Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging[1–3]

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ABSTRACT

Background: High concentrations of homocysteine have been linked to a greater risk of Alzheimer disease, dementia, and cognitive decline.

Objective: We evaluated the association between homocysteine and 4.5-y combined incidences of dementia and cognitive impairment without dementia (CIND) in a cohort of 1779 Mexican Americans aged 60–101 y.

Design: Homocysteine, red blood cell (RBC) folate, and plasma vitamin B-12 were measured at baseline. New cases of dementia or CIND were ascertained by neuropsychological and clinical examinations and expert adjudication. We used proportional hazards models to estimate the risk of homocysteine-associated dementia or CIND and the influence of RBC folate and plasma vitamin B-12 on that association.

Results: High homocysteine concentrations were associated with a greater risk of dementia or CIND: hazard ratio (HR): 2.39; 95% CI: 1.11, 5.16. Plasma vitamin B-12 modified the association between homocysteine and the outcome. The rates of dementia or CIND associated with homocysteine for those in the lowest and highest tertiles of vitamin B-12, respectively, were significantly higher (HR: 1.61, $P = 0.04$) and lower (HR: 0.94, $P = 0.015$) than the risk for those in the middle tertile.

Conclusions: Homocysteine is an independent risk factor for both dementia and CIND. Higher plasma vitamin B-12 may reduce the risk of homocysteine-associated dementia or CIND. *Am J Clin Nutr* 2007;85:511–7.

KEY WORDS Homocysteine, B vitamins, dementia, cognitive impairment without dementia, red blood cell folate

INTRODUCTION

Biomarkers associated with the risks of late-life dementia and Alzheimer disease (AD) are not well described or understood. Longitudinal cohort studies linked high concentrations of homocysteine with a greater risk of AD or dementia (1, 2), whereas others did not find an association (3). Homocysteine, a sulfhydryl amino acid, is a product of the methionine cycle that is derived from dietary protein. The homocysteine concentration is influenced by folate and vitamin B-12 and is modifiable through B vitamin supplementation (4, 5). Homocysteine has been linked to a greater risk of cardiovascular disease in some studies (6, 7),

although others authors have raised questions about the mechanisms underlying these findings (8). A meta-analysis of 30 studies of homocysteine and stroke or ischemic heart disease suggested that low homocysteine could be associated with a lower risk of either outcome (9). No rrandomized clinical trials of folic acid supplementation in relation to dementia or AD as outcomes have yet been published. A small trial reported that B vitamin supplementation lowered homocysteine in AD patients (10). Despite the potential benefits of B vitamin supplementation for lowering homocysteine, a review of folic acid with or without vitamin B-12 supplementation did not find evidence of a benefit for cognition (11). Our group (12, 13) reported in previous cross-sectional analyses of the current study that higher homocysteine was associated with worse cognitive scores and that homocysteine was higher at baseline in participants with dementia than in those without dementia. However, these comparisons are likely to reflect reverse causation. After folate fortification was implemented in the United States (in 1998), red blood cell (RBC) folate concentrations reported by the National Health and Nutrition Examination Survey (NHANES) in 1999–2002 showed less prevalence of folate deficiency than was seen before 1998, and plasma homocysteine declined by ≈15% after 1998 (14). That

study was initiated after 1998 and may reflect postfortification concentrations of folate.

The goal of this analysis is to evaluate the association between baseline plasma homocysteine and the 4.5-y combined incidence of the outcomes of dementia and cognitive impairment without dementia (CIND). The influence of RBC folate and plasma vitamin B-12 on this association was evaluated.

SUBJECTS AND METHODS

Population

The analysis presented here is based on an ongoing cohort study ($n = 1779$) of older (aged 60–101 y), primarily Mexican American, Latinos who were residing in the Sacramento Valley of California from 1997 to 1999. Details of the recruitment and baseline assessment and the protocols for diagnosing baseline dementia and CIND were published elsewhere (15, 16).

