Epidemiological studies suggest that certain micronutrients may improve or maintain cognitive function. Consistent demonstration of benefits in intervention trials has been elusive, possibly because most intervention trials do not select subjects on the basis of nutrient status and/or intake. The objective of this review was to identify levels of intake or markers of nutrient insufficiency that define at-risk older adult populations to determine whether these populations will benefit from nutritional intervention. This review examines evidence from interventional and prospective observational studies that evaluated the effects of folate, vitamin B$_{12}$, and vitamin E on cognitive decline in older populations. The studies suggest that supplementation may protect against cognitive decline when serum folate is $<12$ nmol/L or vitamin E intake is $<6.1$ mg/day. The literature is inadequate to define a level for vitamin B$_{12}$. Epidemiological studies investigating the relations of nutrients to cognitive decline should consider nutrient status in the reporting and interpretation of results. Randomized trials should design inclusion and exclusion criteria to select individuals with low intake and to disallow multivitamin intake. These recommendations may be useful for the design of valid trials and to advance the current understanding of nutrition and neurological diseases.

INTRODUCTION

As the US population ages, cognitive decline is expected to impose significant individual, familial, and societal burdens. Optimizing nutritional status is a highly desirable approach for reducing cognitive decline in the population. However, the current body of literature investigating the relations of nutrients to cognitive decline is plagued by a lack of consideration of nutrient level in study design and interpretation. This has negatively affected the progress of research. In particular, observational studies of populations whose nutrient intake levels range from low/marginal to high tend to show protective benefit with higher nutrient levels. Most randomized trials of nutritional supplements, however, have not targeted individuals with low or marginal nutrient status. Thus, a likely explanation for null findings in the trials is that the nutrient status of participants is already at a protective level, and further supplementation confers no additional benefit. Although the United States has well-established recommended dietary intake levels to avoid frank nutritional deficiency in most of the population, these recommendations do not address the levels required to maintain brain health. Establishing such recommendations would require the reporting of study findings analyzed with special emphasis on indicators of nutrient level, i.e., both dietary intake level and nutritional status level. Currently, this kind of calibration across studies has received little attention. These gaps in the knowledge complicate the design of clinical trials that seek to establish a benefit of vitamin supplementation on cognitive function. In this review, these gaps were addressed by conducting a qualitative assessment of data from prospective observational studies and randomized trials to discern the nutrient levels...
below which cognitive decline is likely to result and supplementation may thus prove beneficial. Such an objective cannot be accomplished through a meta-analysis. In fact, two recently conducted meta-analyses investigating B vitamins and cognition did not find supplementation to be efficacious.

This review is designed to shed light on whether the null findings can be explained by heterogeneity in the baseline nutrient status of intervention participants. The goal is not to recommend dietary reference intake levels to prevent cognitive decline. Rather, the intent is to calibrate the findings of existing studies on B vitamins and vitamin E around possible levels of benefit in order to more effectively direct future studies and, therefore, provide better information upon which to base public health recommendations.

**METHODS**

The PubMed database was searched for longitudinal epidemiological studies and randomized controlled intervention trials on vitamin B₁₂, folate, or vitamin E in relation to cognitive decline. Search terms included combinations of the following words: elderly, cognition, vitamin B₁₂, folate, folic acid, vitamin E, and malnutrition. Published manuscripts identified through these search terms were also searched for additional studies through the references cited.

Studies included in this review were restricted to prospective observational studies and randomized controlled intervention trials that investigated vitamin B₁₂, folate, or vitamin E in relation to cognitive change over time. Studies were further restricted to those that reported baseline data of either nutrient intake and/or blood biomarkers of nutrient status. No restrictions on the number or type of cognitive tests used in the studies were imposed. A statistically significant ($P \leq 0.05$) result for any cognitive test was considered evidence of a nutrient effect. The review did not include cross-sectional studies or studies focused on demented patients. Studies were tabulated to specifically highlight the markers of nutritional status and to show whether there was a cognitive change associated with each nutrient studied (Tables 1–7). Normal reference ranges for each nutritional status were tabulated to specifically highlight the markers of nutritional status. The far right column shows in each table. The right column shows the number of tests administered in each study and the number of tests that showed a benefit or a deleterious outcome. Some cognitive tests are subject to floor and ceiling effects and thus are insensitive measures of cognitive change and are susceptible to bias by attained cognitive ability. For example, the Mini-Mental Status Examination (MMSE), commonly used as a single measure of cognitive ability, is less sensitive to change in higher-educated individuals. Combining the scores of a number of individual tests into one global cognitive measure, as many of the studies have done, helps to minimize these sources of error and provides a more sensitive measure of cognitive change.

**RESULTS**

The B vitamins are of interest for cognition because of their roles in single-carbon and homocysteine metabolism. Given that folate, vitamin B₆, and vitamin B₁₂ are cofactors involved in the metabolism of homocysteine, insufficiency in one or more of these vitamins can increase homocysteine concentrations. Evidence suggests that increased homocysteine concentration is a risk factor for Alzheimer’s disease and cognitive decline.

