Effect of Folic Acid and B Vitamins on Risk of Cardiovascular Events and Total Mortality Among Women at High Risk for Cardiovascular Disease

A Randomized Trial

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Context Recent randomized trials among patients with preexisting cardiovascular disease (CVD) have failed to support benefits of B-vitamin supplementation on cardiovascular risk. Observational data suggest benefits may be greater among women, yet women have been underrepresented in published randomized trials.

Objective To test whether a combination of folic acid, vitamin B_6, and vitamin B_{12} lowers risk of CVD among high-risk women with and without CVD.

Design, Setting, and Participants Within an ongoing randomized trial of antioxidant vitamins, 5442 women who were US health professionals aged 42 years or older, with either a history of CVD or 3 or more coronary risk factors, were enrolled in a randomized, double-blind, placebo-controlled trial to receive a combination pill containing folic acid, vitamin B_6, and vitamin B_{12} or a matching placebo, and were treated for 7.3 years from April 1998 through July 2005.

Intervention Daily intake of a combination pill of 2.5 mg of folic acid, 50 mg of vitamin B_6, and 1 mg of vitamin B_{12}.

Main Outcome Measures A composite outcome of myocardial infarction, stroke, coronary revascularization, or CVD mortality.

Results Compared with placebo, a total of 796 women experienced a confirmed CVD event (406 in the active group and 390 in the placebo group). Patients receiving active vitamin treatment had similar risk for the composite CVD primary end point (226.9/10 000 person-years vs 219.2/10 000 person-years for the active vs placebo group; relative risk [RR], 1.03; 95% confidence interval [CI], 0.90-1.19; $P=.65$), as well as for the secondary outcomes including myocardial infarction (34.5/10 000 person-years vs 39.5/10 000 person-years; RR, 0.87; 95% CI, 0.63-1.22; $P=.42$), stroke (41.9/10 000 person-years vs 36.8/10 000 person-years; RR, 1.14; 95% CI, 0.82-1.57; $P=.44$), and CVD mortality (50.3/10 000 person-years vs 49.6/10 000 person-years; RR, 1.01; 95% CI, 0.76-1.35; $P=.93$). In a blood substudy, geometric mean plasma homocysteine level was decreased by 18.5% (95% CI, 12.5%-24.1%; $P<.001$) in the active group (n=150) over that observed in the placebo group (n=150), for a difference of 2.27 μmol/L (95% CI, 1.54-2.96 μmol/L).

Conclusion After 7.3 years of treatment and follow-up, a combination pill of folic acid, vitamin B_6, and vitamin B_{12} did not reduce a combined end point of total cardiovascular events among high-risk women, despite significant homocysteine lowering.

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HOMOCYSTEINE LEVELS HAVE been directly associated with cardiovascular risk in observational studies[1]; and daily supplementation with folic acid, vitamin B_6, vitamin B_{12}, or a combination have been shown to reduce homocysteine levels to varying degrees in intervention studies.[2] Based on these data, several randomized trials were designed to test the hypothesis that supplementation with folic acid or B vitamins or both would prevent cardiovascular disease (CVD). However, published trials on patients with preexisting vascular disease have not demonstrated a benefit of folic acid or B vitamins on CVD risk.[3] Participants in observational studies were followed up for longer durations then participants in randomized trials[1]; therefore, it is plausible that homocysteine lowering may have had a greater effect if participants were treated and observed for longer periods of time. Meta-analyses of randomized trials sug-

