PLASMA HOMOCYSTEINE AS A RISK FACTOR FOR DEMENTIA AND ALZHEIMER'S DISEASE

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ABSTRACT

Background In cross-sectional studies, elevated plasma homocysteine levels have been associated with poor cognition and dementia. Studies of newly diagnosed dementia are required in order to establish whether the elevated homocysteine levels precede the onset of dementia or result from dementia-related nutritional and vitamin deficiencies.

Methods A total of 1092 subjects without dementia (667 women and 425 men; mean age, 76 years) from the Framingham Study constituted our study sample. We examined the relation of the plasma total homocysteine level measured at base line and that measured eight years earlier to the risk of newly diagnosed dementia on follow-up. We used multivariable proportional-hazards regression to adjust for age, sex, apolipoprotein E genotype, vascular risk factors other than homocysteine, and plasma levels of folate and vitamins B_{12} and B_6.

Results Over a median follow-up period of eight years, dementia developed in 111 subjects, including 83 given a diagnosis of Alzheimer's disease. The multivariable-adjusted relative risk of dementia was 1.4 (95 percent confidence interval, 1.1 to 1.9) for each increase of 1 SD in the log-transformed homocysteine value either at base line or eight years earlier. The relative risk of Alzheimer's disease was 1.8 (95 percent confidence interval, 1.3 to 2.5) per increase of 1 SD at base line and 1.6 (95 percent confidence interval, 1.2 to 2.1) per increase of 1 SD eight years before base line. With a plasma homocysteine level greater than 14 μmol per liter, the risk of Alzheimer's disease nearly doubled.

Conclusions An increased plasma homocysteine level is a strong, independent risk factor for the development of dementia and Alzheimer's disease. (N Engl J Med 2002;346:476-83.)

ALZHEIMER'S disease accounts for more than 70 percent of all cases of dementia, so it is important to identify modifiable risk factors for the disease.[1] During the past decade, there has been growing interest in vascular factors that may underlie Alzheimer's disease. It is now recognized that subjects with cardiovascular risk factors and a history of stroke have an increased risk of both vascular dementia and Alzheimer's disease.[2-4] Plasma total homocysteine has recently emerged as a major vascular risk factor. Elevated total homocysteine levels have been associated with an increased risk of atherosclerotic sequelae, including death from cardiovascular causes,[5,6] coronary heart disease,[6,7] carotid atherosclerosis,[8] and clinical stroke.[9,10] These observations led to the hypothesis that elevated plasma homocysteine may be a risk factor for dementia and Alzheimer's disease. If this hypothesis is valid, it points to a modifiable risk factor, since plasma homocysteine levels can be lowered by supplementation with folic acid.[11]

Previous studies have reported an inverse association between plasma total homocysteine levels and simultaneously assessed cognitive function.[12-16] Two case–control studies have found higher plasma homocysteine levels in persons with Alzheimer's disease.[17,18] However, in a prospective study plasma homocysteine levels were not related to cognitive decline during follow-up in a community-based sample.[19] Elevated plasma homocysteine levels in subjects with cognitive impairment or dementia might be the result of poor nutrition and vitamin deficiencies.[20] A prospective study should be able to show whether elevated plasma homocysteine in cognitively intact adults is associated with an increased risk of dementia and Alzheimer's disease on follow-up. We therefore examined plasma total homocysteine in relation to newly diagnosed dementia and Alzheimer's disease in the elderly, population-based cohort of Framingham Study participants.

METHODS

Subjects

The Framingham Study cohort has been evaluated biennially since 1948. Between 1976 and 1978, a total of 2611 subjects were enrolled in a dementia-free cohort.[21,22] At the 20th biennial examination (between 1986 and 1990), 1592 subjects from this cohort were alive and free of dementia and had follow-up data for at least one year. Of these subjects, 1229 (77 percent) underwent the 20th examination, and in 1092 participants (89 percent of those examined), plasma total homocysteine levels were measured. These 1092 subjects constituted our study sample. There were

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667 women and 425 men, and their mean (±SD) age was 76±6 years (range, 68 to 97). Informed consent was obtained from all study subjects with the use of a consent form approved by the institutional review board for human research at the Boston University School of Medicine.

