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ORIGINAL CONTRIBUTION

Cognitive impairment is associated with elevated serum homocysteine levels among older adults

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Abstract

Purpose The aim of this study was to examine the associations between the risk of cognitive impairment and the serum levels of folate, vitamin B_{12}, and homocysteine (Hcy).

Methods Subjects were persons aged 60–79 years who participated in the Yangpyeong Cohort study between 2011 and 2012. Cognitive impairment and normal subjects consisted of 100 pairs of old adults matched by age, sex, and education levels. Cognitive function was evaluated with the Korean version of the Mini-Mental State Examination for Dementia Screening (MMSE-DS). Pearson's partial correlation coefficients and conditional multiple logistic regression analysis were applied to determine the associations between cognitive function and the serum levels of folate, vitamin B_{12}, and Hcy.

Results Compared with the matched normal group, the cognitive impairment group had higher proportions of folate deficiency (< 3 ng/mL) and hyperhomocysteinemia (≥ 15 μmol/L). Serum Hcy concentrations were inversely associated with serum folate ($r = -0.234$, $p = 0.001$) and MMSE-DS score ($r = -0.150$, $p = 0.037$) after adjusting for age, sex, and education. The high Hcy group showed a higher prevalence of cognitive impairment (4th vs. 1st quartile, OR 3.30, 95% CI 1.12–9.72, p for trend = 0.014) after adjusting for exercise.

Conclusions The present findings suggest a putative protective role of high serum folate and normal Hcy against cognitive impairment among older adults.

Keywords Cognitive impairment · Folate · Vitamin B_{12} · Homocysteine

Introduction

Dementia not only destroys the quality of life for patients and their family, but also introduces a number of health and social problems [1]. In addition, patients with dementia incur higher medical expenditures per head than those with hypertension or diabetes [1]. South Korea has one of the most rapidly aging populations in the world [2]. According to a nationwide study in 2008, the prevalence of patients with dementia and mild cognitive impairment (MCI) aged ≥ 65 years was estimated to increase by 9.6 and 24.4% in 2012, and by 13.2 and 26.6% until 2050 [1]. Because the conversion rate of MCI to dementia is approximately 5–10% [3], early detection and therapy for MCI are important to reduce the population with dementia [4].

Low serum folate and vitamin B_{12} concentrations were reported to be connected with elevated homocysteine (Hcy) in many cross-sectional and longitudinal studies [2, 5, 6]. Several potential mechanisms, such as one-carbon metabolism playing a contributory role in methylation reactions,

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have been proposed to explain the connections among folate and vitamin B_{12} and Hcy [7]. Furthermore, hyperhomocyteinemia (HHcy) has been suggested as a risk factor for cognitive impairment [8]. More recently, it has been demonstrated that increased Hcy serves as a neurotoxin to promote neurodegeneration via apoptosis through DNA breakage [7]. Despite many studies, the relationships of cognitive function to serum levels of folate, vitamin B_{12}, and Hcy are inconclusive.

Thus, the aim of the present study was to examine the associations between cognitive impairment and the levels of serum folate, vitamin B_{12}, and Hcy concentrations in 100 pairs of adults aged 60–79 years matched by age, sex, and education levels.

Methods

Data source and study population

The Yangpyeong cohort was initiated in 2004 to determine the risk factors for cardiovascular disease and obtain genomic data in adults aged ≥ 40 years. A health interview and health examination survey obtained using a questionnaire, as well as anthropometry and blood samples of this cohort data between 2011 and 2012 from adults aged 60–79 years were used. Among 700 participants, we excluded 44 subjects who had not provided education information ($n = 5$) or MMSE-DS scores ($n = 35$) and those who had implausible energy intake (> 4000 or < 500 kcal, $n = 4$) [9]. 656 subjects were categorized into subjects with cognitive impairment ($n = 169$) or normal subjects ($n = 487$) using MMSE-DS score. Cognitive impairment and matched normal group consisted of 100 pairs from 656 subjects matched by age (year of birth, ± 2 years), sex (43 pairs of male, 57 pairs of female) and education level. The present study was approved by the Institutional Review Boards of Hanyang University and was performed in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

