Mortality and Cardiovascular Events in Patients Treated With Homocysteine-Lowering B Vitamins After Coronary Angiography: A Randomized Controlled Trial

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Mortality and Cardiovascular Events in Patients Treated With Homocysteine-Lowering B Vitamins After Coronary Angiography. A Randomized Controlled Trial

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Observational studies have demonstrated that the concentration of total homocysteine in blood is associated with risk of coronary artery disease (CAD) and stroke.1,2 In prospective cohort studies from western Norway, plasma total homocysteine concentration was found to be a strong predictor of mortality both in patients with CAD3 and those with aortic valve stenosis.4 Because plasma total homocysteine levels can be easily lowered by oral administration of folic acid, trials to investigate whether cardiovascular disease (CVD) could be prevented by such homocysteine-lowering therapy were called for. Furthermore, observational studies have found inverse associations between vitamin B₆ intake and risk of future CAD5 and between circulating vitamin B₆ concentration and risk of CVD, independent of plasma total homocysteine concentration.6

Since the late 1990s, a series of secondary prevention trials with folic acid, either alone or in combination with vitamin B₁₂ and/or vitamin B₆, were initiated in cardiovascular and chronic kidney disease patients.7 So far, none of the larger published trials has reported any effect of homocysteine lowering on total mortality or cardiovascular events. Our findings do not support the use of B vitamins as secondary prevention in patients with coronary artery disease.

Trial Registration clinicaltrials.gov Identifier: NCT00354081

Context Observational studies have reported associations between circulating total homocysteine concentration and risk of cardiovascular disease. Oral administration of folic acid and vitamin B₁₂ can lower plasma total homocysteine levels.

Objective To assess the effect of treatment with folic acid and vitamin B₁₂ and the effect of treatment with vitamin B₆ as secondary prevention in patients with coronary artery disease or aortic valve stenosis.

Design, Setting, and Participants Randomized, double-blind controlled trial conducted in the 2 university hospitals in western Norway in 1999-2006. A total of 3096 adult participants undergoing coronary angiography (20.5% female; mean age, 61.7 years) were randomized. At baseline, 59.3% had double- or triple-vessel disease, 83.7% had stable angina pectoris, and 14.9% had acute coronary syndromes.

Interventions Using a 2 X 2 factorial design, participants were randomly assigned to 1 of 4 groups receiving daily oral treatment with folic acid, 0.8 mg, plus vitamin B₁₂, 0.4 mg, plus vitamin B₆, 40 mg (n=772); folic acid plus vitamin B₁₂ (n=772); vitamin B₆ alone (n=772); or placebo (n=780).

Main Outcome Measures The primary end point was a composite of all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke.

Results Mean plasma total homocysteine concentration was reduced by 30% after 1 year of treatment in the groups receiving folic acid and vitamin B₁₂. The trial was terminated early because of concern among participants due to preliminary results from a contemporaneous Norwegian trial suggesting adverse effects from the intervention. During a median 38 months of follow-up, the primary end point was experienced by a total of 422 participants (13.7%): 219 participants (14.2%) receiving folic acid/vitamin B₁₂ vs 203 (13.1%) not receiving such treatment (hazard ratio, 1.09; 95% confidence interval, 0.90-1.32; P=.36) and 200 participants (13.0%) receiving vitamin B₆ vs 222 (14.3%) not receiving vitamin B₆ (hazard ratio, 0.90; 95% confidence interval, 0.74-1.09; P=.28).

Conclusions This trial did not find an effect of treatment with folic acid/vitamin B₁₂ or vitamin B₆ on total mortality or cardiovascular events. Our findings do not support the use of B vitamins as secondary prevention in patients with coronary artery disease.

Trial Registration clinicaltrials.gov Identifier: NCT00354081

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er on risk of cardiovascular disease or total mortality. One smaller trial in patients undergoing percutaneous coronary intervention (PCI) found a reduced rate of target lesion revascularization in patients receiving a combination of folic acid, vitamin B12, and vitamin B6, while another similar trial found an increased risk of in-stent restenosis and need for target vessel revascularization following B vitamin treatment.

