Relation of Higher Folate Intake to Lower Risk of Alzheimer Disease in the Elderly

José A. Luchsinger, MD; Ming-Xin Tang, PhD; Joshua Miller, PhD; Ralph Green, MD; Richard Mayeux, MD

Background: Higher intake of folate and vitamins B6 (pyridoxine hydrochloride) and B12 (cyanocobalamin) may decrease the risk of Alzheimer disease (AD) through the lowering of homocysteine levels.

Objective: To relate intake of folate and vitamins B6 and B12 to AD risk.

Design and Patients: We followed up 965 persons 65 years or older without dementia at baseline for a mean±SD period of 6.1±3.3 person-years after the administration of a semiquantitative food frequency questionnaire. Total, dietary, and supplement intake of folate and vitamins B6 and B12 and kilocalorie intake were estimated from the questionnaire responses. We related energy-adjusted intake of folate and vitamins B6 and B12 to incident AD using the Cox proportional hazards regression model.

Main Outcome Measure: Incident AD.

Results: We found 192 cases of incident AD. The highest quartile of total folate intake was related to a lower risk of AD (hazard ratio, 0.5; 95% confidence interval, 0.3-0.9; P=.02 for trend) after adjustment for age, sex, education, ethnic group, the ε4 allele of apolipoprotein E, diabetes mellitus, hypertension, current smoking, heart disease, stroke, and vitamin B6 and B12 levels. Vitamin B6 and B12 levels were not related to the risk of AD.

Conclusions: Higher folate intake may decrease the risk of AD independent of other risk factors and levels of vitamins B6 and B12. These results require confirmation with clinical trials.

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THE PREVALENCE OF ALZHEIMER disease (AD) is expected to quadruple by the year 2047.1 Delaying its onset would decrease its burden.2 Elevated plasma homocysteine level, a risk factor for cardiovascular disease3 and stroke,4 may be related to higher AD risk.4 Deficiencies of folate and vitamins B6 (pyridoxine hydrochloride) and B12 (cyanocobalamin) intake increase homocysteine levels.5 Folate and vitamin B12 are needed for the conversion of homocysteine to methionine, and vitamin B6 is needed for the conversion of homocysteine to cysteine.3 Thus, high dietary intake or supplementation of folate and vitamins B6 and B12 may prevent cardiovascular disease, stroke, and dementia. Fortification of grain with folate was mandated in the United States by the Food and Drug Administration in 1996 and completed by 1997, resulting in increased folate intake and decreased homocysteine levels in adults.6 Most prospective studies relating vitamin B levels and dementia or cognitive impairment have been conducted in white subjects in Europe7,8 or in white and African American subjects in the United States.9 Some studies do not report ethnic composition.10 Our objective was to relate intake of folate and vitamins B6 and B12 to AD risk in a prospective study in northern New York, NY, with a majority of Caribbean Hispanic and African American elderly participants.

METHODS

PARTICIPANTS AND SETTING

Participants were enrolled in a longitudinal cohort study by a random sampling of Medicare recipients 65 years or older residing in northeastern Manhattan (Washington Heights, Hamilton Heights, or Inwood).11 Participants underwent an in-person interview about general health and function at baseline followed by a standard assessment, medical history, physical and neurological examination, and neuropsychological battery.12 Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at intervals of approximately 18 months. The institutional review board of Columbia-Presbyterian Medical Center, New York, approved the study.
The sample for this study consisted of persons without dementia and with dietary assessments and follow-up. Most dietary assessments were conducted at the first follow-up of the original cohort. Of the 2125 participants, 1772 completed a neuropsychological evaluation, 1469 persons had dietary data, 1070 persons had follow-up after the dietary assessment, and 976 did not have prevalent dementia. Complete data on folate and vitamins B₆ and B₁₂ intake were available in the 965 persons who constituted the final sample. Compared with the 1469 persons with dietary data, persons in the final sample were younger (mean±SD age, 75.8±5.8 vs 76.2±6.2 years; P<.001) and had similar proportions of women (70.2% vs 69.3%) and African American (32.6% vs 32.5%), Hispanic (45.3% vs 45.3%), and white (22.1% vs 22.1%) subjects.

