Cancer Incidence and Mortality After Treatment With Folic Acid and Vitamin B 12

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Cancer Incidence and Mortality After Treatment With Folic Acid and Vitamin B_{12}

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Context Recently, concern has been raised about the safety of folic acid, particularly in relation to cancer risk.

Objective To evaluate effects of treatment with B vitamins on cancer outcomes and all-cause mortality in 2 randomized controlled trials.

Design, Setting, and Participants Combined analysis and extended follow-up of participants from 2 randomized, double-blind, placebo-controlled clinical trials (Norwegian Vitamin Trial and Western Norway B Vitamin Intervention Trial). A total of 6837 patients with ischemic heart disease were treated with B vitamins or placebo between 1998 and 2005, and were followed up through December 31, 2007.

Interventions Oral treatment with folic acid (0.8 mg/d) plus vitamin B_{12} (0.4 mg/d) and vitamin B_6 (40 mg/d) (n=1708); folic acid (0.8 mg/d) plus vitamin B_{12} (0.4 mg/d) (n=1703); vitamin B_6 alone (40 mg/d) (n=1705); or placebo (n=1721).

Main Outcome Measures Cancer incidence, cancer mortality, and all-cause mortality.

Results During study treatment, median serum folate concentration increased more than 6-fold among participants given folic acid. After a median 39 months of treatment and an additional 38 months of posttrial observational follow-up, 341 participants (10.0%) who received folic acid plus vitamin B_{12} vs 288 participants (8.4%) who did not receive such treatment were diagnosed with cancer (hazard ratio [HR], 1.21; 95% confidence interval [CI], 1.03-1.41; $P=.02$). A total of 136 (4.0%) who received folic acid plus vitamin B_{12} vs 100 (2.9%) who did not receive such treatment died from cancer (HR, 1.38; 95% CI, 1.07-1.79; $P=.01$). A total of 548 patients (16.1%) who received folic acid plus vitamin B_{12} vs 473 (13.8%) who did not receive such treatment died from any cause (HR, 1.18; 95% CI, 1.04-1.33; $P=.01$). Results were mainly driven by increased lung cancer incidence in participants who received folic acid plus vitamin B_{12}. Vitamin B_6 treatment was not associated with any significant effects.

Conclusion Treatment with folic acid plus vitamin B_{12} was associated with increased cancer outcomes and all-cause mortality in patients with ischemic heart disease in Norway, where there is no folic acid fortification of foods.

Trial Registration clinicaltrials.gov Identifier: NCT00671346

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FOLATE IS A B VITAMIN ESSENTIAL for nucleotide biosynthesis, DNA replication, and methyl group supply, and thus for cell growth and repair.[1] Folic acid is the synthetic form of folate used in vitamin supplements and in fortified foods. Most epidemiological studies have found inverse associations between folate intake and risk of colorectal cancer,[2] although such associations have been inconsistent or absent for other cancers.[3-8] Experimental evidence suggests that folate deficiency may promote initial stages of carcinogenesis, whereas high doses of folic acid may enhance growth of cancer cells.[9,10]

Since 1998, many countries, including the United States, have implemented mandatory folic acid fortification of flour and grain products to reduce the risk of neural-tube birth defects.[11] In the US population, fortification has resulted in substantial increase in circulating folate[12] and unmetabolized folic acid[13] concentrations. Recently, concerns have emerged about the safety of folic acid, in particular with respect to cancer risk.[1]

Folic acid has been used alone or in combination with other B vitamins in a series of homocysteine-lowering trials ini-

For editorial comment see p 2152.

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tiated in patients with cardiovascular disease to assess whether this treatment may reduce cardiovascular outcomes.[14] So far, none of the larger trials has reported beneficial effects on primary outcomes.[14]

In 2 Norwegian homocysteine-lowering trials among patients with ischemic heart disease, there was a statistically nonsignificant increase in cancer incidence in the groups assigned to folic acid treatment.[15,16] Our study was performed to explore whether folic acid treatment was associated with cancer outcomes and all-cause mortality after extended follow-up. Because there is no folic acid fortification of foods in Norway, this study population was well suited for such an investigation.

METHODS

Study Design, Participants, and Study Intervention

This is a combined analysis of data from 2 randomized, double-blind, placebo-controlled clinical trials (Norwegian Vitamin [NORVIT] trial[15] and Western Norway B Vitamin Intervention Trial [WENBIT][16]) conducted between 1998 and 2005, and an observational posttrial follow-up through December 31, 2007, with respect to cancer outcomes and all-cause mortality. Details and the primary results of the 2 trials have been reported previously.[15,16] The pooling of data is justified by the fact that the 2 trials included similar patients, had identical study design and treatment regimen, had similar follow-up routines, and used the same central laboratory for study-related blood analyses.