All subjects gave written informed consent. The study procedures were approved by the institutional review boards of the University of California, Davis, and the University of Michigan.

Diagnosis

After baseline assessment, the cognitive screening protocol required that any participant should be referred for clinical evaluation who declined from the baseline score by >3 points (SE of measurement) on the Verbal Episodic Memory test or by >8 points on the Modified Mini Mental State Exam (17) or whose current Verbal Episodic Memory or Modified Mini Mental State Exam test score was below the 20th percentile. These participants underwent an expanded neuropsychological test battery and a clinical examination by a geriatrician. Case adjudication was done by an expert panel that included a neurologist, a geriatrician, and a neuropsychologist. Dementia and CIND cases were referred for magnetic resonance imaging for use in assigning diagnoses. Cases were classified as having dementia if they failed one or more cognitive tests on the battery (including one memory test) at the 10th percentile; were limited in daily independent function as measured by the Informant Questionnaire on Cognitive Decline in the Elderly, a standard interview done with informants (18); and were judged by the expert panel to meet the dementia criteria of the *Diagnostic and Statistical Manual of Mental Disorders 3rd edition*, the National Institute of Neurological and Communicative Disease and Stroke, or the Alzheimer's Disease and Related Disorders Association. Cases were diagnosed as having CIND if they failed (≤10th percentile) one additional cognitive test battery after screening but did not meet the criteria for dementia, usually because they were impaired in only one cognitive domain or had a nonmemory multidomain impairment that was judged to be clinically questionable or insignificant. Ten dementia cases that were not previously diagnosed by the study were identified from a mortality search that obtained multiple causes from death certificates. Those cases were assigned a diagnosis of dementia after case review by the same panel, and the year of death was given as the year of diagnosis.

Biomarkers

At baseline, fasting blood was collected from each participant by standard venipuncture into evacuated tubes with and without EDTA. The blood was transported on ice to the Medical Center Clinical Laboratory at the University of California, Davis, for processing within 4 h of collection, and it was isolated and stored at −80 °C until it was analyzed. Plasma homocysteine concentrations were measured by HPLC with postcolumn fluorescence detection (19). RBC folate was measured by using automated chemiluminescence assay [ACS 180; Chiron Diagnostics (now Bayer Diagnostics), Tarrytown, NY]. Plasma vitamin B-12 concentrations were measured by using a radioassay (Quantaphase II; BioRad Diagnostics, Hercules, CA). Serum creatinine was analyzed by using a standard spectrophotometric assay. Glomerular filtration rate (GFR) was calculated by using the formulas GFR = (186 × serum creatinine × age) for males and GFR = (186 × serum creatinine × age) females (20)

Other covariates

Age and education were measured in years. Birthplace was measured by self-report of country of birth (United States, Mexico, or another Latin American country). Self-reported physician diagnoses of renal disease or liver disease were obtained by medical history interview.

Vitamin use was assessed through an inventory done at the home visit. The coding system used was the Centers for Disease Control and Prevention National Center for Health Statistics Ambulatory Care Drug Database System. This procedure coded all nutritional supplements into Group 0913—Vitamins/Minerals. From this coding, the list of ingredients was reviewed to classify the supplement for the presence of B vitamins. Vitamins were coded as multivitamin with vitamin B, only B vitamins, or other vitamins.

Statistical analysis

Analyses were restricted to participants who were free of dementia or CIND at baseline and who had available data on the biomarkers of interest. Biological samples were not obtained for the entire sample because of refusals or technical problems with the sample. After all these exclusions, 1405 participants were available for these analyses.

