Lowering Homocysteine in Patients With Ischemic Stroke to Prevent Recurrent Stroke, Myocardial Infarction, and Death

The Vitamin Intervention for Stroke Prevention (VISP) Randomized Controlled Trial

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Context In observational studies, elevated plasma total homocysteine levels have been positively associated with ischemic stroke risk. However the utility of homocysteine-lowering therapy to reduce that risk has not been confirmed by randomized trials.

Objective To determine whether high doses of folic acid, pyridoxine (vitamin B_6), and cobalamin (vitamin B_{12}), given to lower total homocysteine levels, reduce the risk of recurrent stroke over a 2-year period compared with low doses of these vitamins.

Design Double-blind randomized controlled trial (September 1996–May 2003).

Setting and Participants 3680 adults with nondisabling cerebral infarction at 56 university-affiliated hospitals, community hospitals, private neurology practices, and Veterans Affairs medical centers across the United States, Canada, and Scotland.

Interventions All participants received best medical and surgical care plus a daily multivitamin containing the US Food and Drug Administration's reference daily intakes of other vitamins; patients were randomly assigned to receive once-daily doses of the high-dose formulation (n=1827), containing 25 mg of pyridoxine, 0.4 mg of cobalamin, and 2.5 mg of folic acid; or the low-dose formulation (n=1853), containing 200 µg of pyridoxine, 6 µg of cobalamin, and 20 µg of folic acid.

Main Outcome Measures Recurrent cerebral infarction (primary outcome); coronary heart disease (CHD) events and death (secondary outcomes).

Results Mean reduction of total homocysteine was 2 µmol/L greater in the high-dose group than in the low-dose group, but there was no treatment effect on any end point. The unadjusted risk ratio for any stroke, CHD event, or death was 1.0 (95% confidence interval [CI], 0.8-1.1), with chances of an event within 2 years of 18.0% in the high-dose group and 18.6% in the low-dose group. The risk of ischemic stroke within 2 years was 9.2% for the high-dose and 8.8% for the low-dose groups (risk ratio, 1.0; 95% CI, 0.8-1.3) (P=.80 by log-rank test of the primary hypothesis of difference in ischemic stroke between treatment groups). There was a persistent and graded association between baseline total homocysteine level and outcomes. A 3-µmol/L lower total homocysteine level was associated with a 10% lower risk of stroke (P=.05), a 26% lower risk of CHD events (P<.001), and a 16% lower risk of death (P=.001) in the low-dose group and a nonsignificantly lower risk in the high-dose group by 2% for stroke, 7% for CHD events, and 7% for death.

Conclusions In this trial, moderate reduction of total homocysteine after nondisabling cerebral infarction had no effect on vascular outcomes during the 2 years of follow-up. However, the consistent findings of an association of total homocysteine with vascular risk suggests that further exploration of the hypothesis is warranted and longer trials in different populations with elevated total homocysteine may be necessary.

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HOMOCYSTINURIA, A RARE CONdition in which plasma levels of total homocysteine are very high, was first associated with cerebrovascular disease in 1962.[1,2] In 1969, McCully[3] suggested that more moderate levels of hyperhomocystinemia might be associated with atherosclerosis. Case-control studies have shown higher levels of total homocysteine in patients with premature peripheral and cerebrovascular disease and atherosclerosis.[4,5] Most but not all studies have demonstrated an association between elevated levels of total homocysteine and stroke.[6-11]

In the European Concerted Action Project, the relative risk of vascular disease for participants in the top fifth of fasting homocysteine level distribution (>12 µmol/L) was 2.2 compared with the bottom four fifths.[12] When patients with coronary artery disease were stratified by total homocysteine level, those

See also pp 576 and 621.

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Box. Inclusion and Exclusion Criteria

Inclusion Criteria

Nondisabling ischemic stroke(Modified Rankin Stroke Scale ≤3):
- Onset ≤120 days before randomization
- Focal neurological deficit of likely atherothrombotic origin, classified as ischemic stroke by questionnaire/algorithm or confirmed as new cerebral infarction consistent with symptoms by cranial computed tomography or brain magnetic resonance imaging

Total homocysteine level ≥25th percentile for North American stroke population*

Age ≥35 years

Accessibility for follow-up

Agreement to take study medication and not take other multivitamins or pills containing folic acid or vitamin B_6

Written informed consent

Exclusion Criteria

Potential sources of emboli (atrial fibrillation within 30 days of stroke, prosthetic cardiac valve, intracardiac thrombus or neoplasm, or valvular vegetation)

Other major neurological illness that would obscure evaluation of recurrent stroke

Life expectancy <2 years

Renal insufficiency requiring dialysis

Untreated anemia or untreated vitamin B_{12} deficiency

Systolic blood pressure >185 mm Hg or diastolic blood pressure >105 mm Hg on 2 readings 5 minutes apart at time of eligibility determination

Refractory depression, severe cognitive impairment, or alcoholism or other substance abuse

Use within the last 30 days of medications that affect total homocysteine level (methotrexate, tamoxifen, levodopa, niacin, or phenytoin) or bile acid sequestrants that can decrease folate levels

Childbearing potential

Participation in another trial with active intervention

General anesthesia or hospital stay of ≥3 days, any type of invasive cardiac instrumentation, or endarterectomy, stent placement, thrombectomy, or any other endovascular treatment of carotid artery within 30 days prior to randomization or scheduled to be performed within 30 days after randomization

*Twenty-fifth percentiles were ≥10.5 µmol/L at the beginning of the study (November 1997); ≥9.5 µmol/L after April 8, 1998; and ≥9.5 µmol/L for men and ≥8.5 µmol/L for women after May 5, 1999.

with total homocysteine levels greater than 20 µmol/L had an 8-fold increase in risk.[13] A meta-analysis of epidemiological studies of cardiovascular disease suggested that moderately elevated homocysteine levels are associated with an increased risk of cardiovascular disease independent of other established risk factors.[14] A recent meta-analysis found stronger associations with total homocysteine in retrospective studies of stroke or ischemic heart disease than in prospective studies of individuals with no history of stroke or cardiovascular disease.[15] Boysen et al[16] found a significant difference in total homocysteine levels between patients with ischemic and hemorrhagic stroke, suggesting that elevated total homocysteine is not only a reaction to acute illness but also a risk factor for recurrent stroke.

