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Dietary folate and vitamins B-12 and B-6 not associated with incident Alzheimer's disease¹

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Abstract

Context—It is currently not known whether dietary intakes of folate and vitamins B12 and B6, co-factors in the methylation of homocysteine, protect against Alzheimer's disease.

Objective—To examine the association between risk of incident Alzheimer's disease and dietary intakes of folate, vitamin B-12, and vitamin B-6.

Design—Prospective cohort study.

Setting—Geographically defined biracial Chicago community.

Participants—1,041 residents, aged 65 years and older, initially free of Alzheimer's disease and followed a median 3.9 years for the development of incident disease.

Main Outcome Measure—Probable Alzheimer's disease identified through structured clinical neurological evaluation using standardized criteria.

Results—A total of 162 persons developed incident Alzheimer's disease during follow-up. In logistic regression models adjusted for age, sex, race, education, cognitive activities, APOE-ε4, and dietary intakes of vitamin E in food and total niacin, there was no association between risk of developing Alzheimer's disease and quintiles of folate intake or of vitamin B-12 intake. The adjusted odds ratio was 1.6 (95% confidence interval: 0.5, 5.2) for persons in the highest quintile of total folate intake (median of 752.7 µg/d) compared with persons in the lowest quintile of intake (median, 202.8 µg/d). Compared with persons in the first quintile of total vitamin B-12 intake (median, 3.1 µg/d) the odds ratio was 0.6 (95% confidence interval: 0.2, 1.6) for persons in the fifth quintile of intake (median, 20.6 µg/d). Intake of vitamin B-6 was not associated with incident Alzheimer's disease after control for dietary intakes of vitamin E and total niacin.

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Conclusion—Dietary intakes of folate, vitamin B-12, or vitamin B-6 do not appear to be associated with the development of Alzheimer's disease.

Keywords

Folate; folic acid; vitamin B-12; vitamin B-6; Alzheimer's disease; dementia; aging

1. Introduction

There has been much attention on folate and vitamin B-12 as preventive factors against Alzheimer's disease. The primary theoretical basis for this argument rests on the known relations of folate, vitamin B-12, and vitamin B-6 as co-factors in the methylation of homocysteine, and the importance of deficiencies in these nutrients to increased homocysteine concentration. At least one prospective study has reported increased risk of Alzheimer's disease with elevated plasma homocysteine concentration [1]. Several clinical trials tested the effects of supplementation with one or more of folic acid, vitamin B-12, and vitamin B-6 among older persons and found no effect on cognition [2–4]. Data are limited, however, on the direct relation of dietary intake of these vitamins to incident Alzheimer's disease. One small study reported an inverse association with folate intake, but no association with intakes of vitamins B12 and B6 [5]. In the current investigation, we related food and supplement intakes of these vitamins to 4-year incidence of Alzheimer's disease in a large, biracial community study.

2. Methods

2.1. Subjects

Subjects are participants in the ongoing Chicago Health and Aging Project (CHAP). CHAP is a study of community residents aged 65 years and older from a geographically defined biracial population on the south side of Chicago [6]. A door-to-door census in 1993–1997 identified 7,813 age-eligible residents in the community of whom 6,158 participated in baseline home interviews (78.8% of surviving residents). Self-administered food frequency questionnaires were completed by participants a median of 1.2 years after baseline. Two follow-up interviews were conducted on all surviving participants at three-year intervals over six years. Cases of prevalent and incident Alzheimer's disease were identified based on clinical evaluations performed on stratified random samples from the study population. The stratified sampling design allowed for higher probabilities of selection among the older age groups to ensure a large number of incident cases. The representativeness of the sample was maintained through weighting of all sample participants by the inverse of their sampling probability in the analyses. In the current study, we analyzed 1,041 persons who were clinically evaluated for incident Alzheimer's disease and had complete and valid data on all of the important variables. More detailed descriptions of the CHAP study design were published previously [6,7].

The Institutional Review Board of Rush University Medical Center approved the study, and all participants gave written informed consent.

