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# Reduced risk of Alzheimer's disease with high folate intake: The Baltimore Longitudinal Study of Aging

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## Abstract

**Background:** Study findings have suggested an association between Alzheimer's disease (AD) risk and several vitamins and have speculated about their use as preventive agents. Here, we examine whether total intake (intake from diet plus supplements) of antioxidant vitamins (E, C, carotenoids) and B vitamins (folate, B<sub>6</sub>, and B<sub>12</sub>) is associated with a reduced risk of AD.

**Methods:** Participants were 579 nondemented elderly volunteers from the Baltimore Longitudinal Study of Aging who completed dietary diaries and recorded supplement intake for a 7-day period. Cox regression was used to estimate the relative risk (RR) of AD associated with total vitamin intake categorized into levels above or below the Recommended Dietary Allowance (RDA).

**Results:** After a mean follow-up of 9.3 years, AD developed in 57 participants. Higher intake of folate (RR, 0.41; 95% confidence interval [CI], 0.22 to 0.76), vitamin E (RR, 0.56; 95% CI, 0.30 to 1.06), and vitamin B<sub>6</sub> (RR, 0.41; 95% CI, 0.20 to 0.84) were associated individually with a decreased risk of AD after adjusting for age, gender, education, and caloric intake. When these 3 vitamins were analyzed together, only total intake of folate at or above the RDA (RR, 0.45; 95% CI, 0.21 to 0.97) was associated with a significant decreased risk of AD. No association was found between total intake of vitamins C, carotenoids, or vitamin B<sub>12</sub> and risk of AD.

**Conclusions:** These findings suggest that total intake of folate at or above the RDA is associated with a reduced risk of AD. Additional studies are necessary to further investigate whether folate or other(s) unmeasured factor(s) may be responsible for this reduction in risk.

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## Keywords:

Alzheimer's disease; Antioxidants; B vitamins; Dementia; Folate; Prospective studies; Longitudinal studies

## 1. Introduction

More than 4 million people in the United States suffer from dementia, most with Alzheimer's disease (AD). Given the rapid aging of the population, the number of people suffering from AD is expected to quadruple by the middle of the century unless interventions are found to delay the onset or prevent the disease [1].

There has been much interest in nutrition and specifically

vitamins as potential preventive agents against AD. Data from a handful of cohort studies suggest that the risk of AD may be reduced in people with a high dietary or supplemental intake of antioxidants [2–6]. Furthermore, low serum levels of B vitamins [7] and hyperhomocysteinemia [8] (which can be caused by B-group vitamin deficiencies), have been associated with an increased risk of AD. In fact, interventions that reduce homocysteine levels, such as folic acid supplementation, have been suggested as potential prevention strategies against AD. To date, no prospective study has reported on the effect of intake of folate or other B vitamins on the risk of AD. Moreover, no study has simul-

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Table 1  
Baseline distribution of total intake, dietary intake, and reported supplement use for vitamins E, C, Carotenoids, Folate, B<sub>6</sub>, and B<sub>12</sub>

Vitamin	Total intake		Dietary intake		No. reporting supplement use (%)	RDA
	Median (IQR) <sup>a</sup>	No. at RDA or above (%)	Median (IQR) <sup>a</sup>	No. at RDA or above (%)		
Vitamin E (mg/d)	6.2 (3.9–19.8)	167 (28.8)	4.4 (3.3–6.2)	2 (0.4)	202 (35)	15
Vitamin C (mg/d)	167.0 (112.1–281.1)	503 (86.9)	132.3 (95.3–176.0)	476 (82.2)	220 (38)	Men: 90 Women: 75
Carotenoids <sup>b</sup> (RE/d)	1145.0 (789.0–1728.9)	423 (73.1)	1142.2 (789.0–1723.8)	422 (72.9)	4 (0.7)	Men: 900 Women: 700
Folate (μg/d)	317.8 (224.6–527.5)	203 (35.1)	259.5 (204.8–341.6)	73 (12.6)	181 (31)	400
Vitamin B <sub>6</sub> (mg/d)	2.2 (1.6–4.1)	511 (88.3)	1.8 (1.4–2.2)	468 (80.8)	197 (34)	1.3
Vitamin B <sub>12</sub> (μg/d)	6.3 (3.9–12.2)	531 (91.7)	4.4 (3.1–6.4)	503 (86.9)	194 (34)	2.4

<sup>a</sup> IQR is interquartile range (difference between third and first quartiles).

