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Research Article

Effects of 6-Month Folic Acid Supplementation on Cognitive Function and Blood Biomarkers in Mild Cognitive Impairment: A Randomized Controlled Trial in China

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Abstract

Background. This study is to examine the effects of folic acid supplementation on cognitive function in Chinese older adults with mild cognitive impairment who are unexposed to folic acid fortification and assess cognitive functioning in relation to folate, homocysteine, and vitamin B_{12} values at baseline.

Methods. This was a single-center, randomized, controlled trial in Tianjin, China; 180 individuals aged 65 years and older who had mild cognitive impairment were assigned randomly to one of two groups: (a) those treated with oral folic acid (400 μg/day) and (b) those treated via conventional treatment. Tests of cognitive performance and biomarkers were measured at baseline, 3 months, and 6 months. Analysis was by intention-to-treat. Changes in cognitive or clinical function were analyzed by repeated-measure analysis of variance or mixed-effects models. This trial has been registered with the trial number ChiCTR-TRC-13003227.

Results. Total of 159 participants (intervention group: 80; control group: 79) completed the trial. Repeated-measure analysis of variance showed significant improvements in serum folate ($\eta p^2 = 0.712$, $p = .009$), homocysteine ($\eta p^2 = 0.119$, $p = .017$), serum vitamin B_{12} ($\eta p^2 = 0.144$, $p = .022$), and *S*-adenosylmethionine ($\eta p^2 = 0.117$, $p = .033$) in the intervention group over the control group. Folic acid supplementation improved Full Scale IQ ($p = .031$; effect size $d = 0.168$), Digit Span ($p = .009$; $d = 0.176$), and Block Design ($p = .036$; effect size $d = 0.146$) scores at 6 months in comparison to the control. There were no significant findings for all other cognitive measures.

Conclusion. There was a beneficial effect from relatively short-term folate supplementation on cognitive functioning in later life. Larger-scale, randomized, controlled trials of longer duration in selected age groups are needed.

Key Words: Mild cognitive impairment—Folic acid—Homocysteine—Cognitive function—Randomized controlled trial

Due to the worldwide rapid increase in life expectancy, mild cognitive impairment (MCI) and dementia are among the major public health concerns. Approximately 60% of dementia is due to Alzheimer's disease (AD), which is characterized by progressive cognitive impairment that significantly interferes with activities of daily leaving and behavioral changes (1). Currently, there is no cure for AD, but previously many researchers explored those factors and biochemical markers that are associated with late-life dementia in order to find effective preventive strategies for cognitive impairment and dementia (2).

MCI has been proposed as a possible transitional phase between normal aging and AD, characterized by global cognitive dysfunction and deficits across multiple domains (episodic memory, executive functioning, verbal abilities, visuospatial skill, attention, and perceptual speed), but with largely intact activities of daily living and absence of dementia (3). Elderly people with MCI have a 10%–15% risk of developing AD compared with the 1%–2% risk among non-MCI (4). These individuals are becoming the focus of many prediction studies and early intervention trials. The etiology of MCI and dementia is very complex and is based upon the interplay of genetic and environmental factors. A growing body of epidemiological evidence has suggested that nutrition components may be important in the development of cognitive decline.

B-vitamin status plays an essential role in synthesis reactions for neurotransmitters and structural elements of neurons, and its deficiency has been associated with mental dysfunction such as cognitive impairment and depression (5,6). Low folate concentrations (≤11.8 nmol/L) have been linked to nearly a 90% greater dementia risk than have normal concentrations (7). Decreased B vitamin folate and increased plasma concentrations for the sulfur-containing amino acid homocysteine—two common abnormalities in older individuals—have been associated with poor cognitive performance and dementia syndromes (8). The effects of folate supplementation has been studied as a tool for improving these disorders, with conflicting results (9,10). Methodological issues that hinder comparisons among the randomized controlled trial include the heterogeneity of the study samples, supplement dosage, duration of treatment, and outcome assessment (11–14). Folic acid fortification programs in western countries may mask the relationship between folate status and cognitive decline, and previous findings are limited and inconclusive. The level of folate intake is usually 30%–40% lower than the recommended dietary allowance in China (15) due to a lack of folic acid fortification program and traditional cooking methods that may cause food folate loss in vegetables.

The objectives of the present study were to describe the effects of folic acid supplementation on global cognition, and to assess cognitive abilities in relation to B vitamin status, folate, homocysteine, and vitamin B_{12} values at baseline in a sample of community-dwelling older adults with MCI who were unexposed to mandatory or voluntary fortification of food items with folic acid.