**Longitudinal studies with folate**

Prospective epidemiological studies that examined the relation of folate intake to cognitive outcomes have yielded mixed results (Table 1). Upon close examination of the studies, it appears that population differences in baseline folate status may explain the apparent inconsistencies in study findings. The differences in folate levels could be quite significant, depending on whether and/or when policies of folic acid fortification were implemented in a given country. As outlined below, the data suggest that both low and high folate levels may have deleterious consequences for cognitive function. Furthermore, whether high folate levels prove harmful may depend on vitamin-B₁₂ status.

In the US Veterans Affairs Normative Aging Study (n = 321 subjects), increased folate, both plasma and dietary, was associated with slower cognitive decline. Men in the lowest tertile (median serum folate <20 nmol/L; median dietary folate intake <339 μg/day) showed significantly greater decline compared with men in the highest tertile (serum folate >30 nmol/L; dietary folate intake >523 μg/day). In addition, a higher dietary intake of folate from food and supplements also was associated with slower cognitive decline.

Similarly, in the US MacArthur Studies of Successful Aging (n = 499 subjects), lower quartiles of plasma folate concentrations were associated with increased rates of cognitive decline over 7 years. The lowest quartile of plasma folate in this pre-folic-acid fortification study corresponded to 2.7–7.1 nmol/L, whereas the fourth quartile was >20.0 nmol/L. In the Leiden 85-Plus Study (n = 559 subjects), Mooijaart et al. found that higher levels of serum folate were associated with slower declines in global cognitive scores. Serum folate levels in this study ranged 6.7–19.5 nmol/L. In contrast, the US Nurses’ Health Study (n = 635 subjects) found that baseline...
### Table 1 Summary of longitudinal studies of the relation between B vitamins and cognitive outcomes.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length of follow-up</th>
<th>Measures of potential nutrient insufficiency/excess from reference, if provided [normal reference ranges in plasma]</th>
<th>Daily intake, US RDA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. of cognitive tests</th>
<th>No. of tests showing benefit (+), negative (−), or null (ø) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang et al. (2006)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>10 y</td>
<td>tHcy (&lt;15&lt;sup&gt;μ&lt;/sup&gt;mol/L) Qr 1: 9.5 Folate (nmol/L) [7–45] B&lt;sub&gt;12&lt;/sub&gt; (pmol/L) [81–590] MMA (nmol/L) [&lt;360] holoTC (pmol/L) [&gt;20]</td>
<td>Folic acid, Qr 1: 359 μg B&lt;sub&gt;12&lt;/sub&gt;, Qr 1: 9 μg</td>
<td>6</td>
<td>Folic acid, Qr 1: 359 μg B&lt;sub&gt;12&lt;/sub&gt;, Qr 1: 9 μg</td>
</tr>
<tr>
<td>Tucker et al. (2005)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>3 y</td>
<td>11 Tr 1: &lt;20 Folate: 283</td>
<td>Folic acid, Tr 1: &lt;339 μg B&lt;sub&gt;12&lt;/sub&gt; (mean): 9.57 μg</td>
<td>6</td>
<td>Folic acid, Tr 1: &lt;339 μg B&lt;sub&gt;12&lt;/sub&gt; (mean): 9.57 μg</td>
</tr>
<tr>
<td>Kado et al. (2005)&lt;sup&gt;10&lt;/sup&gt;</td>
<td>7 y</td>
<td>Qr 4: ≥13.4 Folate: 1</td>
<td>Folic acid, Qr 1: &lt;7.1</td>
<td>5</td>
<td>Folic acid, Qr 1: &lt;7.1</td>
</tr>
<tr>
<td>Morris et al. (2005)&lt;sup&gt;11&lt;/sup&gt;</td>
<td>6 y</td>
<td>Qr 1: &lt;7.1 Folate: 1</td>
<td>Qr 1: &lt;217 Folate: 1</td>
<td>4</td>
<td>Folate: 1+ (global score only)</td>
</tr>
<tr>
<td>Mooijaart et al. (2005)&lt;sup&gt;12&lt;/sup&gt;</td>
<td>4 y</td>
<td>13.3 12.1 282</td>
<td>Folic acid, Qn 1: 63–221 μg Folic acid, Qn 4–5: 349–1719 μg</td>
<td>4</td>
<td>Folate: 1+</td>
</tr>
<tr>
<td>Tangney et al. (2009)&lt;sup&gt;13&lt;/sup&gt;</td>
<td>6 y</td>
<td>11.5 336 279</td>
<td>Folic acid, Qn 1: 18.6–186 Folic acid, Qn 5: 435–695</td>
<td>4</td>
<td>B&lt;sub&gt;12&lt;/sub&gt;: 1+ (and global score)</td>
</tr>
<tr>
<td>Clarke et al. (2007)&lt;sup&gt;14&lt;/sup&gt;</td>
<td>10 y</td>
<td>14.5 15.8 280 35 73</td>
<td>Folic acid, Qn 1: 0.54–4.8 Qn 5: 20.2–149</td>
<td>1</td>
<td>holoTC: 1+ MMA: 1–</td>
</tr>
<tr>
<td>Morris et al. (2012)&lt;sup&gt;15&lt;/sup&gt;</td>
<td>8 y</td>
<td>Qn 1: 0.54–4.8 4.8 186–186 Qn 5: 435–695</td>
<td>Folic acid: 320 μg B&lt;sub&gt;12&lt;/sub&gt;: 6.6 μg</td>
<td>1</td>
<td>B&lt;sub&gt;12&lt;/sub&gt;: 1+ Folate: 1–</td>
</tr>
</tbody>
</table>