In 1998, mandatory folic acid fortification of foods was implemented in the United States and Canada with a primary objective of preventing neural tube defects. Circulating folate concentrations substantially increased and plasma total homocysteine concentrations decreased in the North American population following the fortification. This change has been linked to an accelerated decline in stroke mortality observed in the United States and Canada from 1998 to 2002 compared with stroke mortality in England and Wales, where folic acid fortification is not mandatory.

The Western Norway B Vitamin Intervention Trial (WENBIT) is a prospective, randomized, double-blind, placebo-controlled secondary prevention study of the clinical effects of B vitamin treatment in patients having undergone coronary angiography for suspected CAD or aortic valve stenosis. Our main purpose was to evaluate the effects of homocysteine-lowering treatment with folic acid plus vitamin B12 on mortality and cardiovascular events. The 2 × 2 factorial study design also allowed separate assessment of effects of vitamin B6 treatment.

METHODS

Participants

Participants eligible for randomization were men and women aged 18 years or older undergoing coronary angiography for suspected CAD and/or aortic valve stenosis at 2 university hospitals in western Norway. Exclusion criteria were unwillingness to attend study visits, participation in other trials, known alcohol abuse, serious mental illness, or cancer. During the main recruitment period at Haukeland University Hospital, Bergen, Norway (January 2000–April 2004), and the recruitment period at Stavanger University Hospital, Stavanger, Norway (September 2000–April 2004), a total of 5630 patients underwent coronary angiography for stable angina pectoris, 4216 for acute coronary syndromes, and 395 for aortic valve stenosis. For capacity reasons, not all of these possibly eligible patients were consecutively screened.

Patients were informed about the trial at the routine clinical interview and examination before baseline coronary angiography. The first 90 participants in the trial, recruited to the WENBIT-90 substudy, were randomized before undergoing angiography to ensure no effects on blood indexes from the invasive procedure. Subsequent participants were randomized after baseline angiography. Written informed consent was obtained on the day of randomization. The study protocol was in accordance with the principles of the Declaration of Helsinki and the trial was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate.

Study Intervention

Participants were randomly assigned, using a 2 × 2 factorial design, to 1 of 4 groups receiving a daily oral dose of 1 of the following treatments: (1) folic acid, 0.8 mg, plus vitamin B12 (cyanocobalamin), 0.4 mg, and vitamin B6 (pyridoxine), 40 mg; (2) folic acid, 0.8 mg, plus vitamin B12, 0.4 mg; (3) vitamin B6, 40 mg; or (4) placebo. Treatment with folic acid was expected to lower plasma total homocysteine concentration by 23% to 28%. Vitamin B12 was added to folic acid mainly to prevent possible masking of vitamin B12 deficiency by folic acid but also to further lower plasma total homocysteine concentration by 3% to 10%. The study medication (Alpharma Inc, Copenhagen, Denmark) was given in a single capsule. For the first 2 weeks after randomization, the groups allocated to receive folic acid were provided with an extra capsule with a loading dose of 5 mg of folic acid per day, while the other groups were provided with an extra capsule of placebo. The randomization sequence was generated in blocks of 20 by Alpharma Inc, and study nurses assigned boxes of study capsules to participants in numerical order. The different capsules were indistinguishable by color, weight, or ability to dissolve in water. Participants, study and laboratory personnel, and the steering and end-points committees were unaware of the treatment allocation, and the randomization code was kept at Alpharma Inc until data entry was completed.

Participants were requested to abstain from taking supplements containing B vitamins. They were given conventional medical treatment and underwent myocardial revascularization procedures and/or valve surgery at the discretion of the treating physician.

Baseline and Follow-up Evaluations

Demographic, clinical, and routine laboratory data were obtained by study personnel. Participants were scheduled for follow-up visits with an interview, a clinical examination, and blood sampling at 1 month, at 1 year, and at a final study visit. Planned median follow-up time was 4 years. If unable or unwilling to attend study visits, participants were interviewed by telephone or by letter.

Adherence was judged by capsule counts and interviews. Participants were asked about hospital admissions, and copies of hospital records were retrieved by mail. In addition, archives of the hospitals in western Norway were searched for information on all participants' hospital admissions, and copies of records on possible events were collected. Data on deaths were obtained from the Cause of Death Registry and on incident cancer from the Cancer Registry in Norway, using the Norwe-
gian unique 11-digit person number for each participant.