2.2. Clinical evaluation samples

The clinical evaluations were conducted on stratified samples selected at baseline (Cycle 1) and at each of two follow-up assessments (Cycle 2 and Cycle 3). The baseline clinical evaluations ($n = 729$) were used to identify prevalent cases of Alzheimer's disease as well as a disease-free cohort of 3,838 persons to follow for incident Alzheimer's disease at the next cycle. The disease-free cohort included 3,526 persons who had good performance on the

population interview cognitive tests [8,9] and 312 persons whose cognitive performance was intermediate or poor, but were determined to be unaffected by disease at the clinical evaluation. The clinical evaluations for incident disease were conducted in a stratified random sample selected subsequent to the Cycle 2 population interviews from the disease free cohort. Sample selection was within strata defined by age, sex, race, and change in cognitive performance from baseline to the 3-year follow-up (stable or improved, small decline, and large decline). At Cycle 2 a new disease-free cohort of 2,694 persons was identified from which a sample was drawn for incident disease clinical evaluation at Cycle 3 using a stratified random design similar to that of Cycle 2. For the analyses presented here, we combined the two incident disease samples, both of which were clinically evaluated for incident Alzheimer's disease after a median of 3.9 years of follow-up. This included 842 persons evaluated at Cycle 2, and 299 persons evaluated for the first time at Cycle 3. Of the total 1,141 persons in the combined sample, 32 persons had potentially invalid dietary information and 68 persons had incomplete data on important variables leaving 1,041 persons for analysis. More detailed descriptions of the design and combining of the incidence samples were published previously [7,10].

2.3. Dietary assessments

A modified Harvard food frequency questionnaire (FFQ) [11,12] was used to assess usual intake during the past year of 139 food items and vitamin supplements. Nutrient composition of food items was based on the continually updated Harvard nutrient database. For dietary questionnaires completed after 1997, the folate nutrient composition of food items reflected fortified grain levels as mandated by the USDA beginning in January 1998. To estimate daily nutrient intake, the nutrient composition for each food was multiplied by frequency of intake and summed over all food items. The nutrient composition was modified based on specified types of oil used at home, and brand name products of margarine, cereal, and multivitamins. All nutrients were energy-adjusted using the regression residual method [13].

The 32 FFQs deemed potentially invalid and omitted from the analyses either had implausible daily caloric intake (for women <500 kcal or >3800 kcal; for men <700 kcal or >4000 kcal), more than half the food items missing, or were missing an entire food category.

A validity and reproducibility study of the FFQ was conducted in a random sample of 232 CHAP participants using the mean nutrient intake from repeated 24-hour dietary recalls as the comparison method. Spearman correlations for 1-year reproducibility of the FFQ were: 0.70 for total folate, 0.50 for total vitamin B-12, and 0.58 for vitamin B-6. Pearson correlations for validity were: 0.50 for total folate, 0.38 for total vitamin B-12, and 0.51 for total vitamin B-6 [14].

2.4. Alzheimer's disease

Alzheimer's disease was diagnosed on the basis of structured clinical evaluations that were conducted in participants' homes by a team consisting of a neuropsychological technician, nurse clinician, phlebotomist, and neurologist. All team members were blinded to dietary and other participant information. The 2 to 3-hour evaluations included neuropsychological testing (using tests of the Consortium Established for Research on Alzheimer's Disease, CERAD [15]), a complete medical history, medication use, laboratory testing, and neurological examination. Informant interviews were conducted for cognitively impaired participants. The diagnosis of probable Alzheimer's disease was based on criteria of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Associated Disorders Association [16], with the

exception that the diagnosis did not exclude persons with Alzheimer's disease who had a co-existing condition that could cause dementia.

2.5. Other covariates

Most non-dietary variables were collected at participants' baseline interview, and included: age (years), sex, race (black, white), education (years), and frequency of participation in cognitive activities. Frequency of participation in cognitive activities was based on a previously established composite measure representing the average frequency score for seven cognitive activities [17,18] Apolipoprotein E (APOE) genotyping was performed using methods of Hixson and Vernier [19], and the primers described by Wenham et al. [20].

2.6. Statistical analysis

Logistic regression in SAS [21] was used to estimate the odds of incident Alzheimer's disease according to quintiles of vitamin intake using the lowest quintile as the referent category. Similar results were produced when the dietary variables were modeled as continuous variables. All models were weighted for the stratified random sampling design, and variance estimation was computed by jackknife repeated replication [10,22] Effect modification with non-dietary factors was examined in age-adjusted models with terms for quintiles of vitamin intake, the potential effect modifier, and multiplicative terms between each quintile variable and the effect modifier. Statistical significance was determined by $p < 0.05$. In models that examined effect modification among dietary covariates, the dietary variables were modeled as continuous terms. In secondary analyses, models were adjusted for the timing of the dietary assessment in relation to the date of disease free status. This variable was computed as: (diet assessment date – beginning date of time on study)/time on study. All primary models were tested for model assumptions.

