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DIETARY FOLATE, VITAMIN B-12, VITAMIN B-6 AND INCIDENT ALZHEIMER'S DISEASE: THE CACHE COUNTY MEMORY, HEALTH, AND AGING STUDY

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Abstract

Objective—To examine associations between dietary and supplemental folate, vitamin B-12 and vitamin B-6 and incident Alzheimer's disease (AD) among elderly men and women.

Design, Setting and Participants—Data collected were from participants of the Cache County Memory, Health and Aging Study, a longitudinal study of 5092 men and women 65 years and older who were residents of Cache County, Utah in 1995.

Measurements—Multistage clinical assessment procedures were used to identify incident cases of AD. Dietary data were collected using a 142-item food frequency questionnaire. Cox Proportional Hazards (CPH) modeling was used to determine hazard ratios across quintiles of micronutrient intake.

Results—202 participants were diagnosed with incident AD during follow-up (1995–2004). In multivariable CPH models that controlled for the effects of gender, age, education, and other covariates there were no observed differences in risk of AD or dementia by increasing quintiles of total intake of folate, vitamin B-12, or vitamin B-6. Similarly, there were no observed differences in risk of AD by regular use of either folate or B6 supplements.

Conclusion—Dietary intake of B-vitamins from food and supplemental sources appears unrelated to incidence of dementia and AD. Further studies examining associations between dietary intakes of B-vitamins, biomarkers of B-vitamin status and cognitive endpoints are warranted.

Keywords

Folate; B-vitamins; Alzheimer's disease; elderly; dementia

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Introduction

The prevalence of Alzheimer's disease (AD) is expected to more than double in the next fifty years with the aging of the U.S. population. The U.S. Centers for Disease Control and Prevention (CDC) lists AD as the 7th leading cause of death in the US (1). In 2003, AD increased the cost of health care by 80–100 billion dollars (2).

AD is an age-related disease with a strong genetic component, but risk is also likely influenced by modifiable factors, including diet (2, 3). B-vitamins including folate, vitamin B6, and vitamin B12 may be related to cognitive health in several ways. Low levels of B-vitamins have been associated with increased homocysteine levels and have been observed in the cognitively impaired in several large population-based studies (4). In addition, B-vitamins may affect levels of S-adenosyl-methionine (SAM), an important intermediate for key methylation reactions in the brain (4). Finally, folate is essential for nucleic acid formation and deficiency is associated with chromosomal breakage (5).

Research examining associations between B-vitamin intake, cognitive decline, and risk of dementia is inconclusive (6). In large prospective studies high intake of folate has been identified as both harmful (7, 8) and protective (9) in relation to cognitive decline and incidence of AD. Other observational studies have shown no relationship between folate, B-vitamins, the rate of cognitive decline and AD (10–13). The objective of this study is to examine associations between dietary and supplemental folate, vitamin B-12 and vitamin B-6 and incident Alzheimer's disease (AD) among elderly men and women.

Subjects and Methods

Study Population

The Cache County Memory Study (CCMS) is a population-based prospective study of men and women who were living in Cache County, Utah and who were 65 years or older at the baseline assessment in 1995. Of the 5,657 potential participants identified in 1995, 89.7% were enrolled (n = 5092) and provided baseline information. Baseline and subsequent screening interviews included self or proxy reports of demographic variables, medical history, occupational history, smoking or alcohol history, and information on family history, as well as information about usual dietary intake and consumption of dietary supplements. Methods and procedures were reviewed by Institutional Review Boards at Utah State, Duke, and Johns Hopkins Universities and all participants or legal caregivers signed an informed consent to participate.

Incident dementia was assessed in careful multiple staged clinical evaluations over 9 years of follow-up as described in detail in previous CCMS publications (14). Cognitive function was screened at baseline and three subsequent follow-up assessments using a modified version of the Mini Mental State Examination (3MS). Participants who scored lower than 87 on the 3MS and all subjects older than 90 years of age were further assessed for cognitive status using multi-staged clinical assessments of cognitive status. The clinical data included results from the Dementia Questionnaire (DQ) (15) and a clinical assessment conducted in the participant's home or place of residence. The DQ is an inventory of cognitive symptoms, functional impairments, and medical conditions relevant to dementia and was administered by trained nurses. The clinical evaluations were reviewed by a geropsychiatrist and a neuropsychologist, who assigned dementia diagnoses. Participants initially assigned a dementia diagnoses were further examined and completed a magnetic resonance imaging scan and additional laboratory tests. Final diagnoses were obtained via a consensus conference of clinical experts.

