

ORIGINAL ARTICLE

# Homocysteine Lowering with Folic Acid and B Vitamins in Vascular Disease

The Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators\*

## ABSTRACT

### BACKGROUND

In observational studies, lower homocysteine levels are associated with lower rates of coronary heart disease and stroke. Folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> lower homocysteine levels. We assessed whether supplementation reduced the risk of major cardiovascular events in patients with vascular disease.

### METHODS

We randomly assigned 5522 patients 55 years of age or older who had vascular disease or diabetes to daily treatment either with the combination of 2.5 mg of folic acid, 50 mg of vitamin B<sub>6</sub>, and 1 mg of vitamin B<sub>12</sub> or with placebo for an average of five years. The primary outcome was a composite of death from cardiovascular causes, myocardial infarction, and stroke.

### RESULTS

Mean plasma homocysteine levels decreased by 2.4  $\mu\text{mol}$  per liter (0.3 mg per liter) in the active-treatment group and increased by 0.8  $\mu\text{mol}$  per liter (0.1 mg per liter) in the placebo group. Primary outcome events occurred in 519 patients (18.8 percent) assigned to active therapy and 547 (19.8 percent) assigned to placebo (relative risk, 0.95; 95 percent confidence interval, 0.84 to 1.07;  $P=0.41$ ). As compared with placebo, active treatment did not significantly decrease the risk of death from cardiovascular causes (relative risk, 0.96; 95 percent confidence interval, 0.81 to 1.13), myocardial infarction (relative risk, 0.98; 95 percent confidence interval, 0.85 to 1.14), or any of the secondary outcomes. Fewer patients assigned to active treatment than to placebo had a stroke (relative risk, 0.75; 95 percent confidence interval, 0.59 to 0.97). More patients in the active-treatment group were hospitalized for unstable angina (relative risk, 1.24; 95 percent confidence interval, 1.04 to 1.49).

### CONCLUSIONS

Supplements combining folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> did not reduce the risk of major cardiovascular events in patients with vascular disease. (ClinicalTrials.gov number, NCT00106886; Current Controlled Trials number, ISRCTN14017017.)

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**N**UMEROUS STUDIES SUGGEST THAT homocysteine may be a modifiable risk factor for cardiovascular disease. In experimental studies, homocysteine causes oxidative stress, damages endothelium, and enhances thrombogenicity.<sup>1-3</sup> In general, epidemiologic studies show an independent and graded association between homocysteine levels and cardiovascular risk.<sup>4-8</sup> The observational data suggest that even mild-to-moderate elevations in homocysteine increase cardiovascular risk; this observation is important, because such increases are common and can easily be corrected with safe and inexpensive therapy. Folic acid is the most important dietary determinant of homocysteine; daily supplementation with 0.5 to 5.0 mg typically lowers plasma homocysteine levels by about 25 percent. Vitamin B<sub>12</sub> supplementation of at least 0.4 mg daily further lowers levels by about 7 percent, and vitamin B<sub>6</sub> supplements may be particularly important in lowering homocysteine after methionine loading.<sup>9,10</sup>

We report the results of the Heart Outcomes Prevention Evaluation (HOPE) 2 study, a large, prospective, randomized clinical trial designed to determine whether prolonged administration of folic acid combined with vitamins B<sub>6</sub> and B<sub>12</sub> reduces the risk of major vascular events in persons at high cardiovascular risk.

## METHODS

### STUDY DESIGN

HOPE-2 was a randomized, double-blind, placebo-controlled trial evaluating whether therapy with homocysteine-lowering B vitamins reduces the risk of major vascular events in a high-risk population. The trial design has been described previously.<sup>11</sup> The study was coordinated by the Population Health Research Institute at McMaster University in Hamilton, Ontario, and sponsored by the Canadian Institutes of Health Research. Study drug and matching placebo were provided by Jamieson Laboratories, Canada. The study sponsors were not involved in the design, execution, analysis, or reporting of the trial results. An independent data and safety monitoring board monitored the safety of the participants and the overall quality and scientific integrity of the study. The study was approved by the ethics review boards of all participating institutions, and all patients provided written informed consent.

### STUDY POPULATION

Men and women 55 years of age or older who had a history of vascular disease (coronary, cerebrovascular, or peripheral vascular) or diabetes and additional risk factors for atherosclerosis were enrolled, irrespective of their homocysteine levels, from countries with mandatory folate fortification of food (Canada and the United States) and countries without mandatory folate fortification (Brazil, western Europe, and Slovakia). Patients who were taking vitamin supplements containing more than 0.2 mg of folic acid per day were excluded. Detailed eligibility criteria have been published previously<sup>11</sup> and are provided in the Supplementary Appendix (available with the full text of this article at [www.nejm.org](http://www.nejm.org)).

