Heterogeneity and Lack of Good Quality Studies Limit Association Between Folate, Vitamins B-6 and B-12, and Cognitive Function1,2

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Abstract

We conducted a systematic review to evaluate the association between folate, vitamin B-6, vitamin B-12, and cognitive function in the elderly. Our search was conducted in Medline for English-language publications of human subjects from 1966 through November 2006; we supplemented these results with information from article reviews and domain experts. We included longitudinal cohort and case-control studies of B vitamins and analyses of cognitive tests or Alzheimer’s disease. We evaluated the quality and heterogeneity of study outcomes and assessed 30 different cognitive function tests. Of 24 studies that met eligibility criteria, 16 were determined to be of fair quality. A majority of the studies reviewed 2 or more B vitamins. Considerable heterogeneity was found among B-vitamin–level thresholds, comparisons, and data analyses. Six of 10 folate studies reported a significant association between low baseline blood folate concentrations and subsequent poor test performance in the global cognitive domain, and 4 of 9 folate studies found associations between low blood folate concentrations and increased prevalence of Alzheimer’s disease. Studies did not reveal an association of vitamin B-6 and vitamin B-12 blood concentrations with cognitive-test performance or Alzheimer’s disease, nor was B-vitamin dietary intake associated with cognitive function. Higher plasma homocysteine concentrations were associated with poorer cognitive function. Although the majority of studies indicated that low blood folate concentrations predicted poorer cognitive function, data supporting this association were limited because of the heterogeneity in cognition-assessment methodology, and scarcity of good quality studies and standardized threshold levels for categorizing low B-vitamin status. J. Nutr. 137: 1789–1794, 2007.

Introduction

An increasing worldwide prevalence of dementia is a public health concern (1). Age-related cognitive changes include benign memory loss, cognitive impairment, and dementia. Alzheimer’s disease is a progressive dementia and also a disabling health condition in the elderly. B vitamins play an essential role in the maintenance of normal nervous system function (2). The elderly are potentially vulnerable to compromised B-vitamin status due to age-related changes in absorption and metabolism and insufficient dietary intake (3,4). Compromised B-vitamin status, in particular, folate and vitamin B-12, results in elevated homocysteine levels and may cause cognitive decline through neurotoxic and vasotoxic effects (5).

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Proposed mechanisms for the neurotoxic consequences of compromised B-vitamin status and hyperhomocysteinemia include hypomethylation of nucleic acids and neurotransmitters, increased oxidative stress, and increased β amyloid synthesis (6). Elevated homocysteine levels may also directly overstimulate N-methyl-D-aspartate receptors resulting in neurotoxicity due to calcium influx and apoptosis. Folate and vitamin B-12 are essential for methionine synthesis and to the formation of a universal methyl donor, S-adenosylmethionine, important to the generation of neurotransmitters, phospholipids, and myelin (2,7).

Given the complexity of the mechanisms related to cognitive changes in the elderly, and the metabolic relationship between B vitamins and homocysteine levels, the association between B-vitamin status and subsequent cognitive function remains unclear. This systematic review of observational studies investigates the association between blood levels or dietary intake of B vitamins and the risk and progression of neurocognitive deficit as measured by cognitive tests and age-related neurodegenerative disorders such as Alzheimer’s disease in humans. The terms
Methods

We searched Medline and the Commonwealth Agricultural Bureau (CAB) abstracts for English-language publications on B vitamins from 1966 through November 2006. We conducted a comprehensive literature search on all B vitamins on 2 February 2005; an update, limited to folate, vitamin B-6, and vitamin B-12, was performed on 26 November 2006. Search terms included common and chemical names for the B vitamins of interest, including folic acid, folate, pteroylglutamic, folacin, cobalamin, cyanocobalamin, pyridoxine, pyridoxal, pyridoxamine, vitamin B-6, vitamin B-12, and terms related to age-related neurocognitive diseases, including nervous system diseases, cognitive disorders, memory, delerium, amnestic, neurodegeneration, dementia, Alzheimer, Lewy body, brain, neuron, and nerve cell. Additional studies were sought from neurology and vitamin research experts and from reference lists of included articles and selected reviews.