<sup>b</sup> Because there is currently no separate RDA for carotenoids, the RDA for vitamin A was used.

taneously evaluated the effect of antioxidant and B vitamins on the risk of AD. Therefore, we analyzed data from the Baltimore Longitudinal Study of Aging (BLSA) to examine whether total intake (intake from diet as well as supplements) of antioxidant vitamins (E, C, carotenoids) and B vitamins (folate, B<sub>6</sub>, and B<sub>12</sub>) is associated with a reduced risk of AD.

## 2. Methods

### 2.1. Participants

The BLSA is a longitudinal study initiated in 1958 by the National Institute on Aging to prospectively examine the normal aging process [9]. Originally limited to men, the study began enrolling women in 1978. The cohort comprises well-educated, predominantly white, community-dwelling volunteers who return every 2 years for 2½ days of multidisciplinary tests. These procedures include medical, physiologic, and biomedical examinations, medical and family histories, and cognitive evaluations. The inclusion criteria for this study included availability of a dietary intake report, one visit on or after January 1, 1986 for determination of outcome, and the participant being more than 60 years of age at last follow-up. All participants included in this study provided written informed consent, and all procedures performed were approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center.

### 2.2. Diagnosis of Alzheimer's disease

Beginning in 1986, participants of the BLSA received a neurologic examination and a battery of neuropsychological tests in addition to those in the usual BLSA protocol. Medical records, laboratory tests, and informant questionnaires (Dementia Questionnaire) [10,11] were obtained for participants who presented with cognitive problems based on the neurologic examination and neuropsychological tests.

The diagnostic status of each subject was assigned dur-

ing a multidisciplinary conference in which all available information was reviewed. Participants were classified as demented according to criteria from the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised [12]. Diagnosis of AD was based on criteria from the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association [13]. Participants who met criteria for probable AD but who did not undergo neuroimaging studies were categorized as "consistent with AD" [14]. Participants whose clinical diagnosis was probable, possible, or "consistent with AD" were included as AD cases in the current study. For a more detailed description of the procedures, see the article by Kawas et al [14].

### 2.3. Assessment of vitamin intake

Data used to determine vitamin intake were collected between October 1984 and August 1991. During their biennial visit, participants were instructed on how to record food intake and estimate serving amounts on a 7-day dietary record. After returning home, they recorded dietary intake during their first typical week. Nutrient amounts were estimated by one of the authors (J.H.) according to USDA Composition of Food Handbook. Information on supplement intake, including name brands, dosage, and frequency of consumption, was also collected during their biennial visit. Total intake per day at the time of the earliest dietary diary report (baseline) was estimated as the intake from diet and supplements combined. More detailed procedures for the collection of intake data are described in articles by Hallfrisch and colleagues [15,16].

### 2.4. Definition of covariates

Current values of the recommended dietary allowance (RDA) [17–19] were used for the categorization of vitamin intake (Table 1). Total intake of the individual nutrients at baseline was categorized into below the RDA versus at or above the RDA. Because there is currently no separate RDA

for carotenoids, we used the RDA for vitamin A. Variables previously included in other prospective studies of AD and dietary intake that were available in our study were analyzed as potential confounders and are defined below. Education was dichotomized into college degree or higher versus less than college degree. Total caloric intake was estimated as the number of kilocalories per day averaged over the 7-day dietary period. Smoking (current, past, or never smoker) was categorized to indicate status at baseline. Baseline measures of total caloric intake (kcal/day), total plasma cholesterol level (mg/dL), systolic blood pressure (mm Hg), body mass index (BMI; kg/m<sup>2</sup>), and age (years) were analyzed as continuous variables.

### 2.5. Data analysis

We used Cox regression [20] to estimate the association between baseline total nutrient intake and the risk of AD. For the Cox models, chronological age was used as the fundamental time scale [21], age at AD diagnosis was the event of interest, and delayed entry was used with baseline age as the age of entry. This analysis includes visits and follow-up examinations conducted through September 30, 1999. Participants not diagnosed with AD were censored at the age of their last visit, age at death, or age when another dementia was diagnosed. The risk of AD for each vitamin was estimated by using the “below the RDA” group of total intake as the reference group.