Diagnosis of New Cases of Dementia and Alzheimer's Disease

Subjects in the cohort that was free of dementia at inception have been monitored with published surveillance techniques since 1978 for the development of stroke or dementia.[21,22] Methods have included a screening Folstein Mini–Mental State Examination[23] at each biennial evaluation, followed by annual neurologic and neuropsychological assessment of subjects with suspected cognitive impairment.

The final diagnosis of dementia was made by a committee, comprising at least two neurologists and a neuropsychologist, that determined the type of dementia and the date of diagnosis. All available information was used to evaluate participants with suspected dementia, including serial neurologic and neuropsychological assessments, a telephone interview with a family member or care giver, medical records, imaging studies, and autopsy data when available. The review committee was unaware of the subjects' plasma homocysteine levels. The diagnosis of dementia was made according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition[24]; our definition also required a duration of symptoms greater than six months, and a score for severity of dementia of 1 or higher on the Clinical Dementia Rating scale.[25] Alzheimer's disease was diagnosed when subjects met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association for definite, probable, or possible Alzheimer's disease.[26]

Plasma Homocysteine

Plasma total homocysteine levels were measured in all subjects at the 20th biennial examination (base line). An earlier measure from the 16th biennial examination (performed between 1979 and 1982, approximately eight years before base line) was also available for 935 of the subjects (86 percent). All plasma specimens were stored at or below −20°C. Homocysteine levels were determined with the use of high-performance liquid chromatography with fluorometric detection.[27] The coefficient of variation for this assay was 9 percent.[28]

Apolipoprotein E Genotypes

Data on the apolipoprotein E (*APOE*) genotype were available for 1012 of the subjects (93 percent). The presence of particular alleles was determined by means of isoelectric focusing of the plasma and confirmed by DNA genotyping.[29,30] Participants were divided into two groups, one comprising persons with an *APOE* $\epsilon 4$ allele ($\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$, or $\epsilon 4/\epsilon 4$ genotype) and another comprising those without an *APOE* $\epsilon 4$ allele.

Vitamin Levels

Plasma concentrations of folate, cyanocobalamin (vitamin B_{12}), and pyridoxal-5′-phosphate (the coenzyme form of vitamin B_6) were estimated at the 20th biennial examination. Plasma folate was measured by a microbial (*Lactobacillus casei*) assay with a 96-well plate and manganese supplementation[31]; plasma vitamin B_{12} levels were estimated with the use of a radioassay kit (Magic, Ciba–Corning, Medfield, Mass.); and pyridoxal-5′-phosphate was measured by the tyrosine decarboxylase apoenzyme method.[32] Coefficients of variation for these assays were 13 percent for plasma folate, 7 percent for cyanocobalamin, and 16 percent for pyridoxal-5′-phosphate.[28] Because of insufficient plasma samples, the vitamin levels were not determined for all patients. Of the subjects with measurements of plasma homocysteine, 85 percent had measurements of vitamin B_{12}, 92 percent had measurements of vitamin B_6, and 98 percent had measurements of folate.

Definition of Additional Risk Factors

Risk factors that could potentially confound the relation between plasma homocysteine and dementia or Alzheimer's disease were defined with the use of data collected at the 20th biennial examination. When appropriate, data from earlier biennial examinations were also used. Educational status was dichotomized at the level of high-school completion. We adjusted the analyses for cigarette smoking using two variables: current smoking status (smoker or nonsmoker) and lifetime exposure to cigarette smoke (<5.0 pack-years, 5.0 to 29.9 pack-years, or ≥30.0 pack-years). Alcohol intake was categorized in terms of the number of drinks per day: zero, less than one, one to two, or more than two.[33] Diabetes mellitus was defined by a recorded casual blood glucose level of at least 200 mg per deciliter (11.1 mmol per liter), a previous diagnosis of diabetes mellitus, or the use of a hypoglycemic agent or insulin. Systolic blood pressure and body-mass index (the weight in kilograms divided by the square of the height in meters) were treated as continuous variables.