**Abbreviations:** holoTC, holotranscobalamin; MMA, methylmalonic acid; Qn, quintile; Qr, quartile; RDA, recommended dietary allowance; tHcy, total homocysteine; Tr, tertile.

<sup>a</sup>Values are means unless otherwise noted. Blank cells indicate that no values were reported.
plasma folate status was not related to cognitive decline.8 The quartile means for plasma folate ranged 9.5–42 nmol/L. Women in the lowest quartile consumed on average 359 μg of folate per day from food and supplements combined, an amount approaching the recommended dietary allowance (RDA) of 400 μg/day. Thus, a potential explanation for the absence of association or null finding in this study is that the distribution of folate nutriture in this sample did not include levels low enough to negatively affect cognition.

Two population studies suggest that excessive folate intake may contribute to cognitive decline in the aging population.11,15 In the Chicago Health and Aging Project (CHAP), participants (n = 3,718 subjects) in the fourth and fifth quintiles of dietary and supplement folate intake, whose intakes ranged between 349 and 1,719 μg/day, had a greater rate of cognitive decline than subjects in the first quintile (63–221 μg/day).11 In addition, CHAP subjects consuming >400 μg folate/day from supplements displayed greater cognitive decline than those who did not use supplements. The Framingham Heart Study examined the relation of plasma folate and vitamin B12 levels to decline in MMSE scores over 8 years and observed a statistical interaction such that the increased rate of cognitive decline in individuals with low vitamin-B12 status (<258 pmol/L) was significantly greater in individuals who had plasma folate levels above 21.75 nmol/L.15 Overall, faster rates of cognitive decline occurred among those with plasma folate levels below 7.52 nmol/L and those with levels above 20 nmol/L.

**Intervention trials with folic acid**

Three folate intervention trials have been reported; all three showed that folic acid supplementation improves cognitive function in subjects with suboptimal folate and/or B-vitamin status (Table 2).5–7,16–18

In the FACIT trial conducted in the Netherlands (n = 818 subjects), folic acid supplementation significantly improved global cognitive functioning and memory and attenuated decline in information-processing speed compared with placebo.16 In post-hoc analysis, folic acid improved sensorimotor and processing speed in subjects with serum B12 levels between 201 and 249 pmol/L but not in those with serum B12 levels ≥250 pmol/L. Inclusion criteria for this trial targeted individuals with high plasma total homocysteine (13–26 μmol/L) and excluded those with conditions other than suboptimal folate that could possibly increase homocysteine concentrations, including serum vitamin B12 levels <200 pmol/L. Serum folate levels of randomized participants were in the low-normal range (mean serum folate, 12 nmol/L). The placebo and treatment groups consumed similar amounts of folate at baseline, with
<table>
<thead>
<tr>
<th>Reference</th>
<th>Daily dose (mg)</th>
<th>Duration of trial</th>
<th>Measures of potential nutrient insufficiency/excess from reference, if provided</th>
<th>Total no. of cognitive tests</th>
<th>No. of tests showing benefit (+), negative (−), or null (ø) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eussen et al. (2006)</td>
<td>B₁₂: 1.0</td>
<td>24 wks</td>
<td>tHcy (μmol/L) [&lt;15] Folate (nmol/L) [7–45] B₁₂ (pmol/L) [81–590] MMA (nmol/L) [&lt;360] holoTC (pmol/L) [&gt;20]</td>
<td>19</td>
<td>ø</td>
</tr>
<tr>
<td>Seal et al. (2002)</td>
<td>B₁₂: 0.01</td>
<td>1 mo</td>
<td>100–150</td>
<td>1.4–2.5 μg</td>
<td>1</td>
</tr>
<tr>
<td>Bryan et al. (2002)</td>
<td>B₁₂: 0.75</td>
<td>5 wks</td>
<td>0.6–27.4 μg</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>van Asselt et al. (2001)</td>
<td>IM injection: B₁₂: 1.0 mg/wk for 4 wks; B₁: 1.0 mg/mo for 4 mo</td>
<td>5 mo</td>
<td>Median: 15.3</td>
<td>≤150</td>
<td>Median: 340</td>
</tr>
<tr>
<td>Kwok et al. (1998)</td>
<td>IM injection: B₁₂: 1.0 mg 3x/wk for 1 wk; 1.0 mg/wk for 3 mo; 1.0 mg/mo until study end</td>
<td>=4 mo</td>
<td>1.0–200 or 200–300 with MMA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: holoTC, holotranscobalamin; IM, intramuscular; MMA, methylmalonic acid; RDA, recommended dietary allowance; tHcy, total homocysteine.