We evaluated the association between homocysteine concentrations and the combined incidences of dementia and CIND. Statistical power was thought to be too low to permit examination of associations with dementia alone or with a specific diagnosis of dementia. Combined incidence rates of dementia and CIND were calculated by using the cumulative incidence technique, in which the total number of new cases was the numerator and the cumulative number of person-years was the denominator. Time from enrollment in the study until diagnosis, death, or the most recent contact date was used for estimating person-years. For subjects lost to follow-up or who refused follow-up and were not known to be dead, the last date of contact was used in calculations of follow-up time or age at diagnosis. For those alive, free of dementia or CIND, and still active in the study, the most recent visit date was used. For those who died, the date of death was used. Rate ratios within sex by age and between the sexes were calculated. We examined the untransformed mean differences for each of the 4 biomarkers by birthplace, sex, age, baseline stroke, cognitive status, and vitamin use by using a general linear model. The association between education and the 4 untransformed biomarkers was tested by using Spearman's rank correlation.

TABLE 1
Incidence rates per 1000 person-years for a combined diagnosis of dementia and cognitive impairment without dementia by age and sex[1]

	Men			Women		
Age group	Cases	Person-years	Incidence rates	Cases	Person-years	Incidence rates
	n	*no.*		*n*	*no.*	
<70 y	358	1268.31	7.88 (10)[2]	469	1735.98	9.79 (17)
70–79 y	222	1420.81	11.26 (16)	348	2275.69	12.30 (28)
≥80 y	48	493.61	18.23 (9)	76	650.83	23.05 (15)
Total	628	3182.74	11.00 (35)	893	4662.50	12.87 (60)

[1] No significant association was found between sex and incidence, and no interaction was found between sex and age.

[2] The number of cases per 1000 person-years in parentheses (all such values).

Plasma homocysteine, RBC folate, and GFR values were log transformed for regression analyses. For analyses predicting incident dementia or CIND, we used a series of proportional hazards models in which the time variable was the time from age at baseline to age at diagnosis. Covariates were included in the model if they were associated with homocysteine concentrations and if inclusion influenced the association between homocysteine concentrations and the outcome of dementia or CIND by at least ±10%. The fit of the models was tested by using Akaike's Information Criterion (21), a goodness-of-fit test for selecting models. When the square root of plasma vitamin B-12 was included in the models, the Akaike's Information Criterion fit test improved significantly, and therefore this transformation was retained in the analysis. Model 1 was unadjusted and included all covariates separately. Because RBC folate and GFR were not associated with the outcome in the bivariate model, model 2 included only homocysteine and the square root term for plasma vitamin B-12. Only the inclusion of plasma vitamin B-12 influenced the association of homocysteine and outcome. Model 3 included homocysteine, vitamin B-12, baseline stroke, and education. Model 4 excluded participants who had baseline stroke and included all other covariates from model 3. RBC folate, renal function, birthplace, sex, and several vitamin measures were not associated with the outcome and did not influence the association of homocysteine with the outcome; therefore, they were dropped after model 1.

We compared 2 models for vitamin B-12, each with a linear term; one model added a squared term for vitamin B-12 and the other added a square root term for vitamin B-12. The square root of plasma vitamin B-12 was associated with a greater risk of dementia or CIND, and the model fit was better than that for the model with the squared term. To further test this nonlinear association, we used indicator variables representing the highest and lowest tertiles of vitamin B-12 and entered these into a Cox regression model in which the middle tertile was the reference term. To test for interactions between vitamin B-12 and homocysteine, we added to models multiplicative interaction terms including these indicator variables and homocysteine (homocysteine × vitamin B-12 tertile 1; homocysteine × vitamin B-12 tertile 3). The inclusion of covariates followed the same procedure as for the models without interaction terms.

RESULTS

We identified 62 new dementia cases and 55 new CIND cases. Of these 2 groups of cases, 93 had homocysteine values. Forty-four percent (*n* = 27) of the dementia cases were classified as

AD, 10 as vascular dementia, and 9 as mixed AD and vascular dementia. The remainder were either other dementias (*n* = 1) or undetermined (*n* = 15). All diagnoses were combined in these analyses.

Comparison of the new CIND cases with the new dementia cases for baseline cognitive status showed the following: the mean word list scores were 7.05 and 6.15 (*P* = 0.14), and the mean Modified Mini Mental State Exam scores were 81.3 and 75.3 (*P* = 0.091) for CIND and dementia, respectively. New CIND cases did not differ significantly from new dementia cases in mean age (CIND: 74 y; dementia: 76 y; *P* = 0.11).