An estimate of usual dietary intake at the baseline interview in 1995 was collected using a 142-item self-administered food frequency questionnaire (FFQ) that included a section on dietary supplement usage. The FFQ used was modeled after the Harvard Nurses' Health Study FFQ; its design has been tested and validated for replicated use (16, 17). FFQs are often used in large population-based studies to estimate average intake over long periods of time, can be self-administered, are relatively quick to complete and are inexpensive. Participants were asked to report the frequency of consumption of each of the 142-food items. To calculate intake of a specific nutrient, the nutrient content of each food was multiplied by the frequency of consumption for each food and then summed over all food items. Nutrient composition was obtained from the Food Processor (ESHA version 7.02) (18), a nutrient database of approximately 30,000 foods including foods from the USDA nutrient composition data tables and brand-specific information obtained from manufacturers (18). Nutrient intake was adjusted for total energy intake using the residual method (19). Energy-adjusted dietary intake was summed with nutrient intakes from supplements to obtain total intake of nutrients.

Ther were 5092 participants screened using the 3MS at the baseline interview and those who scored > 87 points (n=4737) were invited to complete the FFQ. Of the 4737, 3829 (81%) completed and returned the questionnaire; of those, 197 were excluded because they were identified as having prevalent dementia or were deemed to have provided implausible caloric intake (energy intake ≥ 5000 kilocalories or ≤ 500 kilocalories per day). The final analysis included 3634 participants.

Statistical analysis

The SPSS version 15.0 for Windows software program was used for all statistical analysis. Exposure variables were defined using quintiles of total intakes (food and supplement) of folate, B-12, and B-6 and quintiles of food and beverage sources of folate, B-12 and B-6. Additionally, participants who usually consumed at least 400 μg of folic acid or 2 mg of vitamin B-6 per day were classified as folic acid or B-6 supplement users, respectively. Differences between groups were examined using chi-square tests of independence for categorical variables and one-way ANOVAs for continuous variables. Cox Proportional Hazards models were used to evaluate risk of incident dementia and AD across increasing quintiles of B-vitamin intake. The time variable was defined as the age at diagnosis of dementia, current age if non-demented and remaining in the study or age at death or last follow-up. The outcome variables were incident dementia n=353 and incident AD n=212.

Covariates used in the analysis (model 3) were obtained from the baseline interview and included gender, level of education (less than high school education or greater than high school education), Apo €4 genotype, history of tobacco use (ever/never) and alcohol use (ever/never), physical activity pattern (≥ a few times per month vs. ≤ a few times per month), total caloric intake (kcal/day), self-reported comorbidities [(history of diabetes (ever/never), myocardial infarction (ever/never), and stroke (no/probable) at baseline)], and intake of folate, B-12, and B-6.

The significance of the effects of interest was tested by comparing the difference in the −2 log likelihood ratio statistics from the model with and without the variable. The Likelihood Ratio Test (LRT) was used to examine the fit of the models examining incident AD.

Results

Two-hundred and twelve participants developed AD during the period of observation (1995 – 2003) Table 1 includes characteristics of the population by gender. Women in the CCMS had higher rates of supplement use, were more educated, older, and had higher rates of

incident AD than men (P < 0.001, <0.001, <0.01, respectively). Men consumed more kilocalories, exercised more often, had higher rates of myocardial infarction and were more likely to have ever smoked and or drank alcohol than women (P < 0.001, P < 0.001, P < 0.001, P < 0.001, P < 0.001, respectively).

Table 2 displays population characteristics by quintiles of total folate, B-12, and B-6 intake. Women were more likely to be in the highest quintiles of intake than men. Participants with high intakes of B-vitamins from food and supplements had higher intakes of fruits and vegetables and were more likely to take dietary supplements than were participants with lower intakes.

Cox proportional hazards models were used to examine associations between B-vitamin intakes and risk of incident dementia and AD (Tables 3–5). No associations were observed between increasing quintiles of total folate and incident dementia or AD in either unadjusted or adjusted models that included education and gender. Adding total B-12, total B-6, number of apo €4 alleles, body mass index (BMI), total calories, physical activity, history of alcohol and tobacco use, history of myocardial infarction, diabetes, and stroke in the models did not alter results. These analyses were repeated across quintiles of folate from food only and by folic acid supplements use. No associations were found in either analysis. In similar analyses examining the independent effects of vitamins B-12 and B-6 intakes from food and supplement combined, food only sources and supplemental intake (B-6 only) were not associated with incident dementia or AD.