Not all subjects visiting the BLSA between October 1984 and August 1991 completed a dietary diary. We performed *t* tests and  $\chi^2$  tests to compare characteristics (gender, education, length of follow-up, age at which the dietary intake was or would have been obtained, and diagnosis of dementia or AD at last follow-up) of subjects who completed a dietary diary with those who did not. Although AD risk analyses included only baseline dietary reports, we performed *t* tests and signed rank tests to compare the change in total dietary intake between the first and second report for subjects who completed more than one report of dietary intake during the study period. All statistical analyses were performed using SAS software version 8.01 for Windows (SAS Institute Inc, Cary, NC).

### 3. Results

There were 919 active participants in the BLSA older than 60 years during the period of October 1984 and August 1991 when the dietary diary information was collected. Of those, 579 participants (359 men and 220 women) met the inclusion criteria for these analyses. Among those participants excluded, 313 did not complete the dietary diary, 23 did not undergo adequate follow-up, and 4 had no diagnosis determined. Table 2 shows baseline characteristics of the participants included in the study. The average length of follow-up was 9.3 years, and the average age at baseline was 69.6 years. More than 60% of the participants were

Table 2  
Baseline characteristics of BLSA participants included in the study

Characteristic	Study Subgroup (N = 579)	Participants with AD (N = 57)
	Mean (range)	
Follow-up <sup>a</sup> (yr)	9.3 (0.4–14.6)	7.4 (0.9–13.5)
Age at 1 <sup>st</sup> intake report (yr)	69.6 (49–93)	77.7 (54–93)
Age at last follow- up (yr)	78.9 (61–102)	85.1 (61–102)
Caloric intake (kcal/d)	1941.5 (737.0–3507.8)	1762.3 (964.1–2764.3)
Total plasma cholesterol (mg/dL) <sup>b</sup>	198.5 (107–328)	201.6 (118–283)
Systolic blood pressure (mm Hg) <sup>c</sup>	131.5 (85–210)	135.0 (105–210)
BMI (kg/m <sup>2</sup> ) <sup>d</sup>	25.3 (16.4–46.8)	24.6 (17.6–33.7)
	Number (%)	
Men (%)	359 (62)	29 (51)
Education (%)		
Less than college	150 (26)	16 (28)
College or higher	429 (74)	41 (72)
Smoking (%)		
Never	177 (31)	19 (33)
Past	360 (62)	35 (61)
Current	42 (7)	3 (5)

<sup>a</sup> Follow-up is the time between baseline (the earliest dietary diary report) and diagnosis of AD for those in whom AD developed or time between baseline and last follow-up for those in whom AD did not develop.

<sup>b</sup> Excludes 7 people (including 1 AD case) with missing data;

<sup>c</sup> Excludes 1 person with missing data.

<sup>d</sup> Excludes 2 people (including 1 AD case) with missing data.

men, and nearly three fourths were college graduates. Table 2 also shows demographic characteristics of the 57 participants with AD (29 men and 28 women) identified during the follow-up period.

The median total intake, dietary intake, and the number of participants reporting supplement use are shown in Table 1. For vitamin E and folate, the median total and dietary intakes were below the RDA. In contrast, the median total and dietary intake of vitamins C, B<sub>6</sub>, B<sub>12</sub>, and carotenoids were well above the RDA. Supplement use for all vitamins was around 35%, except for carotenoids for which less than 1% of participants reported supplementation. Table 1 also shows participants classified as above or below the RDA for total intake and dietary intake in all 6 vitamins. Less than 1% of participants (N = 2) reached RDA levels of vitamin E with diet alone; therefore, those who reached RDA levels according to total intake (N = 167) did so with the use of supplements. Only 13% of participants (N = 73) reached RDA levels of folate with diet alone. However, diet alone was sufficient for most participants to reach the RDA for

Table 3  
Analysis of individual vitamins: RR for AD with total intake categorized as above and below the RDA or as tertiles\*