3. Results

A total of 161 cases of incident Alzheimer's disease were diagnosed during a median follow-up of 3.9 years among the 1,041 clinically evaluated CHAP participants. Folate intake ranged from 89.7 to 1660 $\mu\text{g}/\text{d}$, with a median intake level of 338 $\mu\text{g}/\text{d}$, or about the equivalent of the recommended dietary allowance of 400 $\mu\text{g}/\text{d}$. Vitamin B-12 intake ranged from 0.53 to 127.7 $\mu\text{g}/\text{d}$, and vitamin B-6 ranged from 0.58 to 100.9 mg/d . High total intakes of all three B-vitamins were associated with a more favorable risk profile for prevention of Alzheimer's disease including younger age, white race, higher educational level, greater participation in cognitive activities, not having the APOE- $\epsilon 4$ allele, and higher intakes of vitamin E and niacin, which were protectively associated with incident Alzheimer's disease in previous CHAP studies [23,24] The only case of association with a negative risk factor was higher intake of saturated fat among persons with high intake of vitamin B-12.

Intake of folate from food and supplements was not associated with the risk of developing Alzheimer's disease. The age-adjusted odds ratio of 0.5 for persons in the top quintile of intake (median of 752.7 $\mu\text{g}/\text{d}$) compared with persons in the lowest quintile of intake (median, 202.8 $\mu\text{g}/\text{d}$) was not statistically significant. (Table 2) The odds ratio for the fifth quintile was modified to 0.7 with additional adjustment for sex, race, education, cognitive activities, and APOE- $\epsilon 4$ allele status. This was further substantially modified to an increased odds ratio of 1.6 in analyses that accounted for confounding by dietary intakes of vitamin E and total niacin, two dietary factors that were found previously to be strong protective factors for Alzheimer's disease in the CHAP study population, and were also linearly associated with folate intake (see Table 1). The estimated odds ratios did not change in models that further controlled for intakes of saturated and trans fats, or of the ratio of

polyunsaturated to saturated fat intake. (data not shown.) A similar pattern of non-significant but increasing odds ratios was observed for folate intake from food intake only, after adjustments for important dietary and non-dietary risk factors (Table 2).

Persons in the highest quintile of total vitamin B-12 intake (median intake, 20.6 µg/d) had a marginally significant decreased risk of Alzheimer's disease compared with persons in the lowest quintile of intake (median 3.1 µg/d) in the age-adjusted model (Table 2). The effect estimate of 0.4 was not modified with adjustment for the important non-dietary risk factors, however, with further adjustment for dietary intakes of vitamin E and total niacin, the odds ratio for the fifth quintile was increased to 0.6 and no longer statistically significant.

An apparent protective, marginally significant, association of high vitamin B-12 intake from food sources in the basic-adjusted model was due to confounding by intakes of dietary vitamin E and total niacin as evidenced by an increase in the odds ratio for the fifth quintile to 1.0.

Total and food intakes of vitamin B-6 had similar patterns of association with Alzheimer's disease, with apparent linear associations in the age- and basic-adjusted models that were strongly modified and no longer statistically significant once the contribution of vitamin E and niacin were accounted for in the analysis (Table 2).

In further analyses, we re-analyzed the data after excluding 18 of the Alzheimer's disease cases who had a co-existing condition that could cause the dementia. However, the estimated odds ratios for the fifth versus first quintiles were: 2.1 (95% CI: 0.5, 8.1) for total folate, 0.6 (95% CI: 0.2, 1.6) for total vitamin B-12, and 0.8 (95% CI: 0.2, 3.1) for total vitamin B-6.

We also examined the data for potential modifications in the vitamin associations with Alzheimer's disease by age, sex, race, and APOE-ε4 genotype, but there was no evidence of statistical interaction with dietary intakes of folate, vitamin B-12, or vitamin B-6. There was also no statistical interaction among dietary intakes of the three B-vitamins on Alzheimer's disease.

In secondary analyses, we investigated whether the negative findings for intakes of folate, vitamin B-12, or vitamin B-6 could be due to changes in dietary consumption patterns among persons who developed Alzheimer's disease, or to poor recall of diet or supplement use among persons with poor cognitive function. First, we reanalyzed the fully adjusted models for the three B-vitamins after including a variable of the time period between FFQ completion and clinical evaluation for incident Alzheimer's disease, however, there were no material differences in the estimated odds ratios for the quintiles of intake. For example, the odds ratios for the fifth versus first quintiles of intake were: 1.6 for folate, 0.6 for vitamin B-12, and 0.7 for vitamin B-6. And finally, when we deleted 50 persons from the analysis who had poor cognitive performance at the baseline, the fully-adjusted odds ratios for the fifth quintiles of intake were: 1.3 for folate, 0.5 for vitamin B-12, and 0.9 for vitamin B-6.