General characteristics, anthropometric data, and health behavior data

Data from the health interview and health examination survey were used to determine general characteristics, anthropometric data, health behavior data, and disease prevalence. The height (cm) and weight (kg) of the subjects were measured, and the body mass index (BMI) was calculated as weight (kg)/height (m^2). Regular exercise was defined as a weekly frequency of sweat-inducting activity. Medical history of diabetes mellitus, stroke, and hypertension was obtained from face to face interviews by a trained researcher. Supplement users were identified as persons who had taken vitamins (A, C, E, or D), folate, beta-carotene, multi-vitamins, minerals, or fatty acids supplements since the last checkup. Depressive symptoms were investigated using the Center for Epidemiologic Studies Depression (CES-D) scale. The CES-D consists of 20 total items with self-reporting of depression symptoms (0 point; "not at all", 1 point; "1–2 days/week", 2 points; "3–4 days/week", 3 points; "have experience of depression symptoms over 5 days/week"). The highest score in this scale is 60 points, and high scores indicate a high risk of depressive symptoms.

Cognitive functional examination

The Mini-Mental Status Examination (MMSE) is commonly used as a simple tool for screening cognitive impairment [10]. In Korea, the Korean MMSE (K-MMSE) or MMSE in the Korean version (MMSE-KC) is commonly used. The diagnostic accuracy of the Korean version of the Mini-Mental State Examination for Dementia Screening (MMSE-DS) is better than that of the K-MMSE and MMSE-KC [11]. Therefore, the current study employed MMSE-DS to evaluate the cognitive function of the subjects. The diagnostic accuracy of the MMSE-DS was proved using the area under the receiver-operating characteristic curve [11]. The reliability was confirmed by an inter-rater ($r = 0.968$, $p < 0.001$) and test–retest ($r = 0.825$, $p < 0.001$) method, and the validity was identified by the Clinical Dementia Rating ($r = -0.698$, $p < 0.05$) [12]. The MMSE-DS was administered by a trained researcher in a one-on-one manner. The MMSE-DS consists of a total of 19 questions including time orientation (5 questions, 5 points), place orientation (5 questions, 5 points), registration and recall (2 questions, 6 points), attention and calculation (1 question, 5 points), naming and repetition (2 questions, 3 points), three-stage command (1 question, 3 points), copying interlocking pentagons (1 question, 1 point), and judgment and abstract thinking (2 questions, 2 points). The highest score for this screening tool is 30 points, and lower scores show worse cognitive function. Subjects were divided into cognitive impairment and normal subjects according to sex, age, and education levels ('0–3', '4–6', '7–12', ≥ 13 years) using the normative table presented in the MMSE-DS. Cognitive impairment was classified using ≤ − 1.5 standard deviation (SD) of MMSE-DS mean score [13]. SD was calculated from the MMSE-DS mean of 1008 healthy volunteers aged ≥ 60 years that did not have dementia, MCI, physical health problems, or psychoneurotic disease [13].

Biochemical examination

Blood samples were collected from the participants, who did not eat for at least 12 h before the examination. The

coagulated blood was transferred to Eppendorf tubes and serum was extracted using a centrifugal separator. Serum was stored in the refrigerator at − 70 °C before analysis. Serum concentrations of folate, vitamin B_{12}, and Hcy were measured using ADVIA Centaur Folate, VB12, HCY reagent, and Centaur XP (Siemens Healthcare, Inc., Malvern, PA, USA). Coefficients of variation (CV) between and within experimenters were as follows (5.26–7.19% and 4.54–7.93% for folate; 2.7–9.2% and 2.4–5.0% for vitamin B_{12}; 1.5–5.2% and 2.3–4.4% for Hcy). Serum folate (< 3 ng/mL) [14], and vitamin B_{12} (< 80 pg/mL) [14] were considered as deficient, respectively. Levels of Hcy (≥ 15 µmol/L) were considered as hyperhomocysteinemia [15].

