# Plasma folate concentration and cognitive performance: Rotterdam Scan Study[1–3]

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**ABSTRACT**

**Background:** Evidence is increasing for beneficial and independent effects of folate on cognitive function, but the underlying biologic mechanism is as yet unknown.

**Objective:** We examined the independent association of plasma folate concentration with cognitive performance and explored the nature of this association by evaluating brain-imaging markers for cerebrovascular disease and brain cell loss.

**Design:** In the population-based Rotterdam Scan Study, 1033 nondemented participants aged 60–90 y underwent extensive cognitive testing and brain imaging. We cross-sectionally examined the association between plasma folate concentration and cognitive test performance by multivariate linear regression. To evaluate the role of vascular or other mechanisms in this association, we subsequently studied whether plasma folate was related to the presence of white matter lesions and hippocampal and amygdalar volumes.

**Results:** After multivariate adjustment, the mean change in test score per 1-SD increase in plasma folate was 0.05 (95% CI: 0.01, 0.09) for global cognitive function, 0.08 (95% CI: 0.04, 0.13) for psychomotor speed, and 0.02 (95% CI: −0.04, 0.07) for memory function. Adjustment for homocysteine concentration only slightly diminished these associations. The odds ratio relating a 1-SD increase in plasma folate to the presence compared with the absence of severe white matter lesions was 0.79 (95% CI: 0.66, 0.94), whereas no relation was seen between folate status and hippocampal or amygdalar volume.

**Conclusions:** Higher plasma folate concentrations are associated with better global cognitive function and better performance on tests of psychomotor speed, regardless of homocysteine concentration. These associations may be mediated by vascular mechanisms. *Am J Clin Nutr* 2007;86:728–34.

**KEY WORDS** Folate, cognition, white matter lesions, epidemiology, cohort study

## INTRODUCTION

High concentrations of total homocysteine were associated with cognitive decline and an increased risk of dementia in many cross-sectional and prospective studies, although some studies failed to replicate this finding (1–7). However, it is still unclear whether these associations are explained by homocysteine itself, or rather by other factors closely related to homocysteine metabolism, such as vitamin B-12 or folate (8, 9). Interest in the role of folate in relation to cognition has grown since several studies reported an association between low plasma folate and worse cognitive performance (4, 9–16), which in some cases appeared independent of homocysteine concentration (4, 11–15). Although several hypotheses were proposed, a definite biological explanation for these findings is as yet lacking, and it is not known whether folate affects cognitive function through direct effects on brain tissue, through vascular mechanisms, or both. Analysis of the relation between folate concentration and structural brain measures that are generally considered markers for cerebrovascular disease or for brain cell loss may help to elucidate the nature of the link between folate and cognition. In a large population-based study among nondemented elderly in which we had brain imaging for all participants, we examined the relation between plasma folate and cognitive performance, as well as the association between folate concentration and the presence of white matter lesions (WMLs) and hippocampal and amygdalar volumes.

## SUBJECTS AND METHODS

### Study population

In 1995–1996, the cohort for the Rotterdam Scan Study, a prospective study on causes and consequences of age-related brain changes in the elderly (17), was assembled by random selection of 1904 participants aged 60–90 y from 2 ongoing population-based cohort studies, the Rotterdam Study (18) and the Zoetermeer Study (17). Selected subjects who were blind, had contraindications for magnetic resonance imaging (MRI) scanning, or were diagnosed with dementia were excluded ($n$ = 187). Dementia was assessed through a stepwise procedure (5).

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All participants were initially screened with the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule, organic section. Subsequently, participants who scored below a cutoff of 26 on the MMSE or above 0 on the Geriatric Mental Schedule were further evaluated with more extensive neuropsychological tests, an informant interview, and review of medical records. Of the 1717 persons eligible for inclusion, 1077 (63%) agreed to participate. All included subjects, 563 of whom came from the Rotterdam Study and 514 of whom came from the Zoetermeer Study, gave written informed consent to the protocol, which was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam. The current study involves cross-sectional analyses on data and measurements from blood samples collected at baseline (1995–1996). Complete data on MRI measures, cognitive test results, and folate measurements were available for 1033 of the 1077 participants.