Statistical Analysis

The distribution of plasma homocysteine levels in the population was positively skewed. The use of natural-log–transformed values provided the best-fitting model for analyses in which the plasma homocysteine level was treated as a continuous variable. Plasma homocysteine levels were also evaluated with a quartile-based analysis. Since homocysteine levels increase markedly with age,[28,34,35] the quartiles were defined in an age-specific manner for each of several five-year age categories.

Cox proportional-hazards regression models[36] were used to examine the relation between the homocysteine level and the incidence of dementia and Alzheimer's disease during follow-up, after adjustment for age (in one-year increments), sex, and *APOE* genotype (with or without an *APOE* $\epsilon 4$ allele).[37] In supplementary analyses, we also adjusted for vitamin levels and other covariates. Subjects were followed for new cases of dementia from the date of their 20th biennial examination until December 31, 2000. For the analysis of new cases of Alzheimer's disease, data for subjects in whom other types of dementia developed were censored at the date of the diagnosis of dementia, since the diagnostic categories were mutually exclusive. Subjects who had a stroke during the study period were not excluded, since such an event could be part of the causal chain between an elevated plasma homocysteine level and the development of dementia. All statistical analyses were performed with the use of SAS software (SAS Institute, Cary, N.C.).

RESULTS

Base-Line Characteristics

The base-line characteristics of the subjects are presented in Table 1 (further information may be found in Supplementary Appendix 1, available with the full text of this article at http://www.nejm.org). Mild-to-moderate elevation of the plasma homocysteine level (>14 μmol per liter) was present in 30 percent of the subjects. None of the subjects had severe hyperhomocysteinemia (plasma homocysteine, >100 μmol per liter). The mean plasma homocysteine level within each of the five-year age groups is shown in Table 2. The correlation between the base-line plasma homocysteine level in a given subject and the level measured eight years earlier was calculated for the 935 subjects (571 women and 364

men) for whom both measurements were available (Pearson r=0.47, P<0.001).

Dementia, Alzheimer's Disease, and Plasma Homocysteine

Over a median follow-up period of 8 years (range, 1 to 13), dementia developed in 111 subjects (10.2 percent; 74 women and 37 men), and 83 of these subjects (62 women and 21 men) were given a diagnosis of Alzheimer's disease. In five subjects, the clinical diagnosis of Alzheimer's disease was confirmed at autopsy (definite Alzheimer's disease). The diagnosis was probable Alzheimer's disease for 67 subjects and possible Alzheimer's disease for 11 subjects. Other types of dementia diagnosed in the study population included vascular dementia in 11 subjects, non-Alzheimer's degenerative dementias in 11 subjects, and other types of dementia in 6 subjects. The absence of Alzheimer's disease was confirmed at autopsy in 14 subjects.

The overall results relating the plasma homocysteine level to the development of any dementia and to the development of Alzheimer's disease are shown in Tables 3 and 4 and in Figure 1. After adjustment for the age, sex, and *APOE* genotype, the relative risks of dementia and Alzheimer's disease, for each increase of 1 SD in log-transformed base-line homocysteine value, were 1.3 (95 percent confidence interval, 1.1 to 1.6) and 1.4 (95 percent confidence interval, 1.2 to 1.7), respectively. Hyperhomocysteinemia (plasma homocysteine, >14 μmol per liter)[8,18] was correspondingly associated with an increased risk of dementia (relative risk, 1.9; 95 percent confidence interval, 1.3 to 2.8) and Alzheimer's disease (relative risk, 1.9; 95 percent confidence interval, 1.2 to 3.0). An increase in the plasma homocysteine level of 5 μmol per liter increased the multivariable-adjusted risk of Alzheimer's disease by 40 percent (P<0.001). We did not find evidence of modification of this effect by age or sex.