DIET ASSESSMENT

We assessed daily dietary intake with a 61-item semiquantitative food frequency questionnaire (Channing Laboratory, Cambridge, Mass.). Information on daily dietary, supplement, and total intake of folate and vitamins B₆ and B₁₂ was estimated from the semiquantitative food frequency questionnaire data. We adjusted the daily dietary and total intake for daily energy intake using the residuals method. In this method, the total and nonsupplement intake of folate and vitamins B₆ and B₁₂ were regressed on caloric intake, and the residuals from linear regression, uncorrelated with caloric intake, were used in all analyses. The residuals represent the nutrient intake that is independent of caloric intake. Residuals have a mean of zero and may be negative or positive. As suggested by Willett and Stampfer, we added the residuals to a constant (intake of each nutrient predicted by the mean caloric intake).

DEMENTIA DIAGNOSIS

Dementia diagnosis and cause assignment was made by the consensus of 2 neurologists, 1 psychiatrist, and 2 neuropsychologists on the basis of baseline and follow-up information. Dementia diagnosis was based on DSM-IV criteria and required evidence of cognitive deficit on the neuropsychological test battery results with evidence of impairment in social or occupational function (clinical dementia rating of ≥1). The diagnosis of AD was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Associations. A diagnosis of dementia associated with stroke was made when the dementia started within 3 months of the stroke and if the local effects of stroke were thought to be the primary mechanism for dementia. Brain imaging was available in 84 (84.8%) of the cases of stroke; in the remainder, we used World Health Organization stroke criteria.

COVARIATES

Besides demographic variables (age, sex, ethnic group, and years of education), we included variables that are associated with a higher risk of AD in our cohort such as diabetes mellitus, hypertension, heart disease, current smoking, and stroke, defined by self-report. Heart disease included history of arrhythmias, congestive heart failure, myocardial infarction, and angina pectoris. Apolipoprotein E (APOE) genotypes were determined as described by Hixson and Vernier and others. We classified persons as homozygous or heterozygous for the APOE ε₄ allele or not having the ε₄ allele.

STATISTICAL METHODS

Intake of kilocalories, folate, and vitamins B₆ and B₁₂ required logarithmic transformation to resemble a normal distribution. We used bivariate analyses to compare characteristics between persons with and without AD. We compared continuous variables using unpaired t tests and categorical variables using the χ² test. We used Cox proportional hazards regression models in multivariate analyses relating intake of folate and vitamins B₆ and B₁₂ to incident AD. The time-to-event variable was the time from the dietary interview to incident AD; individuals without incident dementia were censored at the last follow-up. Individuals who developed non-AD dementia were censored at the time of diagnosis. We conducted analyses relating total intake of folate and vitamins B₆ and B₁₂ to AD as continuous variables and as quartiles. We conducted secondary analyses examining supplement and dietary intake. We show the results of multivariate analyses for the following 4 models: (1) adjusted for age and sex; (2) adjusted for education and APOE ε₄ allele and stratified by ethnic group using the SAS STRATA statement in PROC PHREG; (3) adjusted for diabetes, hypertension, heart disease, stroke, and current smoking; and (4) adjusted for the intake of other vitamins. All analyses were conducted using SAS version 9.1 for Windows (SAS Institute Inc, Cary, NC). Unless otherwise indicated, data are expressed as mean±SD.

There were 192 cases of AD in 5902 person-years of observation (mean follow-up, 6.1±3.3 years). The mean age was 75.8±5.8 years, and 70.5% of the sample were women, 32.6% were African American, 45.3% were Hispanic, and 22.1% were white. Among the clinical characteristics, 28.2% were homozygous or heterozygous for the APOE ε₄ allele, 19.3% had diabetes, 60.3% had hypertension, 27.8% had heart disease, and 10.3% had a history of stroke. The mean total intake of folate was 446.0±226.8 μg; of vitamin B₁₂, 12.6±18.8 μg; and of vitamin B₆, 7.1±17.3 μg.

Persons who developed incident AD were older and had less education. A higher proportion of Hispanic and a lower proportion of white subjects, a higher proportion of subjects with the APOE ε₄ allele, and a higher proportion of subjects with diabetes, hypertension, heart disease, and stroke also developed incident AD. There were no differences between persons with and without incident AD in the energy-adjusted intake of vitamins B₆ and B₁₂ or in the use of supplements. Compared with those without AD, persons with AD had an energy-adjusted total folate intake that was almost statistically significantly lower (383.8 vs 407.5 μg; P=.09).

