Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun Study¹⁻³

David A Snowdon, Christine L Tully, Charles D Smith, Kathryn Perez Riley, and William R Markesbery

ABSTRACT
Background: Previous studies suggested that low concentrations of folate in the blood are related to poor cognitive function, dementia, and Alzheimer disease–related neurodegeneration of the brain.
Objective: Our aim was to determine whether serum folate is inversely associated with the severity of atrophy of the neocortex.
Design: Nutrients, lipoproteins, and nutritional markers were measured in the blood of 30 participants in the Nun Study from one convent who later died when they were 78–101 y old (X̄: 91 y). At autopsy, several neuropathologic indicators of Alzheimer disease were determined, including the degree of atrophy of 3 lobes of the neocortex (frontal, temporal, and parietal) and the number of neocortical Alzheimer disease lesions (ie, senile plaques and neurofibrillary tangles) as assessed by a neuropathologist.
Results: The correlation between serum folate and the severity of atrophy of the neocortex was −0.40 (P = 0.03). Among a subset of 15 participants with significant numbers of Alzheimer disease lesions in the neocortex, the correlation between folate and atrophy was −0.80 (P = 0.0006). Atrophy may be specific to low folate because none of the 18 other nutrients, lipoproteins, or nutritional markers measured in the blood had significant negative correlations with atrophy.
Conclusions: Among elderly Catholic sisters who lived in one convent, ate from the same kitchen, and were highly comparable for a wide range of environmental and lifestyle factors, low serum folate was strongly associated with atrophy of the neocortex. Definitive evidence for this relation and its temporal sequence awaits the findings of other studies.

KEY WORDS
Folate, folic acid, diet, nutrition, dementia, neocortex, Alzheimer disease, women, Nun Study

INTRODUCTION
Findings from case series and population-based studies have suggested that low concentrations of folate in the blood are related to dementia and to poor cognitive function in older adults (1–7). Because of the role of folate in reducing blood concentrations of homocysteine (8) and the association of homocysteine with vascular disease (7, 9–11), it has been hypothesized that folate’s relation to poor cognitive function may be due to its effects on the vascular system (7). Because folate plays an important role in the development of the central nervous system and in the metabolism of some neurotransmitters (12), low folate concentrations may also be related to dementia and to poor cognitive function through other nonvascular mechanisms.

The results of several studies indicate that inborn errors in the uptake and metabolism of folate can lead to degeneration of the central nervous system, atrophy of the cerebral cortex, and mental retardation in children [see reviews by Erbe (13) and Martin (14)]. Twenty years ago, findings from a case series of 16 folate-deficient older adults led investigators to conclude that “chronic folate deficiency could induce cerebral atrophy” (3). This observation and its possible relevance to the cerebral atrophy associated with Alzheimer disease has gone virtually unnoticed. However, recent findings by Clarke et al (15) indicated that serum folate has a strong negative association with the risk of Alzheimer disease. Findings from that study also indicated that the relation between low folate and Alzheimer disease may be due, in part, to folate’s role in reducing serum homocysteine.

Because Clarke et al reported that elevated concentrations of serum homocysteine are associated with progressive atrophy of the medial temporal lobe in subjects with Alzheimer disease, we decided to investigate the relation between serum folate and the severity of atrophy of the neocortex at autopsy. Because of their findings, we also hypothesized that low folate concentrations would have a strong association with atrophy of the neocortex only among those with an atrophying disease process such as Alzheimer disease. We conducted these analyses in a subset of 30 participants of the Nun Study, a longitudinal study of aging and Alzheimer disease (16–18).
SUBJECTS AND METHODS

Study population

Participants in the Nun Study are members of the School Sisters of Notre Dame religious congregation and live in communities in the midwestern, eastern, and southern United States. The design of this population-based longitudinal study was described in detail elsewhere (16–18). At the beginning of the study, there were 678 participants aged 75–102 y (3± 83 y). In 1993 blood was drawn from 95 sisters living in the Mankato, MN, convent who were aged 77–98 y (3± 86 y) (19). The CERAD (Consortium to Establish a Registry for Alzheimer’s Disease) battery of neuropsychologic tests was administered to these participants (20), including the Mini-Mental State Exam, which assesses global cognitive function. Thirty of the 95 sisters from whom blood was drawn subsequently died and these 30 participants constitute the primary sample of the present analysis. The Nun Study was approved by the University of Kentucky’s Institutional Review Board.