Effect of Vitamin Levels

Low serum levels of certain B vitamins (folate and vitamins B_{12} and B_6) have been associated with elevated plasma homocysteine levels in several studies and with an increased risk of dementia in a few investigations.[38-42] In our study, the observed association between plasma homocysteine and risk of dementia was not significantly altered by adjustment for the plasma levels of these vitamins (Table 3). Furthermore, after adjustment for age, sex, and *APOE* genotype, none of these vitamin levels were independently related to the risk of dementia or Alzheimer's disease (data not shown).

Additional Covariates

The observed association between the plasma homocysteine level and dementia or Alzheimer's disease was not diminished by adjustment for educational status, systolic blood pressure, smoking status, alcohol intake, presence or absence of diabetes, body-mass index, or presence or absence of a history of stroke (Table 3). Serum creatinine was measured at the 15th biennial examination, and cholesterol and

TABLE 1. BASE-LINE CHARACTERISTICS OF STUDY SUBJECTS AT THE 20TH BIENNIAL EXAMINATION.*

CHARACTERISTIC	MEN (N=425)	WOMEN (N=667)
Age (yr)	76±5	77±6
Plasma homocysteine		
Level (μmol/liter)	13.1±6.3	13.0±7.0
Log-transformed value	2.5±0.4	2.5±0.4
>14 μmol/liter (%)†	30	30
>9 μmol/liter (%)‡	81	76
Other plasma levels§		
Folate (ng/ml)	5.9±7.5	6.9±7.1
Vitamin B_{12} level (pg/ml)	416±209	461±233
Pyridoxal-5′-phosphate level (nmol/liter)	74.7±89.0	79.9±94.8
Body-mass index¶	27.0±4.0	26.5±5.0
Systolic blood pressure (mm Hg)	146±22	147±23
Apolipoprotein E genotype (%)		
ε2/ε2 or ε2/ε3	11.0	10.9
ε3/ε3	68.0	68.0
ε2/ε4, ε3/ε4, or ε4/ε4	21.0	21.1
High-school graduate (%)‖	66.1	67.8
History of stroke (%)	7.3	5.1
Current cigarette smoker (%)	10	11
Lifetime smoking (%)**		
<5.0 pack-years	35	63
5.0–29.9 pack-years	22	20
≥30.0 pack-years	43	17
Diabetes (%)	14.4	8.6
Alcohol intake (%)††		
0 drinks/day	37.5	52.7
<1 drink/day	25.9	29.9
1–2 drinks/day	13.7	8.0
>2 drinks/day	22.9	9.5

*Plus-minus values are means ±SD.

†This threshold represents the accepted cutoff point for hyperhomocysteinemia.

‡This threshold represents the mean plasma homocysteine level in the general population.

§To convert values for plasma folate to nanomoles per liter, multiply by 2.266; to convert values for plasma vitamin B_{12} to picomoles per liter, multiply by 0.7378. Data on plasma folate levels were available for 419 men and 657 women; data on plasma B_{12} levels were available for 375 men and 557 women; and data on plasma pyridoxal-5′-phosphate levels were available for 398 men and 611 women.

¶The body-mass index is the weight in kilograms divided by the square of the height in meters; data were available for 411 men and 630 women.

‖Data were available for 413 men and 653 women.

**Data were available for 374 men and 611 women for whom the age when smoking began could be reliably ascertained.

††One drink is defined (according to the criteria established by the National Institute on Alcohol Abuse and Alcoholism) as 12 oz (360 ml) of beer, 5 oz (150 ml) of wine, or 1.5 oz (45 ml) of distilled spirits, each containing approximately 0.5 oz (15 ml) of pure alcohol. Data on alcohol intake were available for 424 men and 666 women.