**Trial Outcomes**

The primary clinical end point was a composite of all-cause death, nonfatal acute myocardial infarction (AMI), acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke (infarction). Secondary end points were fatal and nonfatal AMI, acute hospitalization for angina pectoris, stable angina pectoris with angiographically verified progression, myocardial revascularization procedures, and fatal and nonfatal stroke. Incident cases of newly diagnosed cancer, except basal cell carcinoma, were recorded as a measure of safety.

If death occurred within 28 days after the onset of an event, the event was classified as fatal. Acute MIs were classified according to definitions published in *Reprinted* JAMA, classified according to the diagnostic was classified as fatal. Acute MIs were recorded as a measure of safety.

**Laboratory Analysis**

Blood samples obtained at baseline and during follow-up were collected and processed by study personnel. Routine blood analyses were performed by the hospital laboratories. Gommerulofiltration rates were estimated by the 4-variable Modification of Diet in Renal Disease equations. Low-density lipoprotein cholesterol was calculated using the Friedewald formula. Blood samples for assessment of B vitamins and total homocysteine were stored at −80°C until analyzed in the laboratory.

**Statistical Analysis**

The 2 × 2 factorial design allowed separate assessments of effects from the folate acid plus vitamin B12 and the vitamin B6 interventions. Because we anticipated no effect modification by vitamin B6 on the effect of folic acid plus vitamin B12, we ignored the secondary factor (vitamin B6) in the sample size calculations. Using data from a cohort of patients with CAD in western Norway in 1991-1992 and accounting for the effects of more widespread use of lipid-lowering treatment since the mid-1990s, we estimated a 4-year mortality rate of 7.5% and a total 4-year event rate (fatal and non-fatal events) of 22% in the placebo group. We expected that a 30% reduction in plasma total homocysteine concentration in the groups allocated to folic acid plus vitamin B12 would give an event rate reduction of 20% in these groups compared with the groups allocated to no treatment with folic acid/vitamin B12. Furthermore, we assumed a combined withdrawal and nonadherence rate of 20%.

Thus, we calculated that a sample size of 3088 participants would be needed to detect a 20% reduction in the primary end point during 4 years of follow-up with a statistical power of 80% at a 2-sided significance level of .05.

Differences between intervention groups were tested using the 2 test.
for categorical variables and the t test or analysis of variance for continuous variables. The prespecified analyses were comparison of treatment effect of folic acid plus vitamin B12 (groups 1 and 2) with control (groups 3 and 4) and comparison of treatment effect of vitamin B6 (groups 1 and 3) with control (groups 2 and 4), according to the 2 × 2 factorial study design. We also performed a post hoc overall comparison of treatment effect of the 3 different vitamin interventions with that of placebo. For each participant, only the first of events in the composite primary or secondary end points was included in the survival analyses. Survival curves were constructed using the Kaplan-Meier method, and the differences in survival between groups were analyzed by the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazard regression with separate assessment for the folic acid plus vitamin B12 groups vs control and the vitamin B6 groups vs control. All analyses were performed according to the intention-to-treat principle. We also conducted a per-protocol analysis for the primary end point. Reported P values are 2-sided and unadjusted for multiple comparisons, and P < .05 was regarded as statistically significant. We used the statistical software packages SPSS for Windows, version 15.0 (SPSS Inc, Chicago, Illinois), and S-PLUS, version 7.0 (Insightful Corp, Seattle, Washington).

RESULTS

From April 1999 to April 2004, a total of 3096 patients were randomized (FIGURE 1). In 1999, recruitment was limited to 90 patients for the WENBIT-90 substudy. Six participants withdrew consent immediately after randomization without having started study medication and were excluded from further analyses. Thus, the study population consisted of 3090 patients, of which 2121 (68.6%) were randomized at Haukeland University Hospital and 969 (31.4%) at Stavanger University Hospital. Reasons for referral to baseline angiography were stable angina pectoris (n = 2585 [83.7%]), acute coronary syndromes (n = 461 [14.9%]), and aortic valve stenosis (n = 44 [1.4%]).

Media reports on preliminary results from the Norwegian Vitamin Trial (NORVIT), presented in September 2005, stating no beneficial effects and suggesting increased risk of cancer by B vitamin treatment, made numerous WENBIT participants worried. Although the interim analysis on total mortality did not give any reason for concern, the steering committee was advised by the safety committee to stop the intervention because adherence could be severely compromised. The remaining participants by October 2005 (n = 692) were therefore asked by letter to discontinue study medication and were subsequently called for their final study visit, the last of which was carried out in April 2006.