The cumulative 4.5-y incidence rates per 1000 person-years for the combined outcomes by baseline age group and by sex are shown in **Table 1**. Sex- and age-specific rates along with crude combined incidence rates for dementia and CIND are shown. The crude rate ratio for sex (M/F) was 0.85. In the men, rate ratios (RRs) by age group were 70–79 y versus <70 y: RR: 1.43 and >80 y versus <70 y: RR: 2.31. In the women, the RRs by age group were 70–79 y versus <70 y: RR:1.26 and >80 y versus <70 y: RR:2.35. In analysis with a proportional hazards model, no association was found between sex and the combined incidence of dementia and CIND [hazard ratio (HR): 1.19, 95% CI: 0.78, 1.81]. In addition, no significant interaction was found between sex and age.

Homocysteine was elevated (≥13 umol/L) in 16.5% (252/1529) of the sample. RBC folate (≤160 ng/mL) was low in 0.64% (9/1403), vitamin B-12 was low (≤200 pg/mL) in 6.4% (92/1440), and GFR was low (<60 mL · min^{-1} · 1.73 m^{-2}) in 61.5% (939/1528). The means ± SDs for homocysteine, plasma vitamin B-12, RBC folate, and GFR overall by birthplace, sex, age at baseline, incident dementia or CIND, baseline stroke, and vitamin use are shown in **Table 2**. Homocysteine was significantly lower in women than in men, higher in those at older ages, and higher in those with a baseline stroke; it did not differ for other covariates. Plasma vitamin B-12 was significantly higher in men and lower in those at older ages; it did not differ by dementia or CIND status, baseline stroke, or vitamin use. Further analysis suggested that vitamin B-12 was higher in those with renal disease (447 and 486 pg/mL, respectively; *P* = 0.034) after adjustment for age and sex or in those with liver disease (449.4 and 507.2 pg/mL, respectively; *P* = 0.04) than in those without those conditions. RBC folate was significantly lower in Mexican-born participants than in those born elsewhere and did not differ by age, sex, baseline stroke, use of vitamins, or dementia or CIND status. GFR did not differ significantly for any covariates. Education was significantly associated (Spearman's

TABLE 2
Homocysteine, vitamin B-12, red blood cell (RBC) folate, and glomerular filtration rate (GFR) for study covariates[1]

	Homocysteine (n = 1529)[2]	Vitamin B-12 (n = 1440)	RBC folate (n = 1403)	GFR (n = 1528)
	μmol/L	pg/mL	ng/mL	mL · min^{-1} · 1.73 m^{-2}
Overall descriptive statistics	10.78 ± 6.46 (4.0–129.2)[3]	452.59 ± 203.49 (22.0–1000)	504.69 ± 159.89 (50.0–900.0)	60.48 ± 52.98 (14.56–1123.26)
Covariates				
Birthplace				
United States (n = 776)	10.74 ± 0.23[4]	431.26 ± 7.5[5]	523.55 ± 5.99[5]	61.99 ± 2.00
Mexico or Latin America (n = 753)	10.83 ± 0.24	475.02 ± 7.73	485.56 ± 5.99	58.89 ± 2.03
Sex				
Female (n = 894)	10.07 ± 4.13[5]	478.52 ± 252.63[5]	510.29 ± 158.08	45.55 ± 33.58[5]
Male (n = 635)	11.80 ± 8.76	417.15 ± 190.56	497.05 ± 162.23	81.62 ± 71.94
Baseline age (y)[6]				
60–69 (n = 790)	10.26 ± 0.23[5]	461.40 ± 7.6	502.76 ± 5.95	58.95 ± 1.99
70–79 (n = 597)	10.91 ± 0.26	449.46 ± 8.65	511.88 ± 6.81	61.78 ± 2.28
≥80 (n = 147)	13.24 ± 0.54	415.96 ± 17.92	483.85 ± 13.96	63.37 ± 4.68
Dementia or CIND				
Yes (n = 93)	11.68 ± 4.06	479.53 ± 252.74	481.05 ± 147.60	56.52 ± 22.41
No (n = 1436)	10.73 ± 6.65	450.85 ± 202.04	506.22 ± 9160.62	60.75 ± 57.35
Baseline stroke				
Yes (n = 121)	12.05 ± 4.79[7]	451.71 ± 234.89	513.32 ± 155.90	65.37 ± 36.09
No (n = 1408)	10.68 ± 6.64	452.66 ± 202.84	504.02 ± 160.26	60.06 ± 57.21
Multivitamins with vitamin B				
Yes (n = 374)	10.74 ± 6.24	451.76 ± 206.63	497.82 ± 159.94	59.64 ± 61.55
No (n = 1153)	10.91 ± 7.34	452.86 ± 205.17	506.86 ± 159.93	60.76 ± 53.88
B vitamin alone				
Yes (n = 73)	10.69 ± 4.59	468.08 ± 214.91	495.29 ± 156.59	58.02 ± 28.22
No (n = 1454)	16.79 ± 6.61	451.79 ± 205.02	505.17 ± 160.14	60.61 ± 56.85
Other vitamins				
Yes (n = 412)	10.71 ± 6.49	447.48 ± 214.26	507.72 ± 154.45	62.15 ± 71.41
No (n = 1115)	10.81 ± 6.54	454.47 ± 202.20	503.55 ± 161.99	59.87 ± 48.93