### Measurement of plasma folate concentration

Nonfasting blood samples were collected into citrate tubes at baseline, put on ice immediately, and centrifuged (2000 × *g*, 4 °C, 10 min) within 60 min. Aliquots of plasma were stored at −80 °C. Plasma folate concentrations were measured 7 y after storage by microbiological assays with the use of a chloramphenicol-resistant strain of *Lactobacillus casei*. The assay was adapted to a microtiter plate format and performed by a robotic workstation (Microlab AT plus 2; Hamilton, Bonadus AG, Switzerland) (19)

### Neuropsychological testing

Several neuropsychological tests were administered at baseline of the Rotterdam Scan Study. An abbreviated Stroop test, the Letter-Digit Substitution Task, and a verbal fluency test were used to assess executive function. Attention was measured by a Paper-and-Pencil Memory Scanning Task consisting of 4 subtasks. Memory function was assessed with the use of a 15-word verbal learning test, consisting of 3 immediate recall trials and a delayed recall of words. Individual test scores were transformed into standardized *z* scores [*z* score = (individual test score − mean test score)/SD of the baseline tests]. From these *z* scores, compound scores were constructed for psychomotor speed, memory performance, and global cognitive function (5). Compound scores for psychomotor speed were calculated by averaging the *z* scores of the reading subtask of the Stroop test, the one-letter subtask of the Paper-and-Pencil Memory Scanning test, and the Letter-Digit Substitution Task. Compound scores for memory function were calculated by averaging the *z* scores of the total 3 immediate recall trials and the delayed recall trial of the 15-word verbal learning tests. Compound scores for global cognitive performance were calculated by averaging the *z* scores of the reading subtask of the Stroop test, the one-letter subtask of the Paper-and-Pencil Memory Scanning Test, the Letter-Digit Substitution Task, and the immediate and delayed recall of the 15-word verbal learning test.

### MRI procedure

All 1077 participants underwent axial T1, T2, and proton-density weighted brain MRI scanning in a 1.5-T unit to assess the presence of WMLs (5, 17), which are considered indicators of cerebral small vessel disease (20, 21). Periventricular WMLs were scored semiquantitatively for locations at the frontal and occipital horns and at the lateral walls of the ventricles, which resulted in a total periventricular score (range: 0–9). For subcortical WMLs, a total volume was approximated on the basis of the number and size of the lesions in the frontal, parietal, temporal, and occipital lobes (range: 0–29.5 mL). Subjects originating from the Rotterdam Study additionally underwent a 3-dimensional half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence that was used to reconstruct a series of coronal brain slices (contiguous 1.5-mm slices) perpendicular to the long axis of the hippocampus. All reconstructed slices were transferred to a Magic View 1000 workstation (Siemens, Erlangen, Germany) for volumetric assessment of the left and right hippocampi and amygdalae. The boundaries of both hippocampi and amygdalae were manually traced with the use of a mouse-driven cursor based on a reference atlas, and outline surface areas on each side were multiplied by slide thickness to yield estimates of left and right hippocampal and amygdalar volumes (5), which are thought of as markers of brain cell loss and presymptomatic Alzheimer disease (22). Of the 563 participants originating from the Rotterdam Study, 52 persons developed claustrophobia during scanning, so the HASTE sequence could not be completed, or had severe movement artifacts on their HASTE scans, leaving 511 participants with complete data for the analyses on hippocampal and amygdalar volumes.

### Covariates

We obtained information on the following covariates by interview and physical examination in 1995–1996: pack-years of cigarette smoking, alcohol consumption, use of vitamin supplements, serum creatinine (enzymatic assay), diabetes mellitus, systolic blood pressure, use of antihypertensive medication, presence of depressive symptoms [determined with the Center of Epidemiologic Studies Depression Scale and defined as a score ≥ 16], and highest levels of education achieved. Plasma total homocysteine concentrations were measured 3 y after storage by fluorescence polarization immunoassay on an IMx analyzer (Abbott Laboratories, Chicago, IL) in nonfasting blood samples obtained at baseline. Plasma cobalamin (vitamin B-12) was measured 7 y after storage by a microbiologic assay with the use of a colistin sulfate-resistant strain of *Lactobacillus leishmannii* (23). The assay was adapted to a microtiter plate format and performed by a robotic workstation (Microlab AT plus 2; Hamilton) (19). Intima-media thickness (IMT) of the common carotid artery, a marker of atherosclerotic disease, was measured by longitudinal 2-dimensional ultrasound scans. We calculated the mean common carotid artery IMT as the mean of 4 locations: the near and far walls of both the right and the left common carotid arteries (7).