### STUDY INTERVENTION

Patients were randomly assigned to receive a combined pill containing 2.5 mg of folic acid, 50 mg of vitamin B<sub>6</sub>, and 1 mg of vitamin B<sub>12</sub> (active treatment) or matching placebo daily. The study used central telephone randomization. The randomization code was generated with the use of a fixed block size of four, stratified according to center. All study investigators, personnel, and participants were unaware of the randomization procedure and the treatment assignments.

### FOLLOW-UP AND LABORATORY EVALUATION

After randomization, patients were evaluated every six months to determine their adherence to treatment (by interview and pill count) and identify adverse events and clinical outcomes. Blood samples were collected at randomization, at two years, and at the end of the study in a randomly selected subgroup of patients after an overnight fast, with proportional representation from countries with folate fortification of food and countries without folate fortification and with expected significant differences in dietary habits.

Plasma levels of folate (Roche chemiluminescence method, Roche Diagnostics), vitamin B<sub>6</sub> (Chromsystems kit, Instruments and Chemicals), and vitamin B<sub>12</sub> (Immulite 2000 Analyzer, Diagnostic Products) were measured at randomization and at two years. Total plasma homocysteine levels were measured (Abbott IMX immunofluorescence method, Abbott) at randomization, at two years, and at the end of the study (average, five years).

**TRIAL OUTCOMES**

The primary study outcome was the composite of death from cardiovascular causes, myocardial infarction, and stroke. Secondary outcomes were total ischemic events (defined as the composite of death from cardiovascular causes, myocardial infarction, stroke, hospitalization for unstable angina, and revascularization), death from any cause, hospitalization for unstable angina, hospitalization for congestive heart failure, revascularization, the incidence of cancer, and death from cancer. Other outcomes included transient ischemic attacks, venous thromboembolic events, and fractures. All primary and secondary outcomes were centrally adjudicated.

Deaths classified as due to cardiovascular causes were unexpected deaths presumed to be due to ischemic cardiovascular disease and occurring within 24 hours after the onset of symptoms without clinical or postmortem evidence of another cause, deaths from myocardial infarction or stroke within 7 days after the event, deaths associated with cardiovascular interventions within 30 days after cardiovascular surgery or within 7 days after percutaneous interventions, and deaths from congestive heart failure, arrhythmia, pulmonary embolism, or ruptured aortic aneurysm. Deaths from uncertain causes were presumed to be due to cardiovascular causes.

Myocardial infarction was diagnosed when two of the following three criteria were met: typical symptoms, increased cardiac-enzyme levels, and diagnostic electrocardiographic changes.<sup>12</sup> Stroke was defined as a focal neurologic deficit lasting more than 24 hours. Computed tomography or magnetic resonance imaging was recommended to identify the type of stroke (ischemic or hemorrhagic). When these tools were not available, the stroke was classified as of uncertain type. Cancers (except basal-cell skin cancer) were diagnosed on the basis of pathological (or cytologic) findings or, when pathological data were not available, on the basis of clinical summaries, results of imaging, levels of serum markers, and other diagnostic procedures. Cancers were classified according to the *International Classification of Diseases, 9th Revision*.

**STATISTICAL ANALYSIS**

The study was designed to enroll 5000 patients and to average five years of follow-up to allow the detection of a proportional reduction in the risk of the primary outcome of 17 to 20 percent, with

a statistical power of 80 percent and 90 percent, respectively, given an annual event rate of 4 percent in the placebo group and a two-tailed  $\alpha$  value of 0.05. This enrollment target was also estimated to provide over 80 percent power to detect a 15 percent reduction in the risk of total ischemic events.

All analyses were performed according to the intention to treat and included all randomized patients. Survival curves were estimated according to the Kaplan–Meier procedure and were compared between treatment groups with the log-rank test. Prespecified subgroup analyses involving Cox models were used to evaluate outcomes in patients from regions with folate fortification of food and regions without folate fortification, according to the baseline plasma homocysteine level and the baseline serum creatinine level. Additional exploratory subgroup analyses were conducted to evaluate the consistency of the study results.

**RESULTS****CHARACTERISTICS OF THE PATIENTS**

Between January and December 2000, 5522 patients were recruited at 145 centers in 13 countries: 3982 (72.1 percent) were from countries with folate fortification of food, and 1540 (27.9 percent) were from countries without folate fortification. Of these patients, 2758 were randomly assigned to active treatment with folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> and 2764 were assigned to placebo. Baseline characteristics are shown in Table 1 and were generally well balanced between the study groups.