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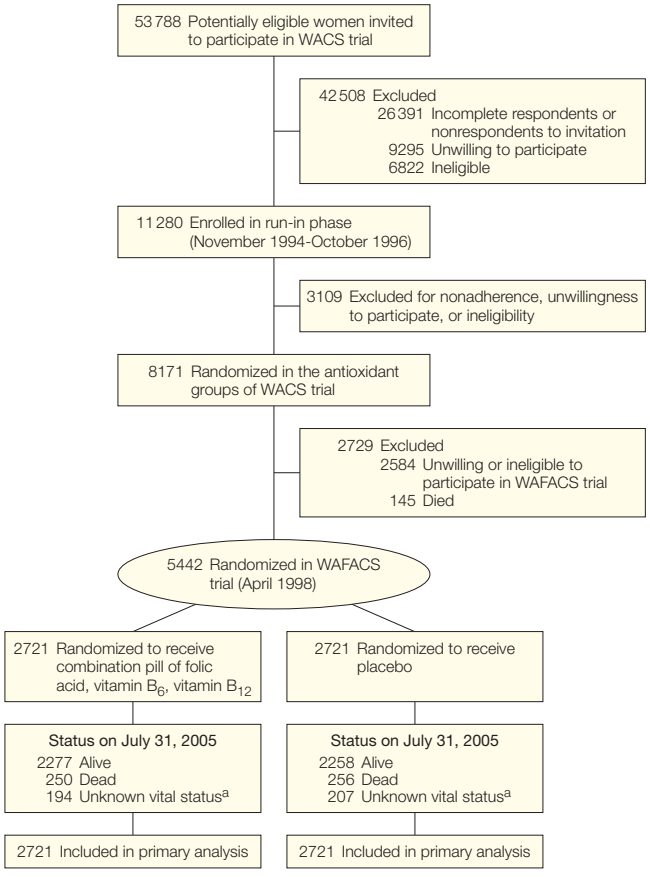
gest that benefits may be greater with longer treatment durations,[4] but the majority of published trials have 2 years or less of follow-up,[3] with only 1 trial having 5 years of follow-up.[5]

Women have been underrepresented both in observational studies and in randomized trials of homocysteine lowering, and limited data from meta-analyses of observational studies suggest that women may benefit from homocysteine lowering to a greater extent. In the most recent meta-analysis of these observational studies, a 25%-lower homocysteine level was associated with a 32% (95% confidence interval [CI], 15%-45%) lower risk of coronary heart disease in women as compared with a 15% lower risk (95% CI, 8%-21%) in men.[1] Although women have been included in meta-analyses of randomized trials,[3,6] the relative risks (RRs) for women have not been separately estimated. Given the paucity of data on women and the known influences of estrogen on homocysteine levels,[7,8] adequately powered randomized trials of homocysteine lowering in women are still needed.[9]

The present study, the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), tested whether a combination of folic acid, vitamin B_6, and vitamin B_{12} would reduce total cardiovascular events among women at high risk for the development of CVD over 7.3 years of follow-up.

Figure 1. Flow Diagram of the Folic Acid, Vitamin B_6, and Vitamin B_{12} Component of WAFACS



WACS indicates Women's Antioxidant Cardiovascular Study; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study.
[a]Mortality and morbidity information was complete for 98.9% and 98.0% of person-years of follow-up, respectively.

METHODS

Study Design

WAFACS was a randomized, double-blind, placebo-controlled trial that evaluated whether a combination pill of folic acid (2.5 mg/d), vitamin B_6 (50 mg/d), and vitamin B_{12} (1 mg/d) reduces the risk of important vascular events among high-risk women with either a history of CVD or at least 3 cardiovascular risk factors. The WAFACS trial began in 1998, when the folic acid, vitamin B_6, and vitamin B_{12} component was added to the Women's Antioxidant Cardiovascular Study (WACS), an ongoing $2 \times 2 \times 2$ factorial trial of 3 antioxidant vitamins (C, E, and beta carotene), which expanded it to a 4-group factorial trial. The factorial design allowed an examination not only of the main effects of each agent but also of potential interactions between agents. Interactions between antioxidant vitamins and folic acid were plausible because homocysteine lowering might have antioxidant effects.[10] Details of the overall trial design[11] and the results for the antioxidant group have been reported previously.[12] This report describes the CVD results of the folic acid, vitamin B_6, and vitamin B_{12} combination pill treatment group (FIGURE 1).

The study was sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health. The study vitamins and matching placebo were provided by BASF Corporation, Mount Olive, New Jersey. The trial was approved by the institutional review board of the Brigham and Women's Hospital, Boston, Massachusetts, and all patients provided written informed consent. An external independent data and safety monitoring board monitored the safety of the participants and the overall quality and scientific integrity of the study.

Study Population

In the WACS parent trial, 8171 female health professionals throughout the United States were randomized in a $2 \times 2 \times 2$ factorial design between June 1995 and October 1996 to receive vitamin C (500 mg/d), vitamin E (600 IU every other day), and beta carotene (50 mg every other day) vs respective matching placebos, yielding 8 treatment groups. Of these women, 5922 (72.5%) returned a blood sample at the beginning of the trial, prior to the initiation of folic acid fortification; and 98.8% completed a semiquantitative food-frequency questionnaire, which was used to assess baseline dietary intake including folate.