We included longitudinal cohort and case-control studies of human adult participants that evaluated blood concentrations or dietary intake of B vitamins with the risk or odds of outcomes related to diagnosis or degree of severity of Alzheimer's disease and performance on cognitive function tests. We excluded studies that focused on mental retardation, Wernicke's encephalopathy, subacute combined degeneration, acute encephalopathy, and evaluations of outcomes related to depression and other psychiatric conditions. Studies of vascular and mixed causes of dementia lacking separate analyses for Alzheimer's disease were excluded. We excluded studies that limited their analyses to homocysteine and did not evaluate the role of B vitamins on tests of cognitive function and Alzheimer's disease. Studies of cross-sectional designs or studies that provided only cross-sectional correlation data (8) and B-vitamin intervention trials (9) were excluded from our review and are discussed elsewhere.

A single reviewer performed data extractions of each accepted article and a second reviewer independently verified the data. Discrepancies were resolved through consensus. We evaluated all study outcomes relevant to Alzheimer's disease, cognitive impairment, or cognitive-function tests.

Study quality. A 3-category grading system was used to denote the quality of each study included in our review (8,10). Each study was graded by at least 2 people, and disagreements were resolved through consensus.

Good: Results were valid without obvious major bias. The study provided a clear description of the population including a description of cases and comparison groups, settings, B-vitamin measurement technique and status, and used appropriate outcome measurement (e.g., diagnosis of Alzheimer's disease using validated clinical criteria and/or histopathological methods), appropriate statistical and analytic methods (including adjustment for other risk factors such as homocysteine and ApoE), had no reporting errors, had <20% dropout, and clearly reported reasons for dropouts.

Fair: The study had some deficiencies or was susceptible to some bias but not major bias. The study might be missing information, making assessment of the limitations and potential problems difficult, but omissions were not sufficient to invalidate the results. This category included studies that did not meet all the criteria in the "good" category.

Poor: Significant bias was present that could invalidate results. Study had serious errors in design, analysis, or reporting, or had large amounts of missing information or discrepancies in reporting.

Results

The search of Medline and CAB Abstracts yielded 6921 citations including human, animal, and in vitro studies. After a manual screening of the titles and abstracts, 304 articles on human studies of B vitamins were retrieved. An additional 17 human studies were identified from review articles, study reference lists, and domain experts. Of these, 24 longitudinal cohort or case-control studies (11–34) met eligibility criteria (Fig. 1). These included 2 publications (21,27) on the same study subjects reported in prior publications by Quadri et al. (20) and Clarke et al. (26). The overall evidence for the studies meeting the review criteria on the association of B-vitamin concentrations or B-vitamin intake and cognitive function is summarized in Table 1. Meta-analyses of the associations between B vitamins
and cognitive deficit could not be performed due to heterogeneity in B-vitamin level thresholds and comparisons because of 30 different cognitive-testing methods and data analyses. Additional sources of heterogeneity in these studies included differences in biological sample (plasma, serum), sample types (fasting, nonfasting), and assay techniques (microbial assay, radioassay).

**Blood folate concentrations and cognitive-test performance.** Ten studies (8 prospective cohort, 1 retrospective cohort, and 1 case-control), of which 8 were graded fair quality and 2 were graded poor quality, evaluated cognitively intact or impaired aging subjects over a 3–8 y period (11–20) (Table 2). Sample sizes ranged from 30 to 700 subjects, and the threshold for defining low blood folate concentrations ranged from 3.6 to 27 nmol/L. Cognitive-test scores were compared among groups of subjects based on predetermined baseline blood folate–concentration categories. Four studies compared high mean folate blood concentrations with low (11,12,14,17), 5 studies used quantile comparisons (13,15,16,19,20); and 1 study reported only qualitative data (18).