4. Discussion

In this older community-based population, dietary intakes of folate, vitamin B-12 or vitamin B-6 were not associated with the risk of developing Alzheimer's disease over 4 years.

The study findings are not likely the result of confounding bias. We adjusted for the important dietary and other risk factors for Alzheimer's disease in the analyses. But more importantly, dietary intakes of the B-vitamins were strongly correlated with protective risk factors for Alzheimer's disease. This would indicate that residual confounding is in the

protective direction, and therefore not a likely explanation of the null results. The CHAP study has a number of features that minimize the potential for bias, including its prospective design of a stratified random sample from the community, case identification through structured clinical evaluation, use of standardized criteria for disease diagnosis, and examiners blinded to dietary behavior data. In addition, nutrient intake was assessed using a validated food frequency questionnaire that was shown to be unbiased in the estimation of nutrient intake among CHAP participants of different ages, sex, race, educational level, and cognitive ability [14]. A limitation of the study is that a number of dietary assessments occurred after the baseline, and this may have influenced the results, especially if the disease process resulted in changed dietary habits or invalid responses. We attempted to address these issues by adjusting for timing of the dietary assessment in relation to disease evaluation, and by deleting persons from the analysis who had poor memory performance at the baseline. Results of these secondary analyses confirmed the overall finding of no association. Another limitation of the study is the absence of biochemical levels of these nutrients that would determine whether nutrient deficiencies may be related to disease. This limitation is most likely to apply to vitamin B-12 deficiency, which is common in the elderly [25] and has a neurologic syndrome that includes symptoms of dementia. The possibility of folate deficiency is much less since the institution of the folate fortification program in 1998. Recent data from the NHANES study [22], a representative sample of the US population, indicates that only 0.5% of the population is currently folate deficient.

We are aware of only one small prospective study, conducted by Corrada et al. [5] that investigated dietary intakes of the B-vitamins and risk of incident Alzheimer's disease. This study of 579 participants of the Baltimore Longitudinal Study of Aging (BSLA) of whom 57 developed Alzheimer's disease during 9 years of follow-up, found a protective association with higher folate intake. Perhaps because these participants were a select group of high-educated volunteers, the lowest referent category of folate intake was comparable to that of the CHAP population, even though the investigation occurred before the folate fortification program. Three prospective studies examined biochemical levels of folate and/or vitamin B-12 to incident dementia. Two reported no association of serum concentrations of the vitamins with incident Alzheimer's disease [1, 26]. The other study, by Wang et al. [27] reported a near doubling in risk among persons who had deficient levels of either vitamin B-12 (< 250 pmol/L) or folate (< 12 nmol/L), but there was no association when either nutrient was considered alone. We found no evidence of interaction between intakes of the B-vitamins in the CHAP study. The Wang et al study did not adjust for levels of other nutrients, such as vitamin E or niacin, thus the results may have been due to confounding by other nutrient levels. In the CHAP study, adjustment for dietary intakes of vitamin E and niacin substantially altered the odds ratios of Alzheimer's disease and B-vitamin intake, indicating strong confounding by these dietary factors. The BLSA study finding for folate was not adjusted for niacin intake.

Other lines of evidence offered in support of the B-vitamin relation to the development of Alzheimer's disease include studies of B-vitamins on cognitive decline, and studies relating homocysteine concentration to Alzheimer's disease or cognitive change. There are many such studies with cross-sectional or case-control designs that are incapable of distinguishing the causes from effects of disease processes. When one considers only the prospective studies (which do have the correct time relation for a causal interpretation), there is little evidence to support the theory, although there is some evidence that vitamin B-12 and homocysteine concentration may be associated with cognitive change. Of the prospective studies that examined the association of folate levels and cognitive change, three found no association with serum levels [28,29,34] two found greater decline among persons who had low plasma levels of folate [30,31] and one (the CHAP study) observed faster decline at dietary supplement levels greater than 400 mcg/d [32]. In two of these four studies, faster