The risk of AD decreased with the increasing quartile of total folate intake (Table 2), and this association was statistically significant after adjustment for intake of vitamins B₆ and B₁₂. The association between dietary folate intake and AD was not statistically significant; the hazard ratio (HR) for the fourth quartile of dietary folate intake was 0.8 (95% confidence interval [CI], 0.5-1.2; P=.25 for trend) in the full model. Intake of folic acid supplements alone was not related to the risk of AD (HR, 1.0; 95% CI, 0.7-1.4). When only intake of high-dose supplements of folic acid (≥400 μg) was considered, the asso-
The association between folic acid intake and AD remained non-significant (HR, 0.7; 95% CI, 0.5-1.2) but in a direction suggesting a lower risk.

Total intakes of vitamin B_6 (Table 3) and B_12 (Table 4) were not related to the risk of AD in any of the models. Secondary analyses stratified by sex, age, categorical by the median, APOE ε4 allele, and diabetes history showed no evidence of interaction.

There was a modest correlation of total folate intake to lower homocysteine levels ($r = -0.1; P = .05; n = 579$) and higher serum folate levels ($r = 0.2; P < .001; n = 460$) and a moderate inverse correlation of serum folate with ho-
creatinine levels \( r = 0.4; P < .001; n = 453 \), indirectly suggesting that a lower homocysteine level is a potential mechanism for the association between higher folate intake and a lower AD risk. There was also a modest correlation of vitamin B\(_12\) intake with lower homocysteine levels \( r = -0.1; P = .04; n = 579 \) and serum vitamin B\(_6\) levels \( r = 0.1; P = .06; n = 460 \) and a moderate correlation of serum vitamin B\(_{12}\) with homocysteine levels \( r = -0.4; P < .001 \). Vitamin B\(_6\) intake had a modest correlation with lower homocysteine and higher serum vitamin B\(_6\) levels \( r = 0.3; P < .001 \), and serum vitamin B\(_6\) was negatively correlated with homocysteine levels \( r = -0.2; P < .001 \).

Our previous report\(^{26}\) detected a weak, nonsignificant association of high plasma homocysteine level with a higher risk of AD, the putative mechanism linking folate level and AD. We replicated our previous results\(^{26}\) in 579 individuals with dietary and homocysteine data. In this group, the logarithm-transformed homocysteine level was related to AD as a continuous variable (HR, 1.9; 95% CI, 1.1–3.5) without adjustment. This association attenuated markedly after adjustment for age (HR, 1.4; 95% CI, 0.7–2.5) and disappeared after adjustment for sex, education, ethnic group, and APOE \(\varepsilon4\) allele. In analyses by homocysteine quartile, only the fourth quartile was associated with a higher AD risk (HR, 1.5; 95% CI, 1.1–2.2) without adjustment, but this was attenuated by adjustment for age (HR, 1.3; 95% CI, 0.9–1.9) and for sex, education, ethnic group, and APOE \(\varepsilon4\) allele (HR, 1.2; 95% CI, 0.8–1.8). We added homocysteine level to the fully adjusted model relating vitamin intake and AD and the results were unchanged. The HR relating the fourth quartile of folate intake to AD was 0.5 (95% CI, 0.2–0.9; \( P = .07 \) for trend).

**COMMENT**

We found that higher total folate intake was independently related to lower AD risk in a predominantly Hispanic and African American cohort of elderly persons with a high prevalence of vascular risk factors. Intake of vitamins B\(_6\) and B\(_{12}\) was not related to the risk of AD.

The putative culprit of AD is the accumulation of intracellular and extracellular amyloid-\(\beta\) in the brain.\(^{27}\) In vitro, homocysteine potentiates the effects of amyloid-\(\beta\) on calcium influx and apoptosis.\(^{28}\) Animal models show that folate deficiencies and high homocysteine impair DNA repair in hippocampal neurons and make them susceptible to amyloid-\(\beta\) toxicity.\(^{29}\) Lower serum concentrations of folate, which increase levels of homocysteine but not vitamins B\(_{12}\) and B\(_6\), are correlated with cerebral atrophy on autopsy.\(^{30}\) Another potential pathway for AD pathogenesis is cerebrovascular disease,\(^{31-33}\) for which elevated homocysteine levels may be a risk factor.\(^{34,35}\) Higher folate intake is related to a lower stroke risk,\(^{36}\) presumably by decreasing homocysteine levels.