[1] CIND, cognitive impairment without dementia. Available biomarker data were from a general linear model.

[2] n after exclusions (all such).

[3] $\bar{x}$ ± SD; range in parentheses (all such values).

[4] $\bar{x}$ ± SD (all such values).

[5] P < 0.0001.

[6] Bonferroni correction applied.

[7] P = 0.03.

correlation) with all 4 biomarkers as follows: homocysteine, −0.095; P = 0.0001; vitamin B-12, 0.093; P = 0.0003; RBC folate, 0.12; P <0.0001; and GFR, 0.085; P = 0.0006. The results from a series of proportional hazards models that test the association between homocysteine and the incidence of dementia or CIND are shown in **Table 3**.

Homocysteine

In model 1 (unadjusted), homocysteine was significantly associated with a greater risk of dementia or CIND. After the addition of a square-root term for plasma vitamin B-12 (model 2), the HR was 55% greater than that in the unadjusted model. The addition of stroke and education (model 3) reduced the HR for homocysteine by 32% compared with model 2, and the confidence limit included 1.0. The association between homocysteine and dementia or CIND was significant in model 4 (excluding baseline stroke), and the HR increased by 29% compared with model 3.

A continuous term for plasma vitamin B-12 (square root) was significantly associated with a greater risk of dementia or CIND in all models. This indicated a U-shaped, nonlinear association between plasma vitamin B-12 and the incidence variable. To further test differences between the comparisons of the low and high tertiles with the middle tertile, a separate proportional hazards model was developed including 2 indicator variables for the lowest (<340 pg/mL) and highest (≥498 pg/mL) tertiles of plasma vitamin B-12 to be used in comparison with the middle tertile (≥340 < 498 pg/mL) as a reference category. The risk of dementia or CIND was significantly higher in the highest than in the middle vitamin B-12 tertile (HR: 2.5; 95% CI: 1.31, 4.54). The risk of dementia or CIND was higher in the lowest than in the middle vitamin B-12 tertile, but the confidence limit included 1.0 (HR: 1.63; 95% CI: 0.88, 3.01). Adjustments for RBC folate, homocysteine, GFR, education, and birthplace did not influence these associations.