**ADHERENCE, ADVERSE EVENTS, AND FOLLOW-UP**

Among those assigned to the active-treatment group, 95.5 percent were still taking the study drug at one year, 94.0 percent were doing so at two years, 92.5 percent at three years, 91.4 percent at four years, and 90.8 percent at five years. The respective figures for the placebo group were 96.0 percent, 93.4 percent, 92.3 percent, 89.9 percent, and 88.5 percent. Use of open-label folic acid supplements ranged from 2.3 to 4.5 percent in the active-treatment group and from 2.2 to 5.5 percent in the placebo group. There were no serious adverse events related to study treatment. The most common reasons for permanently or temporarily discontinuing treatment at any time

**Table 1. Baseline Characteristics of the Patients.\***

Characteristic	Active Group (N = 2758)	Placebo Group (N = 2764)
Age — yr	68.8±7.1	68.9±6.8
Female sex — no. (%)	796 (28.9)	763 (27.6)
Vascular disease history — no. (%)		
Coronary artery disease	2285 (82.8)	2315 (83.8)
Myocardial infarction	1501 (54.4)	1498 (54.2)
Stable angina	1651 (59.9)	1636 (59.2)
Unstable angina	709 (25.7)	730 (26.4)
Coronary-artery bypass grafting	722 (26.2)	779 (28.2)
Percutaneous coronary intervention	565 (20.5)	546 (19.8)
Stroke	241 (8.7)	251 (9.1)
Stroke or transient ischemic attack	341 (12.4)	343 (12.4)
Intermittent claudication	73 (2.6)	60 (2.2)
Peripheral-artery surgery or percutaneous intervention	157 (5.7)†	119 (4.3)
Carotid endarterectomy	82 (3.0)	64 (2.3)
Risk factors — no. (%)		
Hypertension	1542 (55.9)	1497 (54.2)
Diabetes mellitus	1122 (40.7)	1087 (39.3)
Elevated total cholesterol	1333 (48.3)	1306 (47.3)
Low HDL cholesterol	432 (15.7)	454 (16.4)
Current smoking	306 (11.1)	327 (11.8)
Medication use — no. (%)		
Aspirin or antiplatelet agents	2148 (77.9)	2224 (80.5)
Beta-blockers	1270 (46.0)	1294 (46.8)
Lipid-lowering drugs	1627 (59.0)	1690 (61.1)
ACE inhibitors	1818 (65.9)	1827 (66.1)
Angiotensin II-receptor blockers	124 (4.5)	131 (4.7)
Calcium-channel blockers	1045 (37.9)	1012 (36.6)
Diuretics	756 (27.4)	696 (25.2)
Oral hypoglycemic agents	653 (23.7)	647 (23.4)
Insulin	406 (14.7)	361 (13.1)
Hormone-replacement therapy‡	137 (17.2)	130 (17.0)
Multivitamins	331 (12.0)	307 (11.1)

were the patient's decision (11.1 percent in the active-treatment group, vs. 12.6 percent in the placebo group), physician's advice (1.6 percent vs. 2.0 percent), hospitalization (1.0 percent vs. 0.8 percent), and general malaise (1.0 percent vs. 0.7 percent).

Follow-up averaged five years. A total of 37 patients, 21 in the active-treatment group and 16 in the placebo group, did not complete the study (21 declined to continue, and 16 were lost to follow-up). The vital status of 99.3 percent of pa-

tients was ascertained at the end of the study. All patients who declined to continue the study or were lost to follow-up completed at least two clinic visits and were included in the final analysis, with data censored at the time of the last follow-up visit.

#### EFFECTS OF SUPPLEMENTATION ON VITAMIN AND HOMOCYSTEINE LEVELS

Plasma vitamin levels and homocysteine levels for the subgroup of patients in whom they were

Table 1. (Continued.)

Characteristic	Active Group (N = 2758)	Placebo Group (N = 2764)
Findings on physical examination		
Heart rate — beats/min	68.7±11.2	68.9±11.5
Systolic blood pressure — mm Hg	138.8±21.7	138.9±23.4
Diastolic blood pressure — mm Hg	77.4±11.8	77.5±11.7
Body-mass index	29.6±16.4	29.7±21.1
Waist-to-hip ratio	0.95±0.3	0.94±0.1
Ankle-brachial index	1.0±0.2	1.0±0.2
Laboratory results — mg/dl§		
Total cholesterol	186.8±38.8	184.8±38.0
LDL cholesterol	105.7±33.2	103.9±31.9
HDL cholesterol	46.8±14.0†	45.8±13.0
Triglycerides	178.6±119.7	181.1±113.8
Plasma glucose	128.8±57.6	125.7±51.7
Creatinine	1.0±0.3	1.0±0.3

\* Plus-minus values are means ±SD. The body-mass index is the weight in kilograms divided by the square of the height in meters. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129. To convert values for glucose to millimoles per liter, multiply by 0.05551. To convert values for creatinine to micromoles per liter, multiply by 88.4. HDL denotes high-density lipoprotein, ACE angiotensin-converting enzyme, and LDL low-density lipoprotein.

† P<0.05 for the comparison with placebo.

‡ The results include women only.

