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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 vi-

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 × 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.

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tamin 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 low-

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ering on risk of cardiovascular disease or total mortality.⁸⁻¹⁰ 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 B₆¹¹ while another similar trial found an increased risk of in-stent restenosis and need for target vessel revascularization following B vitamin treatment.¹²

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.¹³ Circulating folate concentrations substantially increased and plasma total homocysteine concentrations decreased in the North American population following the fortification.¹³ 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.¹⁴

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

Patients eligible for randomization were men and women aged 18 years or older undergoing coronary angiography for suspected CAD and/or aortic valve stenosis at the 2 university hospitals in western Norway. Exclusion criteria were unavailability for follow-up, 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,^{15,16} 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%.² 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%.² 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-

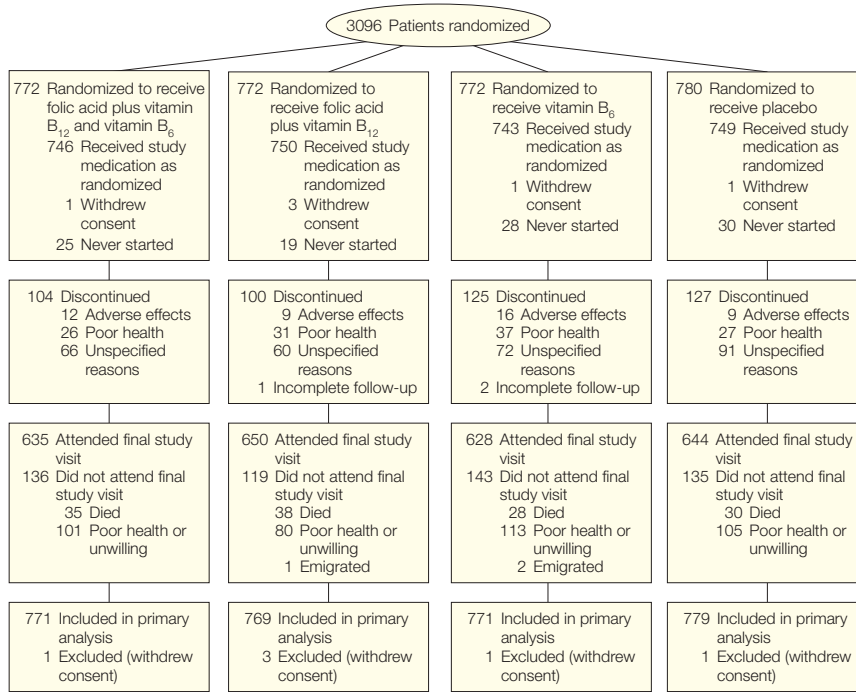
gian unique 11-digit person number for each participant.

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 the diagnostic criteria of the revised definition of MI published in 2000¹⁷ and strokes according to definitions published in 2001.¹⁸ Procedure-related nonfatal AMIs within 24 hours after coronary angiography, after PCI, or after coronary artery bypass grafting (CABG) were not included in the primary end point but were in the secondary end point. Events of unstable angina pectoris were classified as primary end points if patients were urgently admitted to the hospital because of acute onset of typical 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.¹⁸ Events of worsened but not unstable angina pectoris with angiographically verified significant progression of CAD were classified as secondary end points. Nonfatal hemorrhagic strokes were not included in the primary end point but were in the secondary end point. Myocardial revascularization procedures as determined by baseline angiography were not included in the secondary end point. All events were adjudicated by members of the end-points committee.

Figure 1. Flow of Participants Through the Trial



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. Glomerular filtration rates were estimated by the 4-variable Modification of Diet in Renal Disease equations.¹⁹ 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 of Bevitall AS, Bergen, Norway, by published methods.²⁰⁻²³ Results from follow-up measurements of B vitamins and total homocysteine were not disclosed to study personnel or the end-points committee until the randomization code was broken.

Statistical Analysis

The 2 × 2 factorial design allowed separate assessments of effects from the folic 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 1991-1992³ 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 nonfatal 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 χ^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).