Interactions between vitamin B-12 and homocysteine were tested in a model including vitamin B-12 tertiles, homocysteine, 2 indicator variables (lowest and highest tertiles of vitamin B-12), and 2 interaction terms between the vitamin B-12 indicator variables and homocysteine. In those in the lowest tertile of vitamin B-12, compared with those in the middle tertile, homocysteine was associated with a significantly greater risk of dementia or CIND (HR: 1.61; P for interaction = 0.04). In those in the highest vitamin B-12 tertile, compared with those in the middle tertile, homocysteine was associated with a slightly lower risk of dementia or CIND (HR: 0.94; P = 0.015). Adjustment for education, baseline stroke, RBC folate, or renal function did not influence this association; nor did exclusion of baseline stroke. The risk of dementia or CIND associated with homocysteine by low and high vitamin B-12 tertiles compared with the middle tertile for 2 models is illustrated in **Figure 1**. The bivariate model is unadjusted, and model 1 is adjusted for education.

TABLE 3
Hazard ratios (95% CIs) for combined 4.5-y incidence of dementia or cognitive impairment without dementia for each covariate with age at diagnosis or censoring as a time factor[1]

	Bivariate model (model 1) ($n = 95$)	Model 2 ($n = 80$)	Model 3 ($n = 80$)	Model 4 ($n = 66$)
Homocysteine[2]	1.58 (1.88, 2.83)	2.45 (1.26, 4.75)	1.85 (0.93, 3.70)	2.39 (1.11, 5.16)
Plasma vitamin B-12[3]	1.04 (1.01, 1.08)	1.06 (1.02, 1.10)	1.05 (1.10, 1.09)	1.07 (1.02, 1.11)
RBC folate[2]	0.85 (0.57, 1.24)	—[4]	—	—
Glomerular filtration rate[2]	0.93 (0.57, 1.50)	—	—	—
Baseline stroke (0, 1)[5]	2.93 (1.76, 4.86)	—	2.53 (1.43, 4.45)	—
Education (y)	0.94 (0.90, 0.98)	—	0.94 (0.90, 0.99)	0.95 (0.91, 1.00)
Sex (female versus male)	1.14 (0.76, 1.70)	—	—	—
Birthplace (Mexico versus USA)	0.93 (0.62, 1.40)	—	—	—
Multivitamins with vitamin B (0, 1)[5]	0.94 (0.64, 1.48)	—	—	—
Only B vitamins (0, 1)[5]	0.30 (0.07, 1.26)	—	—	—
Other vitamins (0, 1)[5]	1.19 (0.78, 1.81)	—	—	—

[1] RBC, red blood cell. Except for homocysteine, covariates were retained in the model only if they were associated with the outcome and influenced the hazard ratio. Bivariate model, unadjusted coefficients from separate models; model 2, simultaneous adjustment for all biomarkers; model 3, model 2 + age, education, baseline stroke, and sex; model 4, model 3 excluding baseline stroke. The total sample available for analysis after exclusions and missing data on biomarkers was $n = 1519$, 1332, 1332, and 1235 for bivariate model and models 2, 3, and 4, respectively.

[2] Square root.

[3] Natural log.

[4] The covariate was not included in the model (all such).

[5] 0 = absent, 1 = present.

Red blood cell folate and glomerular filtration rate

RBC folate and GFR were not associated with the outcome in any of the models tested. Those values were not retained in models 2–4.

Baseline stroke, demographic factors, and vitamin use

Stroke at baseline was associated with a greater risk of dementia or CIND. Education was significantly associated with a lower risk of dementia or CIND and was included in models 2–4. Sex, birthplace, and vitamin use were not associated with the outcome in the unadjusted model and were not included in models 2–4.