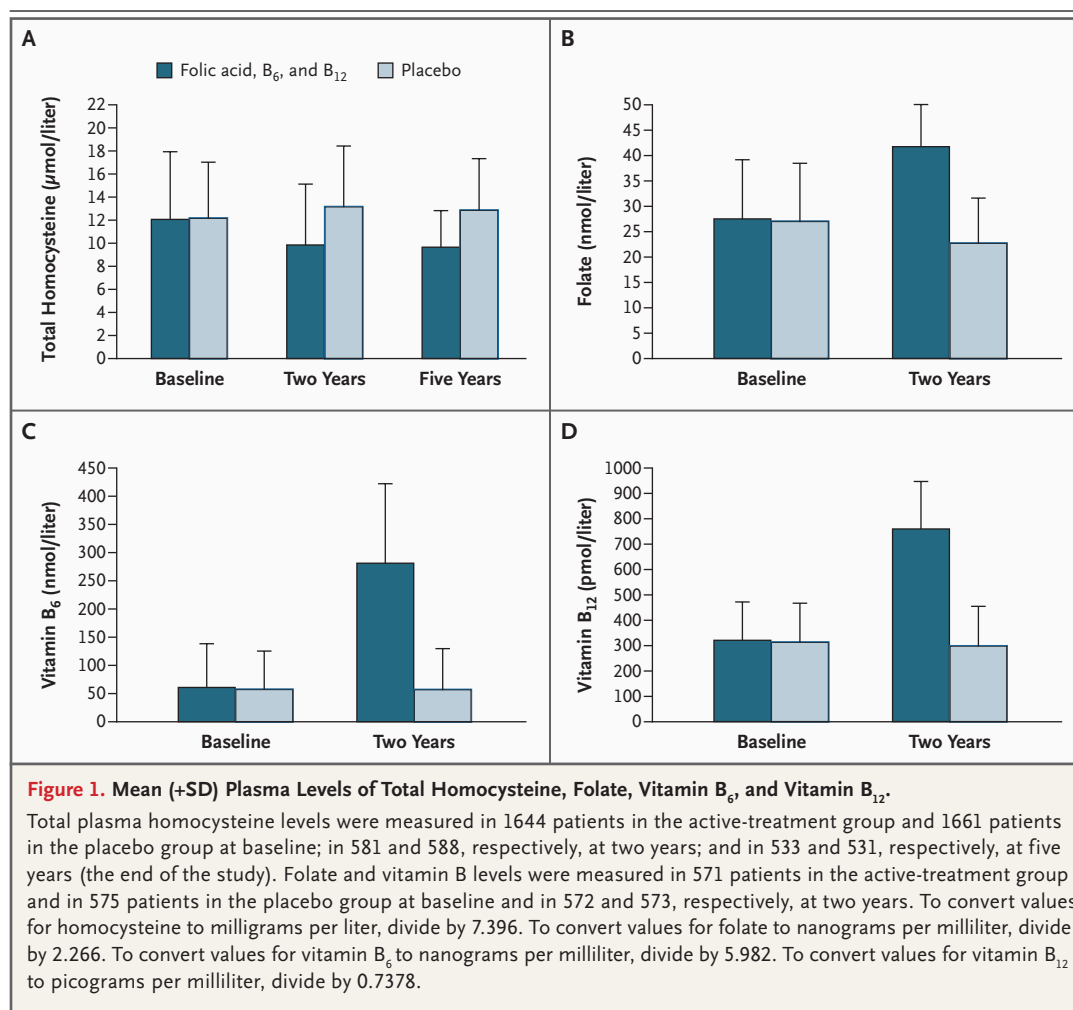
§ These variables were measured in 3311 patients: 1662 in the active-treatment group and 1649 in the placebo group.

measured are shown in Figure 1. At randomization, there were no significant differences between the two groups in plasma levels of folate (27.6 nmol per liter [12.2 ng per milliliter] in the active-treatment group and 27.1 nmol per liter [12.0 ng per milliliter] in the placebo group), vitamin B<sub>6</sub> (pyridoxal) (61.9 nmol per liter [10.3 ng per milliliter] and 58.1 nmol per liter [9.7 ng per milliliter], respectively), or vitamin B<sub>12</sub> (322.2 pmol per liter [436.6 pg per milliliter] and 314.5 pmol per liter [426.1 pg per milliliter], respectively). Mean total plasma homocysteine levels were also similar in both groups (12.2 μmol per liter [1.6 mg per liter] in both). As expected, there were regional differences, with lower folate and higher homocysteine levels in patients from regions that did not require folate fortification of food than in patients from regions that required folate fortification.