Nutrients, lipoproteins, and other nutritional markers

Blood was drawn after subjects had fasted overnight and was separated, refrigerated, and sent immediately to a laboratory for folate measurements. None of the samples hemolyzed before the laboratory determinations. Folate (metabolically active folic acid) was measured in serum by the Lactobacillus casei method (21, 22). Metabolically active vitamin B-12 and thiamine in whole blood and vitamin B-6 in serum were measured by protzoologic analysis (21, 22). As described in detail elsewhere (19), HPLC-based methods were used to measure concentrations of α-tocopherol and carotenoids (β-carotene, α-carotene, lycopene, zeaxanthin and lutein combined, and β-cryptoxanthin) (23). Plasma total cholesterol, HDL cholesterol, and triacylglycerol were measured enzymatically with a CX-5 autoanalyzer (Beckman Instruments, Fullerton, CA) (19) and LDL cholesterol was estimated by the Friedewald equation (24). After blood was collected in element-free tubes, serum zinc, copper, and magnesium concentrations were measured by atomic absorption spectrophotometry (25). Serum albumin and transthyretin were measured nephelometrically (26). Triceps skinfold thickness, an indicator of body fat, was measured with a standard skinfold caliper (27). Homocysteine concentrations in blood were not measured because the blood samples were exhausted before we considered such analyses.

Multivitamin supplement use at the time the blood was drawn was assessed by inspecting the vitamin pill containers that each participant brought to a medical exam. Multivitamin use in cognitively impaired participants was assessed by reviewing medication administration records and vitamin pill containers.

Atrophy of the neocortex

Gross examination of the participants’ brains was performed by one neuropathologist who was blinded to the participants’ nutritional status and cognitive test scores. After formalin fixation, the intact brain was examined by the neuropathologist, who rated the degree of atrophy of the frontal, temporal, and parietal lobes. (The occipital lobes were rarely atrophic.) The degree of atrophy was classified into 4 levels based on the degree of widening of the sulci and narrowing of the gyri in the 3 lobes. Severe atrophy was characterized by a high degree of widening of the sulci and narrowing of the gyri in ≥2 lobes, moderate and mild atrophy by widening of the sulci and narrowing of the gyri in only 1 or 2 lobes, and no atrophy by no noticeable widening of the sulci or narrowing of the gyri in any lobe. In the analysis, the measure of atrophy was given a score ranging from 3 for severe atrophy to 0 for no atrophy.

The measure of atrophy was validated by postmortem magnetic resonance imaging. Brains used in the folate analysis were not scanned because they were sectioned before scanning began in our study. However, brains of 35 other participants from the Nun Study were scanned after formalin fixation by a method similar to that described by Boyko et al (28) and Lamont et al (29). The analyses of the scanned brains indicated strong associations between the severity of atrophy of the neocortex and the volumes (cm³) of gray matter (r = −0.69, P < 0.0001) and white matter (r = −0.65, P < 0.0001) in the brain. The volumes of gray and white matter were based on the whole brain (excluding the brain stem and cerebellum). Had they been based on the volumes in the frontal, temporal, and parietal lobes of the neocortex, the correlations might have been even stronger because our measure of atrophy of the neocortex was based on those 3 lobes.

Quantitation of Alzheimer disease lesions

The numbers of neurofibrillary tangles, senile plaques, and neuritic plaques per mm² microscopic field were counted in Bielschowsky-stained sections. Neurofibrillary tangles were counted in the 5 microscopic fields with the highest numbers of tangles in the middle frontal gyrus (Brodmann area 9), inferior parietal lobule (areas 39/40), and middle temporal gyrus (area 21). Neuritic plaques and total senile plaques (which include both neuritic plaques and diffuse plaques) and were counted in the same regions in the 5 fields with the highest numbers of senile plaques.

The neuropathologic data were used to identify a subset of participants who had significant numbers of Alzheimer disease lesions. Each member of the subset had 1) abundant senile plaques in the frontal, temporal, or parietal lobe, ie, ≥16 senile plaques per mm² microscopic field in any lobe; 2) some neuritic plaques in ≥1 lobe; and 3) some neurofibrillary tangles in ≥1 lobe. These specifications are consistent with the number of senile plaques necessary to meet the neuropathologic criteria for Alzheimer disease described by Khachaturian (30) and with the findings of several studies indicating that the presence of neurofibrillary tangles in the neocortex is a potent marker of the symptoms of Alzheimer disease (17, 31–34).