decline in cognitive ability was associated with low serum [28] and low dietary intake levels [32] of vitamin B-12. Given the absence of association of vitamin B-12 with Alzheimer's disease in the CHAP and other studies, it is possible that the observed relation with cognitive decline is due to the neurologic syndrome of vitamin B-12 deficiency. The occasional finding of an inverse association of folate intake among these prospective studies may be due to lack of control for other dietary risk factors, such as antioxidant nutrients, dietary fats, and niacin. There are limited data to support the association between homocysteine concentration and incident Alzheimer's disease or cognitive decline. For example, only one of two prospective studies of incident Alzheimer's disease found an association [1, 33]. In addition, whereas one study found greater 3-year decline in memory and MMSE scores with higher concentrations of plasma homocysteine [30] no association was observed between homocysteine concentration and change in cognitive ability in four studies that followed participants over approximately two [34], four years [33], six years [29], and seven years [31]. Further, three randomized controlled clinical trials found no effect of B-vitamin supplementation on cognitive function among older participants [2–4].

In summary, we found no association between dietary intakes of vitamin B-12, vitamin B-6, or folate and 4-year risk of incident Alzheimer's disease in a community population of older persons. These data, along with other prospective studies and randomized clinical trials, do not support recommendations of vitamin supplementation with folic acid or vitamin B-12 to prevent Alzheimer's disease, nor do they support folic acid supplementation to slow age-related cognitive decline, at least among persons who are not clinically deficient in these vitamins.

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor dementia and Alzheimer's disease. *NEJM*. 2002; 346:476–483. [PubMed: 11844848]
2. Bryan J, Calvaresi E, Hughes D. Short-term folate, vitamin B-12 or vitamin B-6 supplementation slightly affects memory performance but not mood in women of various ages. *J Nutr*. 2002; 132(6): 1345–1356. [PubMed: 12042457]
3. Clarke R, Harrison G, Richards S. Effect of vitamins and aspirin on markers of platelet activation, oxidative stress and homocysteine in people at high risk of dementia. *J Intern Med*. 2003; 254(1): 67–75. [PubMed: 12823643]
4. Fioravanti M, Ferrario E, Massaia M, et al. Low folate levels in the cognitive decline of elderly patients and efficacy of folate as a treatment for improving memory deficits. *Arch Gerontol Geriatr*. 1997; 26(1):1–13. [PubMed: 18653121]
5. Corrada MM, Kawa CH, Hallfrisch J, Muller D, Brookmeyer R. Reduced risk of Alzheimer's disease with high folate intake: The Baltimore Longitudinal Study of Aging. *Alzheimer's & Dementia*. 2005; 1:11–18.
6. Evans DA, Bennett DA, Wilson RS, et al. Incidence of Alzheimer's disease in a biracial urban community: relation to apolipoprotein E allele status. *Arch Neurol*. 2003; 60:185–189. [PubMed: 12580702]
7. Bienias JL, Beckett LA, Bennett DA, Wilson RS, Evans DA. Design of the Chicago Health and Aging Project (CHAP). *J Alzheim Dis*. 2003; 5:349–355.
8. Albert MS, Smith LA, Scherr PA, et al. Use of brief cognitive tests to identify individuals in the community with clinically-diagnosed Alzheimer's disease. *International Journal Of Neuroscience*. 1991; 57:167–178. [PubMed: 1938160]
9. Smith, A. Symbol Digit Modalities Test Manual- Revised. Los Angeles, CA: Western Psychological; 1984.
10. Bienias, JL.; Kott, PS.; Evans, DA. Alexandria, VA: American Statistical Association; 2003. Applying the Delete-a-Group Jackknife variance estimator to analyses of data from a complex longitudinal survey.