Statistical analysis

The general characteristics of the cognitive impairment and the matched normal group were analyzed using the paired *t* test for continuous variables and the McNemar's Chi-square test for categorical variables. To compare the prevalence of the deficiency of folate (< 3 ng/mL), vitamin B_{12} (< 80 g/mL), and HyperHcy (≥ 15 µmol/L) between cognitive impairment and the matched normal group, Fisher's exact test and McNemar's Chi-square test were performed. The general linear model and the Cochran–Armitage trend test were applied to determine characteristics across serum quartile levels of folate, vitamin B_{12}, and Hcy. The associations among serum folate, vitamin B_{12}, Hcy, and MMSE-DS score were assessed using the partial correlation coefficients after adjusting for age, sex, and education levels. Conditional multiple logistic regression analysis was applied to obtain the odds ratios (OR) and *p* for the trend in models. The first model was a crude model and no variables were adjusted. In the second model (adjusted model), the regular exercise variable was additionally adjusted. In the analyses of serum vitamin B_{12}, three pairs of subjects who had ≥ 10,000 pg/mL were excluded. A *p* value < 0.05 was considered significant. All statistical analyses were performed using SAS 9.3 statistical package (SAS Institute, Inc., Cary, NC, USA) (Fig. 1).

Results

The characteristics of the cognitive impairment and the matched normal group are described in Table 1. Both groups had equal proportions of age, sex, and education levels, because they were matched for these covariates. MMSE-DS scores (mean ± SD) were significantly lower among cognitive impairment (20.8 ± 3.6) as compared to matched normal group (26.0 ± 2.3). Compared with the matched normal subjects, the cognitive impairment had lower proportions of regular exercise (≥ 1 day/week).

Serum concentrations of folate (< 3 ng/mL), vitamin B_{12} (< 80 pg/mL), and Hcy (≥ 15 µmol/L) between the cognitive impairment and the matched normal group are compared in Table 2. The proportions of normal concentrations in folate and Hcy were lower in the cognitive impairment group than the matched normal group.

Table 3 shows the characteristics of matched normal subjects according to serum concentrations of folate, vitamin B_{12}, and homocysteine. The proportions of supplement users increased across the quartiles of serum folate concentrations. Current drinker decreased across the quartiles of serum vitamin B_{12} concentrations. The mean age increased with the quartiles of serum Hcy concentrations, but supplement users decreased across the quartiles of serum Hcy concentrations.

The relationships among serum folate, vitamin B_{12}, Hcy concentrations, and MMSE-DS scores are presented in Table 4. Serum Hcy concentration was inversely associated with serum folate ($r = -0.234$, $p = 0.001$), and MMSE-DS score ($r = -0.150$, $p = 0.037$) after adjusting for age, sex, and education levels.

Associations between the quartiles of serum folate, Hcy, and vitamin B_{12} and the prevalence of cognitive impairment are shown in Table 5. Serum folate concentrations were inversely associated with cognitive impairment in the crude model (4th vs. 1st quartile, OR 0.31, 95% CI 0.12–0.77, *p* for trend = 0.024). However, after additional adjustment for regular exercise, the inverse association between cognitive impairment and serum folate was no longer significant (4th vs. 1st quartile, OR 0.38, 95% CI 0.14–1.01, *p* for trend = 0.096). There was a decreasing trend between serum vitamin B_{12} concentrations and the risk of cognitive impairment, but the associations were not significant. Serum Hcy concentrations showed positive associations with the risk of cognitive impairment in two models.

Discussion

This study included 100 subjects with cognitive impairment and 100 matched normal subjects among older adults aged 60–79 years. The present study was conducted to examine the associations between the risk of cognitive impairment and the serum status of folate, vitamin B_{12}, and Hcy.

Subjects with cognitive impairment had 2 and 16% higher prevalence of deficiency in folate (< 3 ng/mL) and HyperHcy (≥ 15 µmol/L) than the matched normal subjects in the present study. These results were the same as the previous studies [16, 17]. Wahlin et al. reported the association of low levels of serum vitamin B_{12} and folic acid with episodic memory performance in persons aged 75–96 years [16]. The cognitive impairment group with low levels of B vitamins had lower memory performance than the matched normal group with normal levels of B vitamins.