Women were eligible for WACS if they were aged 40 years or older, postmenopausal or had no intention of becoming pregnant, and had a reported history of CVD or had at least 3 cardiac risk factors. CVD was defined as a reported history of myocardial infarction (MI), stroke, coronary or peripheral revascularization, angina pectoris, or transient ischemic attack. Qualifying cardiac risk factors were diagnosed hypertension, high cholesterol, diabetes mellitus, parental history of premature MI (ie, younger than 60 years), obesity (body mass index $\geq$30 kg [calculated as weight in kilograms divided by height in meters squared]), and current cigarette use.[12] Women were excluded if they had a history of cancer (excluding nonmelanoma skin cancer) within the past 10 years, any serious non-CVD illness, or were currently using warfarin or other anticoagulants.

To be eligible for the folic acid, vitamin B_6, and vitamin B_{12} component, potential participants in the ongoing 8-group trial had to be additionally willing to forgo individual supplements of folic acid, vitamin B_6, and vitamin B_{12} at levels beyond the US recommended daily allowance (RDA) of 400 µg of folic acid, 2 mg of vitamin B_6, or 6 µg of vitamin B_{12} during the trial.[2] Multivitamin use at or below these RDA levels was allowed. In April 1998, 5442 of these women were additionally randomized to receive a combination pill containing 2.5 mg of folic acid, 50 mg of vitamin B_6, and 1 mg of vitamin B_{12} (active treatment) or a matching placebo daily, thus creating a total of 16 distinct treatment groups. All study investigators, personnel, and participants were unaware of the participants' treatment assignments.

Follow-up Procedures

Following randomization and annually thereafter, participants were mailed monthly calendar packs containing active agents or placebos. The participants were followed up annually with questionnaires on adherence, use of nonstudy supplements, and occurrence of major illnesses or adverse events. Written permission for medical records was sought from participants who reported cardiovascular end points or from the next of kin in case of death. Death certificates were also obtained. An end points committee of physicians who were blinded to randomized treatment assignment adjudicated all primary and secondary cardiovascular outcome events.

Study medications and end point ascertainment were continued in a blinded fashion until the scheduled end of the trial, July 31, 2005. Follow-up and validation of reported end points were completed in July 2006 for a follow-up duration of 7.3 years. At the scheduled end of the trial on July 31, 2005, morbidity and mortality follow-up was 92.6% complete. If counted in terms of person-time, mortality and morbidity information was complete for 98.9% and 98.0% of person-years of follow-up, respectively.

Blood Substudy

Women in WAFACS who provided a baseline blood sample in 1996, prior to the initiation of background dietary folic acid fortification in the US food supply in 1998, were eligible for this preplanned substudy. From the 2596 eligible participants, 300 (150 in the active treatment and 150 in the placebo group) provided a blood sample at the end of randomized treatment. These women were randomly selected from participants who were adherent with study medications. Plasma levels of folate (chemiluminescence method using the 2010 Elecsys autoimmunoanalyzer, Roche Diagnostics, Basel, Switzerland) and homocysteine (enzymatic assay using the Hitachi 917 analyzer, Roche Diagnostics, Basel, Switzerland) were measured in baseline and follow-up samples in a blinded fashion in the same analytical run.

Study Outcomes

The primary outcome was a combined end point of cardiovascular morbidity and mortality, which included incident MI, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention), and cardiovascular mortality. The individual components of total MI, total stroke, and total coronary heart disease events (MI, coronary revascularization, and death from coronary heart disease) were prespecified secondary end points.

An MI was confirmed if symptoms met World Health Organization criteria and if the event was associated with either diagnostic electrocardiogram changes or elevated cardiac enzymes. Coronary revascularization was confirmed by medical record review. Confirmed stroke was defined as a new neurologic deficit of sudden onset that persisted for more than 24 hours or until death within 24 hours. Clinical information, computed tomographic scans, and magnetic resonance images were used to distinguish hemorrhagic from ischemic events. Coronary revascularization was confirmed if a coronary artery bypass grafting or percutaneous coronary intervention was documented in the medical record. Death due to cardiovascular cause was confirmed by examination of autopsy reports, death certificates, medical records, and information obtained from the next of kin or other family members. Death from any cause was confirmed by the end points committee on the basis of a death certificate. Only confirmed end points were included in

these analyses, except for total mortality, which included an additional 43 reported deaths.