Table 1. Baseline Participant Characteristics

| Characteristics | Intervention Group | | | |
|---|---|--|-------------------------------------|----------------------|
| | Folic Acid + Vitamins B ₁₂ and B ₆ (n = 771) | Folic Acid + Vitamin B ₁₂ (n = 769) | Vitamin B ₆ (n = 771) | Placebo (n = 779) |
| Age, mean (SD), y | 61.7 (10.3) | 61.3 (10.0) | 61.4 (9.7) | 62.0 (9.9) |
| Male sex, No. (%) | 626 (81.2) | 618 (80.4) | 618 (80.2) | 596 (76.5) |
| Body mass index, mean (SD) ^a | 26.8 (3.7) | 26.9 (3.9) | 26.7 (3.5) | 27.2 (3.9) |
| Blood pressure, mean (SD), mm Hg | | | | |
| Systolic | 140 (20) | 141 (20) | 141 (21) | 142 (21) |
| Diastolic | 81 (11) | 81 (11) | 81 (11) | 81 (11) |
| LVEF <50%, No. (%) | 82 (10.6) | 83 (10.8) | 98 (12.7) | 86 (11.0) |
| Hemoglobin, mean (SD), g/dL | 14.4 (1.2) | 14.4 (1.2) | 14.4 (1.2) | 14.4 (1.2) |
| Creatinine, mean (SD), μmol/L | 92 (18) | 91 (14) | 91 (17) | 92 (19) |
| eGFR <60 mL/min/1.73 m ² , No. (%) | 94 (12.2) | 84 (10.9) | 85 (11.0) | 97 (12.5) |
| Total cholesterol, mean (SD), mg/dL | 195.7 (43.9) | 196.8 (44.6) | 196.0 (44.1) | 195.5 (44.7) |
| HDL-C, mean (SD), mg/dL | 48.2 (14.0) | 47.6 (13.3) | 48.4 (13.4) | 48.1 (13.6) |
| LDL-C, mean (SD), mg/dL | 119.5 (38.6) | 120.2 (40.6) | 118.6 (40.0) | 119.8 (40.9) |
| C-reactive protein, median (IQR), mg/L | 1.8 (3.1) | 2.1 (3.6) | 2.0 (3.2) | 2.0 (3.5) |
| Cardiovascular history and risk factors, No. (%) | | | | |
| Myocardial infarction | 292 (37.9) | 323 (42.0) | 331 (42.9) | 334 (42.9) |
| Percutaneous coronary intervention | 152 (19.7) | 156 (20.3) | 162 (21.0) | 167 (21.4) |
| Coronary artery bypass graft surgery | 113 (14.7) | 87 (11.3) | 109 (14.1) | 106 (13.6) |
| Carotid artery stenosis, TIA, or stroke | 52 (6.7) | 49 (6.4) | 50 (6.5) | 41 (5.3) |
| Other peripheral artery disease | 65 (8.4) | 56 (7.3) | 69 (8.9) | 85 (10.9) |
| Family history of premature CAD | 256 (33.2) | 266 (34.6) | 261 (33.9) | 244 (31.3) |
| History of hypercholesterolemia ^b | 449 (58.2) | 453 (58.9) | 470 (61.0) | 465 (59.7) |
| Diabetes mellitus | 84 (10.9) | 92 (12.0) | 77 (10.0) | 103 (13.2) |
| Hypertension | 346 (44.9) | 356 (46.3) | 347 (45.0) | 369 (47.4) |
| Current smoker | 219 (28.4) | 203 (26.4) | 238 (30.9) | 215 (27.6) |
| CAD at baseline angiography, No. (%) | | | | |
| No or nonsignificant coronary stenosis ^c | 84 (10.9) | 81 (10.5) | 86 (11.2) | 88 (11.3) |
| 1-Vessel disease | 224 (29.1) | 228 (29.6) | 241 (31.3) | 227 (29.1) |
| 2-Vessel disease | 208 (27.0) | 205 (26.7) | 198 (25.7) | 215 (27.6) |
| 3-Vessel disease | 255 (33.1) | 255 (33.2) | 246 (31.9) | 249 (32.0) |

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.

^aBody mass index was calculated as weight in kilograms divided by height in meters squared.

^bUntreated total cholesterol values of at least 251.0 mg/dL (≥6.5 mmol/L).

^cNormal 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.

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.^{15,16} 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),²⁴ 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.

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 CABG, among whom 55 (1.8% of all) also had valve surgery, whereas 46 (1.5% of all) had only valve surgery (TABLE 2). Median time from baseline angiography to planned procedures was 21 (5th-95th percentile, 0-84) days.