Atherosclerosis and infarcts in the brain

The neuropathologist classified the degree of atherosclerosis in the major arteries at the base of the brain (circle of Willis), with moderate defined as atherosclerotic plaques present in 25–50% of the vessel wall and severe defined as atherosclerotic plaques in >50% of the vessel wall (17). Brain infarcts visible to the naked eye were identified by examining the intact brain and 2-cm thick coronal sections of the cerebral hemispheres, brain stem, and cerebellum (17). Sections for histopathologic examination were taken from all 4 lobes of the cerebral cortex, entorhinal cortex, hippocampus, amygdala, basal ganglia, thalamus, midbrain, pons, medulla, and cerebellar hemisphere. Microscopic infarcts were identified by reviewing hematoxylin- and eosin-stained sections with a light microscope (Optiphot-2; Nikon, Tokyo). In the present analysis, brain infarcts refer to infarcts of microscopic, lacunar, or larger size.
FOLATE AND ALZHEIMER DISEASE

Statistical methods

Spearman rank correlations and partial (age-adjusted) correlations were derived. \( P \) values for the differences in means were derived from \( t \) tests. All \( P \) values were two-tailed. All data analyses were done by using the SAS statistical software package (SAS Institute Inc, Cary, NC).

RESULTS

Primary sample

Among the 95 participants from whom blood was drawn, the mean (±SD) serum folate concentration of the 30 participants who died was 52 ± 54 nmol/L; that of the 65 survivors was 50 ± 43 nmol/L (\( P \) for difference: 0.73). The 30 participants who died constitute the primary sample of this report.

All 30 participants were white, born in the United States, and of European heritage. Seventy-three percent had attained at least a bachelor’s degree and 90% had been teachers most of their lives. The women died 2–55 mo (\( x \): 24 mo) after blood samples were drawn, when they were 78–101 y old (\( x \): 91 y).

Serum folate concentrations in the primary sample ranged from 5 to 181 nmol/L. Although some of the folate values were above the normal range for our laboratory (11–54 nmol/L), none were implausible for this particular bioassay (given the experience of the laboratory, in which this assay has been used for > 25 y). Seven of the 30 participants were taking multivitamin pills at the time the blood was drawn. The mean folate concentration of these participants was 104 nmol/L, compared with 36 nmol/L in those not taking supplements (\( P = 0.002 \)). Six of the 7 participants taking multivitamin pills had serum folate concentrations > 45 nmol/L, which may partly explain some of the relatively high folate values we observed.

All participants had lived in one convent and had eaten out of one kitchen; those requiring assistance with activities of daily living, such as feeding, had received nursing care from the same staff member. The mean serum folate concentration was 59 nmol/L in the group requiring assistance in feeding and 50 nmol/L in the group able to feed themselves (\( P = 0.74 \)). Furthermore, there was only a small, nonsignificant mean difference in triceps skinfold thickness between those requiring assistance in feeding and those able to feed themselves.

At autopsy, 43.3% of the participants had no atrophy of the neocortex, 30.0% had mild atrophy, 13.3% had moderate atrophy, and 13.3% had severe atrophy. A total of 50% (95% CI: 31%, 69%) of the 30 participants had significant numbers of Alzheimer disease lesions, ie, they met our neuropathologic criteria for Alzheimer disease. This is similar to the 58% prevalence of this neuropathologic condition observed in all 197 autopsies completed for the entire Nun Study during the same time period.

The mean blood nutrient concentrations and other primary variables for the subsets of participants with and without significant numbers of Alzheimer disease lesions in the neocortex are shown in Table 1. Except for senile plaques (\( P = 0.03 \)) and neurofibrillary tangles (\( P = 0.0001 \)), which were the variables used to define the subsets, there were no significant differences between the subsets in any of the variables described in Table 1.

TABLE 1

Mean blood nutrient concentrations and other primary variables in 30 participants in the Nun Study with or without significant numbers of Alzheimer disease lesions in the neocortex