### Data analysis

We evaluated the relation between folate concentration and cognitive performance by multivariate linear regression, with plasma folate as a continuous variable (expressed per SD increase) and in quintiles of the distribution (by creating dummy variables for each quintile). All analyses were initially adjusted for age and sex. We subsequently adjusted for cigarette smoking (in pack-years), alcohol consumption, use of vitamin supplements, serum creatinine concentration, vitamin B-12 concentration, diabetes mellitus, systolic blood pressure, use of antihypertensive medication, presence of depressive symptoms,

**TABLE 1**
Baseline characteristics of the study population ($n = 1033$)

| Characteristic | Value |
|---|---|
| Women [*n* (%)] | 532 (51.5) |
| Age (y) | 72.2 ± 7.4[1] |
| Folate (nmol/L) | 14.2 ± 8.6 |
| Homocysteine (μmol/L) | 11.5 ± 4.1 |
| Vitamin B-12 (pmol/L) | 259 ± 138 |
| Serum creatinine (μmol/L) | 88.9 ± 18.5 |
| Diabetes [*n* (%)] | 59 (5.7) |
| Use of vitamins [*n* (%)] | 59 (5.7) |
| Depressive symptoms [*n* (%)][2] | 78 (7.3) |
| Intima-media thickness (mm) | 0.87 ± 0.14 |
| Alcohol use (units/d) | 0.3 (0.0–2.0)[3] |
| Smoking (pack-years) | 10.0 (0.0–31.4)[3] |
| Primary education only [*n* (%)] | 358 (34.7) |
| Periventricular white matter lesions (grade) | 2.4 ± 2.2 |
| Subcortical white matter lesions (mL) | 1.4 ± 2.9 |
| Hippocampal volume (mL)[4] | 6.4 ± 0.9 |
| Amygdalar volume (mL)[4] | 4.6 ± 0.7 |

[1] $\bar{x}$ ± SD (all such values).
[2] Center of Epidemiologic Studies Depression Scale score ≥ 16.
[3] Median; interquartile range in parentheses.
[4] Measurements were based on 511 participants.

education, and IMT, because these covariates were considered potential confounders of the association between folate concentration and cognitive performance. In a third model, all analyses were additionally adjusted for total plasma homocysteine concentration, to study whether the effects of folate were independent or merely explained through its effect on homocysteine concentration. Finally, to evaluate whether and to what extent the association between folate and cognitive performance is explained by WMLs, we also adjusted all analyses for grade and volume of periventricular and subcortical WMLs.

We subsequently studied the association between folate concentration and WMLs and hippocampal and amygdalar volumes, again with plasma folate as a continuous variable and in quintiles of the distribution. Periventricular and subcortical WMLs were first evaluated as separate outcomes and subsequently were combined into a measure for the presence of severe WMLs, defined as periventricular or subcortical WMLs in the upper quintile of their distribution (periventricular WMLs grade ≥ 4 or volume of subcortical WMLs ≥ 1.5 mL). We used multivariate linear regression to study the association between folate and subcortical and periventricular WMLs and multivariate logistic regression to evaluate the association with the presence of severe WMLs. The analyses for structural brain measures were initially adjusted for age and sex and additionally for the same set of potential confounders that was used in the analyses on cognitive performance. All analyses were performed with the use of SPSS software (version 12.0; SPSS Inc, Chicago, IL).

## RESULTS

### Characteristics of the study population

Baseline characteristics of the study population are shown in **Table 1**. Use of vitamins and B-vitamin status in our cohort were relatively low. Homocysteine concentrations (mean total homocysteine concentration: 11.5 μmol/L) are well within the range of what would be expected for a nonfortified elderly population but higher than those typically found in fortified populations as in the United States (24). Plasma folate ranged from 0.9 nmol/L to 55 nmol/L, and 12% of the participants had a plasma vitamin B-12 concentration below the conventional cutoff for deficiency of 148 pmol/L. Diabetes and depressive symptoms were infrequent in our cohort.