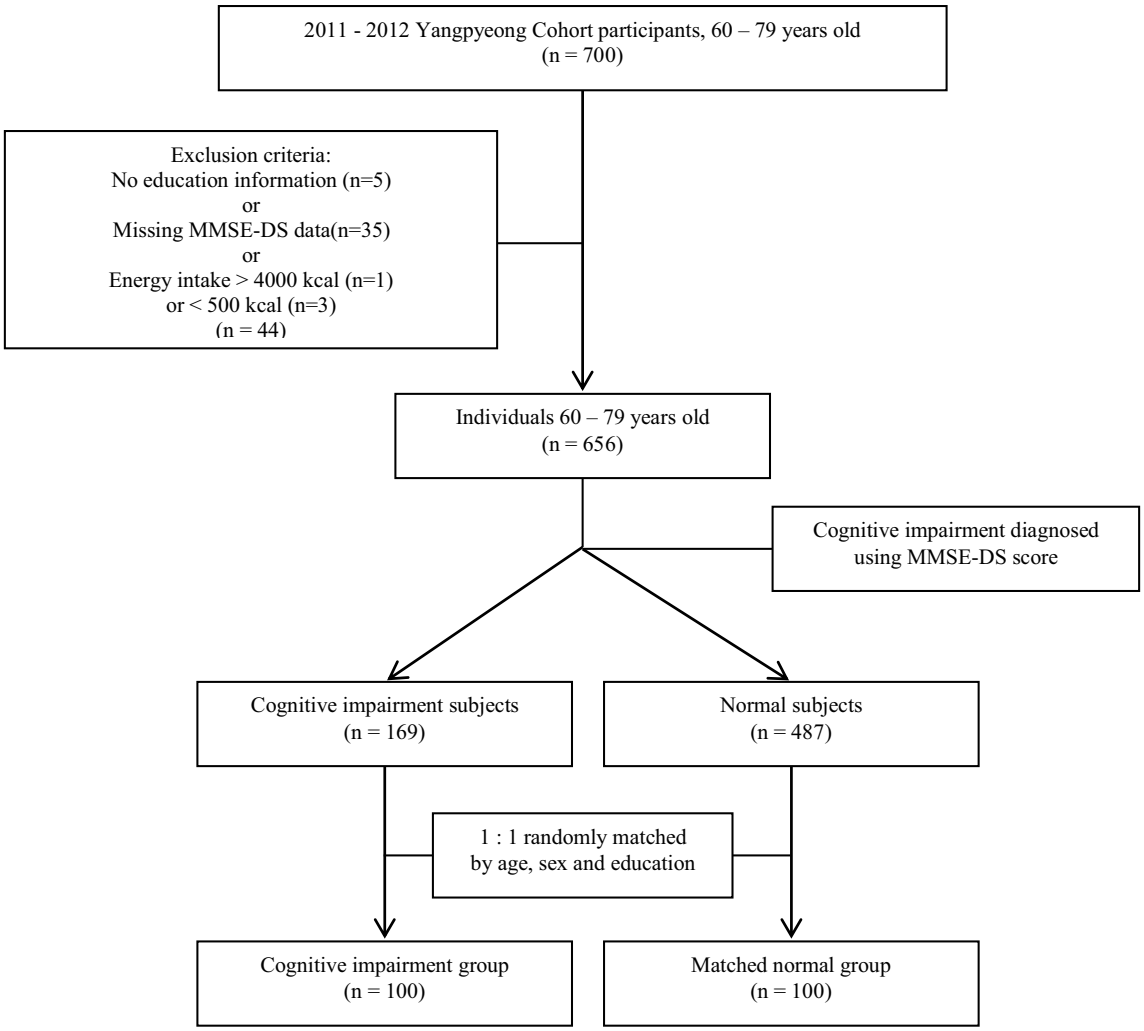

Fig. 1 Flow chart of the study

In the present study, decreased folate levels were associated with raised Hcy levels. Serum folate was inversely associated with cognitive impairment in the crude model, but this association was no longer significant after additionally adjusting for exercise. The association between folate and Hcy can be explained by one-carbon metabolism [7]. N^5-methyl tetrahydrofolate (THF), which is converted to folate in the body, plays a role as a methyl donor [18]. Methionine receives a methyl group from THF, changing into Hcy [18]. Hence, if serum folate concentrations are deficient, Hcy levels are higher [19]. This is similar to the results in the majority of existing studies [5, 6]. Furthermore, a meta-analysis with 25 randomized controlled trials (RCTs) reported a dose-dependent reduction in plasma Hcy concentrations with incremental doses of folate supplementation up to 0.8 mg/d [20]. In an RCT by Durga et al., 3-year folic acid supplementation decreased Hcy concentrations and improved domains of cognitive function [21].

This study found that elevated Hcy concentrations were associated with decreased cognitive score. Moreover, the prevalence of cognitive impairment was elevated across increasing quartiles of Hcy concentrations. The connection between HHcy and cognitive decline such as MCI and AD can be explained by the following potential mechanism. Elevated Hcy enters intracellular sites through a membrane transporter [22]. In the brain, HHcy may promote hypomethylation apoptosis and DNA breakage caused by impaired DNA transmethylation [7, 8, 23]. As a result, HHcy may be connected with cognitive decline [8]. Several studies demonstrated that HHcy was related to cognitive decline or cognitive impairment prevalence [6, 24–28]. However, some studies are inconsistent with our results [26, 29]. The baseline data in other studies revealed associations similar to our results, but the follow-up results were inconclusive [30, 31]. In prospective studies, the findings suggest that high Hcy levels at the