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subset without significant numbers of lesions (( n = 15 ))</th>
<th>Subset with significant numbers of lesions (( n = 15 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (nmol/L)</td>
<td>61 ± 54</td>
<td>45 ± 52</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>128 ± 94</td>
<td>119 ± 58</td>
</tr>
<tr>
<td>Vitamin B-6 (nmol/L)</td>
<td>290 ± 65</td>
<td>319 ± 189</td>
</tr>
<tr>
<td>Thiamine (nmol/L)</td>
<td>148 ± 30</td>
<td>142 ± 36</td>
</tr>
<tr>
<td>α-Tocopherol (μmol/L)</td>
<td>24 ± 10</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>Carotenoids (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>652 ± 559</td>
<td>503 ± 242</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>298 ± 261</td>
<td>261 ± 130</td>
</tr>
<tr>
<td>Lycopene</td>
<td>205 ± 112</td>
<td>224 ± 149</td>
</tr>
<tr>
<td>Zeaxanthin and lutein</td>
<td>210 ± 90</td>
<td>250 ± 70</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>150 ± 110</td>
<td>140 ± 70</td>
</tr>
<tr>
<td>Magnesium (μmol/L)</td>
<td>824 ± 102</td>
<td>819 ± 117</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>11 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>19 ± 6</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>LDL cholesterol (nmol/L)</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>HDL cholesterol (nmol/L)</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol (nmol/L)</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.4 ± 0.9</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Nutritional markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin (μmol/L)</td>
<td>18 ± 6</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Albumin (μmol/L)</td>
<td>606 ± 152</td>
<td>606 ± 152</td>
</tr>
<tr>
<td>Triceps skinfold thickness (mm)</td>
<td>25 ± 4</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of education (y)</td>
<td>16 ± 2</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>Age at death (y)</td>
<td>90 ± 6</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>Alzheimer disease lesions in neocortex (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurofibrillary tangles</td>
<td>0.2 ± 0.5</td>
<td>12 ± 19</td>
</tr>
<tr>
<td>Senile plaques</td>
<td>8 ± 7</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Neuritic plaques</td>
<td>2 ± 2</td>
<td>3 ± 3</td>
</tr>
</tbody>
</table>

\( \bar{x} \) ± SD. Those with abundant senile plaques and some neurofibrillary tangles in the neocortex were classified as having significant numbers of Alzheimer disease lesions, ie, they met our neuropathologic criteria for Alzheimer disease. Folate (ie, metabolically active folic acid), vitamin B-6, magnesium, zinc, copper, transferrin, and albumin were measured in serum; vitamin B-12 and thiamine were measured in whole blood; α-tocopherol, carotenoids, LDL, HDL, total cholesterol, and triacylglycerols (as triolein) were measured in plasma; triceps skinfold thickness was the mean of 3 measures; and neurofibrillary tangles, senile plaques, and neuritic plaques were the mean number per mm² microscopic field, averaged over all fields in the frontal, temporal, and parietal lobes. Except for the number of neurofibrillary tangles and senile plaques, there were no significant differences between subsets.

Among all 30 participants, the age-adjusted correlation of serum folate with the severity of atrophy was −0.40 (\( P = 0.03 \)). (Spearman rank correlations were used in all analyses to reduce the likelihood of 1 or 2 extreme values producing the correlations.) To provide a larger context for the interpretation of the relation between low folate and atrophy, we also investigated the
The mean folate concentration was 39 nmol/L in those with minimal atherosclerosis (P = 0.07) to 0.35 for serum albumin (P = 0.006). In contrast, none of the other nutrients, lipoproteins, or nutritional markers were significantly correlated with the severity of atrophy of the neocortex (Table 2).

The strong negative correlation between serum folate and atrophy was shown in Figure 1. As shown in the figure, the data for 3 participants may have been statistical outliers because of these subjects’ high folate concentrations. When the data for these 3 participants were eliminated from the analysis, the correlation between folate and the severity of atrophy was −0.77 (P = 0.004), however, suggesting that the outliers were not responsible for the association between low folate and atrophy.

The severity of atrophy of the neocortex was significantly correlated with the mean number of neurofibrillary tangles in the neocortex in the subset of subjects with significant numbers of Alzheimer disease lesions (r = 0.54, P = 0.048). This finding is consistent with the strong association between neurofibrillary tangles in the neocortex and low cognitive function observed in our study (17). Serum folate was not significantly correlated with the mean number of neurofibrillary tangles in the neocortex (r = −0.14, P = 0.63), suggesting that the association between low folate and atrophy was not due to an effect of folate on the development of neurofibrillary tangles.

The inverse correlation between folate and atrophy was highly consistent across various subgroups; however, the number of participants in each subgroup was small. For example, the correlation was −0.90 (n = 7; P = 0.006) for those who died during the first half of follow-up and −0.72 (n = 8; P = 0.05) for those who died during the second half of follow-up. The correlation was −0.85 (n = 7; P = 0.02) for those in the bottom half of the distribution of Mini-Mental State Exam scores and −0.77 (n = 8; P = 0.03) for those in the top half of the distribution of scores. The correlation was −0.70 (n = 7; P = 0.08) for those in the bottom half of the distribution of neurofibrillary tangles in the neocortex and −0.83 (n = 8; P = 0.01) for those in the top half of the distribution of neurofibrillary tangles. The correlation was −0.72 (n = 9; P = 0.03) for those with moderate to severe atherosclerosis and −0.85 (n = 6; P = 0.03) for those with minimal atherosclerosis. Last, the correlation was −0.63 (n = 8; P = 0.10) for those with brain infarcts and −0.80 (n = 7; P = 0.03) for those without brain infarcts.