Mechanisms by which total homocysteine may cause vascular disease include propensity for thrombosis, impaired thrombolysis,[17] increased production of hydrogen peroxide,[18] endothelial dysfunction,[19,20] and increased oxidation of low-density lipoprotein.[21]

Folic acid, pyridoxine (vitamin B_6), and cobalamin (vitamin B_{12}) reduce plasma homocysteine levels[22] and may help to reverse endothelial injury associated with elevated total homocysteine.[19,20] Vitamin therapy may lead to regression of carotid plaque, even in patients with normal levels of homocysteine,[23] and may reduce the number of vascular events and revascularization procedures among patients who have undergone coronary angioplasty.[24]

The Vitamin Intervention for Stroke Prevention (VISP) trial was designed to determine whether best medical and surgical management, risk factor modification, and a multivitamin containing high-dose folic acid, pyridoxine, and cobalamin given to lower total homocysteine levels would reduce the incidence of recurrent cerebral infarction (primary outcome) as well as coronary heart disease (CHD) and death (secondary outcomes) in patients with a nondisabling cerebral infarction and fasting total homocysteine levels greater than the 25th percentile for stroke patients.

METHODS

This study was a multicenter, double-blind, randomized controlled clinical trial performed at 56 centers across the United States (n=45), Canada (n=10), and Scotland (n=1). The protocol was approved by the ethics committees of all study institutions and administrative sites. Written informed consent was obtained from every potential participant prior to screening. The administrative sites were an operations center, a statistical coordinating center, a central laboratory for homocysteine and vitamin determinations, and a drug distribution center.

Participants

Volunteers were recruited from university and community hospitals, private neurology practices, and Department of Veterans Affairs medical centers. Screening procedures are described elsewhere.[25] Briefly, patients with a presumptive diagnosis of acute ischemic stroke were screened no sooner than 72 hours following stroke onset per our previous study,[26] which confirmed that poststroke plasma total homocysteine levels are unstable during the first 72 hours following stroke.

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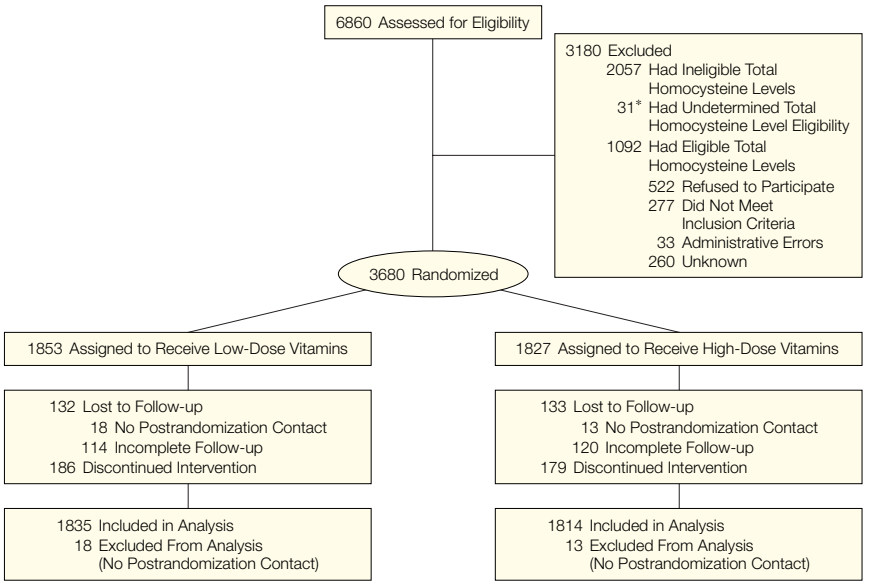
Investigators verified eligibility and obtained written informed consent and a sample of plasma for quantification of total homocysteine by the central laboratory. Total homocysteine includes homocysteine, homocystine, and mixed cysteine-homocysteine disulfide.[27] Efforts were made to obtain fasting plasma samples to standardize testing conditions and total homocysteine measurement. Patients with total homocysteine levels that exceeded thresholds defined in the BOX were qualified for random assignment to high- or low-dose vitamin therapy. These thresholds were adjusted twice during the study as new information was obtained regarding the 25th percentile of total homocysteine levels in stroke patients.

The study inclusion and exclusion criteria are summarized in the Box. Most participants had routine diagnostic tests such as carotid duplex ultrasonography and transthoracic echocardiography. Previous atrial fibrillation was not exclusionary if an electrocardiogram (ECG) performed within the 30 days preceding recruitment showed normal sinus rhythm.

All eligible participants were given the low-dose vitamin formulation for 1 month to determine compliance, assessed by pill counts. Only persons taking at least 75% of the vitamins during the run-in phase were eligible to be randomized.

Other baseline measurements included medical history, current medication and vitamin use, physical and neurological examination, dietary inventory, a stroke symptoms questionnaire, stroke severity determination (including the National Institutes of Health Stroke Scale [NIHSS], Modified Rankin Scale, and Barthel Index),[28] the Mini-Mental State Examination,[29] the Rose angina questionnaire,[30] and blood sampling for central laboratory determination of plasma folate and B_{12} levels and local laboratory determinations of plasma/serum B_{12} and creatinine. Another plasma total homocysteine sample was obtained to serve as the baseline comparison measurement for all subsequent samples. Cranial computed tomography (CT) or magnetic resonance imaging (MRI), ECG, and a current lipid profile were required for randomization.

Figure 1. Flow of Study Participants



Asterisk indicates sex or date or blood drawn not available and patients had values between sex-specific cut points.