Values obtained two years after randomization showed that plasma folate and vitamin B<sub>12</sub> levels had approximately doubled and vitamin B<sub>6</sub> levels had approximately quadrupled in the active-treatment group, with no significant changes in the

placebo group (Fig. 1). In the active-treatment group, the mean homocysteine level had decreased to 9.9 μmol per liter (1.3 mg per liter) at two years (a decrease of 2.2 μmol per liter [0.3 mg per liter] from baseline) and to 9.7 μmol per liter (1.3 mg per liter) at the end of the study (a decrease of 2.4 μmol per liter [0.3 mg per liter] from baseline). In the placebo group, the mean homocysteine level had increased to 13.2 μmol per liter (1.8 mg per liter) at two years (an increase of 1.1 μmol per liter [0.1 mg per liter] from baseline) and to 12.9 μmol per liter (1.7 mg per liter) at the end of the study (an increase of 0.8 μmol per liter [0.1 mg per liter] from baseline) (Fig. 1). As a result, there was a difference of 3.3 μmol per liter (0.4 mg per liter) in the change from baseline in homocysteine levels between the treatment groups at two years and a difference of 3.2 μmol per liter (0.4 mg per liter) at the end of the study. These differences were greater in the regions that did not require folate fortification (3.7 μmol per liter [0.5 mg per liter] at two years and 4.1 μmol per liter [0.6 mg per liter] at the end of the study) than in regions that required folate fortification





(3.2  $\mu\text{mol}$  per liter at two years and 2.9  $\mu\text{mol}$  per liter [0.4 mg per liter] at the end of the study).

#### PRIMARY OUTCOMES AND DEATHS FROM ANY CAUSE

In the active-treatment group, 519 patients (18.8 percent) died of cardiovascular causes or had a myocardial infarction or stroke, as compared with 547 patients (19.8 percent) in the placebo group (relative risk, 0.95; 95 percent confidence interval, 0.84 to 1.07;  $P=0.41$ ) (Fig. 2 and Table 2). When each of the components of the primary composite outcome was analyzed separately, there were no significant differences between the groups in the rates of death from cardiovascular causes or myocardial infarction (Table 2 and the Supplementary Appendix). Fewer patients in the active-treatment group than in the placebo group had a stroke (111 [4.0 percent] vs. 147 [5.3 percent]; relative risk, 0.75; 95 percent confidence interval, 0.59 to

0.97;  $P=0.03$ ). The risk of death from any cause was similar in the active-treatment group and the placebo group (relative risk, 0.99 with active treatment; 95 percent confidence interval, 0.88 to 1.13;  $P=0.94$ ).

#### SECONDARY AND OTHER OUTCOMES

Among the prespecified cardiovascular secondary outcomes, total ischemic events occurred in 900 (32.6 percent) patients in the active-treatment group and in 890 patients (32.2 percent) in the placebo group (relative risk, 1.03; 95 percent confidence interval, 0.94 to 1.13;  $P=0.57$ ) (Table 2). A total of 268 patients (9.7 percent) in the active-treatment group were hospitalized for unstable angina, as compared with 219 (7.9 percent) in the placebo group (relative risk, 1.24; 95 percent confidence interval, 1.04 to 1.49;  $P=0.02$ ). There were no significant differences between the treatment

groups in hospitalization for heart failure and revascularization.

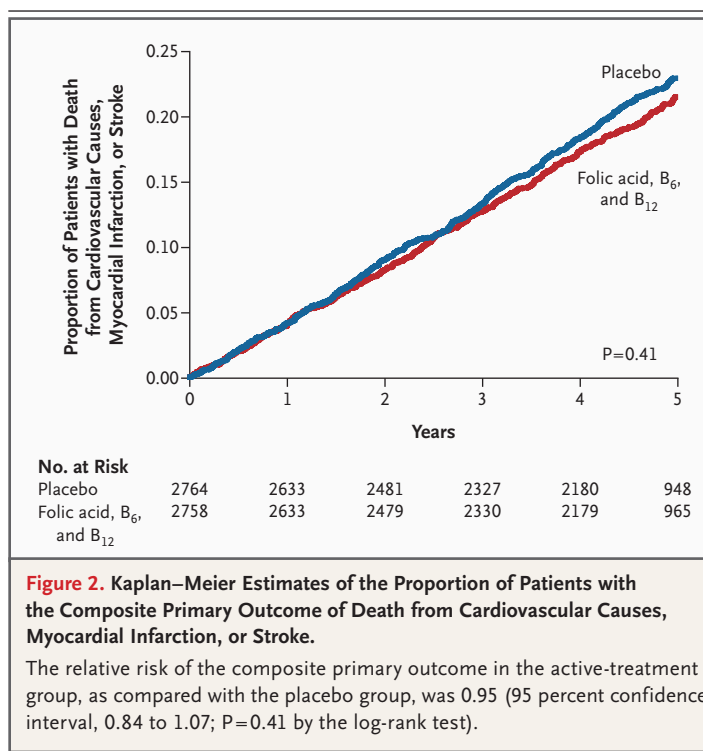
There were no significant differences in incident cancers and deaths from cancer. There were also no significant differences in the rates of transient ischemic attack, venous thromboembolism, or fracture.