Table 1. Baseline Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Folic Acid + Vitamin B12 (n = 771)</th>
<th>Folic Acid + Vitamin B12 (n = 769)</th>
<th>Vitamin B6 (n = 771)</th>
<th>Placebo (n = 779)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>61.7 (10.3)</td>
<td>61.3 (10.0)</td>
<td>61.4 (9.7)</td>
<td>62.0 (9.9)</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>626 (81.2)</td>
<td>618 (80.4)</td>
<td>618 (80.2)</td>
<td>596 (76.5)</td>
</tr>
<tr>
<td>Body mass index, mean (SD)a</td>
<td>26.8 (3.7)</td>
<td>26.9 (3.9)</td>
<td>26.7 (3.5)</td>
<td>27.2 (3.9)</td>
</tr>
<tr>
<td>Blood pressure, mean (SD), mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>140 (20)</td>
<td>141 (20)</td>
<td>141 (21)</td>
<td>142 (21)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81 (11)</td>
<td>81 (11)</td>
<td>81 (11)</td>
<td>81 (11)</td>
</tr>
<tr>
<td>LVEF &lt;50%, No. (%)</td>
<td>82 (10.6)</td>
<td>83 (10.9)</td>
<td>85 (11.0)</td>
<td>86 (11.0)</td>
</tr>
<tr>
<td>Hemoglobin, mean (SD), g/dL</td>
<td>14.4 (1.2)</td>
<td>14.4 (1.2)</td>
<td>14.4 (1.2)</td>
<td>14.4 (1.2)</td>
</tr>
<tr>
<td>Creatinine, mean (SD), µmol/L</td>
<td>92 (19)</td>
<td>91 (14)</td>
<td>91 (17)</td>
<td>92 (19)</td>
</tr>
<tr>
<td>eGFR &lt;60 mL/min/1.73 m², No. (%)</td>
<td>94 (12.2)</td>
<td>84 (10.9)</td>
<td>85 (11.0)</td>
<td>97 (12.5)</td>
</tr>
<tr>
<td>Total cholesterol, mean (SD), mg/dL</td>
<td>195.7 (43.9)</td>
<td>196.8 (44.6)</td>
<td>196.0 (44.1)</td>
<td>195.5 (44.7)</td>
</tr>
<tr>
<td>HDL-C, mean (SD), mg/dL</td>
<td>48.2 (14.0)</td>
<td>47.6 (13.3)</td>
<td>48.4 (13.4)</td>
<td>48.1 (13.6)</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mg/dL</td>
<td>119.5 (38.6)</td>
<td>120.2 (40.6)</td>
<td>118.6 (40.0)</td>
<td>119.8 (40.9)</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/L</td>
<td>1.8 (3.1)</td>
<td>2.1 (3.6)</td>
<td>2.0 (3.2)</td>
<td>2.0 (3.5)</td>
</tr>
</tbody>
</table>