There are conflicting cross-sectional and prospective data on the association of vitamin B and folate with dementia and cognition.\(^{37-41}\) Homocysteine levels greater than 1.9 mg/L (\( > 14 \) \( \mu \)mol/L) doubled the risk of AD in the Framingham study,\(^{4}\) but there was no relation between the plasma levels of folate and vitamins B\(_6\) and B\(_{12}\) and the risk of AD. Another prospective study of subjects older than 55 years found no association between homocysteine levels and cognitive decline.\(^{42}\) Our previous study\(^{26}\) found that the association between elevated homocysteine levels and AD was confounded by age, and that there was no association between a level of homocysteine greater than 1.9 mg/L (\( > 14 \) \( \mu \)mol/L) and the risk of AD. We replicated those results in the sample for this study. Others have also reported that elevated homocysteine levels are related to cognitive decline\(^{43-46}\) and higher dementia risk,\(^{47}\) but not consistently.\(^{7,48,49}\)

Low levels of serum folate, but not of other vitamins, may increase the risk of AD,\(^{50,7,50}\) as seen in our study. Plasma levels and dietary intake of folate and vitamins B\(_6\) and B\(_{12}\) have been reported to be inversely related to cognitive decline,\(^{44}\) but only the association for folate persists after adjustment for vitamins B\(_6\) and B\(_{12}\) levels. Surprisingly, a higher risk of cognitive decline has been reported in persons with a higher intake of dietary folate and supplementary folic acid.\(^{9}\) In 1 study,\(^{8}\) persons with low vitamin B\(_{12}\) or folate levels (vitamin B\(_{12}\) level, \( \leq 203 \) pg/mL \([\leq 150 \) pmol/L\]); folate level, \( \leq 4.4 \) ng/mL \([\leq 10 \) nmol/L\]) had twice the risk of developing AD compared with individuals with higher levels, but another study\(^{51}\) with a longer follow-up showed no increased risk of AD for persons with low vitamins B\(_{12}\) levels. An uncontrolled trial of folic acid and cyanocobalamin treatment in 33 persons with dementia and evidence of deficiency observed improved cognitive scores.\(^{52}\) Cyanocobalamin supplementation was accompanied by improved language and frontal lobe function test results in the patients with cognitive impairment but not dementia in 1 study.\(^{53}\) However, a randomized trial of 60 individuals

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**Table 4. Relation of Quartiles of Energy-Adjusted Total Vitamin B\(_{12}\) Intake to Incident AD**

<table>
<thead>
<tr>
<th>Quartiles of Vitamin B(_{12}) Intake, µg</th>
<th>No. at Risk</th>
<th>No. of Cases (Rate per 100 Person-years)</th>
<th>Model, HR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3.5</td>
<td>242</td>
<td>52 (3.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>3.5-6.6</td>
<td>241</td>
<td>44 (2.9)</td>
<td>0.8 (0.5-1.2)</td>
</tr>
<tr>
<td>6.7-13.5</td>
<td>240</td>
<td>48 (3.6)</td>
<td>1.0 (0.6-1.4)</td>
</tr>
<tr>
<td>&gt;13.5</td>
<td>242</td>
<td>48 (3.2)</td>
<td>1.0 (0.6-1.4)</td>
</tr>
</tbody>
</table>

*Abbreviations: See Table 2.*

*Models 1 through 3 are described in the footnote to Table 2. Model 4 is also adjusted for levels of vitamin B\(_6\) and folate.*
with low vitamin $B_12$ levels found no improvement in cognitive performance in the intervention group (who received intramuscular cyanocobalamin) compared with placebo.54

Our results are consistent with those of studies suggesting that higher intake of folate is related to a lower risk of AD, and that intake of vitamins $B_12$ and $B_6$ is not related to or is not as important to the risk of AD. We found this association for total (dietary and supplement) folate intake, but not for dietary or supplement sources alone, suggesting that what is important is the total cumulative intake of folate from both sources. To our knowledge, ours is the first published study to associate homocysteine-related vitamins and AD in a cohort that is predominantly African American and Caribbean Hispanic.

We postulated that the main putative mechanism relating folate intake and AD risk was homocysteine level. However, the inverse correlation between vitamin $B_12$ and homocysteine levels was stronger than that for folate and homocysteine levels, and the association between folate intake and AD was independent of homocysteine level. Thus, we must consider whether there are mechanisms relating folate intake to AD independent of homocysteine level, which we cannot address.