Power Calculations

Power calculations were performed under the assumption that all 4 agents would have beneficial effects on CVD, which would be additive (on the log scale) when used in combination. This method affects the power calculations by reducing the incidence in all exposed groups and provides a conservative estimate of the study's power to detect the effects of each agent. We assumed additive 10% risk reductions for each of the antioxidant vitamins and the folic acid, vitamin B_6, and vitamin B_{12} combination pill. It was estimated that the 5442 women who were randomized would provide 82% power to detect an observed 20% reduction in the primary end point.

Statistical Analysis

Baseline characteristics were compared by randomized groups using *t* tests, χ^2 tests for proportions, and tests for trend for ordinal categories. Primary analyses were performed on an intent-to-treat basis, including all 2721 randomized participants in each treatment group, as randomized. For both the primary and secondary analyses, person-time was calculated until the first confirmed end point specified by the analysis or to the end of the trial if no end point occurred. Kaplan-Meier curves were used to estimate cumulative incidence over time by randomized treatment group, and the log-rank test was used to compare survival curves. Cox proportional hazards models were used to calculate RRs expressed as hazard ratios and 95% CIs, after adjustment for age and other randomized treatment assignments (vitamin E, vitamin C, and beta carotene). The proportionality assumption was tested using an interaction term for treatment with log time, and was met for each of the primary and secondary analyses. To examine the effect of nonadherence, a post hoc sensitivity analysis censored participants when they stopped taking at least two-thirds of their study medications, reported taking outside supplements, or were missing adherence information.

Prespecified subgroup analyses according to antioxidant treatment assignment(s), presence or absence of prior CVD, dietary folic acid intake, smoking, diabetes, aspirin, hormone therapy, and multivitamin use were performed using stratified Cox proportional hazards models. These analyses used baseline exposure assessments and were restricted to participants with nonmissing subgroup data at baseline. Additional exploratory subgroup analyses were conducted to evaluate the consistency of the results. Tests for effect modification by subgroup used interaction terms between subgroup indicators and randomized assignment, with a test for trend for ordinal subgroup categories. The raw distributions and median values of plasma homocysteine and folate levels in the blood substudy were compared using the nonparametric Wilcoxon rank sum test. For homocysteine, geometric means were compared after natural logarithmic transformation to compare differences between treatment

Table 1. Baseline Characteristics of WAFACS and Blood Substudy Participants

Characteristic	Active Group, No. (%) (n = 2721)[a]	Placebo Group, No. (%) (n = 2721)[a]
Age, mean (SD), y	62.8 (8.8)	62.8 (8.8)
Age, y		
40-54	582 (21.4)	584 (21.5)
55-64	990 (36.4)	970 (35.6)
≥ 65	1149 (42.2)	1167 (42.9)
Prior cardiovascular disease[b]	1764 (64.8)	1728 (63.5)
Risk factors		
Hypertension[c]	2360 (86.7)	2335 (85.8)
Elevated cholesterol[d]	2118 (77.8)	2150 (79.0)
Body mass index ≥30[e]	1341 (49.3)	1349 (49.6)
Parental history of myocardial infarction[f]	1056 (38.9)	1097 (40.5)
Alcohol intake, ≥1 glass/wk[g]	897 (33.0)	889 (32.7)
Diabetes	570 (21.0)	574 (21.1)
Current smoking	311 (11.4)	334 (12.3)
Current medication use		
Aspirin[h]	1446 (51.1)	1385 (48.9)
Hormone therapy	1320 (48.5)	1329 (48.8)
Lipid-lowering drugs	914 (33.6)	938 (34.5)
β-Blockers	684 (26.6)	697 (27.0)
Angiotensin-converting enzyme inhibitors	627 (24.3)	668 (25.8)
Multivitamins[i]	616 (22.6)	631 (23.2)
Median dietary intake (25th-75th percentiles)[j]		
Folic acid, µg/d	424.4 (308.6-664.4)	438.7 (309.7-666.8)
Vitamin B_6, mg/d	2.48 (1.81-3.81)	2.54 (1.81-3.87)
Vitamin B_{12}, µg/d	7.10 (4.62-10.9)	6.98 (4.69-11.0)