Discussion

Dietary intake of folate was not associated with risk for incident dementia or AD among men and women of the Cache Study on Memory, Health, and Aging after nine years of follow-up. In multivariable-adjusted models no associations were observed for either folate, vitamin B-6, or vitamin B-12 from foods or supplements or both combined.

Several plausible biological mechanisms link folate and other B-vitamins to AD risk. Deficiencies of B-vitamins have been known to raise levels of homocysteine as a consequence of disrupted one-carbon metabolism (4). Elevated levels of homocysteine may induce oxidative stress and increase the neurotoxicity (20–22). Damaged or altered environment in the brain may contribute to increased plaques and tangles observed in persons with AD. In addition to elevated homocysteine levels, other consequences of disturbed one-carbon metabolism include decreased levels of S-adenosyl-methionine (SAM), increased S-adenosylhomocysteine (SAH), and limitation of folate metabolites involved in nucleotide synthesis (4, 5). SAM is required for the methylation of DNA, RNA and neurotransmitters and inadequate levels of SAM may impair neurons and damage vital brain structures (4, 5). SAH, the precursor to homocysteine, has also been observed at irregular levels in individuals with AD similar to homocysteine, SAM and B-vitamins (23) and is thought to increase as homocysteine metabolism is inhibited. Increases in SAH have been seen to inhibited normal functions of SAM in the brain (24).

Vitamin B-12 is a cofactor for methionine synthase, the enzyme that transfers a methyl group from 5-methyl-THF to homocysteine to form methionine. The conversion of 5,10 methylene-THF to 5-methyl-THF requires the enzyme, methyltetrahydrofolate reductase. This reaction is irreversible, therefore deficiency of B-12, prevents 5-methyl-THF from being converted to 5,10 methylene-THF, essential for the synthesis of nucleotides. Low levels of nucleotides may result in misincorporation of neucleotides in DNA replication and chromosomal breaks which may facilitate neuronal damage common in patient’s brains (5).

Homocysteine can be metabolized in an alternative pathway that requires B-6 as a cofactor in converting homocysteine to cystathione. Further, more glutathione is produced in a downstream reaction and is a key antioxidant for preventing lipid peroxidation and other oxidative damage. Therefore, disrupted brain function may also be related to the decreased production of glutathione via disordered metabolism of homocysteine to cystathione.

The prevalence of B-vitamin deficiencies, especially B-12, is highest among elderly persons. Gastritis and other conditions that inhibit vitamin B-12 absorption have been estimated to affect 20–50% of the elderly in the US. Depending on the diagnosis criteria, 24% of elders age 60–69 and 37% of those older than 80 were found to have gastritis in the Framingham Studies cohort (24). Deficiency of folate is also suspected to be higher among the elderly and may increase with age due to decreased absorption caused by changes in the gastrointestinal tract. Studies examining folate intake post-fortification have also seen average intakes below recommendations among all age groups (25, 26).

Beginning in 1998, U.S. Federal law required that all cereal grain products to be fortified with folic acid in order to reduce the birth prevalence of neural tube defects. A standard level of 140 μg folic acid per 100 grams of grain was required (27). Some have raised concern that the benefits may not reach all populations and may even be detrimental to subgroups (7, 8). High levels of folic acid may “mask” evidence of vitamin B-12 deficiency (28, 29). A prolonged B-12 deficiency has been associated with cognitive deficits that in certain cases, depending upon severity and duration of the deficiency, appear to be irreversible (25).

Since folate fortification in the U.S., increases in average serum levels of folate have been observed indicating that though many products, like cereals, were already fortified with folic acid, the mandate did meet its goal of increasing average intakes of folic acid. The Framingham group observed a 38% increase of serum RBC folate among participants who did not consume supplements post-fortification; they also estimated that 3% less of their cohort were deficient in folate post-fortification compared to pre-fortification (29). If folate was independently associated with increased risk for cognitive decline and AD, a decrease in incidence would have been expected. No such report or observation has been made. One study, by Morris et al. (8) did report increased risk for anemia and cognitive decline among those with low serum B-12 and high serum folate. The study included 1459 senior participants in the 1999–2002 US National Health and Nutrition Examination Survey. Morris et al. (7) also found high folate intake to be associated with faster cognitive decline among a group of elderly from the Chicago Health and Aging Project. These finding support concerns that high folate may be toxic in certain situations.