Table 1 General characteristics of the study subjects

Characteristic	Cognitive impairment group	Matched normal group	p^a
n	100	100	
MMSE-DS score	20.8 ± 3.6	26.0 ± 2.3	< 0.0001
Age (year)	68.8 ± 5.2	68.8 ± 5.3	0.914
Sex			–
Male	43 (43%)	43 (43%)	
Female	57 (57%)	57 (57%)	
Education level			–
Uneducated	15 (15%)	15 (15%)	
Elementary school	53 (53%)	53 (53%)	
Middle school	12 (12%)	12 (12%)	
High school	14 (14%)	14 (14%)	
College or higher	6 (6%)	6 (6%)	
Height (cm)	155.6 ± 8.7	157.2 ± 8.3	0.052
Weight (kg)	58.6 ± 9.7	60.9 ± 9.6	0.050
BMI (kg/m^2)	24.2 ± 3.2	24.6 ± 3.4	0.283
Alcohol consumption			0.752
Non-drinker/former drinker	58 (58%)	60 (60%)	
Current drinker	42 (42%)	40 (40%)	
Smoking status			0.564
Non-smoker/former smoker	99 (99%)	98 (98%)	
Current smoker	1 (1%)	2 (2%)	
Exercise (≥ 1/week)			< 0.0001
Yes	19 (19%)	45 (45%)	
No	81 (81%)	55 (55%)	
Diabetes mellitus			0.317
Yes	3 (3%)	6 (6%)	
Stroke			0.564
Yes	2 (2%)	1 (1%)	
Hypertension			0.847
Yes	16 (16%)	17 (17%)	
Depressive symptom[b]	13.7 ± 7.3	13.8 ± 6.8	0.779
Supplement use			0.423
Yes	31 (31%)	36 (36%)	
Energy intake (kcal/day)	1395.8 ± 376.8	1461.9 ± 442.3	0.255

Mean ± standard deviation and frequency (%) are reported

Mini-Mental State Examination for Dementia Screening (MMSE-DS) score; body mass index (BMI)

[a]Paired *t* test for continuous variables and McNemar's Chi-square test for categorical variables

[b]CES-D scale (Center for Epidemiologic Studies Depression scale) score

baseline may predict cognitive decline in 5-year follow-up [32]. On the other hand, other longitudinal study reported that red blood cell folate and plasma Hcy were related to better global cognition at the baseline, but they were not associated with the rate of decline over 5 years [31]. In an intervention study, supplement containing folate, vitamin B_6, and vitamin B_{12} during 14 weeks significantly decreased serum total Hcy and improved cognitive function in middle-aged and elderly patients with HyperHcy [33]. However, in a meta-analysis with 11 trials, Hcy lowering using B vitamins did not slow the rate of cognitive aging [34].