Methods

Study Population

This is a single-center, randomized, controlled trial with an intent-to-treat analysis that seeks to investigate the effects of 6-month folic acid supplementation intervention on cognition and plasma biomarkers in older adults with MCI. Participants were enrolled between March 2013 and April 2013, on the basis of the following criteria: (a) aged 65+; (b) absence of terminal illness or mental disorders (ie, major depression, schizophrenia, bipolar disorder, etc); (c) not using any nutritional supplementation known to interfere with nutrition status, folate metabolism, or cognitive function in the 3 months before recruitment; and (d) not living in a nursing home or being on a waiting list for a nursing home. By random cluster sampling, six geographically convenient communities with a high proportion of older residents who were all native Chinese speakers were selected from the Binhai New District, Tianjin, China. Of the 4,215 selected individuals, 2,816 (66.8%) agreed to participate, but only 2,317 who met the inclusion criteria participated in clinical, physical, and neuropsychological examinations.

Using previously determined criteria for MCI, 210 subjects with MCI were selected (Figure 1); 97% of participants in both the intervention and control groups were living in the community at recruitment and all were considered by their family doctor to be suitable for the study. Of the 210 MCI individuals screened, 180 met the study criteria and were assigned randomly into folic acid supplementation or control groups. Cognitive functioning was assessed at baseline and follow-ups at the 3rd and 6th months.

This study fulfils the principles of the Declaration of Helsinki and was approved by the ethics committee at Tianjin Medical University, China. Written informed consent was obtained from all participants. This trial has been registered with trial number ChiCTR-TRC-13003227.

Data Collection

Non-dietary variables were collected at baseline for each participant. The interview included the following information: age (in years), sex, race (black or white), education (in years), marital status, occupation, cigarette smoking (ever or never), smoking pack/years, number of depressive symptoms (16), transient ischemic attack/stroke, heart disease (self-reported history of myocardial infarction, atrial fibrillation, digitalis use, or angina pectoris) (17), hypertension (self-reported history, measured blood pressure ≥160 mm Hg systolic or ≥95 mm Hg diastolic, or use of antihypertensive medications) (18), history of stroke (self-report), and diabetes mellitus (self-report or antidiabetic medication use).

The primary outcome in the current study was IQ and index scores, which is an indicator of cognitive function. Cognitive functioning was assessed by the Chinese version of the Wechsler Adult Intelligence Scale-Revised (*WAIS-RC*) (19). The WAIS-RC includes 11 subtests: Information, Similarities, Vocabulary, Comprehension, Arithmetic, Digit Span, Block Design, Picture Completion, Digit Symbol-Coding, Object Assembly, and Picture Arrangement. Cognitive data were collected at baseline and follow-ups by trained physicians. The same version of each test was use at each time of measurement. WAIS subtests were administered in a random order at baseline and each follow-up and took about 3 hours to be completed. We used age-appropriate norms from the Chinese standardization to calculate IQ and index scores (19). The Mini-Mental State Examination was administered at each participant as measure of general cognitive function.

Definition of Mild Cognitive Impairment

MCI was identified according with the modified Petersen´s criteria (20):

(1) Subjective memory complaint, with at least 2-week duration, was reported by the participant and corroborated by an informant (family or physician).
(2) Objective memory impairment for age and education has been defined by performing at least 1.5 *SD* below age and education-