For subjects in the lowest baseline blood folate quantiles, studies reported significantly increased poor performance in subjects at follow-up than subjects in global composite score or global cognitive test (12,15,17,19,20) or in the visual motor integration subtest (14). Notably, associations remained significant in 2 studies [Quadri et al. and Tucker et al. (14,20)] that adjusted their analyses for other B vitamins and plasma homocysteine levels. One study reported that poorer performance in a single global cognitive test was associated with lowest folate levels both at baseline and follow-up (15). In 3 studies (14,15,17), the lowest baseline blood folate concentrations were associated with increased rates of decline in cognitive-test performance over a period of 3–6 y.

Low baseline blood folate concentration was consistently associated with poorer global cognitive domain test performance at follow-up. Studies used several types of instruments to assess the same cognitive domain and the definition of low folate status was not consistent across studies.

**Blood concentrations of vitamin B-6 and cognitive-test performance.** Two prospective cohort studies evaluated the association between vitamin B-6 blood concentrations and cognitive-test performance in cognitively intact aging subjects over a 3–7 y period (Table 2) (12,14). The studies had 499 and 321 subjects, were graded fair quality, but each evaluated the association of different pyridoxal-5'-phosphate (PLP) thresholds (35.2 and 86.1 nmol/L) and a wide range of cognitive domains.

Neither study reported a significant association between blood concentrations of vitamin B-6 and 6 different cognitive subtests with the exception of 1 subtest assessing a spatial copying score ($P < 0.01$); in this case, low baseline vitamin B-6 concentrations were associated with poorer test scores at follow-up (14). However, this association was no longer significant after adjusting for other B-vitamin levels and homocysteine. Overall, the limited data did not support an association between blood vitamin B-6 levels and cognitive deficit.

**Blood concentrations of vitamin B-12 blood and cognitive-test performance.** Eight studies (7 prospective cohort and 1 case-control) evaluated the association of vitamin B-12 blood concentrations and cognitive-test performance in cognitively intact or impaired aging subjects; the follow-up period ranged from 3 to 8 y (Table 2) (11,12,14–18,20). Sample sizes ranged from 80 to 700 subjects. Six studies were graded fair quality and 2 were of poor quality. The 8 studies evaluated thresholds of low vitamin B-12 concentrations that ranged from 221.4 to 516.6 pmol/L, and analyses were adjusted for a variety of confounders. Four studies compared high and mean blood concentrations of vitamin B-12 (11,12,14,17), 3 studies used quantile comparisons (15,16,20), and 1 study (18) reported only qualitative data.

Two of the 8 studies reported a significant positive association in memory and visual motor integration subtests at follow-up in subjects with baseline vitamin B-12 blood levels (11,14). One of these studies reported that significance did not persist after adjusting for other B vitamins and plasma homocysteine levels (14); the other study [Elias et al. (11)] did not report analyses adjusted for plasma homocysteine levels. The rate of change in cognitive-test performance was analyzed in 4 studies. Lower baseline blood concentrations of vitamin B-12 did not predict rate of cognitive decline (14–17).

There was no discernible pattern of association between vitamin B-12 blood concentrations and changes in performance in any particular cognitive domain. A significant association was attenuated after adjusting for confounders. Vitamin B-12 blood concentrations were not associated with cognitive decline over time.

**Blood concentrations of vitamin B and Alzheimer’s disease.** Ten studies (5 prospective cohort and 5 case-control studies) evaluated the risk of incident or prevalent Alzheimer’s disease with blood concentrations of vitamin B (Table 3) (13, 20,22–26,28,29,34). One study was rated good, 7 fair, and 2 poor. One study evaluated all 3 B vitamins (24); 7 studies evaluated both folate and vitamin B-12 (20,22,23,25,26,29,32); 1 case-control study evaluated only vitamin B-6 levels (34); and 1 prospective cohort evaluated only folate levels (13). The 5 prospective cohort studies included sample sizes ranging from 30 to 700 subjects. Five studies were graded good quality, 3 were graded fair quality, evaluated cognitively intact or impaired status and lower folate blood levels (12–15, 17, 19, 20).

6 Of 10 studies of blood folate levels and 7 of 24 cognitive tests found a consistent association with cognitive function (mostly in global cognitive domain) in people with cognitively intact or impaired status and lower folate blood levels (12–15, 17, 19, 20).