Values are means unless otherwise noted. Blank cells indicate that no values were reported.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Daily dose (mg)</th>
<th>Duration of trial</th>
<th>Measures of potential nutrient insufficiency/excess from reference, if provided</th>
<th>Total no. of cognitive tests</th>
<th>No. of tests showing benefit (+), negative (−), or null (ᴓ) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker et al. (2012)</td>
<td>Folic acid: 0.4 B12: 0.1</td>
<td>2 y</td>
<td>9.7</td>
<td>6</td>
<td>1+(and global score)</td>
</tr>
<tr>
<td>Eussen et al. (2006)</td>
<td>Folic acid: 0.4 B12: 1.0</td>
<td>24 wks</td>
<td>100–200 or 200–300 w/MMA</td>
<td>≥320</td>
<td>19</td>
</tr>
</tbody>
</table>

**Table 4** Summary of randomized controlled trials of the relation between folic acid plus B12 and cognitive outcomes.*

**Abbreviations**: holoTC, holotranscobalamin; MMA, methylmalonic acid; RDA, recommended dietary allowance; tHcy, total homocysteine.

*Values are means unless otherwise noted. Blank cells indicate that no values were reported.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Daily dose (mg)</th>
<th>Duration of trial</th>
<th>Measures of potential nutrient insufficiency/excess from reference, if provided</th>
<th>Total no. of cognitive tests</th>
<th>No. of tests showing benefit (+), negative (−), or null ( قوله) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Jager et al. (2011)</td>
<td>Folic acid: 0.8 B6: 20 B12: 0.5</td>
<td>2 y</td>
<td>&gt;11.3</td>
<td>4</td>
<td>All subjects: 1+ High tHcy: 4+ Brain atrophy: +</td>
</tr>
<tr>
<td>Smith et al. (2010)</td>
<td>Folic acid: 1 B6: 10 B12: 0.5</td>
<td>2 y</td>
<td>≥13</td>
<td>8</td>
<td>1− (and global score)</td>
</tr>
<tr>
<td>McMahon et al. (2006)</td>
<td>Folic acid: 2.5 B6: 50 B12: 1</td>
<td>=7.8 y</td>
<td>Folic acid: &lt;279 μg B6: &lt;1.9 mg B12: &lt;2.4 μg</td>
<td>5</td>
<td>All subjects:  قوله</td>
</tr>
<tr>
<td>Kang et al. (2008)</td>
<td>Folic acid: 2.5 B6: 50 B12: 1</td>
<td>=7.8 y</td>
<td>Folic acid: &lt;279 μg B6: &lt;1.9 mg B12: &lt;2.4 μg</td>
<td>5</td>
<td>All subjects:  قوله</td>
</tr>
</tbody>
</table>

**Table 5** Summary of randomized controlled trials of the relation between combined folic acid, B6, and B12 and cognitive outcomes.*

**Abbreviations**: holoTC, holotranscobalamin; MMA, methylmalonic acid; RDA, recommended dietary allowance; tHcy, total homocysteine.

*Values are means unless otherwise noted. Blank cells indicate that no values were reported.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Length of follow-up</th>
<th>Measure of potential nutrient insufficiency/excess from reference, if provided</th>
<th>Total no. of cognitive tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma tocopherols (μg/L) [normal reference range = 5,000–18,000]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daily intake of vitamin E (US RDA = 15 mg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of tests showing benefit (+), negative (−), or null (ᴓ) effect</td>
<td></td>
</tr>
<tr>
<td>Morris et al. (2002)</td>
<td>3.2 y</td>
<td>Vitamin E (from food)</td>
<td>4</td>
</tr>
<tr>
<td>Morris et al. (2005)</td>
<td></td>
<td>Qn 1: 4.6 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Qn 4–5: 6.5–7.8 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-tocopherol median: 7.1 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ-tocopherol median: 12.0 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ-tocopherol median: 3.5 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-tocopherol median: 0.8 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-tocopherol equivalents, median: 8.6 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-, γ-, and α-tocopherol intake: global score+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ- and β-tocopherol intake: ⬛</td>
<td></td>
</tr>
<tr>
<td>Wengreen et al. (2007)</td>
<td>7 y</td>
<td>Vitamin E</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Qr 1: 4.6 mg</td>
<td>1+</td>
</tr>
<tr>
<td>Kang et al.</td>
<td>4 y</td>
<td>Total tocopherols, Qr 1: 10,704</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total tocopherols, Qr 4: 22,581</td>
<td>⬛</td>
</tr>
<tr>
<td>Devore et al. (2013)</td>
<td>15–20 y</td>
<td>Vitamin E</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Qn 1: 7 mg</td>
<td>⬛</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Qn 5: 159 mg</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** Qn, quintile; Qr, quartile; RDA, recommended dietary allowance.