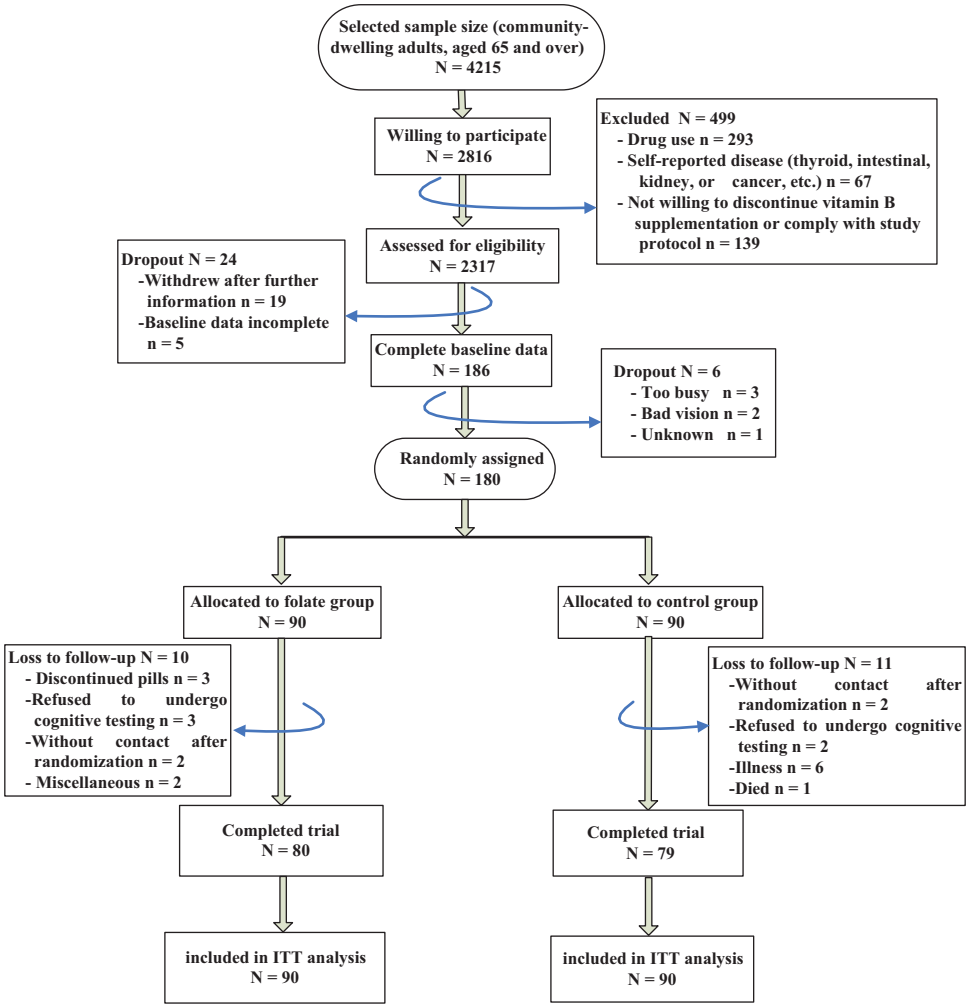

Figure 1. Flow diagram for enrolment, randomization, and follow-up in the trial.

matched controls on Mini-Mental State Examination memory subtask (21).

(3) Normal general cognitive function impairment was defined as a test performance more than 1.5 *SD* below age- and education-specific norms.

(4) Essentially preserved activities of daily living, as measured by Activities of Daily Living scale (ADL), that is, a score <26 (22).

(5) Absence of dementia (*Diagnostic and Statistical Manual of Mental Disorders-IV*), AD (*the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association*), or psychiatric disorders, cerebral damages, or physical diseases that may account for cognitive impairment, and any active neuropsychiatric condition producing disability.

Intervention Implementation

All MCI subjects at baseline live in the same community. They have ever received nonpharmacological interventions for preventing, reducing, or postponing cognitive decline and dietary recommendations based on a booklet (Guides to Enhance Elderly Memory) in late life. In our study, participants randomized to folic acid supplementation received one pill daily. Folic acid tablets were formulated as a daily oral dose of one tablet consisting of 400 μg folic acid (Beijing Scrianen Pharmaceutical Co. Ltd, China; 400 μg/tablet; state medical permit No.: H10970079) for the entire 6-month period. These folic acid supplementations are available on prescription in China. The control group only received conventional treatment and were identical except for the omission of the active substances under investigation. Conventional treatment was above-mentioned. Participants were instructed to supplement with one tablet daily, during, or immediately after a meal. Adherence was encouraged and monitored throughout the trial by telephone assessment at 15 time points, and by blood assay at baseline and at the 3rd- and 6th-month assessments for both groups.

Blood Sampling and Analytical Methods

Blood samples were collected at baseline, 3 months, and 6 months by venipuncture after a 10- to 12-hour overnight fast. Venous blood at 2–3 mL was extracted from the patient on an empty stomach in the morning. Blood samples were centrifuged at 3,000 rpm for 10 minutes immediately after collection. The concentrations of serum homocysteine, *S*-adenosyl-methionine (SAM), and *S*-adenosyl homocysteine (SAH) were determined by a Hitachi 7180 automatic biochemistry analyzer (Japan), using the enzymatic conversion method. The kit was supplied by Beijing Strong Biotechnologies, Inc. (China). Meanwhile, the concentrations of folic acid and vitamin B_{12} were

determined on the same day using the AbbottArchitect-i2000SR automated chemiluminescence immunoassay system and its supporting kit (Abbott, USA).