### Folate concentration and cognitive performance

Increasing folate concentration was associated with higher scores for global cognitive function and psychomotor speed (**Table 2**). Analyses in quintiles suggested a concentration-response relation. The results remained virtually unchanged after adjusting for all selected potential confounders. When we additionally adjusted for total homocysteine concentration, the associations were just slightly attenuated and remained statistically significant. At the same time, this mutual adjustment still yielded statistically significant results on the association between plasma homocysteine and global cognitive function and psychomotor speed (Table 2). Controlling the analyses for WMLs attenuated the results, although the association between folate and psychomotor speed remained statistically significant. No association was seen between plasma folate and memory function. Analyses with scores for the individual neuropsychological tests as endpoints showed a significant association between folate concentration and the reading subtask of the Stroop test and the one-letter subtask of the Paper-and-Pencil Memory Scanning test, whereas no significant association was observed for the Letter-Digit Substitution Task and the 15-word verbal learning test.

### Folate concentration and brain-imaging measures

The volume of subcortical WMLs and the presence of severe WMLs decreased significantly with increasing plasma folate concentration (**Table 3**). Results for subcortical WMLs remained statistically significant after adjustment for all selected potential confounders. However, for periventricular WMLs, additional adjustment for homocysteine resulted in associations that were no longer statistically significant, although size and direction of the estimates did not substantially change. No relation was seen between folate and hippocampal or amygdalar volume (**Table 4**).

## DISCUSSION

In this large population-based study, we observed that a higher plasma concentration of folate was associated with better cognitive performance, in particular psychomotor speed. The association slightly diminished but remained significant after adjustment for total homocysteine concentration. Furthermore, a significant inverse association between plasma folate and the presence of severe WMLs was found, whereas no association was seen with hippocampal and amygdalar volumes. Because periventricular and subcortical WMLs are generally considered markers for cerebral small vessel disease (20, 21) and hippocampal and amygdalar volume losses are early indicators of presymptomatic Alzheimer disease (22), these findings suggest that folate status is related to cognition through vascular mechanisms rather than through a primary neurodegenerative process.

Methodologic strengths of our study are its size, population-based design, and the large number of potential confounders,