Participants were asked to fast for 12 hours before all clinic visits, but blood was drawn regardless of fasting state and plasma total homocysteine levels were determined in duplicate analyses. Concordance between duplicates was within 10% by high-performance liquid chromatography, using the modified method of Smolin and Schneider.[31-33] Plasma aliquots were protected from light for single radioassays of folate and vitamin B_{12} (Bio Rad Quantaphase II, Bio Rad Diagnostics, Hercules, Calif). For quality control, replicate blood aliquots were obtained from a sample of participants (n = 283) and sent to the central laboratory with different identifiers. Interrun coefficients of variation for analytes were as follows: total homocysteine, 0.08; plasma folate, 0.14; and plasma vitamin B_{12}, 0.08. The corresponding intraclass correlations between repeat measurements of blind replicates were 0.94, 0.94, and 0.97, respectively. These results were similar throughout the duration of the study.

Randomization, Intervention, and Follow-up

Participants were randomized to the high-dose or low-dose vitamin groups within strata defined by clinic, sex, and age (≥70 vs <70 years). Permuted block randomization (with block size randomly selected as 4 or 6) was used.[34] The allocation of participants was programmed by the statistical coordinating center, encrypted, and entered into a data entry program installed on a study computer at each site. After computer verification that all eligibility criteria had been met, participants were randomly assigned 1 of 20 medication codes. Allocation information was accessible only to the drug distribution center, which bottled and distributed the vitamins to clinics, and to selected coordinating center personnel who could assist with randomization in case of computer failure. Both pill formulations were manufactured (Magno-Humphries Laboratories, Tigard, Ore) to be indistinguishable by external color, weight, or dissolution in water. No request was ever made to break the blind.

The multivitamin compositions contained the reference daily intakes rec-

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ommended by the US Food and Drug Administration for vitamins,[35] varying only in the content of folic acid, pyridoxine, and cobalamin. The high-dose multivitamin formulation contained 25 mg of pyridoxine, 0.4 mg of cobalamin, and 2.5 mg of folic acid; the low-dose formulation (control) contained 200 µg of pyridoxine, 6 µg of cobalamin, and 20 µg of folic acid.[25] Both doses contained at least 6 µg of cobalamin to minimize the potential for neurological complications resulting from vitamin B_{12} deficiency. Participants received once-daily doses of these formulations.

Physicians provided best available medical and surgical management to prevent recurrent stroke, which included risk factor control education and, usually, administration of aspirin, 325 mg/d.

Participants were contacted every 3 months, alternating between telephone contacts and in-clinic visits for up to 2 years after randomization. At every contact, the stroke symptoms questionnaire was administered and patients were asked about hospitalizations since the last contact. These forms along with discovery of the death of a participant provided the triggers for end-point determination. The 2-year visit (the exit visit) had an expanded clinical examination including CT or MRI. When the study was closed, participants who had not completed the 2-year enrollment or had not had an exit visit were invited for an early exit visit.

Table 1. Baseline Participant Characteristics*

Characteristics	Low-Dose Vitamin Group (n = 1853)	High-Dose Vitamin Group (n = 1827)	*P* Value
Age, mean (SD), y	66.2 (10.8)	66.4 (10.8)	.68
Age group, y			
35-54	300 (16.2)	285 (15.6)	.91
55-64	463 (25.0)	472 (25.8)	
65-74	634 (34.2)	617 (33.8)	
≥75	456 (24.6)	453 (24.8)	
Women	690 (37.2)	689 (37.7)	.77
Race/ethnicity			
Black	286 (15.4)	259 (14.2)	.20
White	1452 (78.4)	1473 (80.6)	
Other	115 (6.2)	95 (5.2)	
Mini-Mental State Examination[29] score, mean (SD)	26.9 (3.3)	26.9 (3.4)	.84
Current smoker	287 (15.5)	334 (18.3)	.02
Taking multivitamins†	419 (22.6)	407 (22.3)	.81
Body mass index, mean (SD)‡			
Men	28.1 (5.3)	28.1 (5.3)	.67
Women	28.5 (5.9)	28.7 (6.7)	.66
Blood pressure, mean (SD), mm Hg			
Systolic	140.6 (18.9)	141.1 (18.6)	.38
Diastolic	77.8 (10.1)	78.0 (10.0)	.46
Cholesterol level, mean (SD), mg/dL			
Total	203.0 (46.5)	200.8 (46.9)	.16
High-density lipoprotein	45.6 (15.7)	45.2 (15.3)	.46
Low-density lipoprotein	123.3 (40.1)	121.6 (40.2)	.22
Triglyceride level, mean (SD), mg/dL	172.7 (108.7)	176.8 (190.1)	.44
Serum creatinine level, mean (SD), mg/dL	1.10 (0.54)	1.13 (0.63)	.23
History of			
Hypertension	1358 (73.4)	1354 (74.3)	.53
Diabetes	571 (30.8)	500 (27.4)	.02
Any cardiac disease§	438 (23.8)	456 (25.1)	.37
Chest pain	646 (34.9)	697 (38.1)	.04
Smoking (ever)	1205 (65.1)	1239 (67.8)	.08
Stroke prior to qualifying stroke	428 (23.1)	428 (23.4)	.81
Carotid endarterectomy	129 (7.0)	118 (6.5)	.55
Angina‖	130 (7.0)	145 (7.9)	.29

SI conversions: To convert total, high-density lipoprotein, and low-density lipoprotein cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113; to convert serum creatinine to µmol/L, multiply by 88.4.

*Data are presented as No. (%) unless otherwise indicated. Seven variables had more than 10 participants with missing data: any cardiac disease (missing 18), Mini-Mental State Examination score (missing 32), body mass index (missing 37), total cholesterol (missing 150), high-density lipoprotein cholesterol (missing 240), triglycerides (missing 263), and low-density lipoprotein cholesterol (missing 371).

†Taking multivitamins, B-vitamin, folate, B_6, or B_{12} at time of qualifying stroke.