Statistical Analysis

Statistical differences were examined with chi-square tests or Fisher's exact test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables, with post hoc comparison using the Bonferroni test for multiple comparisons. The methods of repeated-measures ANOVA and mixed-model repeated-measures ANOVA were used for the primary analysis. Repeated-measures ANOVA was used to evaluate the effects of folic acid and control interventions on biomarker and cognitive performance over 6 months. Data are mean unadjusted scores plus standard deviations, with ηp^2 and p value from repeated-measures ANOVA that included the time-treatment interaction. A mixed-model repeated-measures ANOVA was used to evaluate hypotheses concerning differential change between folic acid and the control. Multiple comparisons were not adjusted for when examining the WAIS subscales and total score because hypotheses were determined a priori. Within-person variation was modeled by using an unstructured covariance matrix; *df* were estimated by using Satterthwaite's approximation. Models were developed for each of the cognitive outcome variables. The critical test of the effectiveness was the presence of an effect of the folic acid supplementation relative to the control over time—that is, showing that folic acid supplementation improved cognitive functioning over time. Mixed effect models yield an intention-to-treat analysis by using all available measurement points for each participant under the assumption that withdrawal data are missing at random. All analyses were performed with the intent-to-treat principle, and subjects lost to follow-up were censored at the time clinical data became no longer available. All analyses were performed using SPSS PASW Statistics for Windows, version 18.0 (SPSS Inc., Released 2009, Chicago).

Results

Characteristics of Participants

Of the 180 participants, 80 participants (88.9%) were assigned to the intervention group and 79 (87.8%) to the control group. Dropout rates were similar between the two groups: 11 (12.2%) dropout in control and 10 (11.1%) in intervention ($\chi^2 = 0.0539$, $p = .421$).

A summary of characteristics of participants before the implementation of intervention are shown in Table 1. The socio-demographics, lifestyle, diabetes, and hypertension were not significantly different between the two groups. Furthermore, the intervention and control groups were similar in the levels of folate, vitamin B_{12}, homocysteine, SAM, and SAH ($p > .05$). Therefore, these biochemical variables were used as covariates in repeated-measures analysis of covariance.

Biomarkers

Repeated-measures ANOVA showed that, over 6 months, serum folate showed substantial percentage increases in both groups ($p = .012$, $\eta p^2 = 0.125$). Participants in intervention group had a greater increase in serum folate level (+27.96%) compared with the controls (+3.11%). Serum SAM also increased in both groups at month 6, it was greater in the intervention group (+28.32%) than in the control group (+18.16%). There was an interaction between time effect and group effect ($p = .022$, $\eta p^2 = 0.144$). Serum vitamin B_{12} showed substantial percentage increases in both groups ($p = .019$, $\eta p^2 = 0.120$), and was greater in the intervention group (+19.70%) compared

Table 1. Baseline Characteristics of the Study Population ($n = 180$)

Profile	Folic Acid Group ($n = 90$)	Control Group ($n = 90$)	p Value*
Demography			
Age at screening (y)	74.82 ± 2.75	74.63 ± 3.21	.81
Female	51 (63.75)	52 (65.82)	.88
Total education (y)	9.21 ± 3.57	9.93 ± 2.72	.16
Lifestyle			
Smoker—ever	47 (58.75)	49 (62.03)	.27
Alcohol (units/wk)	9.12 ± 9.23	7.12 ± 8.52	.11
Systolic BP (mm Hg)	147 ± 23	146 ± 20	.77
Diastolic BP (mm Hg)	80 ± 11	80 ± 11	.92
BMI (kg/m^2)	25.72 ± 3.63	26.12 ± 4.05	.40
Daily dietary intake of folic acid (μg/day)	76.31 ± 4.59	75.29 ± 3.77	.44
Medical history			
Diabetes—ever	5 (6.25)	4 (5.06)	.11
TIA/stroke	8 (10.00)	7 (8.86)	.07
Myocardial infarction	3 (3.75)	4 (5.06)	.15
Atrial fibrillation	6 (7.50)	5 (6.33)	.33
Hypertension	7 (8.75)	6 (7.59)	.66
Biochemical measures			
Folate (ng/mL)	7.01 ± 3.64	5.79 ± 2.67	.15
Homocysteine (mmol/L)	13.65 ± 4.82	12.19 ± 3.85	.74
Vitamin B_{12} (pg/mL)	571.25 ± 256.94	538.82 ± 284.06	.09
folate insufficiency†	9 (10.0.)	10 (11.11)	.08

Notes: Results are shown as n (%) for chi-square or Fisher´s exact test, and mean ± *SD* for independent t test (two-tailed). BMI = body mass index; TIA = transient ischemic attack.