**TABLE 2**
Association between folate and homocysteine concentrations and cognitive performance[1]

| | Q1 | Q2 | Q3 | Q4 | Q5 | Per 1-SD increase | *P* for trend |
|---|---|---|---|---|---|---|---|
| Global cognitive function[2] | | | | | | | |
| Folate[3] | | | | | | | |
| Model 1[4] | (ref) | 0.02 (−0.10, 0.14)[5] | 0.11 (−0.01, 0.23) | 0.05 (−0.08, 0.17) | 0.17 (0.05, 0.30) | 0.05 (0.01, 0.09) | 0.007 |
| Model 2[6] | (ref) | 0.05 (−0.07, 0.17) | 0.13 (0.01, 0.25) | 0.07 (−0.05, 0.18) | 0.19 (0.07, 0.31) | 0.05 (0.01, 0.09) | 0.004 |
| Model 3[7] | (ref) | 0.02 (−0.10, 0.14) | 0.10 (−0.03, 0.22) | 0.02 (−0.10, 0.15) | 0.14 (0.01, 0.26) | 0.04 (0.00, 0.08) | 0.054 |
| Model 4[8] | (ref) | 0.01 (−0.11, 0.14) | 0.08 (−0.04, 0.21) | 0.00 (−0.12, 0.14) | 0.11 (0.00, 0.23) | 0.02 (−0.02, 0.07) | 0.087 |
| Homocysteine[9] | | | | | | | |
| Model 5[10] | (ref) | −0.02 (−0.14, 0.10) | −0.01 (−0.14, 0.11) | −0.01 (−0.14, 0.11) | −0.17 (−0.31, −0.02) | −0.05 (−0.09, 0.00) | 0.107 |
| Psychomotor speed[11] | | | | | | | |
| Folate[3] | | | | | | | |
| Model 1[4] | (ref) | 0.03 (−0.11, 0.17) | 0.15 (0.01, 0.289) | 0.13 (−0.01, 0.27) | 0.25 (0.11, 0.39) | 0.08 (0.04, 0.13) | 0.000 |
| Model 2[6] | (ref) | 0.05 (−0.09, 0.19) | 0.16 (0.03, 0.30) | 0.14 (0.00, 0.28) | 0.26 (0.12, 0.40) | 0.08 (0.04, 0.13) | 0.000 |
| Model 3[7] | (ref) | 0.03 (−0.12, 0.17) | 0.13 (0.01, 0.27) | 0.10 (−0.05, 0.24) | 0.21 (0.07, 0.36) | 0.07 (0.02, 0.11) | 0.004 |
| Model 4[8] | (ref) | 0.02 (−0.13, 0.17) | 0.11 (−0.03, 0.26) | 0.08 (−0.07, 0.23) | 0.19 (0.04, 0.35) | 0.05 (0.00, 0.11) | 0.010 |
| Homocysteine[9] | | | | | | | |
| Model 5[10] | (ref) | −0.02 (−0.16, 0.12) | −0.05 (−0.20, 0.10) | −0.03 (−0.18, 0.13) | −0.22 (−0.39, −0.05) | −0.06 (−0.11, −0.01) | 0.047 |
| Memory function[12] | | | | | | | |
| Folate[3] | | | | | | | |
| Model 1[4] | (ref) | 0.01 (−0.15, 0.18) | 0.04 (−0.12, 0.21) | −0.08 (−0.24, 0.09) | 0.07 (−0.10, 0.24) | 0.01 (−0.04, 0.07) | 0.774 |
| Model 2[6] | (ref) | 0.05 (−0.12, 0.22) | 0.08 (−0.09, 0.25) | −0.03 (−0.19, 0.14) | 0.09 (−0.08, 0.26) | 0.02 (−0.04, 0.07) | 0.581 |
| Model 3[7] | (ref) | 0.02 (−0.15, 0.19) | 0.05 (−0.13, 0.22) | −0.07 (−0.24, 0.11) | 0.04 (−0.14, 0.22) | 0.00 (−0.06, 0.06) | 0.928 |
| Model 4[8] | (ref) | 0.01 (−0.16, 0.19) | 0.04 (−0.15, 0.21) | −0.08 (−0.26, 0.10) | 0.02 (−0.16, 0.21) | 0.00 (−0.06, 0.05) | 0.851 |
| Homocysteine[9] | | | | | | | |
| Model 5[10] | (ref) | −0.02 (−0.19, 0.15) | 0.02 (−0.16, 0.20) | −0.01 (−0.19, 0.18) | −0.08 (−0.29, 0.13) | −0.03 (−0.09, 0.04) | 0.677 |

[1] Q, quintile; ref, reference.
[2] Compound scores were calculated from the reading subtask of the Stroop test, the one-letter subtask of the Paper-and-Pencil Memory Scanning Test, the Letter-Digit Substitution Task, and immediate and delayed recall of the 15-word verbal learning test.
[3] Folate concentration was <8.1 nmol/L for Q1, 8.2–10.9 nmol/L for Q2, 11.0–13.4 nmol/L for Q3, 13.5–17.2 nmol/L for Q4, and >17.3 nmol/L for Q5.
[4] Adjusted for age and sex.
[5] Mean increase in *z* score; 95% CI in parentheses (all such values).
[6] Adjusted for age, sex, alcohol consumption, pack-years of cigarette smoking, serum creatinine, vitamin B-12 concentration, diabetes, score on the Center of Epidemiologic Studies Depression scale, education, intima-media thickness, use of vitamins, use of antihypertensive medication, and systolic blood pressure.
[7] Adjusted as in model 2 with additional adjustment for plasma total homocysteine concentration.
[8] Adjusted as in model 3 with additional adjustment for periventricular and subcortical white matter lesions.
[9] Homocysteine concentration was <8.5 μmol/L for Q1, 8.6–9.8 μmol/L for Q2, 9.9–11.4 μmol/L for Q3, 11.5–13.9 μmol/L for Q4, and >14.0 μmol/L for Q5.
[10] Adjusted for age, sex, alcohol consumption, pack-years of cigarette smoking, serum creatinine, vitamin B-12 concentration, folate concentration, diabetes, score on the Center of Epidemiologic Studies Depression scale, education, intima-media thickness, use of vitamins, use of antihypertensive medication, and systolic blood pressure [analyses of the association between homocysteine concentration and cognitive performance without adjustment for vitamin B-12 and folate concentrations were published previously (5)].
[11] Compound scores were calculated from the reading subtask of the Stroop test, the one-letter subtask of the Paper-and-Pencil Memory Scanning test, and the Letter-Digit Substitution Task.
[12] Compound scores were calculated from a total of 3 immediate recall trials and a delayed recall trial of the 15-word verbal learning tests.