The objective of both trials was to assess whether homocysteine-lowering treatment with folic acid and vitamin B_{12} could improve cardiovascular morbidity and mortality in patients with ischemic heart disease. Patients with known active cancer were excluded, whereas patients with a history of cured cancer were not excluded. All participants gave written informed consent. Participants were randomly assigned to receive a capsule with 1 of the following 4 compositions: (1) folic acid (0.8 mg/d) plus vitamin B_{12} (cyanocobalamin; 0.4 mg/d) and vitamin B_6 (pyridoxine hydrochloride; 40 mg/d); (2) folic acid (0.8 mg/d) plus vitamin B_{12} (0.4 mg/d); (3) vitamin B_6 alone (40 mg/d); or (4) placebo. They were concomitantly requested to abstain from taking over-the-counter supplements containing B vitamins.

Clinical information and blood samples were obtained at baseline, 1 to 2 months after randomization, and at a final study visit in both trials. Adherence was judged by capsule counts and interviews. Analyses of circulating B vitamins, homocysteine and cotinine, and genotyping of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene (NCBI Entrez Gene 4524) 677C>T single-nucleotide polymorphism were performed at the laboratory of Bevital AS, Bergen, Norway, by published methods.[17-20]

The NORVIT trial was terminated in March 2004 and the WENBIT in October 2005. When trial results were available, participants were informed by letter that there was no apparent health benefit from the B vitamin intervention.

Participants residing in Norway at the time of their final study visit were included in the posttrial follow-up, which did not imply any continued study treatment, further blood sampling, or personal contact. Our study was approved by the Regional Committee for Medical and Health Research Ethics, the Data Inspectorate, and the Norwegian Directorate of Health.

Definition and Ascertainment of Outcomes

The primary end points were cancer incidence, cancer mortality, and all-cause mortality through 2007. Data were obtained by linking the unique personal identification numbers to the Cancer Registry of Norway[21] and to the Cause of Death Registry at Statistics Norway.[22] Malignant neoplasms except nonmelanoma skin cancers were considered, and only the first of new primary cancers after randomization was included. A person was regarded deceased from cancer if the underlying cause of death was coded as *International Statistical Classification of Diseases, 10th Revision* (*ICD-10*) codes C00 to C97.

Statistical Analysis

Analyses were conducted among all individuals who participated in the trials. Differences between groups were tested with χ^2 test for categorical variables and 1-way analysis of variance, Kruskal-Wallis, or Mann-Whitney U test were used for continuous variables.

We computed the expected numbers of total incident cancers and incidence of the major cancer subtypes in the study population by applying age (5-year age groups) and sex-specific annual national cancer incidence rates for the actual calendar years from the Cancer Registry of Norway.[23] The 95% confidence intervals (CIs) for the observed vs expected incidence ratios were computed, assuming that cancer incidence had a Poisson distribution.

Cancer and mortality outcomes were analyzed for groups assigned to folic acid plus vitamin B_{12} treatment (folic acid groups) vs no folic acid plus vitamin B_{12} treatment (non–folic acid groups), and for groups assigned to vitamin B_6 treatment (vitamin B_6 groups) vs no vitamin B_6 treatment (non–vitamin B_6 groups), according to the 2×2 factorial design. There were no interaction effects between folic acid plus vitamin B_{12} treatment and vitamin B_6 treatment on the primary end points (all P for interaction ≥ .63).

Neither of the 2 trials was originally designed to address cancer risk. By pooling the data and extending the follow-up of both trial populations, we had a statistical power of 61% to detect the observed difference in cancer incidence between the folic acid and non–folic acid groups at a 2-sided statistical significance level of .05.

Person-time was calculated from the date of randomization to the date of event, date of emigration, or December 31, 2007, whichever occurred first. Participants who declined posttrial follow-up were censored at the date of their final study visit.

Survival curves were constructed using the Kaplan-Meier method and differences in survival between groups were analyzed using the log-rank test. Estimates of hazard ratios (HRs) with 95% CIs were obtained by using Cox proportional hazards regression models stratified by trial. Proportional hazards assumptions were tested by Stata's estat phtest based on Schoenfeld residuals,[24] and evidence of nonproportionality was not found.

For the 3 closely associated primary end points and for noncancer mortality, we obtained HRs with 95% CIs. A 2-sided statistical significance level of .05 was applied throughout and reported *P* values were not adjusted for multiple comparisons.

Because our study was not preplanned, and we performed several outcome analyses, there was an increased risk of type I error. However, to guard against this, for incidence and mortality of cancer subtypes, we reported HRs with 99% CIs.

Effect modifications of folic acid plus vitamin B_{12} treatment by subgroup indicators were assessed by including the relevant interaction terms in the main effects model. Six predefined participant characteristics were examined for the 3 primary end points across folic acid plus vitamin B_{12} treatment. Of the resulting 18 comparisons, there was a 60.3% probability that one or several statistically significant *P* values would appear on the basis of chance alone. No subgroup analyses were performed with respect to vitamin B_6 treatment.