‡Body mass index was calculated as the weight in kilograms divided by the square of height in meters.

§Cardiac disease is defined as myocardial infarction, congestive heart failure, coronary angioplasty, or coronary artery bypass graft surgery.

‖Angina determined by the Rose questionnaire.[30]

End-Point Determination

Data from participants who had a follow-up assessment of likely stroke from the stroke symptoms questionnaire; who had a hospital discharge *International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM)* code of 433, 434, or 436,[36] or a discharge diagnosis of stroke, cerebral infarction, cerebrovascular accident, or other synonyms; who had an increase in score from the previous examination in specified sections of the NIHSS; or who died, with stroke as underlying cause of death, were entered into stroke end-point review.

All relevant information regarding potential stroke end points was reviewed by the local neurologist and 2 external review committee neurologists. Recurrent stroke was diagnosed only with evidence of sudden onset of focal neurologic deficit lasting at least 24 hours accompanied by an increased NIHSS score in an area that was previously normal. When the sudden onset of symptoms lasting at least 24 hours was not accompanied by an increased NIHSS score in an area that was previously normal, then recurrent stroke was diagnosed using cranial CT or MRI evidence of new infarction consistent with the clinical presentation.If the reviewers disagreed, the casewas adjudicated by the full review committee.

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Silent cerebral infarction after treatment was not analyzed as an outcome measure, but each participant underwent cranial CT or brain MRI at exit to assist in the validation of stroke end points with sudden onset of stroke symptoms but without confirmation by increased NIHSS score in areas previously normal.

Coronary heart disease events included myocardial infarction (MI) requiring hospitalization, coronary revascularization, cardiac resuscitation, and fatal coronary heart disease. Coronary heart disease event end-point review was implemented when the hospital discharge diagnosis included terms suggestive of MI, unstable angina, or coronary atherosclerosis. Review was also conducted for deaths with underlying cause of death related to CHD, including sudden death, or with *ICD-9-CM* codes 410, 411, 414.1, 429.2, 36.0, or 36.1.[36] Myocardial infarction was defined by new ECG changes including Q waves or marked ST-T changes plus abnormal cardiac enzymes, cardiac symptoms plus abnormal enzymes, or symptoms plus hyperacute ECG changes resolving with thrombolysis. Fatal CHD was defined by CHD as underlying cause of death on the death certificate and either (1) (a) prior hospitalization with MI, autopsy evidence of MI, or death resulting from an invasive coronary procedure or (b) history of angina, MI, or coronary revascularization when no cause of death other than CHD could be determined; or (2) death was sudden and no cause of death other than CHD could be determined. If the 2 external reviewers who reviewed the data disagreed, a third reviewer adjudicated.

Sample Size

The planned sample of 1800 in each treatment group gave the study 80% power to detect a 30% reduction in recurrent ischemic stroke over 2 years of follow-up at a .05 significance level for a 2-sided test. The calculations assumed probability of recurrent stroke of 8% in the first year and 4% in the second year and that 20% of participants would be lost to follow-up or noncompliant or would die of other causes.

Table 2. Characteristics of Qualifying Stroke*

Characteristics	Low-Dose Vitamin Group (n = 1853)	High-Dose Vitamin Group (n = 1827)	*P* Value
Days from stroke to randomization, mean (SD)	71.2 (30.3)	70.6 (30.7)	.54
National Institutes of Health Stroke Scale[28] Score, mean (SD)	1.7 (2.0)	1.7 (2.0)	.90
Distribution of scores			
0-5	1767 (95.4)	1746 (95.6)	.61
6-13	83 (4.5)	80 (4.4)	
≥14	3 (0.2)	1 (0.1)	
Barthel Index score[28] Mean (SD)	19.4 (1.5)	19.5 (1.3)	.45
Distribution of scores			
0-18	234 (12.6)	211 (11.5)	.32
≥19	1619 (87.4)	1616 (88.5)	
Modified Rankin Scale score[28]			
0	380 (20.5)	374 (20.5)	.46
1	779 (42.0)	806 (44.1)	
2	461 (24.9)	443 (24.2)	
3	233 (12.6)	204 (11.2)	

*Data are presented as No. (%) unless otherwise indicated.

Predetermined interim analyses were conducted when the study had obtained approximately 16%, 30%, 50%, 70%, 85%, and 95% of its total information (ischemic stroke end points). The Lan-DeMets spending rule, which approximates the O'Brien-Fleming stopping rule, was used to guide a decision to stop the study early for efficacy.[34] Conditional power calculations were also presented for consideration of futility.[37,38]

Statistical Analysis

The primary test statistic for interim and final analyses was the log-rank test,[39] based on intention to treat including all randomized patients as randomized.[34] Time was defined as number of days from randomization to the first end point (if one occurred), death, date of last contact, or the last day of 2002. Data collection continued through March 17, 2003, to ascertain and validate events through 2002. In addition, survival curves were fit by the Kaplan-Meier method.[39] In a secondary analysis, tests of treatment group differences in time to end point were also performed by adjusting for stratification variables and baseline covariates using a Cox model.[39] Rate ratios are cited as the ratio between persons in the high-dose group vs those in the low-dose group. SAS software, version 8 (SAS Institute Inc, Cary, NC) was used for all analyses and $P<.05$ was considered statistically significant for all analyses.

RESULTS

Recruitment began in August 1997 and was completed in December 2001. In December 2002, the performance and safety monitoring board recommended to the funding agency that the study be terminated because the chance of showing any difference between the 2 treatment groups in the remaining follow-up period was close to nil. Exit assessments were to be completed by March 17, 2003, after all participants had completed at least 1 year of follow-up. The centers were asked to collect information on potential end points and all hospitalizations that had occurred prior to January 1, 2003.