*$p < .05$ significant difference between groups.

†Based on WHO definitions of folate sufficiency (<4 ng/mL).

with the control group (+18.30%); the difference in change of serum vitamin B_{12} was significant ($p < .05$). Serum homocysteine showed a greater decrease in the intervention group −30.48%) compared with the control group (−10.00%) ($p = .048$, $\eta p^2 = 0.096$). However, there were no significant differences in SAH and SAM/SAH (Table 2).

Cognitive Status

Analysis using repeated-measures analysis of covariance revealed over 6 months few significant interaction effects in all neuropsychological tests except for Full Scale IQ, Digit Span, and Block Design (Table 3). The intervention group showed improved Full Scale IQ, Digit Span, and Block Design. Regarding Full Scale IQ, the intervention group showed a marginally significant improvement over the 6-month score compared with the control group ($p = .048$, $\eta p^2 = 0.096$). Digit Span showed marked increments in both groups at month 6 (+40.78% in the intervention group; +9.92% in the control group). Block Design increased in both groups at month 6 and was greater in the intervention group (+35.93%) than in the control (+14.10%).

In addition to analyzing the data for the Full Scale IQ score, separate mixed-model repeated-measures ANOVAs were used for each WAIS-RC subscale to determine whether there were particular subscales that may further explain in which aspect of cognitive functioning any significant effect may have occurred. Findings for all other cognitive measures were not significant ($p > .05$). Table 4 shows the estimates from the final models for Full Scale IQ score, Digit Span, and Block Design with all of the models taking into account baseline biomarker concentrations. Compared with control group, from baseline to month 6, folic acid group had statistically significant increase in the Full Scale IQ score ($p = .031$, effect size = 0.168); increased performance in Digit Span ($p = .009$, effect size = 0.176); and increased performance in Block Design ($p = .036$, effect size = 0.146). In addition to the intervention effects, baseline homocysteine concentrations had an important association with cognitive performance over time. Elevated homocysteine concentrations at baseline were associated with poorer cognitive performance at 6 months for Full Scale IQ score (estimate value = −0.113, $p = .013$), Digit Span (estimate value = −0.059, $p = .001$), and Block Design (estimate value = −0.079, $p = .000$).

Discussion

The present study aimed to evaluate the impact of folic acid supplementation on cognitive performance in Chinese older people with MCI who are unexposed to folic acid fortification and assess cognitive performance in relation to B vitamin status, folate, homocysteine, and vitamin B_{12} values at baseline. Oral folic acid supplementation (400 μg/day) was associated with improvements in general intellectual function (Full Scale IQ score) and better performance in digit Span and Block Design task, after 6-month intervention.

To date, several randomized controlled trials have evaluated the effect of folic acid as a treatment of cognitive impairment or dementia (23–30). However, there is still no consensus as to its direct impact on dementia, though many studies found an inverse association while others found no association. Our findings are incongruent with some of the existing evidence that found no effect for folic acid supplementation on cognitive performances (13,23,26,29). An important factor for interpreting the results of similar trials is the public policy on folic fortification in a given country. An overwhelming body of evidence for a protective effect of periconceptional folic acid supplementation against neural tube defects led to mandatory folic acid fortification in the United States and Canada in 1998. Thereby mandatory fortification of grains and cereals with folic acid ensured that most of the people could get sufficient amounts of nutrients.

Strong evidence links low folate status to cognitive impairment and decline, but conflicting results weaken the case for boosting folate status to preserve cognition in older age. Randomized trials of folic acid in the prevention of cognitive decline have been carried out in most western countries, but the results are not conclusive (9,10,24). This is one of the first conducted in China. By contrast, studies conducted in China—a country where there is no national fortification program, genetic mutations tied to elevated homocysteine levels are common, and rates of dementia and stroke are high—paint a different picture. Folate is a useful tool, but it is only good when people need it.

In our trial, folic acid supplementation may have significant effects on global cognitive function, although for specific cognitive tasks, the effects were seen in Digit Span and Block Design.