Regression analyses for the subset of 15 participants indicated that folate had a significant inverse association with cognitive function after adjustment for age and the number of neurofibrillary tangles in the neocortex (i.e., the severity of Alzheimer disease in the neocortex): a 10-nmol/L decrease in serum folate was associated with a 1-point decrease in the score for the Mini-Mental State Exam (P = 0.04). This association appeared to be meditated in part by atrophy of the neocortex because further adjustment for atrophy substantially reduced the point decrease on the Mini-Mental State Exam.

**DISCUSSION**

In this relatively small sample of 30 elderly Catholic sisters, serum folate had a strong negative association with the severity of atrophy through its relationship with cognitive function.
of atrophy of the neocortex. Although it may be difficult to generalize our findings in this unique population, many factors that confound most epidemiologic studies were eliminated or minimized in our study. Participants had the same reproductive and marital histories; had similar social activities and support; did not smoke or drink excessive amounts of alcoholic beverages; had similar occupations, income, and socioeconomic status; and had access to similar preventive, nursing, and other medical care services. Our findings are also consistent with findings in case series that suggest that folate deficiency is related to atrophy of the neocortex in children (13, 14) and in older adults (3).

Although folate had a strong, statistically significant negative association with atrophy, none of the other nutrients or nutritional markers examined had significant associations with atrophy in our study. These findings suggest that atrophy may be specific to relatively low folate concentrations. Nonetheless, this relation between low folate concentrations and atrophy needs to be confirmed in other longitudinal studies in which baseline folate concentrations can be related to the subsequent development of brain atrophy. This will help to determine whether the low serum folate concentrations actually preceded the atrophy.

Our study is unique because all participants lived in the same building and ate out of the same kitchen. However, there were wide variations in blood nutrient concentrations, as indicated by the SDs for folate and the other nutrients listed in Table 1. Some of the variation in blood nutrient concentrations reflects individual differences in multivitamin use and may also reflect drug-nutrient and disease-nutrient interactions or differences in the intake, absorption, storage, and metabolism of the nutrients. The finding that many of the participants with atrophy had serum folate concentrations within the reference laboratory range suggests that the optimal folate concentration may in fact be higher in old age or when diseases such as Alzheimer are present.

Folate was related to atrophy only among participants with a significant number of Alzheimer disease lesions in the neocortex. This finding suggests that folate may reduce the likelihood of atrophy only when an atrophying disease process such as Alzheimer disease is present. Assuming that the association between low folate concentrations and atrophy is causal, which was not proven in our study, low folate might relate to atrophy through several mechanisms acting independently or in combination. Because folate can reduce concentrations of homocysteine in the blood (7), and homocysteine is associated with vascular disease (7, 9–11), folate might act through its effect on the vascular system. This is consistent with the findings of Clarke et al (15), who found that elevated serum homocysteine concentrations were associated with progressive atrophy of the medial temporal lobe in subjects with Alzheimer disease. In our study, folate was negatively correlated with atrophy even in subgroups of participants with minimal atherosclerosis and without brain infarcts. This suggests that folate’s negative correlation with atrophy may not have been entirely due to vascular disease. Given the role of folate in the development of the central nervous system and in the metabolism of some neurotransmitters (12), folate may play a role in maintaining the integrity of the brain in late life through nonvascular mechanisms. However, there is no prospective evidence that supplemental folate will benefit the prevention or treatment of Alzheimer disease.

Overall, findings in this unique population of women suggest that relatively low folate concentrations may be related to atrophy of the neocortex, particularly in persons with significant numbers of Alzheimer disease lesions in the neocortex. Definitive evidence for this relation awaits the findings of other longitudinal studies.

This study would not have been possible without the spirited support of the members, leaders, and health care providers of the School Sisters of Notre Dame religious congregation. The following persons were also especially helpful: Sisters Mary Dominic Klaseus, Marlene Manney, Gabriel Mary Spaeth, and Rita Schwalbe; John Belcher; Mark Desrosiers; Huachen Liu; Ann Georgesen; Lydia Greiner; Myron Gross; James Mortimer; Nuwan Nanayakkara; Ela Patel; Gari-Anne Patzwald; Jeanne Ray; Cecil Runyons; Ann Tudor; and Hao Z Wang.