11. Rockett HR, Breitenbach M, Frazier AL, et al. Validation of a youth/adolescent food frequency questionnaire. *Prev Med.* 1997; 26(6):808–816. [PubMed: 9388792]
12. Morris MC, Colditz GA, Evans DA. Response to a mail nutritional survey in an older bi-racial community population. *Ann Epidemiol.* 1998; 8(5):342–346. [PubMed: 9669617]
13. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol.* 1986; 124(17–27)
14. Morris MC, Tangney CC, Bienias JL, Evans DA, Wilson RS. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. *Am J Epidemiol.* 2003; 158:1213–1217.
15. Welsh KA, Butters N, Mohs R, et al. CERAD Part V. A normative study of the neuropsychological battery. *Neurology.* 1994; 44:609–614. [PubMed: 8164812]
16. Anonymous. Criteria for the clinical diagnosis of Alzheimer's disease. Excerpts from the NINCDS-ADRDA Work Group report. *Journal of the American Geriatrics Society.* 1985; 33(1): 2–3. [PubMed: 3965552]
17. Wilson RS, Bennett DA, Beckett LA, et al. Cognitive activity in older persons from a geographically defined population. *J Gerontol Series B-Psych Sci & Soc Sci.* 1999; 54(3):155–160.
18. Wilson RS, Bennett DA, Bienias JL, et al. Cognitive activity and incident AD in a population-based sample of older persons. *Neurology.* 2002; 59(12):1910–1914. [PubMed: 12499482]
19. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res.* 1990; 31(3):545–548. [PubMed: 2341813]
20. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one stage PCR. *Lancet.* 1991; 337:1158–1159. [PubMed: 1674030]
21. SAS/STAT® User's Guide Version 8. Cary, NC: SAS Institute; 2000.
22. Bienias, JL. Replicate-based variance estimation in a SAS macro. *Proceedings of the Fourteenth Annual Meeting of the NorthEast SAS Users Group; Statistics, Data Analysis, and Econometrics Section; 2001. p. 727-735.*
23. Morris MC, Evans DA, Bienias JL, et al. Dietary niacin and risk of incident Alzheimer's disease and of cognitive decline. *J Neurol Neurosurg Psych.* 2004; 75:1093–1099.
24. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer's disease in a biracial community study. *JAMA.* 2002; 287:3230–3237. [PubMed: 12076219]
25. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Elevated serum methylmalonic acid concentrations are common among elderly Americans. *J Nutr.* 2002; 132(9):2799–2803. [PubMed: 12221248]
26. Crystal HA, Ortof E, Frishman WH, Grudber A, Hershman D, Aronson M. Serum vitamin B₁₂ levels and incidence of dementia in a healthy elderly population: A report from the Bronx Longitudinal Aging Study. *J Am Geriatr Soc.* 1994; 42:933–936. [PubMed: 8064100]
27. Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B, Fratiglioni L. Vitamin B(12) and folate in relation to the development of Alzheimer's disease. *Neurology.* 2001; 56(9):1188–1194. [PubMed: 11342684]
28. Starr JM, Pattie A, Whiteman MC, Deary IJ, Whalley LJ. Vitamin B-12, serum folate, and cognitive change between 11 and 79 years. *J Neurol Neurosurg Psychiatry.* 2005; 76(2):291–292. [PubMed: 15654057]
29. Teunissen CE, Blom AH, van Boxtel MP, et al. Homocysteine: a marker for cognitive performance? A longitudinal follow-up study. *J Nutr Health Aging.* 2003; 7(3):153–159. [PubMed: 12766792]
30. Tucker KL, Qiao N, Scott T, Rosenberg I, Spiro A III. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. *Am J Clin Nutr.* 2005; 82(3):627–635. [PubMed: 16155277]
31. Kado DM, Karlamangla AS, Huang MH, et al. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. *Am J Med.* 2005; 118(2):161–167. [PubMed: 15694902]

32. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol*. 2005; 62(4):641–645. [PubMed: 15824266]
33. Luchsinger JA, Tang MX, Shea S, Miller J, Green R, Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. *Neurology*. 2004; 62(11):1972–1976. [PubMed: 15184599]
34. Kalmijn S, Launer LJ, Lindemans J, Bots ML, Hofman A, Breteler MM. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. *American Journal of Epidemiology*. 1999; 150(3):283–289. [PubMed: 10430233]

Table 1

Baseline characteristics* of 1,041 participants of the Chicago Health and Aging Project