Memory is typically altered in amnestic MCI patients (likely to progress to AD). Tests of memory are considered as more sensitive than tests of other cognitive functions for distinguishing between

Table 2. The Levels of Biochemical Parameters at Baseline, 3-Month, and 6-Month Follow-up in the Folate and Control Group

Items	Groups	Cases (n)	Treatment Time*			Repeated Measures†		
			Before Treatment	3 Months	6 Months	Interaction Effect, P (ηp^2)	Time Effect, P (ηp^2)	Group Effect, P (ηp^2)
Homocysteine (μmol/L)	Intervention	90	13.65 ± 4.82	11.35 ± 3.39	9.49 ± 5.72	0.017 (0.119)	0.048 (0.096)	0.775 (0.002)
	Control	90	12.19 ± 3.85	11.38 ± 5.04	10.97 ± 2.42			
Folate (ng/mL)	Intervention	90	7.01 ± 3.64	7.59 ± 4.60	8.97 ± 0.62	0.009 (0.712)	0.012 (0.125)	0.231 (0.043)
	Control	90	5.79 ± 2.67	5.89 ± 3.62	5.97 ± 2.11			
Vitamin B_{12} (pg/mL)	Intervention	90	571.25 ± 256.94	558.31 ± 294.93	683.79 ± 95.91	0.022 (0.144)	0.019 (0.120)	0.039 (0.132)
	Control	90	538.82 ± 284.06	591.37 ± 276.65	637.43 ± 157.39			
SAM (μmol/L)	Intervention	90	13.63 ± 6.39	14.11 ± 7.85	17.49 ± 4.49	0.033 (0.117)	0.278 (0.047)	0.355 (0.046)
	Control	90	14.26 ± 8.32	11.19 ± 6.48	16.85 ± 3.48			
SAH (μmol/L)	Intervention	90	6.89 ± 2.86	6.78 ± 4.44	6.92 ± 2.84	0.737 (0.010)	0.549 (0.020)	0.338 (0.566)
	Control	90	6.62 ± 3.02	6.59 ± 3.78	6.71 ± 3.72			
SAM/SAH	Intervention	90	236.26 ± 137.24	239.99 ± 198.37	238.98 ± 167.38	0.084 (0.079)	0.165 (0.058)	0.550 (0.012)
	Control	90	235.69 ± 189.26	236.62 ± 100.38	239.67 ± 117.37			

SAH = *S*-adenosyl homocysteine; SAM = *S*-Adenosyl-methionine.

Notes: *Presented as mean ± *SD*.

†ηp^2 describes the percentage of variance explained in the dependent variable by a predictor variable. p value for group (intervention vs control) derived from analysis of covariance (ANCOVA) adjusted for respective baseline value and baseline.

Table 3. Neuropsychological Test Results at Baseline, 3-Month, and 6-Month Follow-up

Cognitive Test	Groups	Cases (*n*)	Treatment Time*			Repeated Measures†		
			Before Treatment	3 Months	6 Months	Interaction Effect, *P* (ηp^2)	Time Effect, *P* (ηp^2)	Group Effect, *P* (ηp^2)
Full Scale IQ	Intervention	90	108.11 ± 7.25	112.45 ± 6.94	115.77 ± 6.31	0.048 (0.096)	0.227 (0.051)	0.481 (0.014)
	Control	90	107.53 ± 11.48	107.62 ± 10.84	107.28 ± 9.72			
Information	Intervention	90	10.05 ± 3.44	9.27 ± 3.88	10.35 ± 3.71	0.937 (0.004)	0.717 (0.011)	0.838 (0.007)
	Control	90	11.41 ± 2.66	10.37 ± 2.22	11.09 ± 2.88			
Comprehension	Intervention	90	12.31 ± 1.25	12.07 ± 3.11	12.55 ± 2.35	0.056 (0.101)	0.128 (0.069)	0.427 (0.026)
	Control	90	13.53 ± 1.432	12.97 ± 2.44	13.05 ± 2.66			
Digit Span	Intervention	90	9.27 ± 3.11	11.66 ± 2.49	13.05 ± 3.47	0.000 (0.319)	0.068 (0.087)	0.516 (0.015)
	Control	90	8.87 ± 2.70	10.51 ± 2.86	9.75 ± 3.14			
Vocabulary	Intervention	90	10.95 ± 2.66	11.31 ± 2.48	11.07 ± 2.22	0.179 (0.062)	0.123 (0.069)	0.879 (0.002)
	Control	90	11.72 ± 2.63	10.97 ± 2.55	11.30 ± 2.78			
Arithmetic	Intervention	90	11.77 ± 3.05	10.95 ± 2.44	11.31 ± 3.26	0.337 (0.039)	0.687 (0.014)	0.463 (0.015)
	Control	90	10.93 ± 1.552	11.2 ± 2.92	10.85 ± 3.11			
Similarities	Intervention	90	11.97 ± 2.26	12.58 ± 3.02	11.48 ± 2.46	0.716 (0.011)	0.725 (0.014)	0.523 (0.013)
	Control	90	12.40 ± 1.52	12.26 ± 2.15	12.44 ± 3.18			
Picture Completion	Intervention	90	11.35 ± 2.66	10.97 ± 2.58	12.45 ± 5.66	0.087 (0.086)	0.784 (0.011)	0.868 (0.007)
	Control	90	11.67 ± 1.73	10.51 ± 2.26	11.29 ± 3.77			
Picture Arrangement	Intervention	90	10.34 ± 2.41	11.45 ± 2.65	10.99 ± 2.64	0.516 (0.478)	0.209 (0.053)	0.862 (0.005)
	Control	90	11.72 ± 2.51	10.93 ± 2.47	11.39 ± 2.83			
Block Design	Intervention	90	9.77 ± 5.41	10.33 ± 3.28	13.28 ± 4.21	0.033 (0.107)	0.237 (0.058)	0.364 (0.028)
	Control	90	9.93 ± 2.273	12.52 ± 2.61	11.33 ± 3.11			
Object Assembly	Intervention	90	10.66 ± 2.58	9.77 ± 2.62	10.89 ± 2.91	0.824 (0.002)	0.254 (0.047)	0.962 (0.001)
	Control	90	11.42 ± 2.83	10.39 ± 2.46	10.97 ± 2.66			
Digit Symbol	Intervention	90	13.58 ± 3.61	13.76 ± 2.68	14.07 ± 2.22	0.965 (0.001)	0.255 (0.046)	0.825 (0.002)
	Control	90	14.26 ± 2.631	13.44 ± 2.6	13.29 ± 2.57			