Abbreviation: WAFACS: Women's Antioxidant and Folic Acid Cardiovascular Study.
[a] Column shows No. (%) for all categories except age.
[b] Reported history of myocardial infarction, stroke, coronary revascularization, angina pectoris, transient ischemic attack, carotid endarterectomy, or peripheral artery surgery.
[c] Denotes self-reported systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, self-reported physician-diagnosed hypertension, or reported treatment with medication for hypertension.
[d] Denotes self-reported high cholesterol level of 240 mg/dL or greater, self-reported physician-diagnosed high cholesterol levels, or reported treatment with cholesterol-lowering medication.
[e] Body mass index is calculated as weight in kilograms divided by height in meters squared.
[f] Parental history of myocardial infarction in father younger than 60 years of age or mother younger than 65 years of age.
[g] Denotes beer, wine, or liquor.
[h] Aspirin use at least 4 times per month.
[i] Any multivitamin use in the past month.
[j] Estimated from the semiquantitative food-frequency questionnaire.

groups. Analyses were conducted using SAS version 9 (SAS Institute, Cary, North Carolina), using 2-sided tests with a significance level of .05.

RESULTS

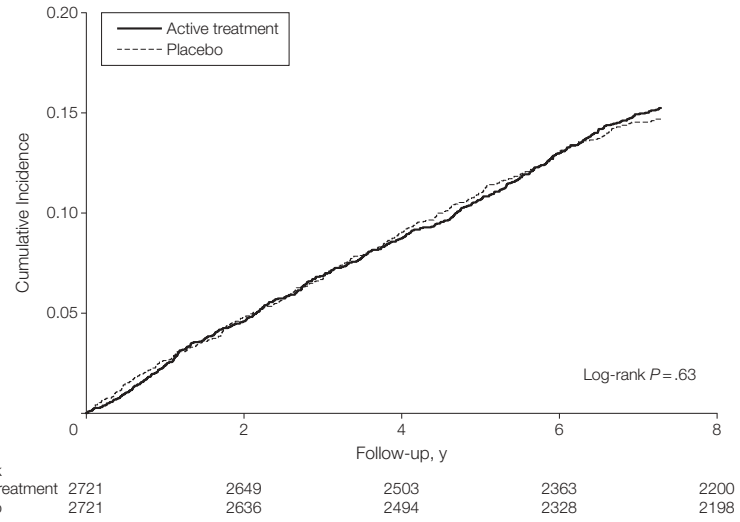
Characteristics of the Participants

In April 1998, 2721 female health professionals participating in the WACS trial were randomly assigned to active treatment with folic acid, vitamin B_6, and vitamin B_{12} and 2721 participants were assigned to placebo. Baseline characteristics at the time of randomization in 1998 and responses to the dietary questionnaire administered prior to randomization in 1996 are displayed in TABLE 1. The mean age of the population was 62.8 years and 64.2% of women had a history of CVD. There were no statistically significant differences in baseline characteristics or in dietary intake of study vitamins between the randomized groups (Table 1). The median daily dietary intake of folic acid including supplements was 432 µg, of which approximately 15% (63 µg) was estimated to be derived from dietary intake of folic acid–fortified grains. The use of permitted multivitamins with less than the RDA of folic acid, vitamin B_6, and vitamin B_{12} at least 4 days per month ranged from 19% at the beginning to 31% at the end of the trial.

Adherence and Adverse Events

Adherence was assessed through self-report on annual study questionnaires and was defined as taking at least two-thirds of the study pills. Average adherence over the course of follow-up was approximately 83% for active and placebo agents with no significant difference between active and placebo groups. Use of open-label folic acid supplements, vitamin B_6, or vitamin B_{12} supplements containing more than the RDA for at least 4 days per month ranged from 2% to 11% in the active group to 2% to 13% in the placebo group over the course of the study. There were no serious adverse events reported that were conclusively related to study interventions.

Figure 2. Cumulative Incidence of Major Cardiovascular Disease by Randomized Folic Acid and B-Vitamin Intervention in WAFACS



WAFACS indicates Women's Antioxidant and Folic Acid Cardiovascular Study. Major cardiovascular disease denotes myocardial infarction, stroke, coronary revascularization, or cardiovascular death.