TABLE 2. DISTRIBUTION OF BASE-LINE PLASMA HOMOCYSTEINE LEVELS WITHIN FIVE-YEAR AGE GROUPS.*

AGE	NO. OF SUBJECTS	PLASMA HOMOCYSTEINE LEVEL		
		MEAN ±SD	RANGE	75TH PERCENTILE
		μmol per liter		
65–69 yr	46	11.5±3.9	5.4–25.5	13.2
70–74 yr	457	12.1±5.9	4.1–66.7	13.8
75–79 yr	315	12.6±5.9	3.5–66.9	14.5
80–84 yr	179	14.2±7.3	4.5–56.1	16.5
85–89 yr	66	15.3±8.0	5.5–59.6	19.3
90–94 yr	29	22.3±12.6	5.4–61.6	26.6

*The difference in mean values between men and women was not significant.

thyrotropin were measured at the 20th biennial examination. Adjustment for these additional variables did not alter our results (data not shown).

Varying the Diagnostic Criteria for Alzheimer's Disease

Higher plasma homocysteine levels have been related to an increased risk of stroke.[8,10] To address the possibility that the association we observed between plasma homocysteine and Alzheimer's disease resulted from the inclusion of subjects who might have vascular dementia rather than Alzheimer's disease, we evaluated separately the association between base-line plasma homocysteine levels and a diagnosis of definite or probable Alzheimer's disease after excluding subjects with a diagnosis of possible Alzheimer's disease. The relative risk per increment of 1 SD in the log-transformed base-line homocysteine value remained essentially unchanged at 1.4 (95 percent confidence interval, 1.2 to 1.7).

Association with Earlier Homocysteine Levels

Unlike stroke or myocardial infarction, clinical dementia begins insidiously. It may therefore be difficult to exclude subjects in whom the disease is incipient at base line. However, subjects who were free of clinical dementia at base line were most likely free of even incipient disease eight years earlier, at the examination from which we derived the previous plasma homocysteine measurement. We examined the relation between the plasma homocysteine level eight years before base line and the risk of newly diagnosed dementia or Alzheimer's disease during the follow-up period between the base-line examination and December 31, 2000. Again, we found a strong association (Table 3), indicating that the elevation of the plasma homocysteine level occurred well before the onset of clinical manifestations.

Quartile-Specific Analysis

Examination of the risks of dementia and Alzheimer's disease in age-specific quartiles of plasma homocysteine levels suggested that subjects with levels in the highest quartile (according to the cutoff points in Table 2) had the highest risk of dementia and Alzheimer's disease. When both measurements of plasma homocysteine were considered, this subgroup had about twice the risk of all other subjects (Table 4 and Fig. 1). Although the effect of the homocysteine level was smaller in the second and third quartiles, we did not find evidence of a specific threshold. When the subjects whose base-line levels were in the lowest age-specific quartile were used as the reference group, the relative risk of Alzheimer's disease was 1.2 (95 percent confidence interval, 0.6 to 2.2) for subjects in the second quartile, 1.3 (95 percent confidence interval, 0.6 to 2.5) for subjects in the third quartile, and 2.2 (95 percent confidence interval, 1.2 to 4.1) for subjects in the fourth quartile. Subjects whose plasma homocysteine levels were consistently high (in the fourth quartile at both the 16th and 20th examinations) had the highest risk.

Population Attributable Risk

In our population, the risk of Alzheimer's disease attributable to a plasma homocysteine level in the highest age-specific quartile was estimated, with the use of standard techniques,[43] at 16 percent. In the same population, 21 percent of subjects had at least one *APOE* ε4 allele, and the age- and sex-adjusted relative risk of Alzheimer's disease associated with the presence of this allele was 2.3 (95 percent confidence interval, 1.5 to 3.7); thus, there was a 21 percent risk of Alzheimer's disease attributable to the presence of an *APOE* ε4 genotype.

