukey's multiple comparison test.
[1] Serum folate concentration was analyzed by Kruskal-Wallis test.
[2] Post hoc test has shown lower limb muscle strength in third tertile was higher than in first ($p=0.061$) and second ($p=0.057$) tertile although not statistically significant.
[3] Post hoc test has shown a significant difference between first and third tertile in handgrip strength ($p=0.002$).

Table 5. Multiple regression analysis to assess determinants for lower limb muscle strength in women.

Variable	Lower limb muscle strength		Handgrip strength	
	Standardized coefficient β	*p*	Standardized coefficient β	*p*
Age (y)	−0.316	0.035	−0.404	0.002
Skeletal muscle mass (%)	0.033	0.801	0.181	0.093
Albumin (g/dL)	−0.116	0.404	0.110	0.343
Hcy (μmol/L)	0.002	0.991	−0.075	0.533
Folate (nmol/L)	0.327	0.024	0.247	0.037
Vitamin B_{12} (pmol/L)	0.066	0.626	−0.049	0.662

Contributing factors for lower limb muscle strength and handgrip strength were analyzed by multiple regression analysis. Adjusted R^2 and *p* value were 0.193 and 0.012, respectively, for limb muscle strength, and they were 0.376 and <0.001, respectively, for handgrip strength.

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

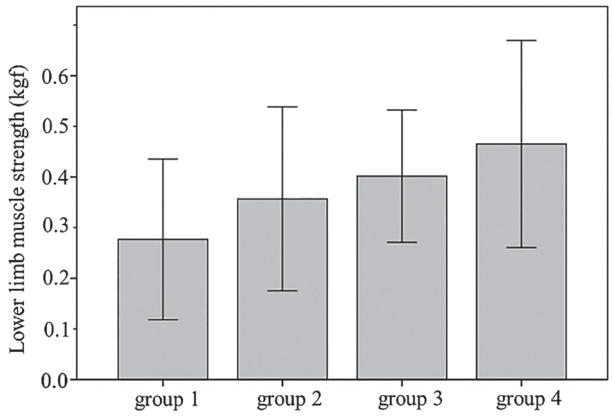


Fig. 1. Subjects were categorized into four groups according to their serum folate and vitamin B_{12} concentrations: group 1 (both lower than median), group 2 (folate lower than, and vitamin B_{12} higher than median), group 3 (folate higher than, vitamin B_{12} lower than median), group 4 (both higher than median). There was a significant difference by one-way ANOVA ($p=0.027$), and lower limb muscle strength was significantly different between group 1 and group 4 according to the post hoc test by Tukey's multiple comparison ($p=0.017$).

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 B_{12} 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 B_{12} and folate, but there was no significant association between folate, vitamin B_{12} 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 B_{12} 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 B_{12} 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 B_{12}, 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 B_{12} 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 B_{12} deficiency (serum vitamin B_{12} 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. Wee 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 B_{12} 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 B_{12} 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 homocysteinylation. 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 B_{12} 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 B_{12} and physical performance in our study does not preclude the involvement of vitamin B_{12}. Compared to subjects with their folate and vitamin B_{12} status both lower than median (folate, 13.6 nmol/L; vitamin B_{12}, 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 B_{12} also seems to have some role in the current population. Despite controversies concerning the mechanisms underlying the relationship among Hcy, folate and vitamin B_{12} 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 B_6 status, its serum concentration could not be measured. Since vitamin B_6 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 B_{12} or folate can be measured by immunoassay, and is easily available. However, serum vitamin B_6 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 methylmalonic acid (MMA). MMA has the advantage that it is influenced by vitamin B_{12} 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.

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