However, serum vitamin B_{12} concentrations were not connected with serum Hcy in the present study. Vitamin B_{12} performs the role of a cofactor in one-carbon metabolism [18]. Thus, if serum vitamin B_{12} concentrations were deficient, serum Hcy concentrations may be increased [19]. However, unlike this mechanism, the results of many

Table 2 Serum concentrations of folate, vitamin B_{12}, and homocysteine between cognitive impairment and matched normal group

	Cognitive impairment group	Matched normal group	*p*
n	100	100	
Serum folate (ng/mL)	8.9 ± 5.3	9.6 ± 4.1	0.267[a]
Serum vitamin B_{12}[b] (pg/mL)	520.7 ± 284.6	741.5 ± 1088.0	0.055[a]
Serum homocysteine (μmol/L)	15.2 ± 6.6	13.0 ± 4.3	0.003[a]
Serum folate (ng/mL)			0.038[c]
Normal (≥ 5.6)	66 (66%)	83 (83%)	
Indeterminate (≥ 3, < 5.6)	32 (32%)	17 (17%)	
Deficient (< 3)	2 (2%)	0 (0%)	
Serum vitamin B_{12}[b] (pg/mL)			0.099[c]
Normal (≥ 80)	97 (96.9%)	97 (100%)	
Deficient (< 80)	3 (3.1%)	0 (0%)	
Serum homocysteine (μmol/L)			0.009[d]
Normal (< 15)	62 (62%)	78 (78%)	
Abnormal (≥ 15)	38 (38%)	22 (22%)	

Mean ± SD and frequency (%) are reported

[a]*p* Value from paired *t* test

[b]Removed three pairs of outlier who had ≥ 10,000 pg/mL of serum vitamin B_{12}

[c]*p* Value from Fisher's exact test

[d]*p* Value from McNemar's Chi-square test

previous investigations like our study also suggested that there was no association between vitamin B_{12} and Hcy levels [35]. In addition, serum vitamin B_{12} concentration was not associated with cognitive score in our results. No definite associations were made with vitamin B_{12}, but there are several speculations. The first reason is the small sample size in the present study. Second, our study did not use newer biomarkers such as holotranscobalamin (holoTC) and methylmalonic acid (MMA) [35]. Systematic review of O'Leary et al. revealed that future studies should use holoTC and/or MMA to establish a clear link between vitamin B_{12} and cognitive decline [35].

In this study, there was an interesting finding about exercise. Matched normal subjects had higher proportions of regular exercise than cognitive impairment subjects. Moreover, the relationship between cognitive impairment and levels of serum folate vanished in the model after adjusting for regular exercise. The existing studies suggested that exercise reduces the risk of cognitive impairment by attenuating neurodegenerative processes and loss of neuropil and synaptic connections [36–38]. These biologic mechanisms may be related to the rise of neurotropic factors such as brain-derived neurotrophic factor, vascular endothelial growth factor, and insulin-like growth factor 1 about exercise [39–48]. In meta-analyses studies of 29 RCTs over 1–12 months, exercise improved cognitive scores in healthy adults without dementia [49]. In addition, exercise in older adults was associated with increased brain volumes after 6-month RCTs compared with sedentary interventions [50].

Our study has the following limitations. First, it is difficult to apply the results to a general population of older adults, because the majority of subjects in the Yangpyeong cohort were farmers and housewives. Second, education level, which is widely known to be associated with cognitive function, differs between the Yangpyeong subjects and the general public aged 60–79 years [51]. Third, the number of subjects was small to detect small-to-medium effects. Fourth, since blood samples and MMSE-DS scores were collected at the same time, we cannot conclude causality of the serum folate, vitamin B_{12}, and Hcy with cognitive function.