DISCUSSION

The results of our prospective, observational study indicate that there is a strong, graded association between plasma total homocysteine levels and the risk of dementia and Alzheimer's disease. An increment in the plasma homocysteine level of 5 μmol per liter increased the risk of Alzheimer's disease by 40 percent. A plasma homocysteine level in the highest age-specific quartile doubled the risk of dementia or Alzheimer's disease. A similar result was found when the single criterion of hyperhomocysteinemia (baseline plasma homocysteine, >14 μmol per liter) was used. The magnitude of this effect is similar to the

TABLE 3. MULTIVARIABLE COX PROPORTIONAL-HAZARDS REGRESSION MODELS EXAMINING THE RELATION BETWEEN THE PLASMA TOTAL HOMOCYSTEINE LEVEL AND THE RISK OF DEMENTIA AND ALZHEIMER'S DISEASE.*

PLASMA HOMOCYSTEINE MEASUREMENT	VARIABLES ADJUSTED FOR	ANY DEMENTIA			ALZHEIMER'S DISEASE		
		NO. OF CASES/NO. OF SUBJECTS	RR (95% CI)	P VALUE	NO. OF CASES/NO. OF SUBJECTS	RR (95% CI)	P VALUE
Base line	Age and sex	111/1092	1.3 (1.1–1.5)	0.007	83/1092	1.4 (1.1–1.7)	0.002
	Age, sex, and *APOE* genotype	105/1012	1.3 (1.1–1.6)	0.003	79/1012	1.4 (1.2–1.7)	<0.001
	Age, sex, *APOE* genotype, and plasma levels of folate and vitamins B_{12} and B_6	77/789	1.4 (1.1–1.8)	0.002	54/789	1.6 (1.2–2.1)	<0.001
	Age, sex, *APOE* genotype, plasma levels of B vitamins, and additional covariates	60/680	1.4 (1.1–1.9)	0.009	44/680	1.8 (1.3–2.5)	<0.001
Eight years before base line	Age and sex	88/935	1.4 (1.1–1.7)	0.02	67/935	1.4 (1.1–1.9)	0.01
	Age, sex, and *APOE* genotype	82/864	1.3 (1.0–1.7)	0.03	63/684	1.4 (1.0–1.8)	0.02
	Age, sex, *APOE* genotype, and additional covariates	72/771	1.4 (1.1–1.9)	0.01	56/771	1.6 (1.2–2.1)	0.004

*The plasma total homocysteine level was analyzed as a continuous variable. The relative risks (RRs) are per increment of 1 SD (0.4) in the log-transformed homocysteine value. The base-line homocysteine level was estimated on the basis of plasma samples collected from nonfasting subjects at the 20th biennial examination (between 1986 and 1990); the level eight years before base line was estimated on the basis of plasma samples collected from nonfasting subjects at the 16th biennial examination (between 1979 and 1982). Log-transformed values were used for plasma folate and plasma vitamin B_6. The "additional covariates" included educational status, history of stroke, smoking status, alcohol intake, diabetes mellitus, body-mass index, and systolic blood pressure (as recorded at the base-line examination in which plasma total homocysteine was measured). CI denotes confidence interval, and *APOE* apolipoprotein E.

magnitude of the increases in the risks of death from cardiovascular causes and stroke associated with a similar increment in the plasma homocysteine level, which have been previously described in the Framingham cohort.[6,10]

The observed association appeared to be independent of age, sex, *APOE* genotype, plasma vitamin levels, and other putative risk factors for dementia and Alzheimer's disease. The prospective nature of this study and the strong association between newly diagnosed dementia and Alzheimer's disease and plasma homocysteine levels measured eight years before base line suggest that the elevation in the homocysteine level preceded the onset of dementia. Finally, subjects with a sustained elevation of plasma homocysteine had the greatest risk of dementia.