Vitamin	No. of AD cases	No. of participants	RR (95% CI)	<i>p</i> value	Median (range)
<b>RDA Groups</b>					
Vitamin E (mg/d)					
<RDA	43	412	1.00 (reference)	-	4.7 (0.4–15.0)
≥RDA	14	167	0.56 (0.30–1.06)	0.07	35.9 (15.1–1137.5)
Vitamin C (mg/d)					
<RDA	9	76	1.00 (reference)	-	Men (M): 66.6 (26.2–88.6); Women (W): 63.6 (23.3–74.7)
≥RDA	48	503	0.68 (0.31–1.49)	0.33	M: 181.9 (90.7–6530.8); W: 197.0 (78.8–5173.0)
Carotenoids (RE/d)					
<RDA	12	156	1.00 (reference)	-	M: 660.4 (209.9–882.7); W: 517.6 (159.6–698.4)
≥RDA	45	423	0.97 (0.50–1.89)	0.93	M: 1526.4 (906.5–5535.1); W: 1280.1 (700.2–4841.2)
Folate (μg/d)					
<RDA	42	376	1.00 (reference)	-	250.9 (79.0–398.9)
≥RDA	15	203	0.41 (0.22–0.76)	0.005	619.0 (403.1–1457.0)
Vitamin B <sub>6</sub> (mg/d)					
<RDA	10	68	1.00 (reference)	-	1.1 (0.5–1.3)
≥RDA	47	511	0.41 (0.20–0.84)	0.01	2.4 (1.3–254.6)
Vitamin B <sub>12</sub> (μg/d)					
<RDA	7	48	1.00 (reference)	-	2.0 (0.2–2.4)
≥RDA	50	531	0.60 (0.26–1.36)	0.22	7.2 (2.4–314.8)
<b>Tertiles</b>					
Vitamin E (mg/d)					
1 <sup>st</sup> tertile	23	194	1.00 (reference)	-	3.3 (0.4–4.5)
2 <sup>nd</sup> tertile	17	192	1.25 (0.63–2.48)	0.53	6.3 (4.5–11.1)
3 <sup>rd</sup> tertile	17	193	0.62 (0.32–1.20)	0.15	26.5 (11.1–1137.5)
Vitamin C (mg/d)					
1 <sup>st</sup> tertile	21	193	1.00 (reference)	-	95.1 (23.3–127.0)
2 <sup>nd</sup> tertile	17	193	0.56 (0.29–1.09)	0.09	167.0 (127.2–221.0)
3 <sup>rd</sup> tertile	19	193	0.61 (0.31–1.18)	0.14	495.1 (225.7–6530.8)
Carotenoids (RE/d)					
1 <sup>st</sup> tertile	14	193	1.00 (reference)	-	663.3 (159.6–920.7)
2 <sup>nd</sup> tertile	20	193	1.20 (0.59–2.44)	0.61	1145.0 (924.7–1502.9)
3 <sup>rd</sup> tertile	23	193	1.38 (0.68–2.79)	0.37	2051.7 (1509.8–5535.1)
Folate (μg/d)					
1 <sup>st</sup> tertile	21	193	1.00 (reference)	-	200.0 (79.0–252.4)
2 <sup>nd</sup> tertile	21	193	0.94 (0.50–1.75)	0.84	317.8 (252.5–416.8)
3 <sup>rd</sup> tertile	15	193	0.44 (0.22–0.89)	0.02	627.8 (419.8–1457.0)
Vitamin B <sub>6</sub> (mg/d)					
1 <sup>st</sup> tertile	20	193	1.00 (reference)	-	1.4 (0.5–1.7)
2 <sup>nd</sup> tertile	18	193	0.69 (0.35–1.36)	0.28	2.2 (1.7–3.1)
3 <sup>rd</sup> tertile	19	193	0.65 (0.34–1.24)	0.19	4.9 (3.1–254.6)
Vitamin B <sub>12</sub> (μg/d)					
1 <sup>st</sup> tertile	20	193	1.00 (reference)	-	3.2 (0.2–4.5)
2 <sup>nd</sup> tertile	16	193	0.80 (0.41–1.59)	0.53	6.3 (4.5–10.0)
3 <sup>rd</sup> tertile	21	193	0.84 (0.45–1.59)	0.60	15.4 (10.1–314.8)

NOTE. Relative risks and confidence intervals were adjusted for age, gender, education, and caloric intake. Each of the 6 vitamins was analyzed separately.

vitamin B<sub>12</sub> (87%), vitamin C (82%), vitamin B<sub>6</sub> (81%), and carotenoids (73%).