To assess separate effects of folic acid treatment and vitamin B_{12} treatment, we stratified the study population by quartiles of serum folate and serum cobalamin (vitamin B_{12}) measured during study treatment, and estimated HRs for the primary end points across these strata, independently of the random treatment assignment.

We used the statistical software packages SPSS version 15.0 (SPSS Inc, Chicago, Illinois), SAS version 9.2 (SAS Institute, Cary, North Carolina), Stata version 10 (StataCorp LP, College Station, Texas), and S-Plus version 8.0 (TIBCO Software Inc, Palo Alto, California).

RESULTS

Patients

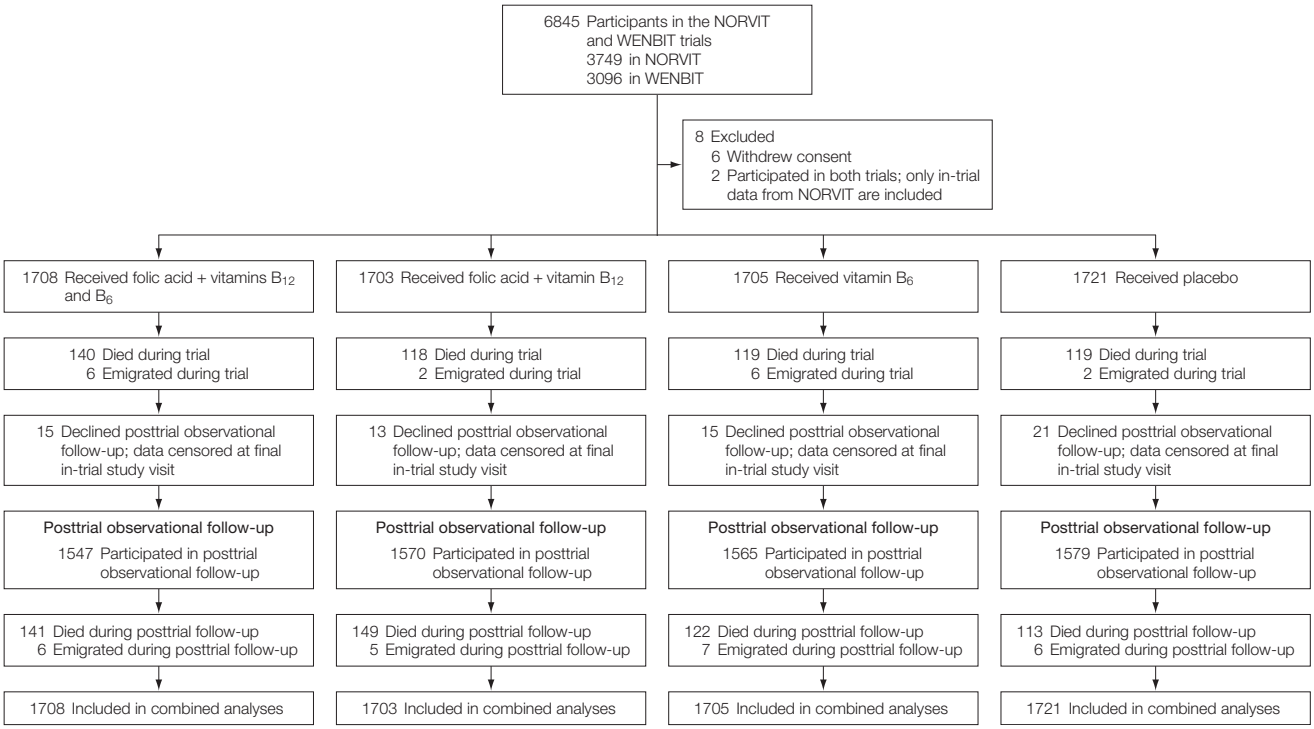
The numbers of participants through in-trial and posttrial follow-up are shown in the FIGURE. A total of 6837 individuals were included in the combined analyses, of whom 6261 (91.6%) participated in posttrial follow-up. Median (interquartile range) duration of extended follow-up through December 31, 2007, was 78 (61-90) months, including median (interquartile range) in-trial follow-up of 39 (31-42) months.