DISCUSSION

We found that homocysteine is associated with a greater risk of dementia or CIND that is independent of RBC folate. The influence of homocysteine on dementia or CIND may be modified by plasma vitamin B-12. The current study has improved on
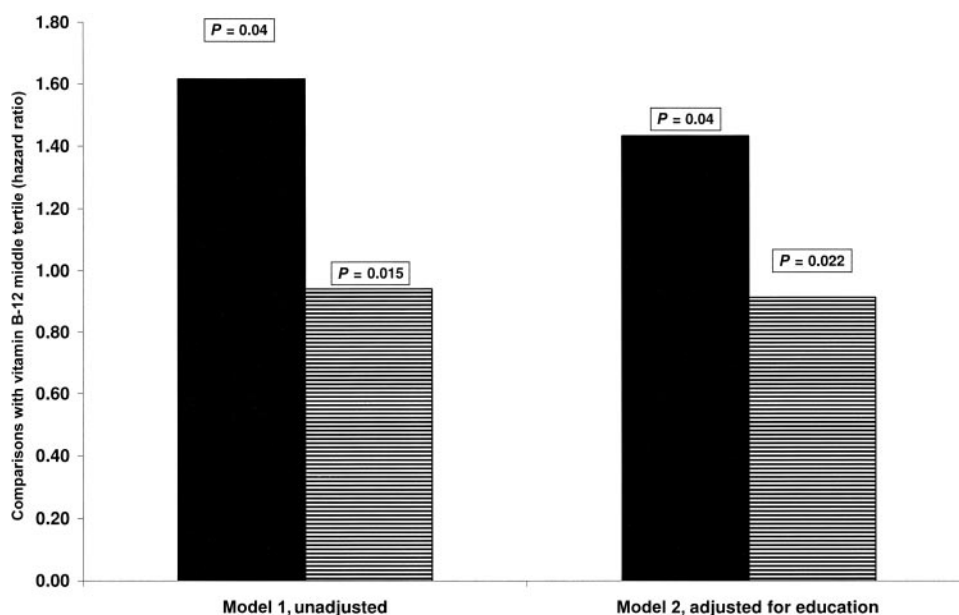

FIGURE 1. Association between homocysteine and dementia or cognitive impairment without dementia (CIND) by low (■; <340 pg/mL) and high (▤; ≥498 pg/mL) vitamin B-12 tertiles compared with the vitamin B-12 middle tertile from 2 proportional hazards models—model 1 including only vitamin B-12 and model 2 adding adjustment for education. *P* values are for the interaction terms between vitamin B-12 indicator variables and homocysteine.

our earlier cross-sectional research by providing evidence of an association between baseline homocysteine and the combined incidence of dementia and CIND in those who were free of disease at baseline. Our findings are consistent with some other work on this topic. For example, Seshadri et al (1) reported that the risk of AD was nearly doubled in association with high concentrations of homocysteine. In contrast, Kado et al (22) reported no association between folate and cognitive decline, Luchsinger et al (3) found no association between homocysteine and cognitive decline or AD, and Ravaglia et al (2) reported a 2-fold increase in the risk of dementia associated with hyperhomocystenemia ($\geq$15 μmol/L) in older Italians. Ellensin et al (23), who summarized studies examining homocysteine and dementia, concluded that the evidence was insufficient to support a randomized trial of folic acid supplementation, but this assessment was based mainly on cross-sectional studies of cognitive status. Stott et al (5) reported that randomized supplementation with vitamin B-12 and folate reduced homocysteine in those at high risk of cardiovascular disease recurrence, but this treatment had no effect on cognitive function.

Several lines of evidence may help to explain the link between homocysteine and the risk of dementia or CIND. The association between vascular disease and homocysteine is an obvious possibility; 2 analyses provided some evidence of greater volumes of white matter associated with elevated homocysteine (24–26). However, Durga et al (8) and Brattstrom and Wilcken (27), in careful critical reviews, found conflicting reports of the evidence supporting an association between white matter and homocysteine. Higher homocysteine has been associated with atrophy of the medial temporal lobe in a small study comparing AD cases with controls (26). In excluding baseline stroke (model 3), we attempted to reduce confounding by vascular disease of the homocysteine-dementia association. Stroke has been associated with homocysteine (28) and is also a strong predictor of dementia in the current study. The fact that an association remains after the exclusion of stroke suggests that pathways may not be restricted to vascular factors.