#### SUBGROUP ANALYSIS

There were no significant treatment benefits with respect to the primary outcome in any of the pre-specified or exploratory subgroups evaluated (Fig. 3). Of particular interest was the treatment effect among patients with high baseline levels of homocysteine. In the top third of the baseline homocysteine distribution (homocysteine  $\geq 12.7 \mu\text{mol}$  per liter [1.7 mg per liter]), 23.9 percent of the patients in the active-treatment group and 24.0 percent of the patients in the placebo group had a primary-outcome event. Primary event rates also did not differ significantly between the treatment groups among patients in the upper fifth of the baseline homocysteine distribution ( $\geq 19.7 \mu\text{mol}$  per liter [2.7 mg per liter]).

We further explored the effect of treatment on stroke. Most strokes (185, or 71.7 percent) were ischemic, 19 (7.4 percent) were hemorrhagic, 48 (18.6 percent) were classified as of uncertain type, and 6 (2.3 percent) were classified as occurring after surgery or an invasive cardiovascular intervention. Ischemic stroke occurred in 81 patients (2.9 percent) in the active-treatment group and 104 (3.8 percent) in the placebo group (relative risk, 0.78; 95 percent confidence interval, 0.58 to 1.04;  $P=0.10$ ). There were no significant differences in the rates of hemorrhagic stroke. Fewer patients in the active-treatment group than in the placebo group had a nonfatal stroke (84 vs. 117; relative risk, 0.72; 95 percent confidence interval, 0.54 to 0.95;  $P=0.02$ ). The incidence of fatal stroke was low and not significantly different between the treatment groups. The apparent effect of treatment on stroke did not differ significantly between regions with mandatory folate fortification of food and regions without mandatory folate fortification and between patients with higher as compared with lower baseline total homocysteine levels (upper vs. middle or lower third of the baseline homocysteine distribution).

The baseline homocysteine level (as a continuous measure) was a predictor of cardiovascular events in analyses adjusted for age, sex, and treat-



**Figure 2.** Kaplan–Meier Estimates of the Proportion of Patients with the Composite Primary Outcome of Death from Cardiovascular Causes, Myocardial Infarction, or Stroke.

The relative risk of the composite primary outcome in the active-treatment group, as compared with the placebo group, was 0.95 (95 percent confidence interval, 0.84 to 1.07;  $P=0.41$  by the log-rank test).

ment assignment. Hazard ratios for these analyses were 1.03 for the primary outcome (95 percent confidence interval, 1.02 to 1.04), 1.04 for death from cardiovascular causes (95 percent confidence interval, 1.02 to 1.05), 1.02 for myocardial infarction (95 percent confidence interval, 1.01 to 1.04), and 1.03 for stroke (95 percent confidence interval, 1.02 to 1.05).

## DISCUSSION

In our study, daily administration of the combination of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> lowered homocysteine levels significantly but did not reduce the incidence of the primary outcome — the composite of death from cardiovascular causes, myocardial infarction, and stroke — during a mean follow-up period of five years. In subgroup analysis, there was no heterogeneity of treatment effects among patients from regions with mandatory fortification of food with folate and regions without mandatory folate fortification and among patients with higher as compared with lower baseline homocysteine levels.

Our findings are consistent with those of the Norwegian Vitamin (NORVIT) trial, reported elsewhere in this issue of the *Journal*.<sup>13</sup> The NORVIT

<b>Table 2. Outcomes.</b>				
<b>Outcome</b>	<b>Active Group (N=2758)</b>	<b>Placebo Group (N=2764)</b>	<b>Relative Risk (95% CI)*</b>	<b>P Value†</b>
	<i>no. of patients (%)</i>			
<b>Primary outcome and its components</b>				
Composite of death from cardiovascular causes, myocardial infarction, or stroke	519 (18.8)	547 (19.8)	0.95 (0.84–1.07)	0.41
Death from cardiovascular causes‡	276 (10.0)	291 (10.5)	0.96 (0.81–1.13)	0.59
Myocardial infarction‡	341 (12.4)	349 (12.6)	0.98 (0.85–1.14)	0.82
Stroke‡	111 (4.0)	147 (5.3)	0.75 (0.59–0.97)	0.03
<b>Secondary outcomes</b>				
Total ischemic events§	900 (32.6)	890 (32.2)	1.03 (0.94–1.13)	0.57
Death from any cause	470 (17.0)	475 (17.2)	0.99 (0.88–1.13)	0.94
Hospitalization for unstable angina	268 (9.7)	219 (7.9)	1.24 (1.04–1.49)	0.02
Hospitalization for heart failure	202 (7.3)	174 (6.3)	1.18 (0.96–1.44)	0.12
Revascularization	458 (16.6)	422 (15.3)	1.10 (0.96–1.26)	0.16
Incident cancer	358 (13.0)	340 (12.3)	1.06 (0.91–1.23)	0.47
Site-specific cancers				
Colon	50 (1.8)	37 (1.3)	1.36 (0.89–2.08)	0.16
Lung	52 (1.9)	45 (1.6)	1.16 (0.78–1.73)	0.47
Breast	11 (0.4)	10 (0.4)	1.11 (0.47–2.61)	0.81
Prostate	70 (2.5)	58 (2.1)	1.21 (0.86–1.72)	0.28
Melanoma	5 (0.2)	12 (0.4)	0.42 (0.15–1.19)	0.10
Death due to cancer	94 (3.4)	95 (3.4)	0.99 (0.74–1.33)	0.94
Other outcomes				
Transient ischemic attack	131 (4.7)	120 (4.3)	1.11 (0.87–1.42)	0.42
Venous thromboembolism (pulmonary embolism and deep-vein thrombosis)	37 (1.3)	40 (1.4)	0.96 (0.61–1.50)	0.86
Fractures	246 (8.9)	235 (8.5)	1.06 (0.88–1.26)	0.55