Primary Analysis

During the 7.3 years of follow-up, 796 participants (14.6%) experienced a confirmed CVD event included in the primary end point, with some individuals experiencing more than 1 event. Overall, 139 MIs, 148 strokes, 508 coronary revascularization procedures, and 190 cardiovascular deaths occurred in the population over the course of the study. There was no difference in the cumulative incidence of the primary combined end point in the active vs placebo treatment groups at any time during study follow-up (FIGURE 2). A total of 406 women (14.9%) in the active treatment group and 390 (14.3%) in the placebo group experienced at least 1 cardiovascular event included in the primary end point (226.9/10 000 person-years vs 219.2/10 000 person-years). This corresponded to an overall RR of 1.03 (95% CI, 0.90-1.19; P=.65) after controlling for age and antioxidant treatment assignment (TABLE 2). There remained no evidence for a treatment effect in sensitivity analysis censoring at nonadherence (RR, 1.05; 95% CI, 0.90-1.23; P=.53) or if coronary revascularization procedures were excluded from the primary end point (RR, 0.96; 95% CI, 0.80-1.17; P=.72).

Secondary and Other Outcomes

Among the prespecified secondary cardiovascular outcomes, total coronary heart disease events occurred in 283 participants (156.5/10 000 person-years) in the active treatment group and in 280 participants (155.8/10 000 person-years) in the placebo group (RR, 1.00; 95% CI, 0.85-1.18; P=.96; Table 2). When analyzed separately, there were no significant differences for each of the components of the primary outcome including MI (34.5/10 000 person-years vs 39.5/10 000 person-years; RR, 0.87; 95% CI, 0.63-1.22; P=.42), stroke (41.9/10 000 person-years vs 36.8/10 000 person-years; RR, 1.14; 95% CI, 0.82-1.57; P=.44), and CVD mortality (50.3/10 000 person-years vs 49.6/10 000 person-years; RR, 1.01; 95% CI, 0.76-1.35; P=.93) between the active treatment and placebo groups. Also, the risk of death from any cause was similar between the active and placebo treatment groups (RR, 0.97; 95% CI, 0.81-1.15; P=.73).

Table 2. Relative Risk of Clinical Outcomes According to Treatment Assignment With Folic Acid, Vitamin B_6, and Vitamin B_{12} vs Placebo

Outcome	No. of Patients (%)		Relative Risk (95% Confidence Interval)[a]	*P* Value
	Active (n = 2721)	Placebo (n = 2721)		
Combined major cardiovascular disease[b]	406 (14.9)	390 (14.3)	1.03 (0.90-1.19)	.65
Myocardial infarction	65 (2.4)	74 (2.7)	0.87 (0.63-1.22)	.42
Stroke	79 (2.9)	69 (2.5)	1.14 (0.82-1.57)	.44
Ischemic[c]	69 (2.5)	62 (2.3)	1.10 (0.78-1.56)	.57
Hemorrhagic[c]	10 (0.4)	6 (0.2)	1.65 (0.60-4.53)	.33
Coronary revascularization[d]	253 (9.3)	255 (9.4)	0.99 (0.83-1.17)	.87
Coronary artery bypass grafting[e]	87 (3.2)	98 (3.6)	0.88 (0.66-1.17)	.38
Percutaneous coronary intervention[e]	192 (7.1)	177 (6.5)	1.08 (0.88-1.33)	.46
Cardiovascular death	96 (3.5)	94 (3.5)	1.01 (0.76-1.35)	.93
Myocardial infarction, stroke, and cardiovascular death	205 (7.5)	211 (7.8)	0.96 (0.80-1.17)	.72
Total coronary heart disease[f]	283 (10.4)	280 (10.3)	1.00 (0.85-1.18)	.96
Total mortality	250 (9.2)	256 (9.4)	0.97 (0.81-1.15)	.73

[a] Estimated from Cox proportional hazards models that adjusted for age and randomized treatment assignment to vitamin E, vitamin C, and beta carotene.
[b] The primary outcome is defined as a composite end point comprising the first of any of these events: nonfatal myocardial infarction, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention), and cardiovascular mortality.
[c] Stroke type was unknown for 1 woman in the placebo group.
[d] Composite end point comprised the first coronary artery bypass grafting or percutaneous coronary intervention.
[e] Includes all incident coronary artery bypass grafting operations and percutaneous coronary intervention, respectively.
[f] Composite end point comprised the first of any of these events: nonfatal myocardial infarction, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention), and coronary heart disease death.