including homocysteine concentration, that were assessed. Moreover, all participants underwent both extensive cognitive testing and brain imaging. A potential limitation of the current study is its cross-sectional design and hence the possibility of reverse causality. Although it might be argued that cognitive impairment may affect dietary habits and thus plasma folate concentration, we think it is unlikely that this influenced our results, given that only nondemented persons were included in our study. Another issue to consider is the relatively low overall response rate of 63%. Persons who agreed to participate were significantly younger, had a lower prevalence of hypertension, and had higher MMSE scores than did nonresponders, whereas cholesterol concentrations, body mass indexes, and proportion of women were equal (17). However, this nonresponse would only have biased our results if it led to a disproportionate loss of persons with the combination of low folate status and good cognition or of high folate status and poor cognition, which we consider unlikely. Finally, because our study population did not include demented persons, we were not able to examine the cross-sectional association between plasma folate concentration and the risk of dementia.

Our finding that higher plasma concentrations of folate were associated with better cognitive performance is in agreement with previously published results. Low folate has been associated with worse cognitive performance, cognitive decline, or an increased risk of dementia in several cross-sectional (3, 4, 9, 10, 15, 16, 25, 26) and prospective (11, 12, 14, 27) studies. However, looking at specific cognitive domains, we found that plasma folate was associated with global cognitive function and psychomotor speed but not with memory performance. This finding

**TABLE 3**
Association between folate concentrations and white matter lesions (WMLs)[1]

| | Q1 (<8.1 nmol/L) | Q2 (8.2–10.9 nmol/L) | Q3 (11.0–13.4 nmol/L) | Q4 (13.5–17.2 nmol/L) | Q5 (>17.3 nmol/L) | Per 1-SD increase | *P* for trend |
|---|---|---|---|---|---|---|---|
| Subcortical WMLs[2] | | | | | | | |
| Folate | | | | | | | |
| Model 1[3] | (ref) | −0.41 (−0.94, 0.12) | −0.67 (−1.19, −0.15) | −0.67 (−1.19, −0.14) | −0.78 (−1.30, −0.25) | −0.27 (−0.43, −0.10) | 0.003 |
| Model 2[4] | (ref) | −0.53 (−1.06, 0.01) | −0.68 (−1.21, −0.14) | −0.68 (−1.22, −0.14) | −0.72 (−1.26, −0.18) | −0.25 (−0.43, −0.07) | 0.009 |
| Model 3[5] | (ref) | −0.47 (−1.02, 0.08) | −0.60 (−1.15, −0.06) | −0.59 (−1.15, −0.04) | −0.62 (−1.19, −0.05) | −0.21 (−0.40, −0.03) | 0.043 |
| Periventricular WMLs[6] | | | | | | | |
| Folate | | | | | | | |
| Model 1[3] | (ref) | 0.05 (−0.33, 0.43) | −0.32 (−0.70, 0.05) | −0.25 (−0.62, 0.13) | −0.36 (−0.74, 0.02) | −0.16 (−0.28, −0.04) | 0.018 |
| Model 2[4] | (ref) | −0.07 (−0.45, 0.31) | −0.37 (−0.75, 0.00) | −0.26 (−0.64, 0.12) | −0.35 (−0.73, 0.04) | −0.15 (−0.28, −0.02) | 0.044 |
| Model 3[5] | (ref) | −0.01 (−0.40, 0.37) | −0.30 (−0.69, 0.09) | −0.17 (−0.57, 0.22) | −0.24 (−0.64, 0.17) | −0.12 (−0.25, 0.01) | 0.176 |
| Severe WMLs[7,8] | | | | | | | |
| Folate | | | | | | | |
| Model 1[3] | 1.00 (ref) | 0.80 (0.52, 1.24) | 0.58 (0.37, 0.91) | 0.72 (0.46, 1.12) | 0.55 (0.35, 0.86) | 0.83 (0.71–0.97) | 0.010 |
| Model 2[4] | 1.00 (ref) | 0.70 (0.44, 1.11) | 0.53 (0.33, 0.86) | 0.68 (0.43, 1.09) | 0.50 (0.31, 0.81) | 0.79 (0.66, 0.94) | 0.010 |
| Model 3[5] | 1.00 (ref) | 0.72 (0.45, 1.17) | 0.56 (0.34, 0.92) | 0.72 (0.44, 1.18) | 0.53 (0.32, 0.89) | 0.81 (0.68, 0.97) | 0.035 |