Participant Recruitment Data

Participant flow is illustrated in FIGURE 1. Of the 6860 potential participants screened for total homocysteine eligibility, 70% (4772) had total homocysteine levels above the eligibility cut point. After adjusting to final total homocysteine cut points (8.5 µmol/L for women and 9.5 µmol/L for

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men), 74% of women (1178/1589) and 73% of men (1711/2336) screened for total homocysteine were eligible, close to the 75% study target. Of those with eligible total homocysteine levels, 77% (n=3680) were randomized (75% of women [1379/1828] and 80% of men [2301/2860]), 1853 to the low-dose vitamin group and 1827 to the high-dose vitamin group. For the 1092 patients with eligible total homocysteine levels who were not randomized, the most frequent reasons included "refusal after screening" (48%); ineligibility, including but not limited to stroke beyond eligibility window and noncompliance with run-in vitamin therapy (25%); administrative errors (3%); and "unknown" (24%).

Baseline Descriptive Data

Selected baseline participant characteristics are shown in TABLE 1 and characteristics of the qualifying stroke are shown in TABLE 2. Of 96 baseline characteristics, only 4 treatment group differences were significant at the .05 level: current cigarette use (16% vs 18%), history of diabetes (31% vs 27%), chest pain or discomfort (35% vs 38%), and distribution of right patellar reflex response (data not shown; $P=.009$).

Patient Follow-up Data

Of the 3680 randomized patients, for the purpose of the primary analysis, 31 had no follow-up after randomization, (high=13, low=18 [high=number in high-dose group, low=number in low-dose group]), 300 proceeded to stroke (high=152, low=148), 161 died without recurrent stroke but with follow-up (high=70, low=91), and 234 had some but not complete follow-up (high=120, low=114). The remaining 2954 exited, reached the end of the study (December 31, 2002), or reached the end of 2 years of follow-up without stroke (high=1472, low=1482) (Figure 1). Mean follow-up time for all participants with follow-up was 20.4 months in the high-dose group and 20.2 months in the low-dose group. For all living participants, 94% of planned contacts were completed for each treatment group.

Follow-up Descriptive Data

Participants returned their vitamins for pill count at approximate 6-month intervals, and the percentage of those doing so was similar between groups (86% for each group), as was compliance among those returning pills, with 94% of each treatment group taking at least 75% of their pills.

Selected patient characteristics after 1 year of follow-up are shown in TABLE 3. More than 150 patient characteristics were tested for treatment group differences during the 6-, 12-, 18-, and 24-month follow-up examinations. Three (not including the expected differences in total homocysteine and plasma vitamin levels) had statistically significant differences between treatment groups at the .05 level at study end: a 1.4-mg/dL higher high-density lipoprotein cholesterol level at the 1-year examination in the high-dose group compared with the low-dose group, a 6% more frequent use at 1 year of estrogen/progestin among women in the high-dose group, and 4.2% more patients with reduced or absent

Table 3. Characteristics of Treatment Groups at 1-Year Follow-up*

Characteristics	No. of Participants Followed Up	Low-Dose Vitamin Group (n = 1696)	High-Dose Vitamin Group (n = 1673)	*P* Value
Tobacco use	3361			
Continuing smoker		196 (11.6)	232 (13.9)	.11
New smoker		62 (3.7)	53 (3.2)	
Nonsmoker		1377 (81.4)	1317 (78.9)	
Quit smoking		56 (3.3)	68 (4.1)	
Current smokers, mean (SD) cigarettes/d	541	14.0 (10.3)	15.1 (10.6)	.21
Endarterectomy after randomization	3369	24 (1.4)	27 (1.6)	.64
Estrogen/progestin use	1266	128 (20.3)	167 (26.3)	.01
Barthel Index score, mean (SD)	3152	19.6 (1.6)	19.6 (1.6)	.90
National Institutes of Health Stroke Scale score, mean (SD)	3145	0.9 (1.5)	0.8 (1.5)	.44
Modified Rankin Stroke Scale score, mean (SD)	3152	1.0 (1.0)	1.0 (1.0)	.61
Mini-Mental State Examination score				
≥2-Point decrease	3073	275 (17.7)	289 (19.1)	.32
Mean (SD)	3097	27.4 (3.2)	27.2 (3.6)	.19
Weight, mean (SD), kg	3048	81.7 (17.3)	82.2 (19.0)	.40
Body mass index, mean (SD)†	2829	28.5 (5.4)	28.6 (6.0)	.68
Blood pressure, mean (SD), mm Hg				
Systolic	3122	140.2 (20.1)	140.1 (19.9)	.91
Diastolic	3121	77.3 (10.8)	77.3 (10.8)	.87
Cholesterol level, mean (SD), mg/dL				
Total	2753	194.0 (40.8)	193.8 (42.5)	.93
High-density lipoprotein	2735	47.8 (15.0)	49.2 (16.7)	.02
Low-density lipoprotein	2583	112.9 (36.2)	111.5 (35.7)	.34
Triglycerides, mean (SD), mg/dL	2687	173.5 (107.0)	170.3 (101.6)	.44
No. of new neurological signs of possible cobalamin deficiency after randomization‡	2834			
0		1017 (70.6)	1002 (71.9)	.34
1		375 (26.0)	331 (23.8)	
2		46 (3.2)	55 (3.9)	
3		3 (0.2)	5 (0.4)	

SI conversions: To convert total, high-density lipoprotein, and low-density lipoprotein cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113.
*Data are presented as No. (%) unless otherwise indicated.
†Body mass index was calculated as the weight in kilograms divided by the square of height in meters.
‡Neurological signs that were suggestive but not diagnostic of cobalamin deficiency included diminished Achilles reflex, reduction of vibration sense in the great toe, or an extensor plantar sign (n = 3).