In 1995, the CCMS participants average intake of folate from food was below the RDA (400 μg/d) at 319 μg/d and 46.9 % received less than the RDA from food and supplements combined. This data was collected pre-folate fortification and folate intake would be expected to be higher post fortification. Associations between dietary intake and AD may be difficult to detect as dietary intake in elderly may not indicate actual nutriture. Increases in malabsorption related to gastritis or pernicious anemia contribute to the problem of using dietary data alone to assess possible associations between B-vitamins and AD.

It is not surprising that results from population-based prospective studies on B-vitamins and cognitive health vary significantly given the variation in study methods. Results from the CCMS study appear consistent with some (10) but not all recently published data (6, 30) similar in design. There is little information on dietary intake of B-vitamins and risk of incident AD (6, 10, 30). Corrado et al. (31) found no independent relation between total B-12 with incident of AD in the Baltimore Longitudinal Study of Aging (BLSA). The BLSA

is a population based cohort study of elderly men and women, although somewhat younger than the elderly of the Cache study, from the Baltimore area followed for 9.3 years. Higher folate was associated with decreased risk for incident AD (RR: 0.45; 95% CI: 0.2, 0.97) in a model that included age, gender, education, and caloric intake. Morris and colleagues conducted longitudinal assessment of incident AD across quintile of increasing folate consumption from the Chicago Health and Aging Project (CHAP) (10). Of 1041 participants of the CHAP study, 162 developed incident AD after 3.9 years of follow-up. Higher B-vitamin intakes from food and food and supplement were not associated with incidence of AD. Average intake of total folate in the CHAP and the Cache study was similar CHAP: 338 μg/d and CCMS: 319 μg/d). The Washington Heights Inwood Columbia Aging Project (n=965) reported a higher incidence of AD than that reported in the Cache study (109 vs. 212, respectively) (30). The highest quartile of folate (food and supplement) in the Washington Heights Study intake (>489.7 μg) (equivalent to quintile four in the CCMS) was related to a decrease risk for AD (HR: 0.5; 95% CI: 0.3, 0.9).

Among studies that explored serum biomarkers and incident dementia and AD the results also vary. Among studies that examined homocysteine two out of three found elevated homocysteine to be related to incident AD (32–34). Wang et al. (35) examined serum folate and B-12 in relation to incident AD and found nearly double the risk for AD among subjects deficient in folate or B-12. The Conselice Study of Brain Aging (CSBA) also found low serum folate to be related to increased risk for AD (6).

Recently, a clinical trial involving 340 participants with mild to moderate AD administered B-vitamin supplements including 5 mg of folate, 25 mg of vitamin B6 and 1 mg of B12 a day for 18 months (36). Although homocysteine levels were significantly decreased, there was no improvement or slowing of cognitive decline among the treatment groups compared to placebo. Supplemented groups experienced a high incidence of depression (36). Another trial involving B-vitamins also reported no improvement in delay of decline with supplementation (37).

The Cache County Study has many strengths including a large long-lived and homogenous population, lengthy follow-up time, high rates of participation and retention, a extensive set of demographic information, medical history, medication usage, detailed and repeated cognitive assessment, dietary intake, lifestyle, occupational history and environment information. A limitation of this study is a lack of biomarkers. The majority of the population is Caucasian and are members of The Church of Jesus Christ of Latter-day Saints, and consequently has low rates of alcohol and tobacco use. Participants also have about an 80% rate of having at least a high school education. Detailed clinical assessment was used to assign diagnosis.

In summary folate intake from food, supplements, or from combined sources was not associated with incident dementia or AD. In addition, this null association was not mediated by intakes of vitamin B6 or vitamin B12, cofactors of folate's role in one-carbon metabolism. The observed average intake of folate from food in 1995 was less than RDAs; although average intake of folate from food and supplement combined exceeded the DRI. Further studies should be done using serum biomarkers to assess the influence and possible interactions of B-vitamin status in relation to incident dementia and AD. Based on results from this study, general recommendations to increase folate intake or other B-vitamin intake in attempt to reduce risk of dementia or AD is not supported. Studies that include examining dietary B-vitamins, associated serum biomarkers, and incident dementia/AD with adequate power and follow-up time would be valuable to clarifying relationships.