[1] Q, quintile; ref, reference.
[2] Values are mean increase in volume; 95% CI in parentheses (except for *P* values).
[3] Adjusted for age and sex.
[4] Adjusted for age, sex, alcohol consumption, pack-years of cigarette smoking, serum creatinine, vitamin B-12 concentration, diabetes, score on the Center of Epidemiologic Studies Depression Scale, education, intima-media thickness, use of vitamins, use of antihypertensive medication, and systolic blood pressure.
[5] Adjusted as in model 2 with additional adjustment for plasma total homocysteine concentration.
[6] Values are mean increase in grade; 95% CI in parentheses (except for *P* values).
[7] Severe WMLs include periventricular WMLs of grade ≥ 4, subcortical WMLs ≥ 1.5 mL, or both.
[8] Values are odds ratio; 95% CI in parentheses (except for *P* values).

is in contrast with most of the previous studies, in which folate was linked specifically to memory function and Alzheimer disease (3, 9, 10, 16). A potential explanation for this discrepancy might be provided by differences in methods of assessing memory function or in composition of the study populations.

In almost all previous studies, a detrimental effect of low folate on cognition was seen, which appeared confined to folate concentrations below a chosen cutoff or in the lowest quintile of the distribution. In agreement with Nurk et al (4), our data provide evidence of a concentration-response relation between folate and cognitive function, showing a clear trend toward better cognitive performance with higher plasma folate concentrations. Our results did not change after adjusting for multiple potential confounders. Furthermore, when we additionally adjusted the analyses for total plasma homocysteine concentration, the associations were only modestly weakened and remained statistically significant. This suggests that the effect of folate on cognition is not, or not solely, through lowering plasma homocysteine. Findings from several previous studies also point toward an independent effect of folate on cognitive performance, contributing to the notion that low folate itself might be a risk factor for cognitive impairment (9, 11–15). It is notable that a recent

**TABLE 4**
Association between folate concentrations and hippocampal and amygdalar volumes ($n = 511$)[1]

| | Q1 (<8.4 nmol/L) | Q2 (8.4–10.9 nmol/L) | Q3 (11.0–13.4 nmol/L) | Q4 (13.5–17.4 nmol/L) | Q5 (>17.4 nmol/L) | Per 1-SD increase | *P* for trend |
|---|---|---|---|---|---|---|---|
| Hippocampal volume | | | | | | | |
| Model 1[2] | (ref) | −0.05 (−0.28, 0.18) | 0.17 (−0.06, 0.40) | −0.02 (−0.25, 0.21) | 0.08 (−0.16, 0.31) | −0.01 (−0.09, 0.07) | 0.511 |
| Model 2[3] | (ref) | −0.04 (−0.28, 0.20) | 0.19 (−0.05, 0.43) | −0.02 (−0.26, 0.22) | 0.10 (−0.15, 0.35) | 0.01 (−0.07, 0.10) | 0.449 |
| Model 3[4] | (ref) | −0.07 (−0.31, 0.18) | 0.15 (−0.10, 0.39) | −0.07 (−0.32, 0.18) | 0.03 (−0.24, 0.30) | −0.01 (−0.10, 0.08) | 0.865 |
| Amygdalar volume | | | | | | | |
| Model 1[2] | (ref) | −0.01 (−0.20, 0.18) | 0.15 (−0.04, 0.33) | 0.08 (−0.11, 0.26) | −0.01 (−0.20, 0.18) | −0.01 (−0.08, 0.05) | 0.656 |
| Model 2[3] | (ref) | −0.01 (−0.20, 0.18) | 0.19 (−0.01, 0.38) | 0.05 (−0.14, 0.24) | −0.04 (−0.20, 0.20) | −0.01 (−0.08, 0.06) | 0.737 |
| Model 3[4] | (ref) | −0.02 (−0.21, 0.18) | 0.18 (−0.02, 0.37) | 0.04 (−0.16, 0.24) | −0.02 (−0.23, 0.20) | −0.02 (−0.09, 0.06) | 0.858 |