After adjusting for, gender, education, and baseline age and caloric intake, increased total intake of folate, vitamin E, or vitamin B<sub>6</sub> were individually associated with a decreased risk of AD (Table 3). Risk among people at the RDA or above of folate (RR, 0.41; *p* = 0.01) and vitamin B<sub>6</sub> (RR, 0.41; *p* = 0.02) was significantly reduced. The risk

reduction for vitamin E approached statistical significance (RR, 0.56; *p* = 0.07). Total intake of vitamin C, carotenoids, or vitamin B<sub>12</sub> showed no association with risk of AD. An additional analysis was performed only with the vitamins that suggested a reduced risk of AD: folate, vitamin E, and vitamin B<sub>6</sub>. When these 3 vitamins were analyzed simultaneously in 1 regression model, only high intake of folate was significantly associated with a decreased

Table 4  
Simultaneous analysis of vitamins: RR for AD with total intake categorized as above and below the RDA or as tertiles

Vitamin	RR (95% CI)	<i>p</i> value
<b>RDA Groups</b>		
Vitamin E		
<RDA	1.00 (reference)	-
≥RDA	1.04 (0.47–2.33)	0.92
Folate		
<RDA	1.00 (reference)	-
≥RDA	0.45 (0.21–0.97)	0.04
Vitamin B6		
<RDA	1.00 (reference)	-
≥RDA	0.55 (0.26–1.15)	0.11
<b>Tertiles</b>		
Vitamin E		
1 <sup>st</sup> tertile	1.00 (reference)	-
2 <sup>nd</sup> tertile	1.44 (0.71–2.92)	0.31
3 <sup>rd</sup> tertile	0.75 (0.30–1.87)	0.54
Folate		
1 <sup>st</sup> tertile	1.00 (reference)	-
2 <sup>nd</sup> tertile	0.98 (0.48–2.01)	0.96
3 <sup>rd</sup> tertile	0.32 (0.11–0.92)	0.03
Vitamin B6		
1 <sup>st</sup> tertile	1.00 (reference)	-
2 <sup>nd</sup> tertile	0.80 (0.37–1.71)	0.56
3 <sup>rd</sup> tertile	2.02 (0.78–5.26)	0.15

NOTE. Relative risks and confidence intervals were adjusted for age, gender, education, and caloric intake. The 3 vitamins were analyzed simultaneously.

risk of AD (Table 4). The correlation among these 3 vitamins was calculated to determine whether a high degree of co linearity may have precluded accurate estimation of RRs. The Pearson correlations between the log of total intake of the vitamins were as follows: vitamin E and folate, 0.50; E and B6, 0.35; and folate and B6, 0.54. Although the correlations were all significant ( $p < 0.01$ ), none were high enough to make co linearity a concern.

We performed additional analyses of total intake categorized into tertiles to explore the consistency of the results. Only the highest tertile of folate intake was associated with a reduced risk of AD, both when all vitamins were analyzed individually (Table 3) and when folate, vitamin E, and vitamin B6 were analyzed simultaneously (Table 4).

All analyses were repeated by further adjusting for total cholesterol, systolic blood pressure, smoking, and BMI, and similar results were obtained (results not shown).

We compared characteristics of the subjects included in our analyses ( $N = 579$ ) with those excluded because they did not complete a dietary diary ( $N = 313$ ). These subjects were similar with respect to gender (male, 62% v 66%;  $p = 0.29$ ), education (college or higher: 74% v 69%;  $p = 0.13$ ), and proportion with a dementia diagnosis (10% v 12%;  $p = 0.29$ ) or an AD diagnosis (14% v 17%;  $p = 0.28$ ). Subjects who did not complete a dietary diary were slightly younger at baseline (mean, 69.6 v 68.2 years;  $p = 0.06$ ), and their

average follow-up was 0.9 years shorter (9.3 v 8.4 years;  $p < 0.001$ ).