Nevertheless, this study had several strengths. First, the accuracy of the study was enhanced by matching the subjects according to age, sex, and education levels, which are reported to be associated with cognitive function [13]. Second, selection bias was reduced, because cognitive impairment subjects were selected with normal subjects from the same region.

Considering the findings above, lower concentrations of serum folate correlate with higher concentrations of serum Hcy, and higher serum Hcy increases the prevalence of cognitive impairment in those 60 years or older living in rural areas. Although this study is limited, because the Yangpyeong cohort resided in rural areas, the findings suggest a putative protective role of high serum folate and normal Hcy against cognitive impairment among older adults. Further studies are necessary to examine these findings in prospective and intervention studies.

Table 3 Characteristics according to quartile groups for serum concentrations of folate, vitamin B_{12}, and homocysteine in matched normal subjects

Characteristic	Serum folate (ng/mL)			Serum vitamin B_{12}[a] (pg/mL)			Serum homocysteine (μmol/L)		
	Q_1	Q_4	p_{trend}[b]	Q_1	Q_4	p_{trend}	Q_1	Q_4	p_{trend}
n	25	25		23	24		25	25	
Related cognitive impairment									
Age (year)	69.4 ± 5.7	68.6 ± 5.8	0.794	71.7 ± 5.2	68.9 ± 5.1	0.363	66.6 ± 4.5	71.6 ± 5.4	0.0002
Sex			0.046			0.656			0.011
Male	13 (52%)	8 (32%)		6 (26%)	8 (33%)		7 (28%)	16 (64%)	
Female	12 (48%)	17 (68%)		17 (74%)	16 (67%)		18 (72%)	9 (36%)	
Education level			–			–			–
Uneducated	4 (16%)	3 (12%)		10 (43%)	2 (8%)		3 (12%)	4 (16%)	
Elementary school	12 (48%)	14 (56%)		9 (39%)	17 (71%)		13 (52%)	13 (52%)	
Middle school	3 (12%)	4 (16%)		1 (4%)	3 (13%)		4 (16%)	2 (8%)	
High school	5 (20%)	3 (12%)		2 (9%)	2 (8%)		5 (20%)	4 (16%)	
College or higher	1 (4%)	1 (4%)		1 (4%)	0		0	2 (8%)	
Alcohol consumption			0.361			0.029			1.000
Non-drinker/former drinker	18 (72%)	15 (60%)		13 (57%)	19 (79%)		15 (60%)	16 (64%)	
Current drinker	7 (28%)	10 (40%)		10 (43%)	5 (21%)		10 (40%)	9 (36%)	
Smoking status			0.523			0.984			0.201
Non-smoker/former smoker	25 (100%)	25 (100%)		23 (100%)	24 (100%)		24 (96%)	25 (100%)	
Current smoker	0	0		0	0		1 (4%)	0	
Exercise (≥ 1/week)			0.323			0.387			0.088
Yes	12 (48%)	14 (56%)		10 (43%)	6 (25%)		14 (56%)	8 (32%)	
No	13 (52%)	11 (44%)		13 (57%)	18 (75%)		11 (44%)	17 (68%)	
Diabetes mellitus			0.132			0.554			0.707
Yes	0	2 (8%)		1 (4%)	2 (8%)		3 (12%)	2 (8%)	
Stroke			0.653			0.659			0.178
Yes	0	0		0	0		0	1 (4%)	
Hypertension			0.905			0.421			0.552
Yes	3 (12%)	2 (8%)		3 (13%)	3 (13%)		4 (16%)	5 (20%)	
Depressive symptoms[c]	11.7 ± 3.6	13.6 ± 6.3	0.458	13.8 ± 5.7	16.3 ± 9.1	0.164	12.6 ± 4.3	14.7 ± 6.9	0.328
Related B vitamins									
Supplement use			0.015			0.571			0.015
Yes	5 (20%)	13 (52%)		6 (26%)	9 (38%)		13 (52%)	4 (16%)	
Energy intake (kcal/day)	1549.7 ± 527.6	1392.5 ± 502.6	0.478	1463.1 ± 525.6	1385.3 ± 440.4	0.345	1345.2 ± 438.4	1442.6 ± 432.4	0.456