Patient baseline characteristics, risk factor levels, and use of concomitant medications are shown in TABLE 1. Mean (SD) age was 62.3 (11.0) years and 23.5% of participants were women. The percentage of current smokers (total 39%) was lower in the folic acid groups (38%) than in the non–folic acid groups (41%) (*P*=.01). Among current smokers, plasma levels of cotinine, a marker of tobacco exposure,[19] did not differ across the intervention groups. A total of 297 participants (4.3%) had been registered with cancer before trial entry. The prevalence of TT homozygotes of the *MTHFR* 677C>T polymorphism was 8.2%, similar to that found in a large sample from the general Norwegian population.[25]

Circulating Vitamin Levels During Study Treatment

Adherence, defined as taking at least 80% of the study capsules throughout in-

Figure. Flow of Participants Through the NORVIT and WENBIT Trials and Posttrial Observational Follow-up



NORVIT indicates Norwegian Vitamin Trial; WENBIT, Western Norway B Vitamin Intervention Trial.

trial follow-up, was 84.7%. eTable 1 (available at http://www.jama.com) shows circulating B vitamin levels at baseline and during study treatment. In the folic acid groups, median serum folate increased from 3.9 to 27.5 ng/mL (to convert to nmol/L, multiply by 2.266) and serum cobalamin from 477 to 761 pg/mL (to convert to pmol/L, multiply by 0.7378) during the intervention. In the vitamin B_6 groups, median plasma pyridoxal 5′ phosphate (the active form of vitamin B_6) increased from 8.2 to 75.4 ng/mL (to convert to nmol/L, multiply by 4.046).

Individuals with the TT genotype of the *MTHFR* 677C>T polymorphism had lower median serum folate concentration than those individuals with the CC or CT genotype, both at baseline (17.7% lower; $P<.001$) and during study treatment (11.7% lower in the folic acid groups and 10.4% lower in the non–folic acid groups, $P<.001$ and $P=.001$, respectively).

Outcome Measures

A total of 629 participants (9.2%) were diagnosed with new cancers during (n=292) or after (n=337) the trials. Diagnoses were based on histological, cytological, or other diagnostic examinations in 90.5%, 4.9%, and 4.1% of the

Table 1. Baseline Participant Characteristics and Use of Concomitant Medications[a]

Characteristics	No./Total No. (%) of Participants			
	Folic Acid + Vitamins B_{12} and B_6 (n = 1708)	Folic Acid + Vitamin B_{12} (n = 1703)	Vitamin B_6 (n = 1705)	Placebo (n = 1721)
Included in NORVIT	937/1708 (54.9)	935/1703 (54.9)	934/1705 (54.8)	943/1721 (54.8)
Included in WENBIT	771/1708 (45.1)	768/1703 (45.1)	771/1705 (45.2)	778/1721 (45.2)
Age, mean (SD), y	62.7 (11.2)	62.3 (10.9)	62.0 (10.9)	62.3 (10.7)
Male sex	1310/1708 (76.7)	1313/1703 (77.1)	1304/1705 (76.5)	1300/1721 (75.5)
BMI, mean (SD)	26.6 (3.9)	26.5 (3.8)	26.5 (3.7)	26.7 (3.8)
Obesity (BMI ≥30)	278/1707 (16.3)	271/1700 (15.9)	283/1702 (16.6)	308/1718 (17.9)
Hypertension	627/1699 (36.9)	605/1687 (35.9)	615/1693 (36.3)	643/1717 (37.4)
Diabetes mellitus	187/1696 (11.0)	175/1693 (10.3)	163/1700 (9.6)	199/1719 (11.6)
Vitamin supplements[b]	401/1708 (23.5)	398/1703 (23.4)	390/1705 (22.9)	392/1721 (22.8)
History of cancer before trial[c]	64/1708 (3.7)	71/1703 (4.2)	83/1705 (4.9)	79/1721 (4.6)
MTHFR 677 genotype				
CC	806/1627 (49.5)	862/1627 (53.0)	810/1636 (49.5)	816/1643 (49.7)
CT	677/1627 (41.6)	636/1627 (39.1)	699/1636 (42.7)	692/1643 (42.1)
TT	144/1627 (8.9)	129/1627 (7.9)	127/1636 (7.8)	135/1643 (8.2)
Smoking status and cotinine				
Never smoker	488/1706 (28.6)	514/1700 (30.2)	449/1702 (26.4)	487/1715 (28.4)
Ex-smoker[d]	553/1706 (32.4)	565/1700 (33.2)	538/1702 (31.6)	552/1715 (32.2)
Current smoker	665/1706 (39.0)	621/1700 (36.5)	715/1702 (42.0)	676/1715 (39.4)
Plasma cotinine in current smokers, median (IQR), μg/L[e]	27.1 (131.0)	27.6 (126.1)	25.7 (119.5)	25.5 (123.7)
Indication for trial entry				
Acute MI	1016/1708 (59.5)	1013/1703 (59.5)	1018/1705 (59.7)	1025/1721 (59.6)
Unstable angina	31/1708 (1.8)	39/1703 (2.3)	35/1705 (2.1)	32/1721 (1.9)
Stable angina	644/1708 (37.7)	645/1703 (37.9)	646/1705 (37.9)	649/1721 (37.7)
Aortic valve stenosis	17/1708 (1.0)	6/1703 (0.4)	6/1705 (0.4)	15/1721 (0.9)
Concomitant medication				
Acetylsalicylic acid	1444/1645 (87.8)	1486/1648 (90.2)	1458/1623 (89.8)	1485/1656 (89.7)
Warfarin	163/1638 (10.0)	124/1640 (7.6)	133/1622 (8.2)	136/1656 (8.2)
Lipid-lowering drugs	1370/1643 (83.4)	1389/1644 (84.5)	1398/1622 (86.2)	1401/1651 (84.9)
β-Blockers	1394/1643 (84.8)	1422/1647 (86.3)	1376/1623 (84.8)	1397/1658 (84.3)
Calcium antagonists	261/1637 (15.9)	244/1639 (14.9)	241/1617 (14.9)	250/1655 (15.1)
ACE inhibitors/ARBs	549/1636 (33.6)	556/1639 (33.9)	537/1623 (33.1)	582/1654 (35.2)
Diuretics	295/1638 (18.0)	262/1638 (16.0)	269/1622 (16.6)	292/1655 (17.6)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; IQR, interquartile range; MI, myocardial infarction; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; NORVIT, Norwegian Vitamin Trial; WENBIT, Western Norway B Vitamin Intervention Trial.