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 4 intervention groups and no report of serious adverse events related to study medication. A total of 2532 (81.9% of all) took 50% 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%; $P = .005$).

Table 2. Conventional Treatments Following Baseline Angiography

| | Intervention Group, No. (%) | | | |
|--------------------------|---|--|-------------------------------------|----------------------|
| | Folic Acid + Vitamins B ₁₂ and B ₆ (n = 771) | Folic Acid + Vitamin B ₁₂ (n = 769) | Vitamin B ₆ (n = 771) | Placebo (n = 779) |
| Medications | | | | |
| Acetylsalicylic acid | 687 (89.1) | 698 (90.8) | 694 (90.0) | 708 (90.9) |
| Clopidogrel | 182 (23.6) | 196 (25.5) | 206 (26.7) | 188 (24.1) |
| Warfarin | 40 (5.2) | 35 (4.6) | 45 (5.8) | 32 (4.1) |
| Statins | 680 (88.2) | 669 (87.0) | 692 (89.8) | 690 (88.6) |
| β -Blockers | 597 (77.4) | 615 (80.0) | 608 (78.9) | 596 (76.5) |
| ACE inhibitors/ARBs | 230 (29.8) | 250 (32.5) | 241 (31.3) | 273 (35.0) |
| Calcium channel blockers | 172 (22.3) | 169 (22.0) | 165 (21.4) | 187 (24.0) |
| Loop diuretics | 82 (10.6) | 62 (8.1) | 78 (10.1) | 86 (11.0) |
| Oral antidiabetics | 48 (6.2) | 51 (6.6) | 45 (5.8) | 55 (7.1) |
| Insulin | 24 (3.1) | 26 (3.4) | 22 (2.9) | 34 (4.4) |
| Medication for COPD | 33 (4.3) | 39 (5.1) | 27 (3.5) | 40 (5.1) |
| Procedures | | | | |
| PCI | 344 (44.6) | 350 (45.5) | 334 (43.3) | 331 (42.5) |
| PCI with use of stent(s) | 292 (37.9) | 316 (41.1) | 302 (39.2) | 298 (38.3) |
| CABG | 177 (23.0) | 198 (25.7) | 187 (24.3) | 174 (22.3) |
| Valve surgery | 31 (4.0) | 22 (2.9) | 24 (3.1) | 24 (3.1) |

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; PCI, percutaneous coronary intervention.

Effect of Intervention on Circulating B Vitamins and Homocysteine Levels

FIGURE 2 shows circulating B vitamin and total homocysteine levels in the 4 treatment groups at baseline, at follow-up visits, and at the final study visit. At baseline, plasma total homocysteine levels were similar in the 4 groups ($P = .72$). None of the participants had severe hyperhomocysteinemia (total homocysteine $>100 \mu\text{mol/L}$), 25 (0.8%) had intermediate (total homocysteine 30-100 $\mu\text{mol/L}$), and 271 (8.8%) had mild hyperhomocysteinemia (total homocysteine 15-30 $\mu\text{mol/L}$) as currently defined.²

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) $\mu\text{mol/L}$ at baseline to 7.6 (SD, 2.2) $\mu\text{mol/L}$ 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 $\mu\text{mol/L}$ 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 4 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 B₆, 200 participants (13.0%) experienced the primary end point compared with 222 (14.3%) in the groups not receiving vitamin B₆ (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 B₆, 139 participants (11.0%) who adhered to study

medication experienced the primary end point vs 164 (12.9%) of those not receiving vitamin B₆ (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 non-fatal, including procedure-related), or unstable angina pectoris (Table 3). The incidence of total stroke (fatal and non-fatal, 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 B₁₂ (HR, 1.34; 95% CI, 1.03-1.75; $P=.03$) compared with placebo.