Cardiovascular history and risk factors, No. (%)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Folic Acid + Vitamin B12 (n = 771)</th>
<th>Folic Acid + Vitamin B12 (n = 769)</th>
<th>Vitamin B6 (n = 771)</th>
<th>Placebo (n = 779)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>292 (37.9)</td>
<td>323 (42.0)</td>
<td>331 (42.7)</td>
<td>344 (42.9)</td>
</tr>
<tr>
<td>Percutaneous coronary intervention</td>
<td>152 (19.7)</td>
<td>156 (20.3)</td>
<td>162 (21.0)</td>
<td>167 (21.4)</td>
</tr>
<tr>
<td>Coronary artery bypass surgery</td>
<td>113 (14.7)</td>
<td>87 (11.3)</td>
<td>109 (14.1)</td>
<td>106 (13.6)</td>
</tr>
<tr>
<td>Coronary artery stenosis, TIA, or stroke</td>
<td>52 (6.7)</td>
<td>47 (6.4)</td>
<td>50 (6.5)</td>
<td>41 (5.3)</td>
</tr>
<tr>
<td>Other peripheral artery disease</td>
<td>65 (8.4)</td>
<td>56 (7.3)</td>
<td>69 (8.9)</td>
<td>85 (10.9)</td>
</tr>
<tr>
<td>Family history of premature CAD</td>
<td>256 (33.2)</td>
<td>266 (34.6)</td>
<td>261 (33.9)</td>
<td>244 (31.3)</td>
</tr>
<tr>
<td>History of hypercholesterolemia</td>
<td>449 (58.2)</td>
<td>453 (58.9)</td>
<td>470 (61.0)</td>
<td>465 (59.7)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>84 (10.9)</td>
<td>92 (12.0)</td>
<td>77 (10.0)</td>
<td>103 (13.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>346 (44.9)</td>
<td>356 (46.3)</td>
<td>347 (45.0)</td>
<td>369 (47.4)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>219 (28.4)</td>
<td>203 (26.4)</td>
<td>238 (30.9)</td>
<td>215 (27.6)</td>
</tr>
<tr>
<td>CAD at baseline angiography, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No or nonsignificant coronary stenosisc</td>
<td>84 (10.9)</td>
<td>81 (10.5)</td>
<td>86 (11.2)</td>
<td>88 (11.3)</td>
</tr>
<tr>
<td>1-Vessel disease</td>
<td>224 (29.1)</td>
<td>228 (29.6)</td>
<td>241 (31.3)</td>
<td>227 (29.1)</td>
</tr>
<tr>
<td>2-Vessel disease</td>
<td>208 (27.0)</td>
<td>205 (26.7)</td>
<td>198 (25.7)</td>
<td>215 (27.6)</td>
</tr>
<tr>
<td>3-Vessel disease</td>
<td>255 (33.1)</td>
<td>255 (33.2)</td>
<td>246 (31.9)</td>
<td>249 (32.0)</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; TIA, transient ischemic attack.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259.

Body mass index was calculated as weight in kilograms divided by height in meters squared.b Untreated total cholesterol values of at least 251.0 mg/dL (6.5 mmol/L).c Normal vessels or plaque(s) with less than 50% luminal narrowing. A total of 61 participants (2.0%) had normal vessels, and 26 of these had aortic valve stenosis.
Median follow-up was 38.4 (5th-95th percentile, 19.3-55.0) months. A total of 131 participants (4.2%) died, and 2557 participants (86.4% of participants alive) attended the final study visit. Of the participants who had premature termination of study intervention, 650 (94%) attended the final study visit. Three patients had incomplete follow-up because of emigration and were censored by the time of last contact. Data entry was completed by June 2007.

### Baseline Characteristics

The randomization procedure resulted in well-balanced intervention groups with no differences in baseline demographics or clinical characteristics. The study population included 20.5% women, 9.6% of participants were older than 75 years, and 59.3% had double- or triple-vessel disease (Table 1). Regular use of over-the-counter supplements containing B vitamins was reported by 329 participants (10.8%) prior to randomization. Following baseline angiography, 92% of participants used antiplatelet drugs, 88.4% used statins, and 78.2% used β-blockers. A total of 2072 patients (67.1%) underwent PCI and/or PCI with use of stent(s), 25% (8.8%) had mild hyperhomocysteinemia (total homocysteine 15-30 µmol/L), 25% (8.8%) had mild hyperhomocysteinemia (total homocysteine 15-30 µmol/L) as currently defined.

### Adherence and Adverse Events

A total of 102 participants (3.3%) never started study medication, and 456 (14.8%) stopped taking it during follow-up; among these, 46 (1.5% of all) reported adverse effects as the reason for stopping. There was no difference in adverse effect rates among the four intervention groups and no report of serious adverse events related to study medication. A total of 2532 (81.9% of all) took 70% to 100% of their study medication throughout follow-up. The proportion of patients adhering to study medication was somewhat higher in the folic acid plus vitamin B12 groups than in the non–folic acid groups (83.9% vs 80.0%; P = .005).

### Effect of Intervention on Circulating B Vitamins and Homocysteine Levels

Figure 2 shows circulating B vitamin and total homocysteine levels in the four treatment groups at baseline, at follow-up visits, and at the final study visit. At baseline, plasma total homocysteine levels were similar in the four groups (P = .72). None of the participants had severe hyperhomocysteinemia (total homocysteine >100 µmol/L), 25 (0.8%) had intermediate (total homocysteine 30-100 µmol/L), and 271 (8.8%) had mild hyperhomocysteinemia (total homocysteine 15-30 µmol/L) as currently defined.