Subgroup Analyses

There were no significant treatment effects with respect to the primary outcome in any of the prespecified or exploratory subgroups evaluated (TABLE 3). Of particular interest, there was a similar lack of benefit among participants without prior CVD as compared with those with prior CVD ($P=.93$ for interaction), although the trial was not powered to detect a specific benefit in this subgroup. Also, there was no evidence that either dietary folate intake or multivitamin use modified the treatment effect, although power was limited for these subgroups as well. The test for interaction was significant for treatment with angiotensin-converting enzyme inhibitors ($P=.03$); however, this was not a prespecified subgroup analysis.

With respect to the antioxidant vitamins, there was evidence for an interaction between randomized treatment assignment to vitamin C and the combination therapy with folic acid, vitamin B_6, and vitamin B_{12} on the primary end point (Table 3). As compared with those randomized to both placebos, RR for folic acid was lower among those receiving placebo vs active vitamin C ($P=.03$ for interaction). There were no other significant 2-way or 3-way interactions among the agents for the primary end point.

Effect of Supplementation vs Fortification on Folate and Homocysteine Levels

The 300 participants in the blood substudy were similar with respect to all the clinical characteristics outlined in Table 1, except that smokers (8.0% in the blood group vs 12.1% in the nonblood group; $P=.003$) and participants with a history of diabetes (16.7% in the blood group vs 21.3% in the nonblood group; $P=.06$) were underrepresented among the adherent participants in the blood substudy. The distributions of baseline and follow-up folate and homocysteine levels among 150 participants in the placebo group and 150 participants in the active group are displayed in TABLE 4. Prior to the initiation of fortification and randomization, median folate levels were similar in the active treatment group (8.9 ng/mL; interquartile range, 6.0-13.4 ng/mL) and the placebo group (8.8 ng/mL; interquartile range, 6.4-12.8 ng/mL; $P=.94$), with 34% of the study population having levels considered inadequate (<7 ng/mL). Median plasma homocysteine levels were also similar at baseline in the active treatment group (12.1 µmol/L; interquartile range, 10.2-15.0 µmol/L) as compared to the placebo group (12.5 µmol/L; interquartile range, 9.6-15.5 µmol/L; $P=.96$), with 27.7% of the study population having a level greater than 15.0 µmol/L; Table 4).

At the end of study follow-up, the median folate level increased significantly in the placebo group to 15.4 ng/mL (interquartile range, 11.5-22.6 ng/mL; $P<.001$); however, the relative increase in folate level was greater in the active treatment group, in which 49.3% of participants had a folate level greater than 40 ng/mL (the upper limit of the assay) as compared to 4.7% in the placebo group (Table 4). Despite significant increases in folate levels among the placebo group, there was no apparent reduction in homocysteine levels at the end of the study as compared with participants measured at the beginning of the study in the placebo group (median = 11.8 µmol/L; interquartile range, 9.8-14.9 µmol/L; $P=.99$). In comparison, homocysteine levels were significantly reduced in the active treatment group (median level, 9.8 µmol/L; interquartile range, 7.9-12.4 µmol/L; $P=.001$), and the number of participants with significantly elevated homocysteine levels of greater than 15 µmol/L was reduced to 10%.

In order to directly compare the degree of additional homocysteine lowering observed in the active over the placebo group, we computed the difference between treatment groups in the change in the natural logarithm of homocysteine level from baseline to follow-up, adjusting for baseline levels. The geometric mean homocysteine level was decreased by 18.5% (95% CI, 12.5%-

24.1%; $P<.001$) in the active group over that observed in the placebo group for a difference of 2.27 µmol/L (95% CI, 1.54 -2.96) from the placebo geometric mean homocysteine level of 12.28 µmol/L.