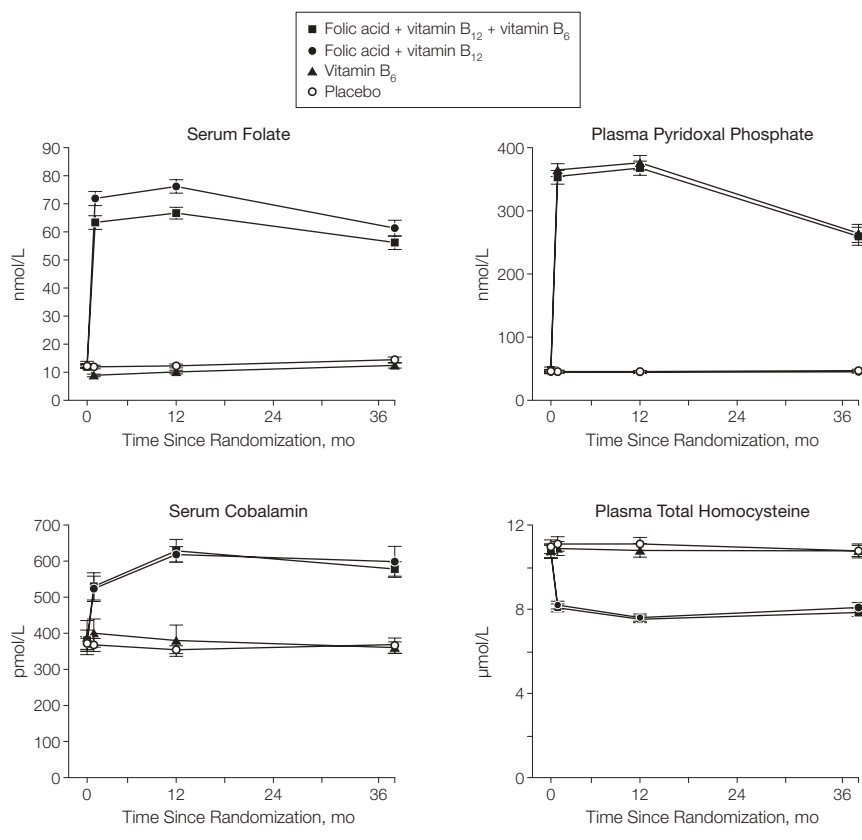
The baseline level of total homocysteine in plasma was a significant predictor of the primary end point (HR associated with a 3- $\mu\text{mol/L}$ difference in total homocysteine level, 1.07; 95% CI, 1.02-1.13; $P=.01$) after adjustment for age, sex, smoking status (never, ex-, or current smoker), and 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 B₁₂ or with vitamin B₆ 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 AMI²⁴ 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

Figure 2. Mean Circulating B Vitamin and Total Homocysteine Levels at Baseline and During Follow-up



Mean concentration of B vitamins and total homocysteine in serum or plasma. Error bars represent 95% confidence intervals. Data for the final study visit (median, 38.4 months) are the final data points shown in each graph. Samples for determination of serum folate, serum cobalamin, and plasma total homocysteine were available for 3083 participants at baseline, 2765 participants at 1 month, 2704 participants at 12 months, and 2557 participants at the end of the intervention. Samples for determination of plasma pyridoxal phosphate were available for 3054 participants at baseline, 2726 participants at 1 month, 2665 participants at 12 months, and 2533 participants at the end of the intervention.

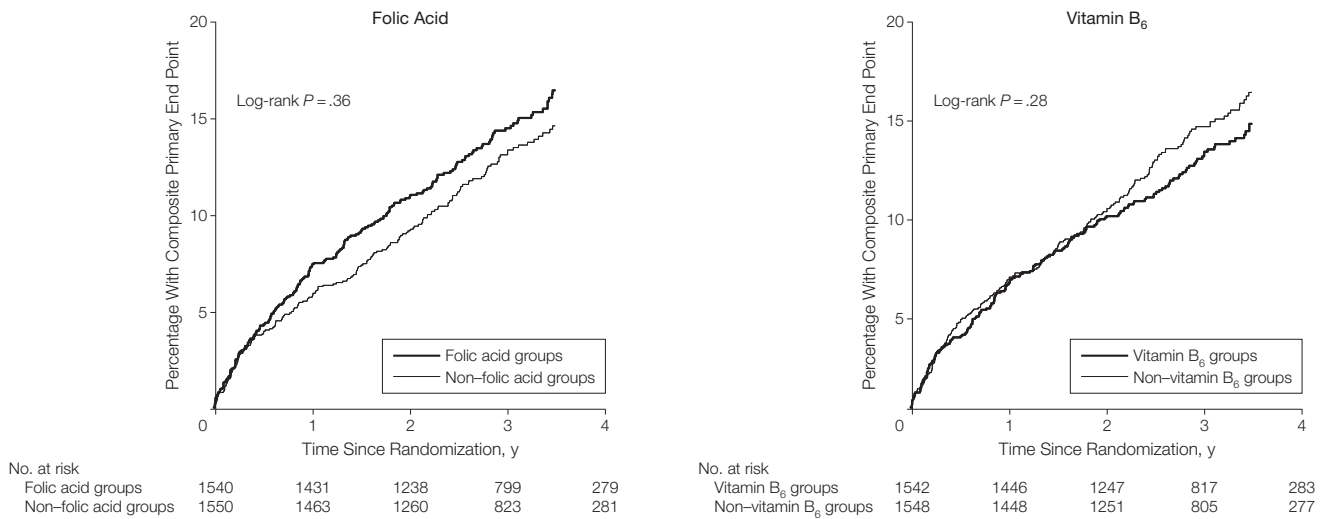
was greater than 80% and was corroborated by assessment of B vitamin concentrations in serum and plasma. There is no mandatory folic acid fortification of foods in Norway, and the self-reported use of over-the-counter B vitamin supplements was modest in this study population. Thus, the homocys-