\* CI denotes confidence interval.

† P values were calculated with the use of the log-rank test.

‡ All patients with this outcome are included.

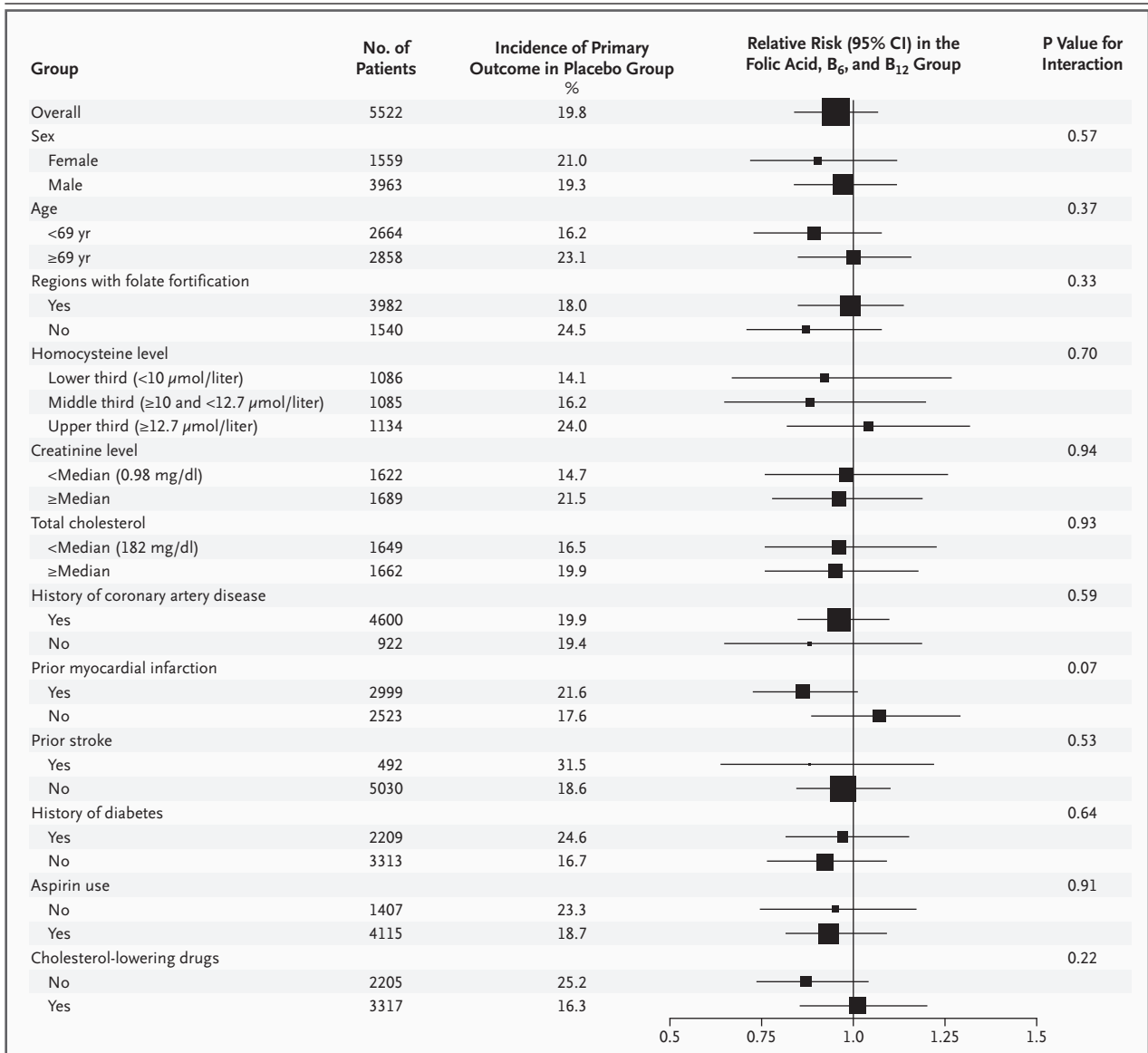
§ This outcome is a composite of death from cardiovascular causes, myocardial infarction, stroke, hospitalization for unstable angina, or revascularization.