Two case–control studies have specifically addressed the relation between homocysteine levels and the risk of Alzheimer's disease.[17,18] Both studies found a significant elevation of the serum homocysteine level in patients with Alzheimer's disease as compared with age-matched controls. A report from the Rotterdam Study did not show an association between the base-line homocysteine level and a decline in the score on the Mini–Mental State Examination, perhaps because the follow-up period was only 2.7 years.[19] In our study population, an elevated homocysteine level at base line was related to a decline in the scores on the Mini–Mental State Examination, but only after a follow-up period of at least four years (data not shown).

Elevated plasma homocysteine levels are associated with carotid atherosclerosis and an increased risk of stroke.[8,10] Atherosclerosis and stroke, in turn, increase the risk of clinical Alzheimer's disease.[2,4] Hyperhomocysteinemia has been related to cerebral microangiopathy,[44] endothelial dysfunction,[45] impaired nitric oxide activity,[46] and increased oxidative stress[47] — all factors associated with the aging of the brain.[48,49] Increased concentrations of homocysteic acid, an *N*-methyl-D-aspartate receptor agonist and a metabolite of homocysteine, may result in excitotoxic damage to neurons.[50] Homocysteine promotes copper-mediated and β-amyloid-peptide–mediated toxic effects in neuronal cell cultures[51] and induces apoptosis in hippocampal neurons in rats.[52]

The strengths of our investigation include its prospective design, the large community-based sample, the long follow-up period, and the availability of pre-

TABLE 4. MULTIVARIABLE COX PROPORTIONAL-HAZARDS REGRESSION MODELS FOR THE RISK OF DEMENTIA AND ALZHEIMER'S DISEASE ACCORDING TO AGE-SPECIFIC QUARTILE OF PLASMA TOTAL HOMOCYSTEINE LEVEL.*

QUARTILE OF PLASMA HOMOCYSTEINE LEVEL	ANY DEMENTIA			ALZHEIMER'S DISEASE		
	NO. OF CASES/NO. OF SUBJECTS†	RR (95% CI)	P VALUE	NO. OF CASES/NO. OF SUBJECTS	RR (95% CI)	P VALUE
4 at base line (reference group, 1, 2, and 3 at base line)	105/1012	1.9 (1.3–2.9)	0.003	79/1012	1.9 (1.2–3.1)	0.008
With additional adjustment for plasma levels of folate and vitamins B_{12} and B_6‡	77/789	2.5 (1.5–4.4)	<0.001	54/789	2.8 (1.4–5.4)	0.003
4 at 8 yr before base line (reference group, 1, 2, and 3 at 8 yr before base line)	82/864	1.7 (1.0–2.8)	0.04	63/864	1.7 (1.0–3.1)	0.06
1, 2, or 3 at 8 yr before and at base line	48/555	1.0		38/555	1.0	
4 at 8 yr before base line and 1, 2, or 3 at base line	7/88	1.4 (0.6–3.1)	0.44	5/88	1.5 (0.6–3.8)	0.44
1, 2, or 3 at 8 yr before base line and 4 at base line	12/116	1.7 (0.9–3.3)	0.09	9/116	1.7 (0.8–3.6)	0.15
4 at 8 yr before and at base line	15/105	2.2 (1.2–4.1)	0.009	11/105	2.2 (1.1–4.4)	0.03

*All analyses were adjusted for age, sex, and apolipoprotein E genotype. The relative risks (RRs) indicate the risk as compared with that in the reference group during the follow-up period between the 20th biennial examination and December 31, 2000. The base-line plasma homocysteine level was estimated on the basis of plasma samples collected from nonfasting subjects at the 20th biennial examination; the level eight years before base line was estimated on the basis of plasma samples collected from nonfasting subjects at the 16th biennial examination. The 75th percentile of the plasma homocysteine level (the cutoff point for quartile 4) was 13.2 μmol per liter for subjects 65 to 69 years old, 13.8 μmol per liter for subjects 70 to 74 years old, 14.5 μmol per liter for subjects 75 to 79 years old, 16.5 μmol per liter for subjects 80 to 84 years old, 19.3 μmol per liter for subjects 85 to 89 years old, and 26.6 μmol per liter for subjects 90 to 95 years old. CI denotes confidence interval.