*Values are means unless otherwise noted. Blank cells indicate that no values were reported.*
averages approaching half of the RDA (mean intake of $195\,\mu g/day$ and $192\,\mu g/day$ in the placebo and treatment groups, respectively).

Bryan et al.\(^{17}\) reported that folic acid supplementation improved memory performance ($n=75$ subjects), although this was the only significant outcome of 20 cognitivetests administered and the only one that showed an effect of folic acid supplementation. There was no attempt to recruit participants into the trial who had evidence of low B-vitamin status, and no biochemical measures were reported. The study did report dietary intake levels that ranged from $134.8$ to $678.2\,\mu g/day$ ($203.1\,\pm\,26.4$ for lowest quartile, and $467.1\,\pm\,79.1$ for highest quartile).

Baseline folate intake was not used as a factor in the analysis, so it is difficult to determine whether there was a treatment effect among those with low intake.

A small interventional study ($n=30$ subjects) conducted in Italy found that folic acid supplementation significantly improved attention efficiency scores from baseline, while placebo did not.\(^{18}\) The study included subjects with low serum folate levels ($<6.8\,nmol/L$). However, it is interesting to note that, when baseline serum folate concentration was used as a covariate in the analysis, folic acid supplementation also significantly improved scores on acquisition and recall, delay recall, memory index, and encoding factor compared with placebo.

In summary, although the number of studies is limited, randomized controlled trials (RCTs) suggest that individuals with serum homocysteine $>13\,\mu mol/L$, plasma folate $<12\,nmol/L$, and/or those consuming $<350\,\mu g/day$ are likely to respond to folic acid supplementation with biochemical folate levels $<7.5\,nmol/L$. Although the data are limited, there is epidemiological evidence to suggest that individuals with low vitamin B$_6$ status may be at risk for negative cognitive effects from excess folic acid intake ($>400\,\mu g/day$).\(^{11,\,15}\)

### Table 7  Summary of randomized controlled trials of the relation between vitamin E and cognitive outcomes.\(^{a}\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose of vitamin E (mg)</th>
<th>Duration of trial</th>
<th>Measure of potential nutrient insufficiency/excess from reference, if provided</th>
<th>Total no. of cognitive tests</th>
<th>No. of tests showing benefit (+), negative (−), or null (ᴓ) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang et al. (2006)(^{14})</td>
<td>402 mg every other day</td>
<td>=9 y</td>
<td>-</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Kang et al. (2009)(^{15})</td>
<td>402 mg every other day</td>
<td>=9.6 y</td>
<td>-</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

**Abbreviations:** RDA, recommended dietary allowance.

\(^{a}\)Values are means unless otherwise noted. Blank cells indicate that no values were reported.
another study of CHAP participants (n = 3,718 subjects), higher total dietary intake of B₁₂ (from food and supplements) was associated with slower cognitive decline, but only among the oldest individuals (Table 1).¹¹ Based on the model used in the CHAP study, an 80-year-old consuming 20 μg of vitamin B₁₂ per day had a significantly slower rate of decline than an 80-year-old consuming the RDA of 2.4 μg/day.

Four additional longitudinal studies examined the association between blood markers of vitamin-B₁₂ status and cognitive change. In the Framingham Study, plasma vitamin B₁₂ levels were positively associated with a slower rate of decline in MMSE scores. Participants in the lowest two-fifths of the distribution, in whom vitamin B₁₂ levels were <258 pmol/L, had a significantly faster decline in scores compared with participants in the upper three-fifths.¹⁵ Clarke et al.¹⁴ found that a doubling in homocysteine or methylmalonic acid correlated with a slower rate of decline in MMSE scores. Participants in the lowest two-fifths of the distribution, in whom vitamin B₁₂ levels were ≤250 μmol/L, had a significantly faster decline in scores compared with participants in the upper three-fifths.

The Nurses’ Health Study (n = 635 subjects) found no association between plasma vitamin B₁₂ levels and cognitive decline.⁸ The means of the lowest plasma quartiles were 200 pmol/L and 236 pmol/L, indicating that a fair proportion of the study population had low B₁₂ status. The Leiden 85+ study (n = 559 subjects) also found that neither total homocysteine nor B₁₂ levels predicted cognitive change in MMSE over 4 years.¹² Reported serum vitamin B₁₂ levels in this study ranged from 180 to 490 pmol/L. These studies are difficult to interpret because, as demonstrated in the Clark et al.¹⁴ study, vitamin B₁₂ plasma or serum levels alone may not be sufficient to characterize vitamin-B₁₂ status.