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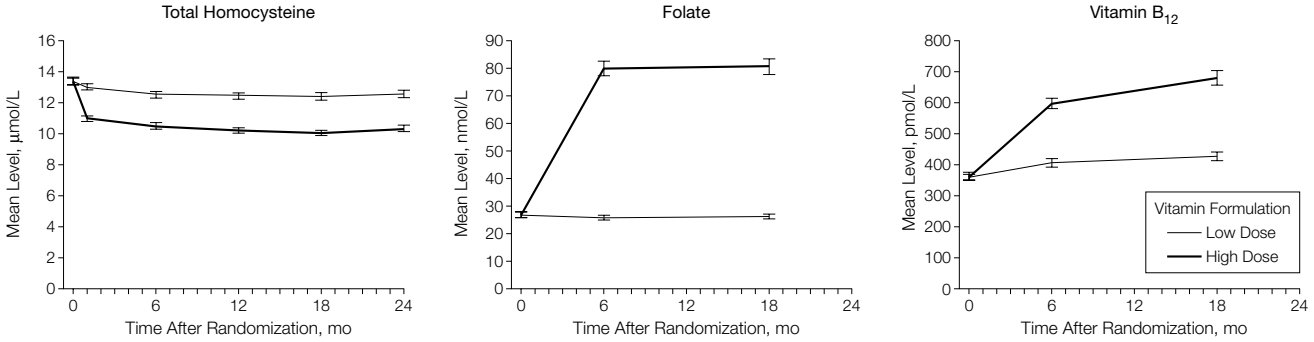
Figure 2. Mean Total Homocysteine, Folate, and Vitamin B_{12} Levels Over Time, by Treatment Group



Error bars indicate 95% confidence intervals.

right-side temperature sensation in the high-dose group compared with the low-dose group from the neurological examination at 18 months ($P = .02$; data not shown).

FIGURE 2 shows the mean levels of plasma total homocysteine, folate, and B_{12} at each clinic examination, by treatment group. Mean values of each were virtually identical between treatment groups at randomization (13.4 µmol/L for total homocysteine in each group). After randomization, both groups experienced a decrease in total homocysteine, but the decrease was greater for the high-dose group by 2.0 µmol/L at 1 month (2.4 µmol/L in the high-dose group and 0.3 µmol/L in the low-dose group), by 2.2 µmol/L at 1 year, and by 2.3 µmol/L at 2 years.

Between-group differences in decrease in total homocysteine for a particular visit relative to baseline were somewhat smaller in late 2001: the mean 1-month difference was 2.7 µmol/L in 1997 through early 1998 and 1.5 µmol/L in late 2001. Likewise, the 12-month difference decreased from 2.4 µmol/L in 1997 through early 1998 to 1.8 µmol/L in late 2001. The large treatment group differences in plasma vitamin levels did not vary substantially over time.

Seventy-five participants had a baseline B_{12} level of less than 150 pmol/L. After study centers were notified and patients received treatment, all but 2 had subsequent levels greater than 150 pmol/L. At 6 or 18 months of follow-up, an additional 9 participants (all but 1 were in the low-dose group) had a B_{12} level at or below the alert threshold of 150 pmol/L. Site investigators were notified of these low values, the affected participants were treated with replacement cobalamin, and all subsequent B_{12} levels exceeded 150 pmol/L when assayed locally or in the central laboratory. At 12 months, 29% of participants had neurological findings (diminished Achilles reflex, reduction of vibration sense in the great toe, or an extensor plantar sign) that could represent cobalamin deficiency but also might be observed in stroke, diabetes, or other conditions. The percentage of participants showing 1 or more of these neurological signs after randomization did not differ significantly between treatment groups, confirming that physical evidence suggestive of cobalamin deficiency was not confined to the low-dose group and was of no consequence (Table 3).

At each follow-up clinic visit, participants were asked about potential adverse effects of the vitamins. There were no statistically significant differences between treatment groups for itching, skin rash, gastrointestinal upset, for the overall question on any adverse effects, or for any of the most frequently cited other adverse effects. No statistically significant differences were found for any additional self-reported adverse effect thought to be due to the vitamins, hospital admissions overall and by diagnostic category, or death overall and by underlying cause.

Efficacy of Treatment

In an intention-to-treat analysis of the primary end point, 8.1% of the low-dose group (148/1835) and 8.4% of the high-dose group (152/1814) had a recurrent ischemic stroke (TABLE 4). The Kaplan-Meier curves by treatment group were nearly identical (FIGURE 3), with $P = .80$ by log-rank test. The high-dose group had a 0.4% (95% confidence interval [CI], –1.6 to 2.4) greater probability of ischemic stroke within 2 years (by Kaplan-Meier method), and the 2-year risk ratio was 1.0 (95% CI, 0.8-1.3). Analysis of fatal or disabling ischemic stroke gave similar results.

The intention-to-treat analysis for CHD events included 6.7% of cases in the low-dose group (123/1835) and 6.3% (114/1815) in the high-dose group. The high-dose group had a 0.5% (95% CI, –1.3 to 2.2) lower probability of CHD events within 2 years (by Kaplan-Meier method), and the 2-year risk ratio was 0.9 (95% CI, 0.7-1.2). Results of separate analyses of hospitalized MI and fatal CHD were similar.

In the low-dose group, 6.3% (117/1847) died compared with 5.4% of the high-dose group (99/1821). The high-dose group had a 1.0% (95% CI, –0.7% to 2.7%) lower probability of death within 2 years (by Kaplan-Meier method); the 2-year risk ratio was 0.9 (95% CI, 0.7-1.1).

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In all analyses, adjusting for characteristics in which the treatment groups differed at baseline or accounting for the stratification in randomization had little effect on the results.

Analysis of an end point combining the ischemic stroke, CHD events, and death end points (whichever event came first) yielded an observed 17.2% event rate in the low-dose group (316/1838) and 16.7% in the high-dose group (303/1819), with a relative risk of 1.0 (95% CI, 0.8-1.1). In similar analyses for ischemic stroke, CHD events, and death within various participant subgroups defined at baseline (eg, age ≥70 vs <70 years, race/ethnicity, sex, current smoking, diabetes, history of stroke prior to qualifying stroke, history of MI, blood pressure, history of chest pain, baseline total homocysteine level, and fruit, vegetable, or grain intake), no effect of treatment was found. Of particular interest is the treatment effect among those who began with high baseline total homocysteine levels. In the top third of the baseline total homocysteine distribution (total homocysteine >14 µmol/L), the 2-year risk ratios were 0.9 (95% CI, 0.7-1.3) for stroke, 0.9 (95% CI, 0.6-1.3) for coronary events, 0.9 (95% CI, 0.6-1.3) for death, and 1.0 (95% CI, 0.8-1.2) for the combined end point including all 3 outcomes. Analyses limited to participants with compliance of at least 75% showed similar results as the intention-to-treat analyses.