SI conversion factor: To convert plasma cotinine to nmol/L, multiply by 5.675.

[a] Because of rounding, percentages may not total 100. For BMI, data were available for 6827 participants (99.9%).

[b] Daily or often use of vitamin supplements at trial entry.

[c] Included any cancer except nonmelanoma skin cancer.

[d] Defined as participant quit smoking more than 1 month before trial entry.

[e] Samples were available for 2634 of 2677 current smokers (98.4%) at baseline. Median values were low at trial entry due to smoking restrictions for participants randomized during hospitalization for acute coronary syndromes.

incident cases, respectively. Three cases of cancer were based on information from death certificates only.

The total cancer incidence and cancer pattern in the study population were similar to what was expected in the general Norwegian population, except for a 25% higher lung cancer incidence (HR, 1.25; 95% CI, 1.01-1.53) in the study population vs the general population. A total of 496 participants (7.3%) died during the trials and 525 (8.4%) died during posttrial follow-up. Of the total 1021 deaths through December 31, 2007, 236 (23.1%) were classified as cancer deaths and 75 of 236 (31.8%) were lung cancer deaths.

TABLE 2 shows cancer and mortality outcomes in the 4 intervention groups and the HRs and CIs for the comparisons between folic acid vs non–folic acid groups and between vitamin B_6 vs non–vitamin B_6 groups. Treatment with folic acid and vitamin B_{12} was associated with a statistically significant increase in cancer incidence during extended follow-up of both trial populations through December 31, 2007 (folic acid vs non–folic acid groups: HR, 1.21; 95% CI, 1.03-1.41; P=.02). This treatment was also associated with a statistically significant increase in cancer mortality (HR, 1.38; 95% CI, 1.07-1.79; P=.01) and all-cause mortality (HR, 1.18; 95% CI, 1.04-1.33; P=.01).

The cumulative incidence and mortality curves in the eFigure (available at http://www.jama.com) indicate that the increase in cancer incidence, cancer mortality, and all-cause mortality associated with folic acid plus vitamin B_{12} treatment emerged after approximately 1, 2, or 3 years of follow-up, respectively. Treatment with vitamin B_6 was not associated with the primary end points (Table 2 and eFigure).

Adjusting for age (continuous), sex, baseline smoking status, and use of acetylsalicylic acid, or for baseline smoking status alone, did not essentially alter the results. Excluding the 297 participants with registered cancer before trial entry, of whom 23 (7.7%) also had new cancer diagnosis after randomization, or excluding those participants with diagnosed new cancer within 1 year after randomization (n=97), gave similar results.

When restricting analyses to participants who took study capsules for more than 6 months following randomization (n=6218, 90.9% of all), HRs for cancer incidence, cancer mortality, and all-cause mortality in the folic acid vs non–folic acid groups increased to 1.27, 1.43, and 1.24, respectively.

Cancer Subtypes

Results for the 4 most common cancer subtypes in the study population are shown in Table 2. Estimated HRs for colorectal cancer incidence and colorectal cancer mortality in the folic acid vs non–folic acid groups were close to 1. For the other cancer subtypes, estimated HRs for folic acid vs non–folic acid groups were more than 1, but 99% CIs included the value of 1.