After 1 year of intervention, mean serum folate concentration increased 7-fold and mean serum cobalamin concentration increased by 65% in the groups receiving folic acid plus vitamin B12. At the same time, mean plasma pyridoxal phosphate concentration increased 9-fold in the groups receiving vitamin B6.

Mean plasma total homocysteine level was decreased by 30%, from 10.8 (SD, 4.5) µmol/L at baseline to 7.6 (SD, 2.2) µmol/L after 1 year of intervention in the groups receiving folic acid and vitamin B12 (P < .001). Plasma total homocysteine concentration remained unaltered in the groups receiving vitamin B6 alone or placebo. At the final study visit, mean plasma total homocysteine level was 2.8 µmol/L lower in the folic acid plus B12 groups than in the non–folic acid groups (a difference of 26%; P < .001).

### Clinical End Points

Figure 3 shows the Kaplan-Meier curves for the event rates of the prespecified primary composite end point comparing groups receiving folic acid or no folic acid and comparing groups receiving vitamin B6 or no vitamin B6. Table 3 shows the number and rates of events in the composite primary as well as secondary end points in the four intervention groups and the HRs for the prespecified comparisons.

During follow-up, 422 participants (13.7% of all) experienced an event in the composite primary end point of death, AMI, unstable angina pectoris, or thromboembolic stroke. A total of 219 participants (14.2%) in the groups receiving folic acid vs 203 participants (13.1%) in the groups not receiving fo-
lic acid experienced the primary end point (HR, 1.09; 95% CI, 0.90-1.32; \( P = .36 \)). In the groups receiving vitamin B\(_6\), 200 participants (13.0%) experienced the primary end point compared with 222 (14.3%) in the groups not receiving vitamin B\(_6\) (HR, 0.90; 95% CI, 0.74-1.09; \( P = .28 \)).

In the prespecified per-protocol analysis performed among patients adhering to the study medication throughout follow-up, 157 participants (12.2%) in the groups receiving folic acid groups vs 146 (11.8%) of those not receiving folic acid experienced the primary end point (HR, 1.04; 95% CI, 0.83-1.30; \( P = .75 \)). In the groups receiving vitamin B\(_6\), 139 participants (11.0%) who adhered to study medication experienced the primary end point vs 164 (12.9%) of those not receiving vitamin B\(_6\) (HR, 0.85; 95% CI, 0.68-1.06; \( P = .15 \)).

There were no differences in treatment response for the separate end points of death, total AMI (fatal and nonfatal, including procedure-related), or unstable angina pectoris (Table 3). The incidence of total stroke (fatal and nonfatal, including hemorrhagic) was lower in the groups receiving folic acid, but this observation was not statistically significant. The incidence of acute hospitalization due to angina pectoris was lower in the folic acid groups reaching borderline statistical significance (HR, 0.82; 95% CI, 0.67-1.00; \( P = .05 \)). There was no difference in the incidence of stable angina pectoris with angiographically verified progression of CAD or in the incidence of PCI or CABG. The incidence of cancer was higher, but not statistically significantly so, in the groups receiving folic acid.

**Figure 4** shows the Kaplan-Meier curves for the composite primary end point in the 4 different intervention groups. In the post hoc overall survival analysis, there were no differences between the groups (\( P = .07 \)) but there was an increased risk of the composite primary end point in the group receiving folic acid plus vitamin B\(_{12}\) (HR, 1.34; 95% CI, 1.03-1.75; \( P = .03 \)) compared with placebo.

The baseline level of total homocysteine in plasma was a significant predictor of the primary end point (HR associated with a 3-µmol/L difference in total homocysteine level, 1.07; 95% CI, 1.02-1.13; \( P = .01 \)) after adjustment for age, sex, smoking status (never, ex-, or current smoker), and serum creatinine concentration.

**COMMENT**

In this randomized, double-blind, placebo-controlled trial, we could not detect any preventive effect of intervention with folic acid plus vitamin B\(_{12}\) or with vitamin B\(_6\) on mortality or major cardiovascular events among patients with mainly stable CAD undergoing intensive conventional treatment. We found a numerically lower incidence of stroke and higher incidence of cancer in the groups receiving folic acid, but these observations were not statistically significant. Our results are in concordance with findings in other large secondary prevention trials with homocysteine-lowering B vitamins in patients surviving an AMI\(^{24}\) and in patients with established or at high risk of CVD\(^{10,25}\).