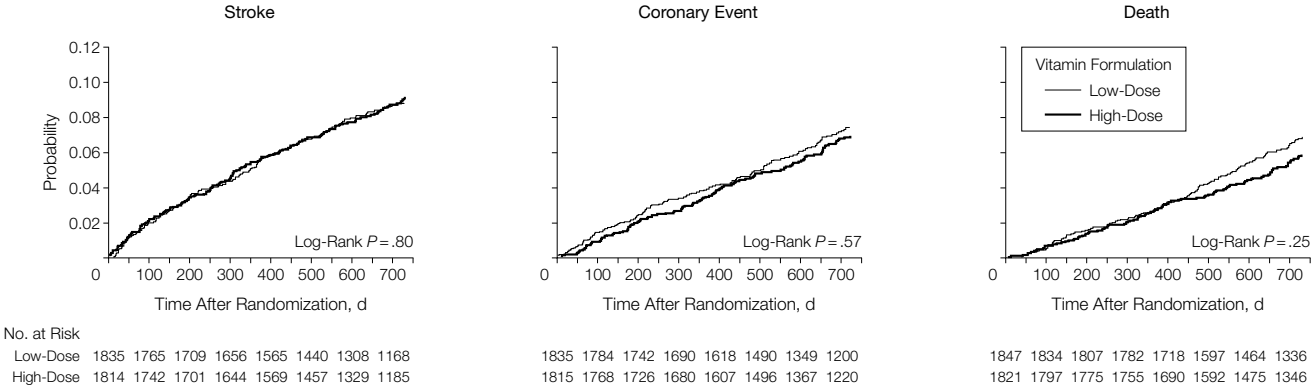
To determine whether treatment effect might occur only after a longer interval, we conducted an analysis limited to participants with at least 1 year of follow-up. These results were not sig-

Table 4. Intention-to-Treat Analysis of Treatment Group Difference in Primary and Secondary End Points

End Points	No. of End Points		*P* Value (Log-Rank Test) for Treatment Effect	Observed % With Event Within 2 y		Kaplan-Meier % With Event Within 2 y		Event Reduction by Kaplan-Meier Analysis (95% CI)	*P* Value for Difference at 2 y (Kaplan-Meier)	Relative 2-y Risk (95% CI)	Adjusted Hazard Rate Ratio (95% CI)*
	Low-Dose	High-Dose		Low-Dose	High-Dose	Low-Dose	High-Dose				
Ischemic stroke											
Any†	148	152	.80	8.1	8.4	8.8	9.2	−0.4 (−2.4 to 1.6)	.68	1.0 (0.8 to 1.3)	1.1 (0.8 to 1.3)
Fatal or disabling	18	21	.63	1.0	1.2	1.2	1.3	−0.1 (−0.9 to 0.7)	.77	1.1 (0.6 to 2.1)	1.1 (0.6 to 1.2)
CHD											
Any	123	114	.57	6.7	6.3	7.4	7.0	0.5 (−1.3 to 2.2)	.62	0.9 (0.7 to 1.2)	0.9 (0.7 to 1.1)
MI or fatal CHD	81	72	.48	4.4	4.0	4.9	4.4	0.5 (−0.9 to 2.0)	.49	0.9 (0.7 to 1.2)	0.9 (0.6 to 1.2)
Ischemic stroke or CHD											
Any	257	249	.74	14.0	13.7	15.2	15.0	0.3 (−2.2 to 2.7)	.83	1.0 (0.8 to 1.2)	1.0 (0.8 to 1.2)
Fatal or disabling stroke or MI or fatal CHD	98	89	.53	5.3	4.9	6.0	5.4	0.6 (−1.0 to 2.2)	.47	0.9 (0.7 to 1.2)	0.9 (0.7 to 1.2)
Death	117	99	.25	6.3	5.4	6.9	5.9	1.0 (−0.7 to 2.7)	.24	0.9 (0.7 to 1.1)	0.9 (0.6 to 1.1)
Ischemic stroke, CHD, or death											
Any	316	303	.61	17.2	16.7	18.6	18.0	0.6 (−2.0 to 3.3)	.65	1.0 (0.8 to 1.1)	1.0 (0.8 to 1.1)
Fatal or disabling stroke, MI, or death	170	156	.45	9.3	8.6	10.3	9.4	0.9 (−1.1 to 3.0)	.38	0.9 (0.7 to 1.1)	0.9 (0.7 to 1.2)

Abbreviations: CHD, coronary heart disease; CI, confidence interval; MI, myocardial infarction.
*Hazard rate ratio calculated by the proportional hazards model for high-dose compared with low-dose treatment, adjusted for characteristics that differed between treatment groups at baseline, including current smoking, history of diabetes or chest pain, and absent patellar reflex or impaired distal temperature sensation.
†Included 1835 and 1814 participants in the low-dose and high-dose groups, respectively, in the primary analysis, excluding the 18 and 13, respectively, with no postrandomization follow-up. These data vary slightly by end point because of missing values for instruments used to determine end points.