teine-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 pro-

cess 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



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

| Events | No. (%) of Patients With Event ^a | | | | Folic Acid vs Non-Folic Acid Groups | | Vitamin B ₆ vs Non-Vitamin B ₆ Groups | |
|--|--|--|----------------------------------|-------------------|-------------------------------------|------------------|---|------------------|
| | Folic Acid + Vitamins B ₁₂ and B ₆ (n = 771) | Folic Acid + Vitamin B ₁₂ (n = 769) | Vitamin B ₆ (n = 771) | Placebo (n = 779) | Hazard Ratio (95% CI) | P Value | Hazard Ratio (95% CI) | P Value |
| | Primary end point ^b | 94 (12.2) | 125 (16.3) | 106 (13.7) | 97 (12.5) | 1.09 (0.90-1.32) | .36 | 0.90 (0.74-1.09) |
| Death | 35 (4.5) | 38 (4.9) | 28 (3.6) | 30 (3.9) | 1.27 (0.90-1.79) | .18 | 0.94 (0.66-1.32) | .70 |
| Acute myocardial infarction ^c | 59 (7.7) | 76 (9.9) | 55 (7.1) | 58 (7.4) | 1.21 (0.95-1.56) | .13 | 0.85 (0.66-1.09) | .20 |
| Unstable angina ^d | 22 (2.9) | 29 (3.8) | 24 (3.1) | 27 (3.5) | 1.00 (0.68-1.48) | .99 | 0.83 (0.56-1.22) | .34 |
| Stroke ^e | 11 (1.4) | 17 (2.2) | 20 (2.6) | 19 (2.4) | 0.72 (0.44-1.17) | .19 | 0.87 (0.54-1.40) | .56 |
| Acute hospitalization for angina ^f | 84 (10.9) | 92 (12.0) | 104 (13.5) | 109 (14.0) | 0.82 (0.67-1.00) | .05 | 0.93 (0.76-1.14) | .48 |
| Angina with verified progression of CAD ^g | 69 (8.9) | 62 (8.1) | 72 (9.3) | 61 (7.8) | 1.00 (0.78-1.27) | .98 | 1.16 (0.91-1.48) | .22 |
| Myocardial revascularization procedures ^h | 118 (15.3) | 120 (15.6) | 119 (15.4) | 108 (13.9) | 1.07 (0.89-1.29) | .46 | 1.05 (0.88-1.26) | .58 |
| Cancer ⁱ | 46 (6.0) | 39 (5.1) | 38 (4.9) | 31 (4.0) | 1.25 (0.91-1.71) | .18 | 1.20 (0.87-1.64) | .27 |

Abbreviations: CAD, coronary artery disease; CI, confidence interval.

^aThe first event in the category for each participant.

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

^cFatal and nonfatal acute myocardial infarction, including procedure-related acute myocardial infarction.

^dTypical 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.