[1] Q, quintile; ref, reference.
[2] Adjusted for age and sex.
[3] Adjusted for age, sex, alcohol consumption, pack-years of cigarette smoking, serum creatinine, vitamin B-12 concentration, diabetes, score on the Center of Epidemiologic Studies Depression Scale, education, intima-media thickness, use of vitamins, use of antihypertensive medication, and systolic blood pressure.
[4] Adjusted as in model 2 with additional adjustment for plasma total homocysteine concentration.

trial of folic acid treatment during a 3-y period found an improvement in memory, information processing speed, sensorimotor speed, and global cognitive function compared with the placebo group (28). A significant association between plasma homocysteine and cognitive performance was previously reported in the Rotterdam Scan Study (5). Associations were particularly pronounced for psychomotor speed and less so for memory function, which is similar to the findings from the present study. At the time the analyses on homocysteine and cognition were done, measurements of plasma folate were not yet available. Given that in the current study the relation between plasma folate and cognitive performance appeared independent of total homocysteine concentration, the question is raised whether the previously reported association between homocysteine and cognition may be explained by plasma folate. However, when both folate and homocysteine were included in the model, the associations between homocysteine and global cognitive function and psychomotor speed remained statistically significant. This suggests that folate and homocysteine may have effects on cognition, independent of each other and possibly through different pathways. Mechanisms that were proposed to explain the association between homocysteine and cognition include the effect of homocysteine on cerebrovascular disease, direct neurotoxic effects of homocysteine, or the possibility that elevated homocysteine is merely a marker for deficiency of B vitamins, which may be related to cognition (6).

The biologic mechanisms that might underlie the relation between plasma folate and cognition are as yet unclear. Most researchers point out the central role of folate in one-carbon metabolism and methylation reactions. Folate is required for the conversion of homocysteine to methionine, which is then converted to *S*-adenosylmethionine. *S*-adenosylmethionine is the primary methyl donor in many reactions required for normal brain function, such as the production of cell membrane phospholipids, neurotransmitters, and myelin. Folate deficiency is hypothesized to cause cognitive dysfunction through impaired methylation reactions in the central nervous system (9, 12).

Alternatively, a vascular explanation was proposed. There is evidence for a beneficial effect of folate on endothelial dysfunction, a key process in atherosclerosis that is considered a surrogate endpoint for cardiovascular risk (29–31). Interestingly, in a number of studies reporting beneficial effects of folate on endothelial function, these effects appeared independent of plasma homocysteine concentration (31, 32), which fits our observation of an independent effect of folate on cognition. Folate deficiency reportedly is also associated with increased carotid IMT, a marker of atherosclerosis and vascular disease (33, 34). Again, this association was independent of homocysteine concentration. Although our data showed no clear trend for the association between plasma folate and carotid IMT, mean IMT was significantly lower in participants in the highest quintile of folate than in those in the lowest quintile (mean difference: −0.03 mm; 95% CI: −0.06, −0.01 mm). Cerebrovascular disease is an established risk factor for cognitive impairment and dementia (17, 35, 36), and the effect of folate on cognition might thus be through reducing vascular risk. We tried to elucidate the nature of the observed association between folate and cognitive performance by evaluating the relation between folate concentration and structural brain measures on MRI scans. WMLs are considered subtle markers for cerebrovascular disease and are associated with an increased risk of dementia and cognitive decline in several studies (17, 20, 37, 38). Consequently, our findings that link folate status to both cognitive test performance and WMLs support the hypothesis that low folate status is linked to cognitive impairment by a vascular mechanism. The association between folate concentration and cognition diminished, but it did not disappear after control of the analyses for WMLs, which suggests that the relation is at least partially mediated through a vascular pathway. Also consistent with our findings, psychomotor speed is known to be adversely affected by vascular disease, whereas memory function supposedly is more influenced by neuronal function and hippocampal size (39). In conclusion, our results indicate that high plasma folate concentrations are associated with better cognitive performance, regardless of homocysteine concentration, and that this association might be explained by effects of folate on vascular pathology.

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