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Table 1

Population characteristics of participants in the Cache County Memory, Health, and Aging Study by gender; baseline interview conducted in 1995

	Male (n=1564)	Female (n=2070)
Age (yrs)	74.19 (6.53)	75.00 (6.75)***
Education (% >High School)	81.5%	86.6%***
Dementia (%)	8.9%	10.3%*
Alzheimer's (%)	4.7%	6.7%**
Total energy intake (kcals/d)	2049 (786)	1882 (764)***
Food folate intake (μg/d)[2]	303 (124)	331 (128)***
Multivitamin supplement users (%)	38.6%	45.8%***
BMI (kg/m^2)	26.40 (3.87)	26.05 (4.82)*
Use Tobacco (% ever)	34.8%	6.9%***
Use Alcohol (% ever)	27.3%	7.4%***
Moderate Physical Activity (% Yes[3])	89.0%	83.0%***
Stroke (% Probable/Uncertain)	4.5%	3.5%
MI (% at baseline)	17.5%	8.8%***
Diabetes (% at baseline)	14.0%	12.0%
ApoE alleles (% 1–2 copies)	32.3%	30.9%

[1] χ ± SD (all such values);

[2] Energy adjusted nutrients from food;

[3] Yes = a few times a month or more;

* p<0.05;

** <0.01;

*** p<0.001

Table 2

Population characteristics of participants in the Cache County Memory, Health, and Aging Study by quintiles of folate, B-12, and B-6 intake from food and supplemental sources; baseline interview conducted in 1995[1]

	Quintiles of Total Folate Intake				
	1 (n=727)	**2 (n=727)**	**3 (n=727)**	**4 (n=727)**	**5 (n=726)**
Age (baseline)	74.6 (7.1)	74.5 (6.4)	74.5 (6.4)	75.0 (6.6)	75.0 (6.9)
Male (%)	52.6%	49.5%	41.1%	35.4%	36.6%**
Education (% > High School)	80.0%	84.5%	84.0%	86.2%	87.3%**
Dementia (%)	9.9%	10.2%	8.8%	10.1%	9.6%
Alzheimer's (%)	5.2%	6.6%	5.2%	6.2%	5.9%
Average 3MS (baseline)	90.2 (7.2)	91.5 (6.4)	91.6 (5.9)	91.4 (6.2)	91.2 (6.6)***
Total energy (kcals/d)	2212.5 (874)	1867 (775)	1797 (687)	1815 (661)	2083 (784)***
Total folate (μg/d)[1]	333 (216)	430 (225)	476 (219)	524 (214)	698 (274)***
Food folate (μg/d)[1]	209 (47.2)	299 (23.0)	374 (100)	285 (96.1)	429 (172)***
Food B-12 (μg/d)[1]	5.2 (2.8)	6.1 (3.4)	6.6 (4.4)	6.0 (4.4)	6.9 (5.9)***
Food B-6 (μg/d)[1]	1.7 (0.5)	2.2 (0.4)	2.4 (0.6)	2.1 (0.6)	2.7 (0.9)***
MVM supp users (%)	1.7%	3.0%	18.7%	91.9%	95.0%***
Folic acid supp users (%)	0.1%	0.1%	7.7%	45.6%	46.5%***
Servings of fruit/day	1.9 (1.5)	2.4 (1.4)	3.0 (1.7)	2.5 (1.8)	3.6 (2.6)***
Servings of vegetable/day	2.5 (1.5)	3.3 (1.9)	4.3 (2.6)	3.2 (2.3)	5.0 (3.5)***

	Quintiles of Total B-12 Intake				
	1 (n=727)	**2 (n=727)**	**3 (n=727)**	**4 (n=727)**	**5 (n=726)**
Age (baseline)	74.9 (7.1)	74.0 (6.4)	74.8 (6.8)	74.6 (6.5)	74.9 (6.6)**
Male (%)	47.7%	46.9%	43.3%	39.5%	37.8%***
Education (% > High School)	81.8%	83.5%	85.4%	87.7%	83.6%*
Dementia (%)	7.3%	5.7%	8.5%	5.7%	6.5%
Alzheimer's (%)	6.0%	4.8%	7.2%	5.1%	6.0%
Average 3MS (baseline)	90.1 (7.0)	91.1 (6.6)	91.2 (6.5)	92.0 (5.8)	90.8 (6.4)*