Mean ± SD and frequency (%) are reported

Quartile, Q_1 (the lowest) and Q_4 (the highest); body mass index (BMI)

[a]Removed three pairs of outlier who had ≥ 10,000 pg/mL of serum vitamin B_{12}

[b]*p* Values for linear trend. General linear model for continuous variables and Cochran–Armitage trend test for categorical variables

[c]CES-D scale (Center for Epidemiologic Studies Depression scale) score

Table 4 Partial correlation coefficients among serum folate, vitamin B_{12}, homocysteine, and MMSE-DS score

	Serum folate (ng/mL)	Serum vitamin B_{12} (pg/mL)	Serum homocysteine (μmol/L)	MMSE-DS score
	r^a (p)	r (p)	r (p)	r (p)
n	200	194[b]	200	200
Serum folate (ng/mL)	–	−0.085 (0.248)	−0.234 (0.001)	0.104 (0.148)
Serum vitamin B_{12} (pg/mL)	−0.085 (0.248)	–	−0.052 (0.478)	0.035 (0.633)
Serum homocysteine (umol/L)	−0.234 (0.001)	−0.052 (0.478)	–	−0.150 (0.037)
MMSE-DS score	0.104 (0.148)	0.035 (0.633)	−0.150 (0.037)	–

MMSE-DS (Mini-Mental State Examination for Dementia Screening) score

[a]r from partial correlation test after adjusting for age, sex, and education levels

[b]Removed three pairs of outlier who had ≥ 10,000 pg/mL of serum vitamin B_{12}

Table 5 Odds ratio (OR) and 95% confidence interval (95% CI) for the prevalence of cognitive impairment according to quartiles of serum folate, vitamin $B_{12,}$ and homocysteine concentrations

	Cognitive impairment subjects	Matched normal subjects	Prevalence of cognitive impairment			
			Crude model		Adjusted model[b]	
	n	n	OR (95% CI)	p_{trend}[a]	OR (95% CI)	p_{trend}
Serum folate (ng/mL)				0.024		0.096
Q_1	33	17	1.00 (referent)		1.00 (referent)	
Q_2	23	27	0.45 (0.19–1.08)		0.47 (0.18–1.22)	
Q_3	23	27	0.37 (0.14–0.93)		0.38 (0.14–1.03)	
Q_4	21	29	0.31 (0.12–0.77)		0.38 (0.14–1.01)	
Serum vitamin B_{12}[c] (pg/mL)				0.111		0.214
Q_1	28	20	1.00 (referent)		1.00 (referent)	
Q_2	24	24	0.68 (0.28–1.62)		0.76 (0.30–1.97)	
Q_3	26	24	0.69 (0.28–1.67)		0.94 (0.35–2.53)	
Q_4	19	29	0.48 (0.20–1.14)		0.56 (0.22–1.40)	
Serum homocysteine (μmol/L)				0.002		0.014
Q_1	22	28	1.00 (referent)		1.00 (referent)	
Q_2	18	32	0.49 (0.20–1.23)		0.37 (0.14–1.01)	
Q_3	25	25	1.36 (0.58–3.19)		1.06 (0.41–2.70)	
Q_4	35	15	4.43 (1.59–12.33)		3.30 (1.12–9.72)	

Quartile; Q_1 (the lowest) and Q_4 (the highest)

[a]p Values for linear trend. Conditional logistic regression analysis

[b]Adjusted model was adjusted for exercise by conditional multiple logistic regression analysis

[c]Removed three pairs of outlier who had ≥ 10,000 pg/mL of serum vitamin B_{12}

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Compliance with ethical standards

Conflict of interest The corresponding authors declare no conflict of interest.

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