WENBIT is one of the larger randomized, placebo-controlled trials to evaluate the clinical effects of homocysteine-lowering treatment with B vitamins in patients with established CVD. Inclusion criteria were wide, and the extent of CAD was precisely ascertained by coronary angiography at baseline. Adherence to study medication...
was greater than 80% and was corroborated by assessment of B vitamin concentrations in serum and plasma. There is no mandatory folic acid fortification of foods in Norway, and the self-reported use of over-the-counter B vitamin supplements was modest in this study population. Thus, the homocysteine-lowering effect of the intervention was substantial. Follow-up for clinical end points was complete in all but 3 participants.

There are several limitations to this trial. The insufficient capacity to consecutively screen all possible eligible participants made the inclusion process nontransparent. However, we find no reason to believe that this biased the randomization. Ultimately, 3006 (29.4%) of all possible eligible participants (N=10,241) were randomized from January 2000 to April 2004, and our study population was quite similar to contemporary populations who

![Figure 3. Kaplan-Meier Curves for the Composite Primary End Point in the Folic Acid vs Non–Folic Acid Groups and the Vitamin B6 vs Non–Vitamin B6 Groups](image)

The composite primary end point consisted of all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke.

### Table 3. Primary and Secondary End-Point Events and Cox Proportional Hazards for End Points

<table>
<thead>
<tr>
<th>Events</th>
<th>No. (%) of Patients With Eventa</th>
<th>Folic Acid vs Non–Folic Acid Groups</th>
<th>Vitamin B6 vs Non–Vitamin B6 Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end pointb</td>
<td>94 (12.2) 125 (16.3) 106 (13.7) 97 (12.5)</td>
<td>1.09 (0.90-1.32) .36 0.90 (0.74-1.09) .28</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>35 (4.5) 38 (4.9) 28 (3.6) 30 (3.9)</td>
<td>1.27 (0.90-1.79) .18 0.94 (0.66-1.32) .70</td>
<td></td>
</tr>
<tr>
<td>Acute myocardial infarctionc</td>
<td>59 (7.7) 76 (9.9) 55 (7.1) 58 (7.4)</td>
<td>1.21 (0.95-1.56) .13 0.85 (0.66-1.09) .20</td>
<td></td>
</tr>
<tr>
<td>Unstable anginad</td>
<td>22 (2.9) 29 (3.8) 24 (3.1) 27 (3.5)</td>
<td>1.00 (0.68-1.48) .99 0.83 (0.56-1.22) .34</td>
<td></td>
</tr>
<tr>
<td>Strokea</td>
<td>11 (1.4) 17 (2.2) 20 (2.6) 19 (2.4)</td>
<td>0.72 (0.44-1.17) .19 0.87 (0.54-1.40) .56</td>
<td></td>
</tr>
<tr>
<td>Acute hospitalization for angina1</td>
<td>84 (10.9) 92 (12.0) 104 (13.5) 109 (14.0)</td>
<td>0.82 (0.67-1.00) .05 0.93 (0.76-1.14) .48</td>
<td></td>
</tr>
<tr>
<td>Angina with verified progression of CAD2</td>
<td>69 (8.9) 62 (8.1) 72 (9.3) 61 (7.8)</td>
<td>1.00 (0.78-1.27) .98 1.16 (0.91-1.48) .22</td>
<td></td>
</tr>
<tr>
<td>Myocardial revascularization procedures3</td>
<td>118 (15.3) 120 (15.8) 119 (15.4) 108 (13.9)</td>
<td>1.07 (0.89-1.29) .46 1.05 (0.88-1.26) .58</td>
<td></td>
</tr>
<tr>
<td>Cancer4</td>
<td>46 (6.0) 39 (5.1) 38 (4.9) 31 (4.0)</td>
<td>1.25 (0.91-1.71) .18 1.20 (0.87-1.64) .27</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; CI, confidence interval.
1The first event in the category for each participant.
2Primary end point was a composite of all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke.
3Primary end point was a composite of all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke.
4Typical ischemic symptoms accompanied by electrocardiographic ST-T findings of myocardial ischemia at rest and/or accompanied by coronary angiography during the same hospital stay verifying significant progression of CAD.
5Fatal and nonfatal stroke, including hemorrhagic stroke.
6Unstable or acute angina pectoris.
7Angiographically verified progression of coronary stenosis.
8Percutaneous coronary intervention or coronary artery bypass graft surgery.
9All cancers except basal cell carcinoma.
Figure 4. Kaplan-Meier Curves for the Composite Primary End Point in the 4 Intervention Groups