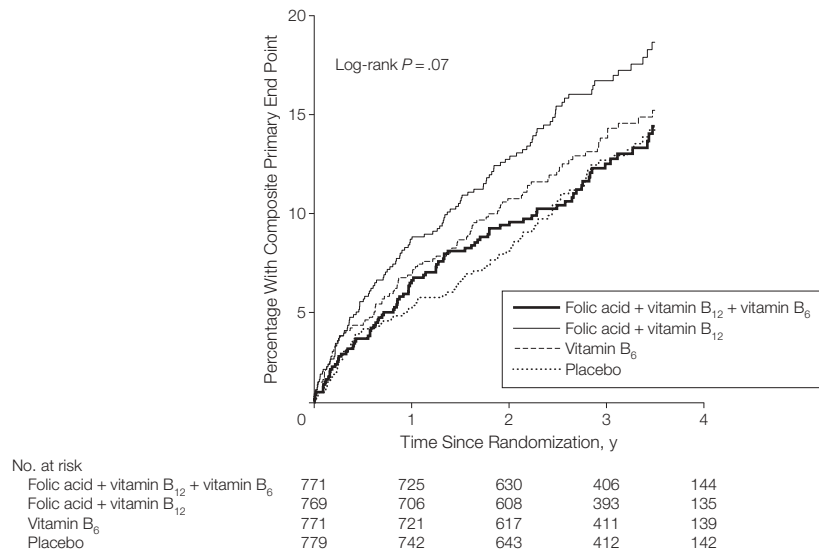
^eFatal and nonfatal stroke, including hemorrhagic stroke.

^fUnstable or acute angina pectoris.

^gAngiographically verified progression of coronary stenosis.

^hPercutaneous coronary intervention or coronary artery bypass graft surgery.

ⁱAll 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.^{26,27} 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.⁷

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 un-

stable 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.^{1,2} However, more recent prospective observational studies have demonstrated that plasma total homocysteine concentration is at most a modest risk factor of CVD.⁷ Mendelian randomization studies, which cannot be subject to confounding or reverse causality, have yielded some evidence that an elevated total ho-

mocysteine level could cause CVD, especially stroke,²⁸ but a meta-analysis found only a weak overall association between the methylenetetrahydrofolate reductase (*MTHFR*) 677C→T polymorphism and CAD in populations in Europe, North America, and Australia.²⁹ A recent large study in white American women found no association between the variant genotype and future risk of CVD.³⁰

The focus of our trial was an intervention to prevent or delay occurrence of occlusive vascular disease in patients with mainly stable CAD. Inflammation plays a key role in CAD and other manifestations of atherosclerosis.³¹ Intervention with B vitamins in WENBIT¹⁶ and in other trials in populations with established CVD³² or elevated plasma total homocysteine levels³³ has not led to significant reduction in circulating concentrations of inflammatory markers involved in atherogenesis. Thus, the failure to reverse inflammatory processes could explain the lack of effect of the B vitamin intervention on cardiovascular events in our trial.

We could not demonstrate any separate effect of the vitamin B₆ intervention on clinical outcomes. Observational studies from the late 1990s demonstrated an association between vitamin B₆ status and risk of CAD and other CVD,^{5,6} whereas more recent studies have shown that circulating concentrations of vitamin B₆ are strongly and inversely associated with C-reactive protein.^{34,35} In a large prospective study, the association between plasma pyridoxal phosphate concentration and risk of future AMI was abolished after adjustment for low-grade inflammation and smoking.³⁶ Hence, low circulating levels of vitamin B₆ 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.³⁷ 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.^{12,37} During the last few years, the assumption that folate prevents cancer has been challenged. One randomized trial with folic acid to prevent colorectal adenomas found increased risk of cancer in the group receiving folic acid,³⁸ but another similar trial found no such increased risk.³⁹ In both WENBIT and NORVIT, the incidence of cancer was higher in the groups receiving folic acid, but in none of these trials was this of statistical significance. Furthermore, it has been postulated that folic 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.⁴⁰ The view prevails that folate may prevent early stages of carcinogenesis but may enhance the growth of established cancer cells.⁴¹

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.^{10,24,25} B vitamin interventions might have a different influence on cerebrovascular disorders, as suggested by meta- and reanalyses of published trials^{42,43} and by accelerated decline in stroke mortality in populations supplied with folic acid fortified foods.¹⁴ 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.⁷ Future studies should also evaluate the possible adverse effects from folic 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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