3 Three of 12 studies of blood folate found a consistent association with Alzheimer’s disease among subjects with lowest blood folate levels (20, 23, 26).

### TABLE 1 Summary of observational studies evaluating the association of B vitamins with neurocognitive outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total studies</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
<th>Total participants</th>
<th>Blood or intake levels of vitamin B-6 or B-12</th>
<th>Low blood folate concentration</th>
<th>High folate intake levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive-test performance</td>
<td>12</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>7343</td>
<td>No association</td>
<td>Association with worse outcome</td>
<td>Association equivalent</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>12</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>5709</td>
<td>No association</td>
<td>Association with worse outcome</td>
<td>No association</td>
</tr>
</tbody>
</table>

1 Six of 10 studies of blood folate levels and 7 of 24 cognitive tests found a consistent association with cognitive function (mostly in global cognitive domain) in people with cognitively intact or impaired status and lower folate blood levels (12–15, 17, 19, 20).

2 Of 2 studies, 1 found a worse outcome in global cognitive function measured as the mean of 4 subtest scores (30), whereas the 2nd study found an improved test performance in 1 of 6 cognitive subtests assessed (14).

3 Three of 12 studies of blood folate found a consistent association with Alzheimer’s disease among subjects with lowest blood folate levels (20, 23, 26).
350 to ~1100 cognitively intact or impaired subjects followed over a 2–8 y period. The 5 case-control studies included 618 subjects with Alzheimer’s disease and 535 controls. Three studies compared high and low mean blood levels of vitamin B (22,23,34), 3 studies used quantile comparisons (13,20,26,28,29), and 2 studies reported qualitative data (24,29). Alzheimer’s disease was determined by validated clinical criteria in all studies; in addition, 2 studies utilized brain imaging and/or histopathology when available (24,26).

Significant associations between blood levels of vitamin B-6 and vitamin B-12 and Alzheimer’s disease or dementia were not found. Whereas significant associations were reported in 4 of 9 studies between low blood folate levels and an increased risk for Alzheimer’s disease or dementia (20,23,26,29).

**Vitamin B intake and cognitive function.** Two prospective cohort studies, of good and fair quality evaluated associations between dietary intake of all 3 B-vitamins and cognitive-test performance (14,30). A third poor quality prospective cohort study evaluated only vitamin B-6 intake in association with cognitive-test performance (31). Sample sizes ranged from 90 to 3700, mostly of cognitively intact subjects with follow-ups of 6 mo to 3 y. Dietary intakes of food and supplements were assessed through FFQ. The fair study, by Tucker et al. (14), reported that among 6 different cognitive function subtests, significant associations with B-vitamin intake were not found, with the exception of the construction praxis spatial copying score ($P < 0.05$), where better cognitive-test performance was associated with increased B-vitamin intake. The good quality study, by Morris et al. (30), reported a slower decline in cognitive-test performance over a 6-y period in subjects with high vitamin B-12 intake but faster decline among subjects with a high folate intake (>400 µg/d) from either food sources or supplements. The poor quality study, by Deijen et al. (31), reported no association between vitamin B-6 intake and changes in cognitive function.

Dietary intakes of B vitamins and incidence or prevalence of Alzheimer’s disease were evaluated in 2 studies: 1 prospective cohort, graded as fair, and 1 case-control, graded as poor (32,33). Mizrahi et al. (32) reported lower dietary intakes of folate and vitamin B-6 among subjects with Alzheimer’s disease than among normal controls, whereas Morris et al. (33) reported no significant association between intakes of all 3 B vitamins and the incidence of Alzheimer’s disease over a 4-y period.

Overall, these limited data do not support an association between vitamin B-6 intake and changes in cognitive function.