Figure 3. Probability of Stroke, Coronary Event, or Death Over Time, by Treatment Group



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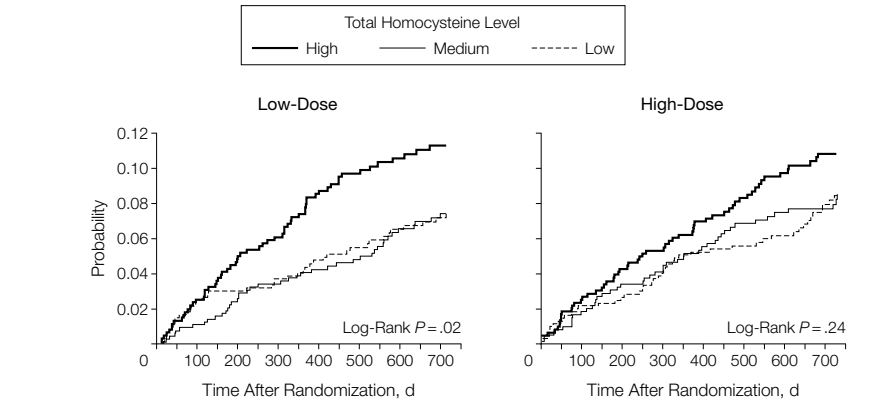
nificant (hazard rate ratios for stroke, 1.0; 95% CI, 0.7-1.5; $P=.81$]; for CHD events, 1.0; 95% CI, 0.7-1.5; $P=.97$; and for death, 0.7; 95% CI, 0.5-1.1; $P=.12$).

Because we found no treatment effect despite several observational studies that found an association between baseline total homocysteine and cardiovascular disease in follow-up, we considered such associations within each treatment group. Based on baseline measurements we found persistent and graded associations between baseline total homocysteine level and outcomes (FIGURE 4), which were significant for stroke ($P=.02$) for the low-dose group but not significant ($P=.24$) for the high-dose group; for CHD events ($P=.001$ for the low-dose and $P=.002$ for the high-dose group); and for death ($P=.001$ for the low-dose and $P=.001$ for the high-dose group). For comparison with other observational studies, the above model was recomputed using baseline total homocysteine as a continuous variable. For the low-dose group, a 3-µmol/L lower total homocysteine level was associated with a 10% lower risk of stroke ($P=.05$), a 26% lower risk of CHD events ($P<.001$), and a 16% lower risk for death ($P=.001$). For the high-dose group, the risk was lowered by 2% for stroke, 7% for CHD events, and 7% for death, but these effects were not significant.

Figure 4. Probability of Stroke Over Time, by Treatment Group and Total Homocysteine Level at Baseline



No. at Risk Total Homocysteine Level																
High	586	559	531	506	476	435	389	344	597	565	546	524	497	462	422	382
Medium	621	600	585	571	541	510	459	413	589	571	556	537	516	476	431	383
Low	596	574	562	549	522	474	441	392	601	579	573	557	532	495	452	398

COMMENT

In this randomized double-blind trial, high-dose vitamin therapy had no effect on the outcome measures of stroke, CHD events, or death. Noncompliance cannot explain the null findings because the reported good compliance was corroborated by the consistently higher blood levels of vitamins and the consistently lower total homocysteine in the high-dose group vs the low-dose group. In addition, the results were similar when limited to high compliers.

One possible reason our treatment was not effective may have been that patients enrolled in this study had levels of total homocysteine that were too low to show a large effect. The very high stroke risk associated with hyperhomocystinemia involves total homocysteine levels in the hundreds of micromoles per liter. At levels approaching the normal range, there is a steep relationship between total homocysteine and risk: a prospective study in Norway[40] showed that levels of total homocysteine above 20 µmol/L carried a 9-fold increase in risk, whereas the European Concerted Action Project[41] showed that levels above 10.2 µmol/L were associated with a doubling of risk.

Wald et al[42] estimated that reducing total homocysteine by 3 µmol/L is associated with a 24% reduced risk of stroke (95% CI, 15%-33%) and a 16% reduced risk of ischemic heart disease (95% CI, 11%-20%). This implies a 13% combined stroke/coronary event reduction for a difference of 2 µmol/L, which our trial had 31% power to detect compared with 80% power for the 30% effect size used in the sample size calculations. To detect a statistically significant 10% reduction in all-cause mortality (the nonsignificant result we observed), a sample size of 20 000 participants would be required for 80% power, assuming 10% dropouts.

The modest reduction in total homocysteine observed in our study may be due in part to the folate fortification of the US grain supply that coincided with the initiation of our trial. Folate fortification, which began in 1996 and was mandated by January 1998, profoundly reduced the prevalence of low folate and high total homocysteine levels. For example, in the Framingham Offspring Study, the proportion with folate deficiency declined from 22% before fortification to 1.7% after fortification.[43] During the course of our trial, the mean difference in total homocysteine levels between the treatment groups narrowed: the 1-month difference was 2.7 µmol/L at baseline and 1.5 µmol/L at the end of the trial. Fortification probably reduced the number of participants with high total homocysteine who might be most likely to benefit.[44] Furthermore, the correction of low serum B_{12} levels in the low-dose group may have blunted the vitamin effect. Thus, other determinants of total homocysteine may have been more important in this setting, suggesting that other regimens, including betaine (trimethylcholine) and higher doses of B_{12}, might be more effective.

Another consideration is that a longer duration of treatment may be necessary. The baseline levels of total homocysteine that were linked to risk in this trial and in many observational studies likely represent many years of elevated total homocysteine; the 2 years

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of treatment in this trial may have been insufficient to reverse those effects.

An alternative interpretation is that elevated total homocysteine levels are a marker but not a cause for vascular disease risk. A previous randomized trial of total homocysteine lowering with vitamins found a significant reduction in adverse outcomes among patients with successful angioplasty.[24] However, another trial, using folate alone, showed no reduction in adverse outcomes for patients with coronary artery disease.[45] That trial, like ours, also found baseline total homocysteine to be an independent predictor of outcome.

In summary, the VISP trial showed that moderate reduction of total homocysteine level after ischemic stroke had no effect on vascular outcomes during the 2 years of follow-up. However, because of the consistent findings of an association of total homocysteine level with vascular risk, further exploration of the hypothesis is warranted and longer trials in different populations with elevated total homocysteine may be necessary.

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