	Quintiles of Total B-12 Intake				
	1 (n=727)	**2 (n=727)**	**3 (n=727)**	**4 (n=727)**	**5 (n=726)**
Total energy (kcals/d)	2207 (761)	1682 (702)	2054 (775)	1796 (658)	2030 (834) ***
Total B-12 (μg/d) [1]	3.4 (1.3)	5.5 (0.5)	8.1 (1.1)	11.1 (0.6)	20.1 (18.5) ***
Food folate (μg/d) [1]	280 (114)	303 (83.1)	325 (123)	316 (109)	374 (171) ***
Food B-12 (μg/d) [1]	3.3 (1.4)	5.4 (0.9)	5.8 (2.5)	6.2 (2.5)	10.1 (7.4) ***
Food B-6 (μg/d) [1]	1.9 (0.6)	2.2 (0.5)	2.2 (0.7)	2.2 (0.6)	2.5 (0.9) ***
MVM supp users (%)	2.6%	3.3%	39.0%	80.8%	85.3% ***
Folic acid supp users (%)	1.2%	1.6%	3.9%	6.4%	8.9% ***
Servings of fruit/day	3.0 (2.5)	2.2 (1.6)	2.8 (2.0)	2.5 (1.7)	2.9 (1.9) ***
Servings of vegetables/day	3.9 (2.9)	3.1 (2.2)	3.7 (2.6)	3.5 (2.4)	4.0 (2.9) ***

	Quintiles of Total B-6 Intake				
	1 (n=727)	**2 (n=727)**	**3 (n=727)**	**4 (n=727)**	**5 (n=726)**
Age (baseline)	74.8 (6.7)	74.6 (6.7)	75.0 (7.0)	74.5 (6.5)	74.74 (6.4)
Male (%)	54.1%	45.2%	41.1%	36.2%	38.5% ***
Education (% > High School)	81.3%	83.5%	85.3%	88.7%	83.4% **
Dementia (%)	7.8%	6.3%	6.8%	6.3%	6.5%
Alzheimer's (%)	6.3%	5.5%	6.3%	5.4%	5.8%
Average 3MS (baseline)	90.8 (7.1)	91.5 (6.2)	90.7 (6.7)	91.3 (6.4)	91.6 (5.9) *
Total energy (kcals/d)	2101 (853)	1792 (689)	1975 (752)	1876 (742)	2025 (805) ***
Total vitamin B-6 (μg/d) [1]	1.57 (0.33)	2.16 (0.14)	2.96 (0.41)	4.24 (0.30)	25.8 (39.9)
Food folate (μg/d) [1]	236 (73.0)	302 (63.5)	352 (121)	338 (114)	371 (181) ***
Food B-12 (μg/d) [1]	5.1 (3.2)	6.3 (3.2)	6.6 (5.2)	6.1 (3.3)	6.6 (5.8) ***
Food B-6 (μg/d) [1]	1.6 (0.3)	2.2 (0.2)	2.4 (0.7)	2.3 (0.6)	2.6 (1.0) ***
MVM supp users (%)	2.13%	2.42%	28.7%	93.9%	84.4% ***
Folic acid supp users (%)	0.58%	0.60%	1.05%	2.1%	17.8% ***
Servings of fruit/day	3.0 (2.5)	2.2 (1.6)	2.8 (2.0)	2.5 (1.7)	2.9 (1.9) ***
Servings of vegetables/day	3.9 (2.9)	3.1 (2.2)	3.7 (2.6)	3.5 (2.4)	4.0 (2.9) ***

[1] Energy adjusted nutrients from food and supplement;

χ^2 ± SD (all such values);

* p<0.05 for difference across quintiles;

** <0.01 for difference across quintiles;

*** p<0.001 for difference across quintiles

Table 3

Hazard ratios (95% CIs) for quintiles of total folate, vitamin B-12, and vitamin B-6 intake over nine years of dementia or AD incidence