**Plasma homocysteine concentrations and cognitive function.** Six of 10 studies that evaluated blood concentrations of vitamin B and cognitive-test performance also reported an association between plasma homocysteine concentrations and cognitive function (11,12,14,15,17,20). Five of the studies were graded as

### TABLE 2 Characteristics of observational studies of blood concentrations of B vitamins associated with decreased cognitive function

| Study, year, design, location, source | Follow-up | Baseline cognition | Age | Total | Study quality | Metric | Cognitive tests | B-vitamin association
|--------------------------------------|-----------|--------------------|-----|-------|--------------|--------|----------------|----------------------|
| Elias 2005, PC, US (11)              | y         | Normal             | ≥60 | 705   | Fair         | $\beta$-coefficient | Global composite score; memory subtests | Folate: no association B-12: <330* vs. ≥330
| Kado 2005, PC, US (12)               | 7         | Normal             | >74 | 370   | Fair         | Risk ratio | Combined tests for multiple cognitive domains | Folate: ≤19.6* vs. >19.6 B-6; B-12: no association
| Maxwell 2002, PC, Canada (13)       | 5         | Normal             | >65 | 243   | Fair         | OR      | Modified mini-mental state examination (3 MS) | Folate: Lower 3 quartile combined vs. ≥14.1
| Tucker 2005, PC, US (14)            | 3         | Normal, all men    | 67  | 284   | Fair         | $\beta$-coefficient | Constructional praxis; verbal fluency; working, recall memory; Mini-Mental State Examination | Folate: ≥26.1* vs. >26.1 B-6; B-12: no association
| Nurk 2005, PC, Norway (15)          | 6         | Cognitive impaired | 72  | 235   | Fair         | Risk ratio | Kendrick Object Learning Test (KOLT) score ≤25 | Folate: (quintile): <8.6* vs. 14.5; B-12 (quintile): no association
| Kang 2006, PC, US (16)              | 4         | Normal, all women  | 63  | 391   | Fair         | Rate of decline | Telephone interview; verbal; global | Folate: B-12 (quartile): no association
| Moinjaart 2005, PC, Netherlands (17)| 4         | Normal             | >85 | 341   | Poor         | Rate of change | Global function; multiple tests | Folate: (range): 9.1–14.1**; B-12 (range): no association
| Jones 2002, PC, Sweden (18)         | 3         | Preclinical AD     | >75 | 230   | Poor         | Rate of decline | Mini-Mental State Examination | Folate: B-12: no association
| Snowden 2000, RC, US (19)           | nd        | AD, all women      | 91  | 30    | Fair         | Rate of decline | Mini-Mental State Examination | Folate: 10.0*
| Quadri 2004, 2005, CC, Switzerland(20, 21) | —       | Cognitive impaired; normal | >79 | 81    | Fair         | OR      | Mini-Mental State Examination; Clinical Dementia Rating (CDR) 0.5 | Folate: (Tertile); Folate <6.0** v 19.5 B-12: no association

1 Includes folate, nmol/L; vitamin B-6, nmol/L; and vitamin B-12, pmol/L concentrations. Asterisk indicates association with worse cognitive tests, *$P < 0.05$; **$P < 0.01$; no asterisk, $P ≥ 0.05$.
2 PC, prospective cohort; AD, Alzheimer’s disease; RC, retrospective cohort; nd, not documented; CC, case control.
3 Visual-Immediate and delayed recall, Logical Memory-Immediate and delayed recall.
4 Including language, memory, conceptualization, and visuospatial ability.
5 Only low blood folate concentrations predicted worse test performance in 1 of the subtests. Construction praxis after analyses adjusted for plasma homocysteine levels.
6 Rate of cognitive-test performance for increase in blood folate concentration was associated with only global cognitive function.
fair and 1 study was graded as poor. Two studies reported no association (12,17) and 4 (3 cohort and 1 case-control) reported a significant association between poor cognitive-test performance at follow-up in subjects with higher baseline plasma homocysteine concentrations after adjusting the analyses for baseline blood concentrations of vitamin B (11,14,15,20).

Among the 10 studies that assessed the association between B vitamins and Alzheimer’s disease, 6 studies assessed the association between homocysteine levels and Alzheimer’s disease (20,23–26,28). Two studies reported no association (25,28), and 4 studies (20,23,24,26) (2 cohort and 2 case-control) reported an association between higher baseline homocysteine levels and an increased risk for Alzheimer’s disease that persisted after adjusting for B-vitamin blood concentrations.