We did not find an association between RBC folate and dementia or CIND. Ravaglia et al (2) found a greater risk of dementia to be associated with low folate. RBC folate values from population-based studies of Mexican Americans aged $\geq$65 y have not been published. Ganji and Kafai (14) reported, for all age groups in NHANES, a 56% increase in mean RBC folate concentrations (from 361 to 564 ng/mL) between 1988–1994 and 1999–2000 and a 2.3% increase between 1999–2000 and 2001–2002 (from 564 to 577 ng/mL). Other evidence from NHANES suggests that RBC folate changes with age, the subjects in the current study have an average age of 75 y at the time of this writing. Our ability to observe an association between RBC folate and dementia or CIND may be influenced by the lack of low folate values. Because we adjusted for folate and found no effects, it is unlikely that the results are due to underlying deficiency. The U-shaped association between plasma vitamin B-12 and dementia or CIND is not surprising, because vitamin B-12 deficiency is an established risk factor for dementia, and excessive vitamin B-12 is a marker for renal and liver disease. The modification by vitamin B-12 of the association between homocysteine and dementia or CIND suggests that higher concentrations of vitamin B-12 can reduce the risk of dementia or cognitive impairment associated with homocysteine.

CIND is an entity conceptually similar to mild cognitive impairment (MCI), a condition felt to be intermediate between normal cognition and dementia (29). Our definition of CIND is not precisely the definition that is currently being applied for classifying MCI, but it is similar. Few population-based studies exist of MCI and the rates of conversion from MCI to dementia, and none exist in Mexican Americans. Larrieu et al (30) reported that conversion from MCI at baseline to dementia was 3.5% and that nearly 19% of new MCI cases found at baseline converted to dementia during follow-up. Amieva et al (31) reported a 32% conversion rate. The conversion rate in the current study was lower than the rates in those reports, but CIND was a predictor of earlier mortality and attrition in the current study, so that some cases that may have progressed to dementia had, instead, died. Moreover, the studies of Larrieu et al and Amieva et al are not comparable with the current study, because they are based on European samples of comparatively well-educated participants. Most other published work on this topic has relied on samples of patients or other nonrandom sources of participants and are not comparable.

The current study has some limitations, in that it does not yet include sufficient numbers of AD cases to allow separate examination of diagnoses. As is the case for all cohort studies of elderly populations, attrition from the current study because of death and loss to follow-up may attenuate our estimates by selecting out more vulnerable participants and those who are more likely to develop cognitive impairment. Exclusion of baseline stroke cases may help account for the effects of subclinical disease on homocysteine at baseline. However, we do not have direct measures of underlying vascular or AD pathology in the entire sample.

No direct way exists to evaluate the effect of folic acid fortification on these results, because the study started after fortification was implemented and we have no historical dietary data. Furthermore, no population-based studies of older Mexican Americans have been published that reflect changes in population folate concentrations before and after fortification that would be appropriate for comparison. The low prevalence of folate deficiency in the current analysis suggests that the contribution of folate deficiency to the population risk of dementia may be lower than expected and that the contribution of homocysteine to dementia risk may derive from sources other than folate. Three recent, well-powered, randomized clinical trials, designed to test the effects of B vitamins on homocysteine, cognitive outcomes and cardiovascular events, respectively, did not find any effects on the specified outcomes, although the supplements did lower homocysteine (32–34). The findings of the current study and of these recent trials have implications for potential B vitamin supplementation as a treatment for dementia. It may be that B vitamin supplementation influences homocysteine but does not strongly influence the risk of cognition or of vascular diseases that are potential pathways for the link between homocysteine and dementia. Homocysteine may be an early marker of a subclinical pathologic condition leading to dementia, or it may be that the pathway by which homocysteine influences cognitive performance is not influenced by folate. Most studies have not had sufficient follow-up time to separate subclinical pathologic conditins from cause or diagnosis. However, findings from the Framingham Study (1, 34) and the current study, both of which have

longer follow-up times, suggest that homocysteine may be an independent predictor of dementia outcomes. The role of vitamin B-12 in dementia risk should be further evaluated.

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