	Quintiles of intake				
	1	2	3	4	5
<i>Total folate</i>					
N	210	205	221	195	210
Total Folate Intake (range, µg/d)	90–240	240–304	304–392	392–620	621–1660
Age (mean years)	72.6	73.3	72.6	71.7	73.
Male (%)	39.6	43.1	36.8	38.8	34.4
Black (%)	68.4	60.2	45.8	40.7	34.6
Education (mean years)	11.5	12.8	12.8	13.7	13.8
APOE-ε4 (% at least one allele)	36.0	34.8	37.8	35.8	33.8
Cognitive Activities (mean)	3.1	3.3	3.2	3.4	3.4
Vitamin E from food (mean IU/d)	5.5	5.9	5.9	7.0	6.9
Niacin Intake (mean mg/d)	16.8	18.9	19.3	30.7	50.4
Total Vitamin B-12 (mean µg/d)	7.4	6.9	8.7	12.2	20.5
Total Vitamin B-6 (mean mg/d)	1.5	1.8	2.3	3.6	7.8
Saturated Fat (mean mg/d)	20.0	18.7	18.2	18.2	17.8
<i>Total Vitamin B-12</i>					
N	213	215	202	209	202
Total B-12 Intake (range, µg/d)	0.5–4.4	4.4–6.4	6.4–9.8	9.8–16.3	16.4–127.8
Age (mean years)	73.3	72.2	72.5	72.8	72.5
Male (%)	41.5	45.7	39.7	29.1	34.2
Black (%)	57.0	59.0	43.3	54.7	34.7
Education (mean years)	12.3	12.8	13.1	13.1	13.2
APOE-ε4 (% at least one allele)	40.0	29.0	34.4	41.0	32.4
Cognitive Activities (mean)	3.2	3.2	3.3	3.3	3.4
Vitamin E from food (mean IU/d)	5.7	5.8	6.3	6.6	6.5
Niacin Intake (mean mg/d)	17.5	18.1	27.0	30.4	42.3
Total Folate (mean µg/d)	275.1	299.0	417.8	489.1	639.3
Total Vitamin B-6 (mean mg/d)	1.6	1.8	2.6	3.7	7.0

	Quintiles of intake				
	1	2	3	4	5
Saturated Fat (mean mg/d)	17.0	18.8	19.1	18.4	19.5
N	191	213	227	189	221
Total B-6 Intake (range, mg/d)	0.6–1.4	1.4–1.7	1.7–2.2	2.2–3.9	3.9–100.9
Age (mean years)	72.4	73.0	73.2	71.9	72.7
Male (%)	38.9	29.4	51.0	35.2	31.9
Black (%)	61.5	65.8	45.1	44.4	29.6
Education (mean years)	11.9	11.9	13.1	13.6	14.0
APOE-ε4 (% at least one allele)	42.1	35.1	29.8	39.9	32.2
Cognitive Activities (mean)	3.1	3.1	3.4	3.4	3.4
Vitamin E from food (mean IU/d)	5.4	5.7	6.1	7.3	6.6
Niacin Intake (mean mg/d)	14.7	17.4	19.3	29.5	54.5
Total Folate (mean µg/d)	245.4	280.6	321.6	526.8	756.4
Total Vitamin B-12 (mean µg/d)	6.7	7.4	7.8	11.7	22.1
Saturated Fat (mean mg/d)	19.8	18.6	18.6	18.0	17.8

* All variables (except age) are standardized to the age distribution of the total CHAP population.

Table 2

Odds ratios for incident Alzheimer's disease by quintiles of intake of folate, vitamin B-12, and vitamin B-6 among 1,041 persons after 4.0 years of follow-up, Chicago Health and Aging Project, 1993–2002

	Quintiles of intake					P for Trend*
	1	2	3	4	5	
Total folate						
Median µg/d	202.8	275.2	338.2	474.5	752.7	
Incident Cases (%)	11.5	9.1	15.6	12.1	6.8	
Odds Ratio (95% Confidence Interval)						
Age-Adjusted**	1.0 (referent)	0.7 (0.3, 1.8)	1.4 (0.5, 3.7)	1.2 (0.5, 2.8)	0.5 (0.2, 1.2)	0.12
Basic-Adjusted ^a	1.0 (referent)	0.9 (0.4, 2.1)	1.7 (0.6, 4.4)	1.8 (0.7, 4.6)	0.7 (0.3, 1.7)	0.47
Basic-and Nutrient-Adjusted ^b	1.0 (referent)	1.0 (0.4, 2.3)	1.9 (0.7, 5.0)	2.7 (1.0, 7.1)	1.6 (0.5, 5.2)	0.21
Folate in food						
Median µg/d	184.0	240.2	279.6	330.3	405.2	
Incident Cases (%)	11.0	12.6	8.8	11.8	10.7	
Odds Ratio (95% Confidence Interval)						
Age-Adjusted**	1.0 (referent)	1.3 (0.5, 3.4)	0.7 (0.2, 2.0)	1.1 (0.4, 2.7)	0.8 (0.4, 1.7)	0.55
Basic-Adjusted ^a	1.0 (referent)	1.5 (0.6, 4.0)	0.8 (0.3, 2.2)	1.3 (0.5, 3.2)	1.2 (0.5, 2.5)	0.84
Basic- and Nutrient-Adjusted ^b	1.0 (referent)	1.7 (0.7, 4.1)	0.9 (0.4, 2.4)	1.5 (0.6, 3.5)	1.8 (0.8, 4.1)	0.29
Total Vitamin B-12						
Median µg/d	3.1	5.3	7.8	12.7	20.6	
Incident Cases (%)	14.1	13.0	6.6	15.2	6.1	
Odds Ratio (95% Confidence Interval)						
Age-Adjusted**	1.0 (referent)	1.0 (0.4, 3.0)	0.5 (0.2, 1.1)	1.2 (0.5, 3.0)	0.4 (0.2, 1.0)	0.12
Basic-Adjusted ^a	1.0 (referent)	1.0 (0.4, 2.6)	0.5 (0.2, 1.3)	1.4 (0.6, 3.3)	0.4 (0.2, 1.1)	0.20
Basic- and Nutrient-Adjusted ^b	1.0 (referent)	1.0 (0.4, 2.7)	0.6 (0.3, 1.5)	1.7 (0.7, 4.4)	0.6 (0.2, 1.6)	0.67
Vitamin B-12 in food						
Median µg/d	2.5	4.2	5.6	7.7	14.0	
Incident Cases (%)	11.5	12.3	9.0	12.8	9.4	
Odds Ratio (95% Confidence Interval)						