There were 56 cases of lung cancer in the folic acid groups compared with 36 cases in the non–folic acid groups (HR, 1.59; 99% CI, 0.92-2.75). Of all patients diagnosed with lung cancer, 64 (69.6%)

Table 2. Cancer and Mortality Outcomes and Hazard Ratios[a]

Outcome	Total No.	No. of Cases (Rate per 1000 Observation-Years)				HR (95% CI)	
		Folic Acid + Vitamins B_{12} and B_6 (n = 1708)	Folic Acid + Vitamin B_{12} (n = 1703)	Vitamin B_6 (n = 1705)	Placebo (n = 1721)	Folic Acid vs Non–Folic Acid Groups	Vitamin B_6 vs Non–Vitamin B_6 Groups
Cancer incidence[b]	629	172 (17.3)	169 (16.7)	151 (14.9)	137 (13.3)	1.21 (1.03-1.41)	1.07 (0.92-1.26)
Colorectal cancer	95	25 (2.5)	22 (2.2)	26 (2.6)	22 (2.1)	1.00 (0.59-1.69)[c]	1.18 (0.69-2.00)[c]
Lung cancer	92	31 (3.1)	25 (2.5)	16 (1.6)	20 (1.9)	1.59 (0.92-2.75)[c]	1.06 (0.62-1.82)[c]
Prostate cancer	165	45 (5.9)	45 (5.8)	36 (4.7)	39 (5.0)	1.21 (0.81-1.81)[c]	0.98 (0.66-1.46)[c]
Hematologic cancer	53	15 (1.5)	16 (1.6)	11 (1.1)	11 (1.1)	1.43 (0.70-2.93)[c]	0.98 (0.48-1.98)[c]
Other cancer	224	56 (5.6)	61 (6.0)	62 (6.1)	45 (4.4)	1.11 (0.79-1.57)[c]	1.13 (0.80-1.60)[c]
Cancer mortality[d]	236	63 (6.1)	73 (7.0)	49 (4.7)	51 (4.8)	1.38 (1.07-1.79)	0.92 (0.71-1.19)
Colorectal cancer	24	3 (0.3)	9 (0.9)	5 (0.5)	7 (0.7)	1.02 (0.36-2.91)[c]	0.51 (0.17-1.55)[c]
Lung cancer	75	26 (2.5)	19 (1.8)	13 (1.2)	17 (1.6)	1.53 (0.83-2.80)[c]	1.10 (0.61-2.00)[c]
Prostate cancer	28	5 (0.6)	10 (1.2)	6 (0.8)	7 (0.9)	1.15 (0.43-3.06)[c]	0.67 (0.25-1.82)[c]
Hematologic cancer	19	5 (0.5)	7 (0.7)	2 (0.2)	5 (0.5)	1.75 (0.51-5.94)[c]	0.59 (0.17-2.02)[c]
Other cancer	90	24 (2.3)	28 (2.7)	23 (2.2)	15 (1.4)	1.39 (0.80-2.41)[c]	1.11 (0.65-1.91)[c]
Noncancer mortality	785	218 (21.1)	194 (18.5)	192 (18.4)	181 (17.0)	1.12 (0.97-1.29)	1.11 (0.97-1.28)
All-cause mortality	1021	281 (27.2)	267 (25.5)	241 (23.0)	232 (21.8)	1.18 (1.04-1.33)	1.06 (0.94-1.20)

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Primary end points were cancer incidence, cancer mortality, and all-cause mortality throughout the year 2007. HRs were estimated by Cox proportional hazard regression, unadjusted, stratified by trial. Non–folic acid groups or non–vitamin B_6 groups were the reference category. Folic acid groups were assigned to treatment with folic acid plus vitamin B_{12} or folic acid plus vitamin B_{12} in combination with vitamin B_6; non–folic acid groups were assigned to treatment with vitamin B_6 alone or placebo. Vitamin B_6 groups were assigned to treatment with vitamin B_6 alone or in combination with folic acid plus vitamin B_{12}; non–vitamin B_6 groups were assigned to treatment with folic acid plus vitamin B_{12} or placebo. For incidence and mortality of prostate cancer, rates and HRs are calculated among men.

[b] Cancer incidence included any new cancer, except nonmelanoma skin cancer. Only the first of new cancers after randomization in each individual are included. Colorectal cancer denotes cancer classified with *International Statistical Classification of Diseases, 10th Revision* (*ICD-10*) codes C18 to C21. Lung cancer denotes cancer classified with *ICD-10* code C34. Prostate cancer denotes cancers classified with *ICD-10* code C61. Hematologic cancer denotes cancers classified with *ICD-10* codes C80 to C96.

[c] 99% CIs.

[d] Deaths classified with *ICD-10* codes C00 to C97 as underlying cause of death.

were current smokers, 22 (23.9%) were former smokers, and 6 (6.5%) were never smokers at trial entry. After removal of the lung cancer cases, HR for cancer incidence in folic acid vs non–folic acid groups was 1.16 (95% CI, 0.98-1.37).

Subgroups

TABLE 3 shows the primary end points for folic acid vs non–folic acid groups across patient characteristics. Results were consistent in both trial populations, among patients aged younger than or older than the median (62.5 years), in both sexes, among never and ever smokers, and among patients with baseline serum folate levels of less than or more than the median (3.9 ng/mL) (all *P* for interaction ≥ .06). HRs for folic acid vs non–folic acid groups were higher among individuals with TT genotype than among those with CC or CT genotypes of the *MTHFR* 677C>T polymorphism. For cancer mortality, we observed a statistically significant interaction between TT vs CC or CT genotypes and folic acid plus vitamin B_{12} treatment (*P* = .03).