†The denominator (number of subjects at risk) is lower than the total number of subjects because 80 of the 1092 subjects evaluated at base line and 71 of the 935 subjects evaluated eight years before base line did not have *APOE* genotype data available and were excluded from the analyses shown.

‡Log-transformed values were used for plasma folate and plasma vitamin B_6.

study plasma homocysteine levels and base-line values for plasma B vitamins and other covariates. A limitation of this study is the lack of racial diversity in the overwhelmingly white Framingham cohort. It is possible that our use of samples obtained from nonfasting subjects resulted in estimates of plasma homocysteine levels that were up to 20 percent higher than they would have been in fasting subjects,[53] but any increase in the variability in plasma homocysteine values caused by this approach is likely to be random and is unlikely to have altered the results.

Vitamin therapy with folic acid, alone or in combination with vitamins B_6 and B_{12}, and dietary supplementation with enriched cereal-grain products and breakfast cereals containing folate can reduce plasma homocysteine levels.[54-56] The U.S. government now mandates folic acid fortification of the food supply.[55] Current plasma homocysteine levels in the Framingham Study population are significantly lower than those that were estimated at the 16th and 20th biennial examinations.[56] However, only 20 cases of dementia were diagnosed between 1997 and the time the levels were remeasured, and therefore it is not possible to assess the effect of recent increases in folic acid fortification on the risk of dementia in this cohort. Furthermore, since there have been no prospective trials of the effect of vitamin supplementation on the incidence of dementia, our findings cannot be used as a basis for setting health policy or treatment recommendations.

The relation between elevated plasma homocysteine levels and dementia must be evaluated in other cohort studies. If such studies confirm our findings, proof of a causal association between plasma homocys-

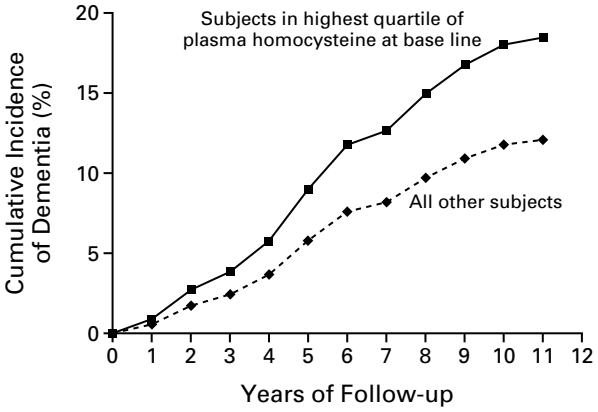

Figure 1. Crude Cumulative Incidence of Dementia among Subjects with Base-Line Plasma Homocysteine Levels in the Highest Age-Specific Quartile and among All Other Subjects.

The 75th percentile of the plasma homocysteine level (the cutoff point for quartile 4) was 13.2 μmol per liter for subjects 65 to 69 years old, 13.8 μmol per liter for subjects 70 to 74 years old, 14.5 μmol per liter for subjects 75 to 79 years old, 16.5 μmol per liter for subjects 80 to 84 years old, 19.3 μmol per liter for subjects 85 to 89 years old, and 26.6 μmol per liter for subjects 90 to 95 years old.

teine and the development of dementia and Alzheimer's disease will require further elucidation of the pathophysiologic mechanisms and direct evidence from controlled clinical trials in humans that interventions that reduce plasma homocysteine levels can reduce the risk of clinical dementia and Alzheimer's disease.

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