The composite primary end point consisted of all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and nonfatal thromboembolic stroke.

had undergone invasive evaluation for angina pectoris or acute coronary syndromes and who had verified CAD in other European hospitals. Hence, our findings should be generalizable to other CAD populations, primarily to patients with stable CAD. The power of the trial was less than planned because of lower event rates than anticipated and shorter follow-up than planned, the latter due to premature study termination of 692 participants (22%). Thus, WENBIT had a power of 80% to detect a 24% reduction in risk of the primary end point and a power of 62% to detect the prespecified, hypothesized 20% reduction in risk of the primary end point by the homocysteine-lowering therapy. Limited statistical power has also been a problem in several other homocysteine-lowering clinical trials.

Our finding of a modestly lowered risk of being urgently hospitalized for angina pectoris when treated with folic acid plus vitamin B₁₂ is not consistent with the nonappearance of beneficial effects on other cardiovascular outcomes in our trial. In particular, we found no difference in the risk of unstable angina between the groups receiving and not receiving folic acid (HR, 1.00; 95% CI, 0.68-1.48; \( P = .99 \)) (Table 3). That we did not adjust for multiple comparisons adds further support to the notion that the reduced risk of hospitalization for angina might be a spurious observation. The apparent elevated risk of the composite primary end point in the group receiving folic acid plus vitamin B₁₂ compared with the group receiving placebo could also be a chance finding due to multiple comparisons.

The null results in this and other homocysteine-lowering B vitamin intervention trials suggest that homocysteine is not a modifiable cause but rather a biological marker of CVD risk. The homocysteine hypothesis of vascular disease emerged from results of mainly retrospective observational studies. However, more recent prospective observational studies have demonstrated that plasma total homocysteine concentrations of vitamin B₁₂ are strongly inversely associated with C-reactive protein. In a large prospective study, the association between circulating concentrations of vitamin B₁₂ and risk of future AMI was abolished after adjustment for low-grade inflammation and smoking. Hence, low circulating levels of vitamin B₁₂ might be a consequence of low-grade inflammation accompanying CAD and/or smoking rather than a cause of CAD itself.

Conceivably, B vitamins in pharmacological doses may exert some deleterious effect in patients with established CVD that could counteract their potential benefits. Harmful effects of high serum folate concentration could
be mediated through mechanisms involving direct effects on vascular smooth muscle cell proliferation and matrix formation in coronary arteries. During the last few years, the assumption that folate prevents cancer has been challenged. One randomized trial with folate acid to prevent colorectal adenomas found increased risk of cancer in the group receiving folate acid, but another similar trial found no such increased risk. In both WENBIT and NORVIT, the incidence of cancer was higher in the groups receiving folate acid, but in none of these trials was this of statistical significance. Furthermore, it has been postulated that folate acid fortification of foods may have been wholly or partly responsible for the observed increase in colorectal cancer rates in the United States and Canada in the late 1990s. The view prevails that folate may prevent early stages of carcinogenesis but may enhance the growth of established cancer cells.

In conclusion, we could not demonstrate an effect of homocysteine-lowering therapy with B vitamins on mortality or major cardiovascular events in patients with CAD during 38 months of follow-up. These results are essentially in agreement with published results from similar larger secondary intervention trials. B vitamin interventions might have a different influence on cerebrovascular disorders, as suggested by meta- and reanalyses of published trials and by accelerated decline in stroke mortality in populations supplied with folic acid fortified foods. More fulfilling answers on the role of B vitamins in secondary prevention of CVD must await the results of ongoing trials and meta-analyses of pooled data. Future studies should also evaluate the possible adverse effects from folate acid supplementation and fortification of foods and especially on the risk of cancer. At this stage, use of B vitamin supplements to improve prognosis in patients with CAD is not justified.

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**REFERENCES**