Primary End Points by Serum Folate or Cobalamin Levels

eTable 2 (available at http://www.jama.com) shows the primary end points across strata defined by serum folate and serum cobalamin levels measured during study treatment. Participants in the second serum folate quartile (range, 3.81-10.56 ng/mL) or in the second cobalamin quartile (range, 456.4-595.9 pg/mL) had the lowest cancer incidence, cancer mortality, and all-cause mortality through extended follow-up; therefore, quartile 2 was used as the reference category. HRs were statistically significantly higher for participants in the fourth serum folate quartile (>27.66 ng/mL) compared with those in the second folate quartile. There were no such differences across quartiles of serum cobalamin.

COMMENT

We investigated cancer and mortality outcomes after extended follow-up of 6837 individuals who participated in 2 similar randomized B vitamin trials. Study treatment with 0.8 mg/d of folic acid and 0.4 mg/d of vitamin B_{12} during a median of 39 months was associated with increased cancer incidence and cancer mortality after an additional median of 38 months of posttrial follow-up. These findings were mainly driven by increased lung cancer incidence. Furthermore, folic acid plus vitamin B_{12} treatment was associated with higher all-cause mortality. The latter finding was driven by the higher cancer mortality, but also by statistically nonsignificant higher noncancer mortality.

Our study is the first to our knowledge to report results from extended follow-up of trial participants after years of treatment with folic acid and other B vitamins. In this large well-described population, adherence was high and corroborated by substantial increase in B vitamin concentrations during study treatment. Loss to follow-up was minimal (1.3%) and ascertainment of outcomes close to complete by linkage to the population-based Cancer Registry[23] and Cause of Death Registry.[22]

Table 3. Hazard Ratios for the Primary End Points in Subgroups[a]

		Folic Acid vs Non–Folic Acid Groups					
		Cancer Incidence		Cancer Mortality		All-Cause Mortality	
Characteristics	Total No.	Hazard Ratio (95% CI)	*P* Value[b]	Hazard Ratio (95% CI)	*P* Value[b]	Hazard Ratio (95% CI)	*P* Value[b]
Trial							
NORVIT	3749	1.29 (1.05-1.58)	.31	1.29 (0.95-1.76)	.42	1.19 (1.03-1.37)	.74
WENBIT	3088	1.09 (0.85-1.40)		1.62 (1.01-2.60)		1.13 (0.88-1.46)	
Age, y							
<62.5	3419	1.34 (1.00-1.78)	.37	2.20 (1.23-3.91)	.06	1.24 (0.94-1.65)	.46
≥62.5	3418	1.14 (0.94-1.37)		1.19 (0.89-1.59)		1.11 (0.97-1.28)	
Sex							
Male	5227	1.27 (1.07-1.51)	.17	1.36 (1.02-1.81)	.84	1.14 (0.99-1.31)	.40
Female	1610	0.96 (0.67-1.37)		1.46 (0.80-2.68)		1.27 (1.00-1.62)	
Ever smoker							
No	1938	1.01 (0.75-1.37)	.18	1.21 (0.71-2.07)	.55	1.22 (0.98-1.52)	.63
Yes	4885	1.29 (1.07-1.55)		1.45 (1.08-1.94)		1.14 (0.99-1.33)	
Baseline serum folate, ng/mL							
<3.9	3385	1.23 (0.99-1.52)	.91	1.47 (1.04-2.07)	.80	1.10 (0.93-1.29)	.19
≥3.9	3388	1.21 (0.96-1.53)		1.37 (0.92-2.05)		1.30 (1.07-1.57)	
MTHFR 677 genotype							
CC	3294	1.08 (0.86-1.36)	.30[c]	1.49 (1.03-2.17)	.03[c]	1.21 (1.01-1.44)	.06[c]
CT	2707	1.24 (0.97-1.60)		1.08 (0.72-1.62)		1.08 (0.89-1.31)	
TT	535	1.55 (0.90-2.68)		4.57 (1.55-13.5)		1.79 (1.14-2.81)	

Abbreviations: CI, confidence interval; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; NORVIT, Norwegian Vitamin Trial; WENBIT, Western Norway B Vitamin Intervention Trial.
SI conversion factor: To convert serum folate to nmol/L, multiply by 2.266.
[a] Folic acid groups were assigned to treatment with folic acid plus vitamin B_{12} or folic acid plus vitamin B_{12} in combination with vitamin B_6; non–folic acid groups were assigned to treatment with vitamin B_6 or placebo. Hazard ratios were estimated by Cox proportional hazard regression, unadjusted, stratified by trial. Non–folic acid groups were the reference category.
[b] For interaction between folic acid plus vitamin B_{12} treatment and the subgroup characteristic.
[c] For interaction between *MTHFR* 677 TT and CC or CT genotypes.