Notes: *Presented as mean ± *SD*.

†ηp^2 describes the percentage of variance explained in the dependent variable by a predictor variable; *p* value for group (intervention vs control) derived from analysis of covariance (ANCOVA) adjusted for respective baseline value and baseline.

individuals with and without amnestic cognitive impairment and, specifically, for predicting progression to AD (31). Digit Span is a common task of attention/short-term memory. Short-term memory is the cognitive domain typically altered in MCI patients. When adjusted for each other, low-dose plasma folic acid supplementation was significantly associated with improvement in short-term memory. This finding conforms to most of the previous studies in which folate was linked specifically to memory function and AD. Folate deficiency is known to influence neurotransmitter production through its role in the methyl cycle (32). Serum folate levels were increased and may have contributed to the observed improvements to short-term memory via these mechanisms. Elevated homocysteine has been associated with shrinkage of the hippocampus, an important brain region for memory (33). Reducing homocysteine may therefore reduce the risk of memory loss and dementia in the later decades of life (34).

The Block Design test is a subtest of WAIS-RC that is considered a measure of visuospatial organization and its performance depends on multiple factors (35). The Block Design test involves different abilities that include perception, decision making, problem-solving, and processing speed. In our trial, MCI people improved in Block Design test after 6 months of folic acid supplementation. These changes might reflect structural or functional brain changes, especially in the postero-parietal region, prefrontal cortex, or in the connectivity across those regions (36). Another explanation might be that the improvement of performance in Block Design test associated with the active intervention could be attributed to an increased folate intake and absorption in the brain (10).

The biological mechanisms that might underly the relationship between plasma folate and cognition are currently unclear. Most researchers point out the central role of folate in one-carbon metabolism and methylation reactions (37). Homocysteine is a potentially harmful sulfur-containing amino acid derived from methionine and it may have detrimental effects on cognitive function through endothelial dysfunction, arteriosclerosis, thrombosis, and cellular damage (38). Folate is required for the conversion of homocysteine to methionine. Our results showed that homocysteine levels decreased with oral folic acid supplementation after six months, and even after 3 months. There is an inverse relationship between folate and homocysteine concentrations through the methionine cycle. Under folic acid intervention, serum vitamin B_{12} level in intervention group increased more significantly than that in control group; which is intriguing. The most likely mechanism that explains covariation of serum folate and vitamin B_{12} may relate to be homocysteine (Hcy) lowering. Vitamin B_{12} deficiency is often an important contributor to elevated tHcy concentrations [39]. Reduction of Hcy levels can be readily achieved with high doses of vitamin B_{12} in the general population [40–42]. There is mutual restriction relationship between vitamin B_{12} and Hcy [43,44]. Folic acid and vitamin B_{12} act as coenzymes and show a close molecular interaction on the basis of Hcy metabolism (Figure 3) Folic acid supplementation may also affect cognitive function through its effect on homocysteine (45). A relatively short-term folic acid intervention is capable of significantly lowering homocysteine level in individuals with MCI. Reducing homocysteine may therefore reduce the risk of cognitive decline and dementia in the later decades of life.