	Quintiles of intake					P for Trend [*]
	1	2	3	4	5	
Age-Adjusted ^{**}	1.0 (referent)	1.1 (0.5, 2.4)	0.8 (0.3, 2.2)	1.1 (0.4, 3.0)	0.8 (0.3, 2.4)	0.66
Basic-Adjusted ^a	1.0 (referent)	1.0 (0.4, 3.0)	0.4 (0.2, 1.1)	1.2 (0.5, 3.0)	0.4 (0.2, 1.0)	0.73
Basic- and Nutrient-Adjusted ^b	1.0 (referent)	1.4 (0.7, 2.7)	0.8 (0.3, 2.3)	1.5 (0.6, 4.0)	1.0 (0.3, 2.7)	0.87
<i>Total vitamin B-6</i>						
Median mg/d	1.2	1.6	1.9	3.1	5.5	
Incident Cases (%)	14.0	12.6	12.1	11.6	4.9	
<i>Odds Ratio (95% Confidence Interval)</i>						
Age-Adjusted ^{**}	1.0 (referent)	0.8 (0.3, 1.9)	0.8 (0.4, 1.7)	0.9 (0.3, 2.2)	0.3 (0.1, 0.6)	0.002
Basic-Adjusted ^a	1.0 (referent)	1.2 (0.5, 2.5)	0.6 (0.3, 1.4)	1.1 (0.4, 3.0)	0.4 (0.2, 0.8)	0.02
Basic- and Nutrient-Adjusted ^b	1.0 (referent)	0.9 (0.4, 2.1)	1.3 (0.6, 3.1)	1.9 (0.7, 5.1)	0.7 (0.2, 2.4)	0.80
<i>Vitamin B-6 in food</i>						
Median mg/d	1.2	1.4	1.6	1.9	2.2	
Incident Cases (%)	14.4	8.9	15.7	10.7	5.3	
<i>Odds Ratio (95% Confidence Interval)</i>						
Age-Adjusted ^{**}	1.0 (referent)	0.6 (0.2, 1.3)	1.2 (0.5, 2.5)	0.6 (0.3, 1.4)	0.3 (0.2, 0.6)	0.005
Basic-Adjusted ^a	1.0 (referent)	0.7 (0.3, 1.6)	1.3 (0.6, 2.8)	0.8 (0.4, 2.0)	0.4 (0.2, 0.8)	0.10
Basic- and Nutrient-Adjusted ^b	1.0 (referent)	0.7 (0.3, 1.7)	1.6 (0.8, 3.4)	1.0 (0.4, 2.3)	0.7 (0.3, 1.4)	0.66

^{*} P-value for linear trend is based on logistic regression models with the nutrient variable modeled as a continuous variable with persons in each quintile assigned the median value for that quintile.

^{**} Age-adjusted models include terms for age (years), time period of observation (years), and indicator variables for quintiles of nutrient intake.

^a Basic-adjusted models include terms from the age-adjusted model plus sex, race (black/white), education (years), APOE-ε4 (any ε4 versus none), an interaction term between race and APOE-ε4, and frequency of participation in cognitive activities.

^b Basic- and Nutrient-Adjusted models include terms from the basic model plus intake of vitamin E from food sources (mg/d) and total intake of niacin (mg/d).