**Discussion**

Our review highlights a substantial degree of heterogeneity in the published literature evaluating the association of B vitamins with cognitive-test performance or Alzheimer’s disease. In addition, the evidence has limitations due to the lack of good-quality studies. Comparisons among studies were difficult, because most of the B-vitamin association studies failed to report definitions of normal B-vitamin ranges for the elderly and varied in their outcome measures such as methylmalonic acid (MMA) and holotranscobalamin. Thus, any associations between B-vitamin status and cognitive function would not be expected to be biologically independent of homocysteine or folate status. The 3 B vitamins (folate, vitamin B-6, and vitamin B-12) are involved in the metabolism of homocysteine and thus any associations between B-vitamin status and cognitive function would not be expected to be biologically independent of homocysteine. Notably, studies that reported a significant association between low blood folate and poor cognitive performance also reported an association between increased plasma homocysteine concentrations and decreased performance on cognitive tests or an increased prevalence of Alzheimer’s disease. However, our review did not evaluate the potential association of homocysteine alone on cognitive deficit.

The evidence from longitudinal cohort and case-control studies suggests that there is no significant association between blood concentrations or the dietary intake of vitamin B-6 and B-12 and cognitive-test performance or Alzheimer’s disease. Although 25% of the studies reported higher vitamin B-12 blood concentrations to be associated with better cognitive-test performance, no consistent pattern of association with a particular cognitive domain was reported, or else the positive association was attenuated after adjusting the analyses for confounders.

The studies of dietary folate intake and cognitive-test performance were equivocal. One study, graded as good (30), reported an increased rate of cognitive decline with increased folate intake, whereas another study, graded as fair, reached the opposite conclusion (14). The clinical significance of this effect remains unclear because the former study found a poorer outcome in global composite score measured as the mean of 4 subtests scores, whereas the latter found an improved test performance in 1 of 5 cognitive subtests.

Approximately 66% of the B-vitamin association studies reported data on homocysteine or adjusted the B-vitamin analyses for homocysteine levels. The 3 B vitamins (folate, vitamin B-6, and vitamin B-12) are involved in the metabolism of homocysteine and thus may have associations with vitamin status and cognitive function that were not expected to be biologically independent of homocysteine. Notably, studies that reported a significant association between low blood folate and poor cognitive performance also reported an association between increased plasma homocysteine concentrations and decreased performance on cognitive tests or an increased prevalence of Alzheimer’s disease. However, our review did not evaluate the potential association of homocysteine alone on cognitive deficit.

Future research endeavors are necessary to evaluate a threshold level for low concentrations of vitamin B that are associated with poorer test performance in the different cognitive domains. Because blood concentrations of the B vitamins may not be a good indicator of their concentrations in tissues or cells, additional outcome measures should include biomarkers of B-vitamin status, such as methylmalonic acid (MMA) and holotranscobalamin. When possible, ongoing and future research should implement...
suggestions generated from our review with regard to the measurement of B vitamins and the assessment of cognitive function.

In our previous systematic review of B-vitamin supplement trials, we reported that the evidence remains inconclusive, although folic acid supplementation may benefit specific populations and age groups (9). However, firm conclusions were not possible because of the small number of trials and participants. Thus, the analyses of both trial and cohort studies failed to support any associations between blood concentrations, dietary intake, or supplementation with vitamin B-6 or vitamin B-12 and neurocognitive outcomes, and showed inconsistent associations with folate.

In conclusion, data supporting an association between low blood concentrations of the B vitamins and increased risk for, or progression of, poor neurocognitive outcomes is limited due to the absence of good-quality studies, heterogeneity in assessment methodology, and a lack of standardized threshold levels for categorizing low B-vitamin status. There are, at best, minimal data to support an association between vitamins B-6 and B-12 and cognitive function. Despite the fact that low baseline blood folate concentrations did predict poorer cognitive function at follow-up in the majority of studies, the heterogeneity of the evidence diminished the validity of an overall association.

**Literature Cited**