trial evaluated 3749 patients with recent myocardial infarction from Norway, a country without folate fortification of food, and found no significant beneficial effect of combined treatment with folic acid and vitamin B<sub>12</sub>, with or without vitamin B<sub>6</sub>, in spite of adequate homocysteine lowering. Similarly, there was no treatment benefit in the Vitamin Intervention for Stroke Prevention (VISP) study<sup>14</sup> and in a smaller trial conducted in 593 patients with stable coronary heart disease in the Netherlands.<sup>15</sup>

On the basis of epidemiologic studies conducted before our study was initiated, many of which were retrospective, our trial was adequately pow-

ered to allow the detection of a proportional reduction in the risk of the primary outcome of 17 to 20 percent. More recent prospective observational studies and a meta-analysis of these studies found the magnitude of the association between homocysteine and cardiovascular risk to be lower. After adjustment for known cardiovascular risk factors and regression dilution bias, a 25 percent decrease in the homocysteine level (about 3  $\mu$ mol per liter [0.4 mg per liter]) was associated with an 11 percent decrease in the risk of coronary heart disease and a 19 percent decrease in the risk of stroke.<sup>8</sup> Our findings cannot definitively exclude the possibility that B vitamin supplements





**Figure 3. Effect of Folic Acid and Vitamins B<sub>6</sub> and B<sub>12</sub> on the Primary Outcome in Prespecified and Exploratory Subgroups.**

There was no beneficial treatment effect in any of the subgroups evaluated. The size of each symbol is proportional to the number of patients in each subgroup. To convert values for homocysteine to milligrams per liter, divide by 7.396. To convert the value for creatinine to micromoles per liter, multiply by 88.4. To convert the value for cholesterol to millimoles per liter, multiply by 0.02586.

have a very small beneficial effect on coronary heart disease, of a magnitude similar to these more recent estimates of the strength of the epidemiologic association (for example, a reduction in risk of 10 percent or less). However, this appears unlikely, considering the consistency of our findings across various coronary heart disease outcomes and subgroups, the lack of effect of treatment on total ischemic events — for which the trial was well powered to detect even a 13 percent reduc-

tion in risk — and the concordant findings of the NORVIT and VISP trials. The apparent increase in the rate of unstable angina in the active-treatment group is inconsistent with the neutral findings for all other coronary heart disease outcomes evaluated and may be related to the difficulty in establishing this diagnosis and to the play of chance.

With regard to the risk of stroke, we observed an absolute reduction of 1.3 percentage points

and a relative reduction of 24 percent among patients assigned to the active-treatment group. However, these results must be interpreted with caution. The number of strokes in our study was much lower than the number of coronary events, the confidence intervals around the estimated risk reduction are wide, and the results are not adjusted for the multiplicity of outcomes compared. Also, we found no effect of treatment on transient ischemic attacks. From a biologic perspective, a treatment benefit restricted to stroke would be difficult to explain. Furthermore, the two other large trials of homocysteine-lowering vitamins that have been completed did not show a beneficial effect of treatment on stroke.<sup>13,14</sup> Therefore, we believe that the apparent beneficial effect of B vitamin supplements on stroke in our trial may represent either an overestimate of the real effect or a spurious result due to the play of chance. Ongoing trials and a meta-analysis of all homocysteine-lowering trials<sup>16</sup> should be able to clarify this issue.

The discordance between the epidemiology of homocysteine and the results of the clinical trials completed to date is similar to that noted for antioxidant vitamins<sup>17</sup> and estrogen<sup>18</sup> and may be related to inherent limitations of observational studies. Indeed, homocysteine levels are related

to renal dysfunction, smoking, elevated blood pressure, and other cardiovascular risk factors and are higher in people with atherosclerosis than in those without.<sup>4</sup> Therefore, homocysteine could be a marker, but not a cause, of vascular disease, and the epidemiologic data could be the result of residual confounding that cannot be fully adjusted for, of reverse causality, or of both. Our findings may also relate to exposure to folate-fortified food in over 70 percent of the study patients. This exposure probably reduced the number of patients with substantially increased homocysteine levels, the subgroup that might be most likely to benefit from B vitamin supplementation. Several large trials are further exploring these questions.<sup>16</sup>

In conclusion, combined daily administration of 2.5 mg of folic acid, 50 mg of vitamin B<sub>6</sub>, and 1 mg of vitamin B<sub>12</sub> for five years had no beneficial effects on major vascular events in a high-risk population with vascular disease. Our results do not support the use of folic acid and B vitamin supplements as a preventive treatment.

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#### APPENDIX

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