We found that the association between higher folate intake and lower AD risk became stronger and statistically significant after adjusting for intake of vitamins $B_12$ and $B_6$, which indicates negative confounding. This effect was particularly driven by vitamin $B_12$. We believe that the explanation for this negative confounding is that dietary vitamin $B_12$ comes primarily from animal sources, whereas folate comes primarily from vegetable sources.55 We recently found that a diet with a higher content of vegetables and a lower content of meats was associated with a lower risk of AD.56 A diet richer in meats has a higher vitamin $B_12$ content, whereas a diet richer in vegetables has a higher folate content.56 It is possible that higher vitamin $B_12$ intake is a marker of an unhealthy diet and a negative confounder, which could explain our results. Given the inherent measurement error that is common in dietary studies,53 it is possible that we could not adjust properly for the dietary sources of vitamin $B_12$ and folate, resulting in residual confounding. Thus, our results have 2 potential explanations: folate has a beneficial association with AD that is unmasked by adjusting for vitamin $B_12$ intake, or folate intake is a marker of a more healthy diet and does not have an independent association with AD.

Another explanation is that the relation between a higher folate intake and a lower AD risk could be due to confounding by socioeconomic or lifestyle factors. Persons who take vitamin supplements are better educated, and education in turn is related to a lower risk of AD.57 However, intake of folic acid was not in itself related to a lower AD risk. In addition, adjusting for education and stratification of the analyses within ethnic groups did not change the results. The negative findings for vitamins $B_6$ and $B_12$ could be the result of regression dilution bias secondary to measurement error. We had only a single measurement of dietary intake and could not account for individual variability; this could have resulted in underestimation of the associations assuming nondifferential misclassification. Our semiquantitative food frequency questionnaire had 61 items, less than other questionnaires, and this likely resulted in underestimation of nutrient intake. It is also possible that the association between total folate intake and a lower risk of AD is due to chance in the setting of multiple comparisons. However, the association was in the hypothesized direction and followed a dose-response pattern, and correlations of folate and homocysteine levels were in the expected direction.

Our study has several strengths. We used a standard research diagnosis of AD. We excluded persons with prevalent dementia, which prevented findings that were due to dietary changes after dementia onset. We also adjusted for energy intake, which is an important confounder in dietary studies13 and is related to a higher risk of AD in our cohort.58

It is important to point out that 98% of the dietary questionnaires were obtained during or before 1996, prior to the implementation of folate fortification of grain.5 Folate deficiency is less common now, and our results apply only to the time before folate fortification. These results may not be reproduced in more recent studies.

Another important consideration is that the cohort in this study consists of subjects 65 years or older, with a high prevalence of vascular risk factors, and the results should be interpreted in this context. For example, in the Framingham homocysteine study sample,5 who were comparable to our subjects in age, the prevalence of diabetes was approximately 11% compared with 19.3% in our sample, and the proportion of persons with a homocysteine level greater than 1.9 mg/L (>14 µmol/dL) was 30% compared with 61.8% in our sample. These differences underline the fact that our sample had a higher burden of cardiovascular disease, which may affect the generalizability of our findings. The relationship between dietary factors in middle age and AD in later life is likely to be different than what we report because of biases related to survival and to changes in diet with aging.19

Finally, it is important to point out that this study is observational, that it is in conflict with another study9 showing an association of higher folate intake with cognitive decline, and that definitive conclusions about the value of higher folate intake in the prevention of AD cannot be made at this time. A trial of folic acid, pyridoxine, and cyanocobalamin in the secondary prevention of stroke found no benefit.7 In addition, there have been sobering examples of the lack of translation of apparent benefit in epidemiological data to clinical trials, such as the case of hormone therapy and dementia,50 in which the risk of dementia was increased in the intervention group. Thus, the decision to increase folate intake to prevent AD should await clinical trials.

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Correspondence: José A. Luchsinger, MD, Division of General Medicine, Columbia University, 630 W 168th St, PH9E-105, New York, NY 10032 (jal94@columbia.edu).
Author Contributions: Study concept and design: Luchsinger, Tang, and Mayeux. Acquisition of data: Mayeux. Analysis and interpretation of data: Luchsinger, Tang, and Mayeux. Drafting of the manuscript: Luchsinger and Tang. Critical revision of the manuscript for important intellectual content: all authors. Administrative, technical, and material support: Luchsinger, Tang, and Mayeux. Study supervision: Luchsinger, Tang, and Mayeux.
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**Announcement**

**Trial Registration Required.** In concert with the International Committee of Medical Journal Editors (ICMJE), *Archives of Neurology* will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. This policy applies to any clinical trial starting enrollment after July 1, 2005. For trials that began enrollment before this date, registration will be required by September 13, 2005, before considering the trial for publication. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorial by DeAngelis et al in the January issue of *Archives of Dermatology* (2005;141:76-77). Also see the Instructions to Authors on our Web site: www.archneurol.com.