Because use of vitamin supplements was modest (23%) at trial entry and there is no folic acid fortification in foods in Norway, baseline serum folate levels were lower than in populations from areas where use of vitamin supplements is more widespread[26] and fortification is voluntary[27,28] or mandatory.[12] The intervention dose of 0.8 mg/d of folic acid was 4 to 6 times higher than the average dose delivered by the mandatory fortification in the United States[29] and twice the recommended daily allowance.[30] Still, the intervention dose was below the tolerable upper intake level of 1 mg/d as set by the US Institute of Medicine.[30] Our findings are therefore relevant across folic acid intake readily obtained from consumption of fortified foods and dietary supplements.

This study has several limitations. First, we do not have data on participants' family history of cancer or on occupational or environmental exposure to cancer-promoting factors. However, due to the large sample size, we expected that these risk factors were evenly distributed across the randomly assigned treatment groups. Second, we do not have information on the use of B vitamin supplements during posttrial follow-up. Because patients were discouraged from such supplement use when trial results were available, we assumed that this was moderate with similar proportions of posttrial B vitamin users across initial treatment groups. Third, the study design implied that all patients assigned to folic acid treatment also received vitamin B_{12}. However, the observed associations between the primary end points and vitamin concentration measured during study treatment were confined to serum folate, suggesting that the adverse effects were mediated by folic acid.

Experimental findings suggest that excess folic acid may stimulate the growth of established neoplasms (ie, the so-called acceleration phenomenon).[9,10] There is also the question of potential adverse effects of circulating unmetabolized folic acid.[13,31] A recent cross-sectional study[32] reported reduced natural killer cell cytotoxicity associated with folic acid in plasma. Thus, folic acid may impair cancer immune defense.[32]

Epidemiological evidence suggests that large relative increase in the incidence of solid cancers in humans over a short period by chemical causes is unlikely.[33] However, it is plausible that folic acid given for a median of 39 months may have influenced growth in cancers that were silent at baseline or during trials,[9,10] leading to excess subsequent clinical surfacing and diagnosis in the folic acid groups during extended follow-up.

The observed risk modification by the *MTHFR* 677C>T polymorphism may reflect its pronounced effect on the metabolic distribution of folate species. In individuals with the TT genotype, more of the intracellular folate is retained as 5,10-methylenetetrahydrofolate needed for nucleotide biosynthesis.[34,35] Folic acid treatment may further expand the 5,10-methylenetetrahydrofolate pool and thereby enhance DNA replication and neoplastic growth in these individuals.

The high lung cancer incidence in the study population could readily be explained by the high percentage of former and current smokers. However, the higher incidence observed in the folic acid groups cannot be explained by imbalance in baseline smoking habits, because there were fewer smokers in these groups but no difference in baseline cotinine levels among smokers across the groups. Epidemiological studies have demonstrated no associations between intakes of folate or folic acid and lung cancer risk[3,4]; therefore, our findings need confirmation in other populations.

Lack of association between folic acid treatment and colorectal cancer outcomes in this study is noteworthy and may reflect the postulated dual effects of folate/folic acid on colorectal carcinogenesis.[9,10] For prostate cancer outcomes, estimated HRs were more than 1 for the folic acid vs non–folic acid groups, but although we observed 5 times as many prostate cancer cases (n=165) as in the Aspirin/Folate Polyp Prevention Study[36] (n=33), we found no statistically significant association between folic acid treatment and these outcomes.

Reports on cancer outcomes from other homocysteine-lowering B vitamin trials do not support our findings.[37,38] Notably, these trials used larger folic acid doses and had longer in-trial follow-up than the NORVIT and WENBIT trials. However, 72% of participants in the Heart Outcomes Preventive Evaluation (HOPE) 2 study[37] and all participants in the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS)[38] resided in North America where fortification had been implemented before trial entry. Therefore, these participants had considerably higher circulating folate concentrations at baseline,[37,38] which may have obscured effects from the intervention. Moreover, the HOPE 2 and WAFACS populations had fewer smokers (11.5 and 11.9%, respectively)[37,38] than the NORVIT and WENBIT populations did (39.0%). Also, the prevalence of smokers in the general adult US population (approximately 20.9% in 2005)[39] is lower than in our study population.

In conclusion, combined analyses and extended follow-up of 2 vitamin B intervention trials among patients with ischemic heart disease in Norway, where there is no folic acid fortification, suggest that treatment with 0.8 mg/d of folic acid was associated with increased cancer incidence, cancer mortality, and all-cause mortality. Our results need confirmation in other populations and underline the call for safety monitoring following the widespread consumption of folic acid from dietary supplements and fortified foods.[1,13,40]

Author Contributions: Drs Ebbing and Arnesen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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Additional Information: Online eTable 1, eTable 2, and eFigure are available at http://www.jama.com.

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