	Quintiles of Total Folate Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	1.07 (0.78, 1.49)	0.89 (0.64, 1.25)	0.99 (0.72, 1.37)	1.01 (0.73, 1.40)	0.86
Model 2	Ref	1.05 (0.76, 1.45)	0.90 (0.64, 1.33)	0.96 (0.69, 1.33)	0.98 (0.70, 1.36)	0.71
Model 3	Ref	1.02 (0.67, 1.57)	0.87 (0.53, 1.45)	1.13 (0.60, 2.12)	1.42 (0.69, 2.88)	0.45
AD						
Model 1	Ref	1.26 (0.83, 1.94)	0.94 (0.60, 1.47)	1.10 (0.72, 1.70)	1.16 (0.75, 1.80)	0.76
Model 2	Ref	1.21 (0.79, 1.85)	0.91 (0.58, 1.43)	1.02 (0.66, 1.58)	1.07 (0.69, 1.67)	0.93
Model 3	Ref	1.14 (0.71, 1.84)	0.95 (0.54, 1.66)	1.36 (0.68, 2.72)	1.75 (0.80, 3.83)	0.23
	Quintiles of Total B-12 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	1.00 (0.66, 1.52)	1.28 (0.88, 1.86)	0.88 (0.58, 1.33)	0.94 (0.63, 1.40)	0.60
Model 2	Ref	0.98 (0.65, 1.49)	1.24 (0.85, 1.81)	0.85 (0.56. 1.28)	0.90 (0.60, 1.35)	0.47
Model 3	Ref	0.93 (0.60, 1.46)	1.14 (0.74, 1.74)	0.83 (0.49, 1.39)	0.87 (0.52, 1.46)	0.56
AD						
Model 1	Ref	0.96 (0.62, 1.47)	1.00 (0.63, 1.57)	1.28 (0.85, 1.92)	0.92 (0.59, 1.44)	0.98
Model 2	Ref	1.01 (0.64, 1.59)	1.29 (0.86, 1.95)	0.92 (0.59, 1.44)	0.99 (0.64, 1.53)	0.84
Model 3	Ref	0.95 (0.58, 1.54)	1.14 (0.71, 1.81)	0.87 (0.50, 1.53)	0.91 (0.52, 1.60)	0.72
	Quintiles of Total B-6 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	0.79 (0.53, 1.17)	0.78 (0.53, 1.15)	0.80 (0.54, 1.19)	0.82 (0.55, 1.21)	0.36
Model 2	Ref	0.76 (0.51, 1.14)	0.75 (0.51, 1.11)	0.75 (0.50, 1.12)	0.78 (0.52, 1.15)	0.24
Model 3	Ref	0.74 (0.48, 1.14)	0.62 (0.38, 1.03)	0.69 (0.36, 1.34)	0.67 (0.34, 1.30)	0.28
AD						
Model 1	Ref	0.86 (0.56, 1.32)	0.87 (0.57, 1.32)	0.85 (0.55, 1.31)	0.85 (0.55, 1.31)	0.56

	Quintiles of Total B-6 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Model 2	Ref	0.82 (0.53, 1.27)	0.82 (0.54, 1.25)	0.79 (0.51, 1.22)	0.82 (0.53, 1.26)	0.37
Model 3	Ref	0.79 (0.49, 1.27)	0.62 (0.36, 1.07)	0.61 (0.30, 1.27)	0.58 (0.27, 1.21)	0.16

Model 1- unadjusted; Model 2- adjusted for gender, education; Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and the other B-vitamins in quartiles and treated as a continuous variables (17 = df).

Table 4

Hazard ratios (95% CIs) for quintiles of food folate and vitamin B-6 intake over nine years of dementia or AD incidence