Although only baseline dietary data were used in the analyses, we compared multiple dietary reports for those subjects who completed it more than once. Of the 579 subjects included in the analyses, 160 (28%) had more than one dietary intake report. The average time between the first and second report was 3.9 years. On average, total intake between the 2 reports increased by 4.4 mg (SE, 9.9) for vitamin E, 107.2 mg (SE, 110.4) for vitamin C, 43.2  $\mu\text{g}$  (SE, 22.4) for folate, 3.5 mg (SE, 2.5) for vitamin B<sub>6</sub>, and 0.3  $\mu\text{g}$  (SE, 1.6) for vitamin B<sub>12</sub>. Total intake decreased between first and second reports for carotenoids by 46.0 retinol equivalents (RE; SE, 93.1). Paired *t* tests as well as signed rank tests showed that for all vitamins these differences were not significantly different from zero.

#### 4. Discussion

This prospective study provides evidence of a reduced risk of AD among people with a high intake of folate. The risk of AD for participants at or above the RDA was reduced nearly 60% compared with participants below the RDA. Although a reduced risk was seen among people with a total intake at or above the RDA of vitamin E or vitamin B<sub>6</sub>, this effect disappeared when folate, vitamin E, and vitamin B6 were analyzed simultaneously. In contrast, we did not observe an association between intake of vitamin C, carotenoids, or vitamin B<sub>12</sub> and development of AD. To our knowledge, this is the first prospective study to report the association between folate intake and risk of AD and to analyze antioxidants and B vitamins simultaneously.

Most prospective studies looking at nutrients and risk of AD have focused on antioxidant vitamins, particularly vitamins E and C. Summarizing the results from prospective studies that have looked at this association is complex because studies have looked at different sources of antioxidants: supplements only, diet only, total intake, or one or more of these sources. In addition, the results from these studies have not been consistent. Some studies of antioxidant supplements have found no protective effect [22], or a significant protective effect only with vitamin C [2], or only when both vitamins E and C are taken together [6]. Regarding dietary intake, studies have found a protective effect with intake of vitamin E [4,5] or vitamin C [4], whereas others have not found an effect [23,24].

In contrast with studies of antioxidant vitamins, no prospective study has looked at the association between intake of B vitamins and AD. To our knowledge only one small case-control study has reported the association between intake of folate and AD; this study found a lower intake of folate in AD cases than in controls [25]. This lower intake, however, could have been caused by dietary changes in cases of AD. Most studies reporting the association between AD and B vitamins have looked at plasma or serum levels,

not intake, and most have been small case-control studies [25–29]. Only 2 prospective studies have looked at this association [7,8]. One showed increased levels of plasma homocysteine as a strong risk factor for the development of AD [8], whereas another smaller study [7] found that subjects with low serum levels of folate or vitamin B<sub>12</sub> had double the risk of an AD diagnosis 3 years later.

Several potential mechanisms can be postulated to relate folate intake to the development of AD, some of which may be related to homocysteine. Folate intake is associated with homocysteine levels, a well-known risk factor for vascular disease [30–35]. It is possible that homocysteine levels contribute to vascular disease through a direct effect on vascular endothelial cells [36]. Supporting this notion are studies that show a relation between vascular disease and AD [37–39]. Homocysteine, however, has also been shown to be a risk factor in patients with neuropathologically confirmed AD without significant cerebrovascular disease or atherosclerosis [27]. More recent animal studies [40] provide evidence that folic acid deficiency and homocysteine may be directly related to amyloid toxicity by impairing DNA repair in neurons, which results in sensitization to oxidative damage induced by  $\beta$ -amyloid (a protein that is accumulated in excess in AD brains). This suggests a non-vascular mechanism by which folate intake may be related to the development of AD. Another nonvascular mechanism is supported from evidence that homocysteine may cause direct toxicity to neuronal cells [41]. Nonhomocysteine mechanisms involving methylation reactions in the brain [42] have also been postulated to explain the association between folate intake and AD development. Of interest, 2 studies have associated atrophy in different areas of the brain to serum folate levels [43] or homocysteine levels [27], but the mechanisms for these results are not known.