**Intervention trials with vitamin B₁₂**

Vitamin-B₁₂ status is particularly sensitive to impaired absorption, which further aggravates inadequate dietary intake. Decreased bioavailability of naturally occurring vitamin B₁₂ is common in older persons because production of gastric acid, which is required for absorption, decreases (achlorhydria).⁴² Achlorhydria increases with older age because of the increased prevalence of gastritis and other digestive and metabolic conditions in this age group as well as the increased use of medications.⁵⁷,⁴² Consequently, trial subjects consuming vitamin B₁₂ at the recommended level from food may still be deficient secondarily to impaired absorption. Impaired acid production affects absorption of the protein-bound (food) form of vitamin B₁₂ to a much greater degree than absorption of the crystalline form used for fortification and in supplements. However, subclinical deficiency in intrinsic factor, a glycoprotein required for vitamin B₁₂ absorption, may blunt the effectiveness of supplementation. Adequate intake may suggest sufficiency, but, when paired with chronically inefficient absorption, may still leave subjects at risk of irreversible cognitive impairment. Furthermore, the only method for quantifying absorption is to measure isotopically labeled vitamin.⁴¹ Parenteral administration, while more invasive, eliminates the variability introduced with oral interventions. Each of these elements may contribute to the variable efficacy reported in trials of vitamin B₁₂ supplementation.

Five intervention trials have assessed the effect of vitamin B₁₂ supplementation on cognitive decline (Table 3).⁵–⁷,¹⁷,¹⁸–²² The two trials that used intramuscular administration of B₁₂ reported decreased cognitive decline,²²,²³ whereas the three other trials, which used oral administration of B₁₂, showed no positive effects.¹⁷,²⁰,²¹ van Asselt et al.²² found that intramuscular B₁₂ supplementation (n = 16 subjects) improved cognitive performance compared with placebo. The intervention also decreased plasma methylmalonic acid from a median of 340 nmol/L to 170 nmol/L and plasma total homocysteine from 15.3 μmol/L to 12.4 μmol/L. Baseline plasma B₁₂ concentrations in this study were low, at ≤150 pmol/L.

In a similar RCT (n = 50 subjects), performance IQ scores were significantly increased in subjects who received intramuscular B₁₂, whereas scores in the placebo group remained the same.²¹ Of note, B₁₂ administration reduced performance on motor skill assessment compared with baseline. This study was conducted in subjects with serum B₁₂ levels <120 pmol/L.

The most recent intervention study investigating oral B₁₂ supplementation did not detect an effect of B₁₂ supplementation on cognitive decline, despite stringent criteria to identify subjects. Specifically, Eussen et al.²⁰ supplemented 111 subjects with mild B₁₂ deficiency (serum B₁₂ 100–200 pmol/L, or serum B₁₂ 200–300 pmol/L and plasma methylmalonic acid ≥320 nmol/L). In another intervention trial, oral B₁₂ supplementation did not affect MMSE scores after a brief intervention in 31 subjects with
low serum B_{12} levels (<150 pmol/L) at baseline.\textsuperscript{21} In the trial conducted by Bryan et al.\textsuperscript{17} (n = 75 subjects), oral B_{12} treatment in women did not improve performance on cognitive tests.

Although limited in number, these trials suggest that parenteral B_{12} therapy at 1,000-\mu g doses may be required to prevent cognitive decline in vitamin B_{12}-deficient older adults. Intramuscular injection eliminates the need for digestion and absorption of vitamin B_{12}, which may be impaired with older age, when increased medications may adversely absorb. To date, the randomized trials of oral B_{12} supplementation have not been sufficiently powered to adequately test the beneficial effects on cognition. Furthermore, the dose levels and length of treatment were likely too low and too brief to correct the deficient state. This is especially critical, given the irreversible nature of cognitive decline secondary to long-term B_{12} deficiency. Taken together, these studies indicate that vitamin B_{12} serum concentrations <120–150 pmol/L and possibly even higher (e.g., 250 pmol/L) are likely to increase the risk of cognitive decline. Plasma levels of methylmalonic acid and holotranscobalamin that predict risk of cognitive decline have not been established.

**Intervention trials with folic acid and vitamin B_{12}**

Two trials\textsuperscript{20,24} tested the combination of folic acid and B_{12} (Table 4),\textsuperscript{5–7,20,24} with only one showing that the combination was effective.\textsuperscript{24} Folate nutrient may explain the findings of the negative trial, as the reported levels of red blood cell folate suggest that subjects were folate sufficient and not in need of supplementation.

The Beyond Ageing Project (n = 752 subjects) found that folic acid and vitamin B_{12} treatment improved scores on a summary measure of combined cognitive tests and on tests of immediate and delayed recall in models adjusted for total homocysteine and depression levels.\textsuperscript{24} Exclusion criteria in this trial included the use of B vitamin supplements and biochemical evidence of pronounced B vitamin deficiency (red blood cell folate levels <250 nmol/L, and vitamin B_{12} levels <130 pmol/L).