The strengths of this trial were its randomized controlled design, the relatively large number of carefully selected participants, low dropout, and a broad assessment of different cognitive functions.

This study has some important issues. First, in the whole study, the same version of the cognitive tests has been used, since the time

Table 4. Mixed-model Analysis for Describing the Association Between Full Scale IQ, Digit Symbol, Block Design, and Laboratory Variables at the Start of the Study

Cognition Score	Estimate	*SEM*s	*t* Test	*p* Value	95% CI		Effect Size, *d*
					Lower	Upper	
Full Scale IQ							
Intercept	21.351	0.478	22.579	.001	20.642	23.683	
Folate (baseline)	−0.001	0.001	−0.452	.673	0.010	0.010	
Homocysteine (baseline)	−0.113	0.052	−2.873	.013	−0.223	−0.003	
Vitamin B_{12} (baseline)	−0.582	0.213	−1.771	.097	−0.715	−0.442	
SAM (baseline)	−0.293	0.272	−1.155	.249	−0.591	0.012	
FA × wave							
FA × Baseline*	0.001	0.001					
FA × 3 months	−0.264	0.247	−1.082	.277	−0.775	0.239	0.074
FA × 6 months	0.615	0.272	2.174	.031	0.081	1.151	0.168†
Digit Span							
Intercept	4.738	0.287	15.983	.001	4.163	5.314	
Folate (baseline)	−0.001	0.001	−0.775	.434	0.010	0.010	
Homocysteine (baseline)	−0.059	0.028	−3.422	.001	−0.093	−0.037	
Vitamin B_{12} (baseline)	−0.001	0.001	−0.491	.631	−0.010	−0.010	
SAM (baseline)	−0.162	0.128	−1.583	.122	−0.376	0.053	
FA × wave							
FA × Baseline*	0.001	0.001					
FA × 3 months	0.028	0.158	0.213	.839	−0.211	0.263	0.007
FA × 6 months	0.343	0.149	2.562	.009	0.095	0.585	0.176†
Block Design							
Intercept	6.159	0.265	22.478	.001	5.634	6.698	
Folate (baseline)	−0.001	0.001	−0.133	.877	0.010	0.010	
Homocysteine (baseline)	−0.079	0.016	−4.831	.000	−0.118	−0.048	
Vitamin B_{12} (baseline)	−0.001	0.002	−0.372	.749	0.010	0.010	
SAM (baseline)	−0.089	0.179	−0.864	.382	−0.262	0.131	
FA × wave							
FA × Baseline*	0.001	0.001					
FA × 3 months	−0.072	0.124	−0.522	.591	−0.308	0.173	0.029
FA × 6 months	0.237	0.119	2.013	.036	0.012	0.477	0.146†

Notes: FA = folic acid; SAM = *S*-Adenosyl-methionine; SEM = standard error of the mean.

*Reference category.

†Cohen's *d* refers to the magnitude of the standardized mean effect (ie, the mean difference between two groups in *SD* units). Significant at $p < .05$.

between measurements is short, the detected improvements may be due to a learning effect (test–retest effect). To decrease learning effect during data analysis, we fitted all models by maximum likelihood, incorporating longitudinal correlations in participants by using unstructured covariance structures. For statistical testing, we used Wald tests. Otherwise, in the subsequent follow-up, to minimize the effect of learning and practice, we repeated cognitive testing at the end of the study using variations of the tests used at baseline (parallel versions, eg, various paragraphs, letters, word categories, or matrices). We wish to decrease the effect of learning and practice to the limit. Second, the individual effects of folic acid on cognitive function were not taken into consideration. Therefore, prolonged observation time and modified statistical measures are needed in further studies. Third, a longer follow-up period of observation was absent. Last, the current study used methods that determined the blood concentrations of folate, which might not reveal the folate status in tissues. It is still uncertain whether normalization of plasma folate status reflects folate status in the cerebrospinal fluid and cells in the central nervous system. Two new markers, plasma homocysteine and serum methylmalonic acid, which are considered to show the functional status of folate in tissues, have attracted growing interest in our following study.

In summary, we found evidence for a beneficial effect of folic acid supplementation on two tests of cognitive function among people with MCI. These trials need to be larger and include dementia as an outcome. Longer-term randomized trials of folic acid are needed.

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