	Quintiles of Food Folate Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	0.73 (0.51, 1.04)	1.08 (0.79, 1.48)	1.04 (0.75, 1.42)	0.93 (0.67, 1.28)	0.68
Model 2	Ref	0.71 (0.50, 1.01)	1.07 (0.78, 1.47)	1.01 (0.73, 1.38)	0.89 (0.64, 1.24)	0.84
Model 3	Ref	0.63 (0.40, 1.01)	0.97 (0.64, 1.47)	0.99 (0.64, 1.51)	0.86 (0.55, 1.35)	0.82
AD						
Model 1	Ref	0.86 (0.57, 1.32)	0.72 (0.46, 1.13)	1.03 (0.69, 1.55)	0.99 (0.66, 1.49)	0.23
Model 2	Ref	0.80 (0.50, 1.27)	1.15 (0.75, 1.75)	1.07 (0.69,1.64)	1.04 (0.68, 1.60)	0.47
Model 3	Ref	0.76 (0.46, 1.27)	1.10 (0.69, 1.75)	1.16 (0.72, 1.86)	1.02 (0.62, 1.67)	0.43
	Quintiles of Food B-12 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	1.07 (0.77, 1.48)	0.95 (0.67, 1.34)	1.33 (0.96, 1.83)	1.00 (0.72, 1.38)	0.58
Model 2	Ref	1.11 (0.80, 1.54)	0.94 (0.67, 1.34)	1.36 (0.99, 1.87)	1.01 (0.73, 1.40)	0.58
Model 3	Ref	1.13 (0.73, 1.75)	0.84 (0.52, 1.36)	1.30 (0.84, 2.01)	0.90 (0.60, 1.37)	0.79
AD						
Model 1	Ref	0.95 (0.61, 1.46)	0.72 (0.45, 1.16)	1.36 (0.91, 2.04)	1.09 (0.73, 1.63)	0.26
Model 2	Ref	1.10 (0.65, 1.55)	0.72 (0.45, 1.16)	1.37 (0.92, 2.06)	1.09 (0.73, 1.63)	0.31
Model 3	Ref	1.08 (0.67, 1.76)	0.83 (0.49, 1.40)	1.46 (0.91, 2.33)	0.95 (0.61, 1.49)	0.81
	Quintiles of Food B-6 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Dementia						
Model 1	Ref	0.94 (0.67, 1.31)	0.99 (0.72, 1.36)	0.93 (0.68, 1.29)	0.92 (0.67, 1.27)	0.64
Model 2	Ref	0.91 (0.65, 1.28)	0.97 (0.70, 1.33)	0.93 (0.67, 1.28)	0.89 (0.64, 1.22)	0.52
Model 3	Ref	0.79 (0.50, 1.24)	1.06 (0.70, 1.61)	0.82 (0.53, 1.28)	0.80 (0.51, 1.23)	0.41
AD						
Model 1	Ref	0.78 (0.49, 1.24)	0.93 (0.61, 1.41)	0.90 (0.59, 1.37)	1.07 (0.73, 1.58)	0.60

	Quintiles of Food B-6 Intake					
	1	**2**	**3**	**4**	**5**	**p-trend**
Model 2	Ref	0.73 (0.46, 1.16)	0.88 (0.58, 1.34)	0.85 (0.56, 1.60)	0.96 (0.65, 1.43)	0.93
Model 3	Ref	0.78 (0.47, 1.28)	1.06 (0.67, 1.68)	0.91 (0.57, 1.45)	0.84 (0.53, 1.35)	0.70

Model 1- unadjusted; Model 2- adjusted for gender, education; Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and the other B-vitamins in quartiles treated as a continuous variables (17 = df).

Table 5

Hazard ratios (95% CIs) for supplemental folate and vitamin B-6 intake over nine years of dementia or AD incidence

	Supplemental Folate Intake*		
	1	2	p-trend
Dementia			
Model 1	Ref	1.01 (0.81, 1.25)	0.94
Model 2	Ref	1.00 (0.80, 1.23)	0.97
Model 3	Ref	1.14 (0.75, 1.74)	0.54
AD			
Model 1	Ref	0.98 (0.74, 1.29)	0.86
Model 2	Ref	0.94 (0.71, 1.25)	0.68
Model 3	Ref	1.13 (0.69, 1.87)	0.58

	Supplemental B-6 Intake**		
	1	2	p-trend
Dementia			
Model 1	Ref	0.90 (0.69, 1.17)	0.43
Model 2	Ref	0.88 (0.68, 1.14)	0.33
Model 3	Ref	0.95 (0.62, 1.46)	0.83
AD			
Model 1	Ref	0.91 (0.69, 1.21)	0.53
Model 2	Ref	0.89 (0.67, 1.17)	0.40
Model 3	Ref	0.84 (0.53, 1.35)	0.45

*Defined by supplemental intake of folic acid 1= <400μg/d, 2= >/= 400μg/d;

**Defined by supplemental intake of B-6 1= <2mg, 2 >2mg; Model 1- unadjusted; Model 2- adjusted for gender, education; Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and the other B-vitamins in quartiles treated as a continuous variables (15 = df).