In another arm of the Eussen et al.\textsuperscript{20} trial described above (n = 108 subjects), the combination of folic acid and B_{12} did not improve cognitive function. According to the red blood cell folate levels outlined in the manuscript (average of 616 nmol/L), these participants were folate sufficient and not in need of supplementation.

**Intervention trials with folic acid, vitamin B_{6}, and vitamin B_{12}**

The trio of folic acid, B_{6}, and B_{12} has been tested in three RCTs (Table 5).\textsuperscript{5–7,25–27,28} This combination treatment reduced brain atrophy and/or improved cognitive function in two of the three trials.\textsuperscript{25,26,28}

A recent double-blind RCT found that B vitamin supplementation significantly decreased the rate of brain atrophy by 29.6% compared with placebo. This study, by Smith et al.\textsuperscript{30} excluded potential subjects (n = 266 subjects) if they consumed B vitamin supplements at levels >300 \mu g of folic acid, >1.5 \mu g of B_{12}, and >3 mg of B_{6}. Nevertheless, baseline nutrient status influenced the results: an elevated level of total homocysteine, a marker of B vitamin insufficiency, at baseline predicted a faster rate of atrophy in the placebo group but not the treatment group. In contrast, an elevated level of holotranscobalamin, a marker of adequate B_{12} status, at baseline predicted a slower rate of brain atrophy in the treatment group. Subjects reporting multivitamin use had higher baseline plasma concentrations of folate and B_{12} and a lower concentration of total homocysteine, indicating adequate B-vitamin status. Consistent with the hypothesis that baseline nutrient status determines response to intervention, these B-vitamin-sufficient subjects did not show a reduced rate of brain atrophy with additional B vitamin supplementation. Although the study was not originally powered to detect an effect of B vitamin supplementation on cognitive decline, the B vitamin intervention, compared with placebo, significantly improved performance on a measure of executive function.\textsuperscript{25} In subgroup analysis, B vitamin supplementation significantly increased scores on episodic memory/delayed recall, category fluency, and the MMSE among trial participants with elevated total homocysteine (>11.3 \mu mol/L) only. This is consistent with the hypothesis that baseline nutrient status influences response to supplementation.

In the Women’s Antioxidant and Folic Acid Cardiovascular Study (n = 1,888 subjects), B vitamin supplementation significantly slowed the rate of cognitive decline only in participants with low baseline intake (defined by the authors as <279 \mu g folate per day, <1.9 mg B_{6} per day, or <2.4 \mu g B_{12} per day) of at least 1 B vitamin compared with those with adequate intakes of all 3 vitamins.\textsuperscript{28} All trial participants were allowed to consume multivitamin supplements up to the RDA level, a common feature of US supplement trials that has a strong potential to eliminate positive effects of nutrient supplementation interventions.

Another trial of B vitamin supplementation versus placebo unexpectedly found that the intervention (1 mg folic acid, 10 mg B_{6}, and 0.5 mg B_{12}) worsened cognitive performance on a global cognitive score that encompassed all tests administered. This study included New Zealanders (n = 253 subjects) with plasma total homocysteine concentrations \geq13 \mu mol/L.\textsuperscript{27} No marker of vitamin-B_{12} status was measured in the trial, so it is possible that B_{12} malabsorption in the subjects hindered or prevented a response to oral supplementation. Excess
folate intake has been associated with accelerated cognitive decline. Baseline folate intake for these subjects was not reported; however, if it was high, supplementation may have resulted in excess folate intake.

The mixed findings of these clinical trials highlight the need to determine the plasma concentrations of total homocysteine, B₁₂, holotranscobalamin, and/or methylmalonic acid at which B vitamin supplementation is most likely to improve cognition. Intervention resulted in cognitive improvement in subjects with total homocysteine levels >11.3 μmol/L. Individuals with low B vitamin intake – but not those with adequate intake – showed decreased cognitive decline with supplementation. The potential for older trial participants to have irreversible cognitive damage and impaired B₁₂ absorption may have influenced these conflicting results.

**Longitudinal studies with vitamin E**

Vitamin E is of interest because of its potent antioxidant activity. Oxidative stress is a primary factor in the pathology of both Alzheimer’s disease and age-related cognitive decline. This fat-soluble vitamin is also implicated in the pathogenesis of Alzheimer’s disease and age-related cognitive decline. Most recently, Devore et al. analyzed the association between vitamin E intake and cognitive decline over 6.4 years in 16,010 participants from the Nurses’ Health Study. The authors adjusted for polyunsaturated fat intake in their statistical model, which is problematic because plant oils high in polyunsaturated fat are a prominent source of dietary vitamin E. Thus, the analyses were overcontrolled. Median intakes in the lowest two vitamin E quintiles (7 mg/d and 12 mg/d, respectively) were less than the RDA of 15 mg/day. Upon initial analysis, the authors report a trend for a protective effect of vitamin E and global cognitive score (P = 0.07). Adjustment for polyunsaturated fat intake eliminated this effect, which the authors conclude to be a null finding. The initial findings, however, show evidence of the protective effect of vitamin E against cognitive decline.