The RDA for folate of 400  $\mu\text{g}/\text{d}$  was established by considering the amount of folate intake necessary to lower homocysteine levels [19]. Data from the Framingham Study show that homocysteine levels reach a plateau and do not significantly decrease further with additional folate intake [44]. When we performed additional analyses to explore whether folate intake beyond the RDA resulted in increased protection, we found that total folate intake much above the RDA did not confer additional protection (results not shown). This last observation gives weight to the hypothesis that folate supplementation may lower the risk of AD through lowering of homocysteine levels. It should be noted, however, that more recent studies suggest that homocysteine levels can be reduced even in subjects who do not have a vitamin deficiency [45,46].

Mandatory folate fortification of grain products began in the United States in 1998 with the intention of reducing neural tube defects [47]. It has been estimated that dietary intake of folate will increase by about 100  $\mu\text{g}/\text{d}$  as a result of mandatory fortification [19]. The dietary data collection for our study occurred before this event. We estimated that

increase owing to fortification would result in an additional 18% of the subjects in our study reaching a level above the RDA, where we believe protection is conferred. Still, almost half (47%) of the participants would be deficient, suggesting that additional supplementation would nevertheless be needed to reach the RDA and levels of protection.

Our study has several strengths. The cohort is well characterized in terms of dementia and AD with standard criteria and methodology. Participants were followed up for up to 14 years, making it one of the longest follow-up periods in this literature. We used a valid and reliable dietary assessment instrument often used to validate other instruments, such as food frequency questionnaires [48,49]. We quantified vitamin intake from both diet and supplements allowing us to estimate the effect of their combined contribution. We also included in our analyses other potential confounders that might influence the results such as caloric intake, cholesterol levels, blood pressure, smoking, and BMI.

One of the limitations of our study is the relative homogeneity of the BLSA cohort in education and ethnicity. The cohort consists of well-educated, mostly white volunteers. Therefore, the results of this study may not be generalizable to other populations. This relative homogeneity, however, may minimize the possible confounding effects of education and ethnicity. We also had a limited ability to assess the stability of total intake over time because few participants had multiple intake reports. Among those with 2 reports ( $N = 160$ ) collected on average 3.9 years apart, there were no significant changes in total intake of the 6 vitamins. It is still possible, however, that dietary intake patterns may have changed during follow-up, but we were unable to measure or analyze them. Another possible limitation of this study is the relatively low number of AD cases that developed over the course of follow-up, thereby potentially limiting our power to detect other significant associations.

The possibility still exists that people in the early pre-clinical stages of dementia may have had difficulty accurately completing the diary. Thus, we also performed an analysis that excluded all participants who completed the dietary diary less than 3 years before their last follow-up. This resulted in the exclusion of 28 participants, including 8 AD cases, and produced similar results; only high intake of folate was associated with a significant reduction in risk of AD (results not shown). We also analyzed intake of vitamins in relation to age at onset rather than age of diagnosis of AD, with total intake of folate remaining as the only significant association with AD risk reduction when analyzing the vitamins together.

There was a potential for bias resulting from the exclusion of those participants who did not complete and return the dietary diary ( $N = 313$ ). However, we found no major differences (in age, education, proportion of men, proportion with a diagnosis of dementia or AD) except a small significant difference in length of follow-up. Given that procedures in the BLSA are voluntary, it is likely that

people elected not to complete the dietary diary because collecting the record is arduous and not because of reasons related to their cognitive abilities.

It is important to note that there was a suggestion of risk reduction with high intake of vitamin E. However, when folate, vitamin E, and vitamin B<sub>6</sub> were analyzed together, only folate remained as the vitamin with a significant risk reduction. It would be interesting to examine if other studies that reported a protective effect with antioxidants could replicate their results once folate intake is analyzed simultaneously. Given the observational nature of this and other previous studies, it is still possible that unmeasured factors may be responsible for the reduction in risk. People with a high intake of one nutrient are likely to have a high intake of several nutrients and may generally have a healthy lifestyle.

This prospective study found an inverse association between the risk of AD and total intake of folate. Protection was observed among participants with a total folate intake at or above the RDA, which was reached by most of the participants only after folate supplementation. A clinical trial would be necessary to minimize the effect of unmeasured or unknown confounders and to establish a protective role for folate in AD.

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