**Intervention studies with vitamin E**

There are only two intervention trials of vitamin E and cognitive decline that consider measures of vitamin-E status or intake at enrollment (Table 7). In the Women’s Health Study (n = 5,845 subjects) randomized trial, no significant effect on cognitive decline was detected after an average of 9.6 years of 600 IU/day of vitamin E. In post hoc analysis, however, vitamin E supplementation was significantly associated with a slower rate of decline in global cognitive score.
compared with placebo in women whose dietary intake levels of vitamin E were below the median of 6.1 mg/day, a level well below the RDA of 15 mg/day. No significant vitamin E effect was detected in women whose baseline dietary consumption was ≥6.1 mg/day.34

In the Women’s Antioxidant and Cardiovascular Study (n = 705 subjects), 600 IU of vitamin E supplementation for an average of 9 years produced no significant effect on cognitive change overall or when women were stratified by baseline dietary intake levels above or below the median intake of 15 mg/day.35 Participants in both this trial and the Women’s Health Study were allowed to consume multivitamins throughout the intervention phase. As noted earlier, if the level of nutrient sufficiency for cognition falls at or below the recommended dietary levels, this would likely neutralize any effect of the nutrient intervention.

These studies demonstrate that individuals consuming less than half of the RDA of 15 mg/day (approx. 6–7 mg/d) may experience a cognitive benefit from increased vitamin E intake, either through food or vitamin supplementation.19,30,34 In addition, the cognitive effects reported here may be differentially impacted by the tocopherol subtype. Longitudinal data suggest both α-tocopherol and γ-tocopherol are important for brain function.31,32,46 Specific protective levels of non-α-tocopherols have not yet been determined.

CONCLUSION

In this review, the hypothesis was that vitamin supplementation with specific nutrients would prevent cognitive decline or improve cognitive function in elderly individuals who have low intake or biochemical indications of insufficiency in those nutrients. Furthermore, these levels may be above previously defined levels of deficiency. A review of the available literature indicates that the strongest evidence for cognitive benefit with vitamin supplementation is for folate and vitamin E, but only among individuals with low status of these vitamins. Low nutrient status for folate may be indicated by plasma levels <12 nmol/L or intake below approximately 350 μg/day. Future trials investigating folate and cognition in the elderly should consider nutrient status at recruitment and select for subjects with folate levels <12 nmol/L and total homocysteine levels >11.3 μmol/L and for nonsupplementing subjects with intakes <350 μg. Indeed, subjects with total homocysteine as low as 10 μmol/L may benefit from B vitamin supplementation. In addition, folic acid supplementation must be carefully considered in tandem with B12 status, as deleterious effects are possible.

The literature on the relation between vitamin B12 and cognition is not as clear. The prospective epidemiological studies do not consistently include populations consuming <2.4 μg/day and occasionally rely only on serum B12 levels to assess status, which is a poor indicator of marginal deficiency. Intervention trials recruiting elderly subjects with very low serum B12 levels may inadvertently include those with irreversible neurological damage and/or impaired absorption. In addition, many of the RCTs testing oral B12 supplementation recruited small numbers of subjects and administered relatively low doses (10–50 μg/d) for short study periods of approximately 1 month. Future intervention trials should measure specific markers of B12 status, such as methylmalonic acid, holotranscobalamin, and total homocysteine, in addition to B12 intake levels to better target nutrient insufficiency and depletion. Parenteral administration eliminates issues arising from impaired B12 absorption and is an important consideration.

The RCT and prospective epidemiological studies investigating vitamin E and cognition suggest that individuals consuming less than approximately 6 mg/day are most likely to experience cognitive benefit from supplementation. Further investigation is required to determine whether a specific tocopherol or a combination of tocopherols is primarily responsible for this effect.

Future studies would be improved by adding sensitive and well-defined measures of nutrient intake and/or biochemical markers to assess insufficiency and deficiency. Studies that report associations should outline the specific levels at which associations with cognitive function occur.

Finally, it is critically important for all studies of the relationship between nutrition and cognitive decline to consider baseline nutrient status in the analyses, reporting, and interpretation of study findings. This will help to reconcile seemingly inconsistent results and provide more reliable data for the design of randomized trials, thereby having greater impact on the advancement of science and health policy. It is hoped that the results of this qualitative review will encourage investigators to focus on nutrient intake and biochemical markers of insufficiency, both in recruitment for future investigations and in the analysis and reporting of results. Ideally, randomized trials should be designed to target subjects with insufficient nutrient status, and findings should be reported according to preintervention levels of serum markers or intake. With these considerations in mind when future studies are designed, more precise recommendations for supplementation can be established to support optimal cognitive function in aging populations.

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