COMMENT

In this large-scale, placebo-controlled, randomized trial among high-risk women participants, we found no overall effects of a combination of folic acid, vitamin B_6, and vitamin B_{12} on the primary outcome of total CVD events over the largest number of person-years and the longest follow-up period reported to our knowledge (7.3 years). In subgroup analyses, there was no heterogeneity of treatment effect among those

Table 3. Effect of Randomized Treatment Assignment on the Primary Outcome in Prespecified and Exploratory Subgroups

Characteristic	No. of Patients[a]		No. of Events (%)[a]		Relative Risk (95% Confidence Interval)	*P* Value for Interaction
	Active	Placebo	Active	Placebo		
Overall	2721	2721	406 (14.9)	390 (14.3)	1.03 (0.90-1.19)	
Age, y						
40-54	582	584	50 (8.6)	43 (7.4)	1.17 (0.78-1.76)	.55
55-64	990	970	119 (12.0)	133 (13.7)	0.85 (0.67-1.09)	
≥65	1149	1167	237 (20.6)	214 (18.3)	1.12 (0.93-1.34)	
Prior cardiovascular disease[b]						
Yes	1764	1728	329 (18.7)	314 (18.2)	1.03 (0.89-1.21)	.93
No	957	993	77 (8.1)	76 (7.65)	1.01 (0.73-1.39)	
Diabetes						
Yes	570	574	142 (24.9)	143 (24.9)	0.99 (0.78-1.24)	.67
No	2151	2147	264 (12.3)	247 (11.5)	1.06 (0.89-1.26)	
Hypertension[c]						
Yes	2360	2335	373 (15.8)	363 (15.6)	1.01 (0.87-1.16)	.30
No	361	386	33 (9.1)	27 (7.0)	1.32 (0.80-2.20)	
Elevated cholesterol[d]						
Yes	2118	2150	340 (16.1)	331 (15.4)	1.03 (0.88-1.19)	.77
No	603	571	66 (11.0)	59 (10.3)	1.07 (0.76-1.52)	
Current smoking						
Yes	311	334	56 (18.0)	70 (21.0)	0.87 (0.61-1.24)	.34
No	2410	2387	350 (14.5)	320 (13.4)	1.07 (0.92-1.25)	
Parental history of myocardial infarction[e]						
Yes	1056	1097	165 (15.6)	167 (15.2)	1.03 (0.83-1.27)	.90
No	1656	1610	238 (14.4)	219 (13.6)	1.04 (0.87-1.26)	
Body mass index[f]						
<25	616	566	100 (16.2)	82 (14.5)	1.12 (0.84-1.50)	.41
25-<30	764	806	126 (16.5)	127 (15.8)	1.07 (0.84-1.37)	
≥30	1341	1349	180 (13.4)	181 (13.4)	0.97 (0.79-1.20)	
Alcohol intake, glass/mo[g]						
≤1	1494	1499	247 (16.5)	234 (15.6)	1.05 (0.88-1.26)	.73
>1	1227	1222	159 (13.0)	156 (12.8)	1.00 (0.80-1.25)	
Folate intake[h]						
Below median, ≤432 µg/d	1322	1263	207 (15.7)	183 (14.5)	1.07 (0.87-1.30)	.88
Above median, >432 µg/d	1263	1323	181 (14.3)	183 (13.8)	1.04 (0.85-1.28)	
Aspirin[i]						
Yes	1446	1385	270 (18.7)	244 (17.6)	1.07 (0.90-1.28)	.37
No	1274	1336	136 (10.7)	146 (10.9)	0.93 (0.73-1.17)	
β-Blockers						
Yes	684	697	125 (18.3)	123 (17.7)	1.04 (0.81-1.34)	.93
No	1887	1883	263 (13.9)	249 (13.2)	1.03 (0.87-1.23)	
Lipid-lowering drugs						
Yes	914	938	178 (19.5)	171 (18.2)	1.05 (0.85-1.30)	.83
No	1807	1783	228 (12.6)	219 (12.3)	1.02 (0.85-1.23)	
Angiotensin-converting enzyme inhibitors						
Yes	627	668	102 (16.3)	125 (18.7)	0.81 (0.63-1.06)	.03
No	1957	1920	287 (14.7)	247 (12.9)	1.15 (0.97-1.36)	
Hormone therapy						
Yes	1320	1329	172 (13.0)	191 (14.4)	0.89 (0.72-1.09)	.05
No	1401	1392	234 (16.7)	199 (14.3)	1.18 (0.97-1.42)	

(continued)

Table 3. Effect of Randomized Treatment Assignment on the Primary Outcome in Prespecified and Exploratory Subgroups (cont)

Characteristic	No. of Patients[a]		No. of Events (%)[a]		Relative Risk (95% Confidence Interval)	*P* Value for Interaction
	Active	Placebo	Active	Placebo		
Multivitamins[j]						
Yes	616	631	94 (15.3)	87 (13.8)	1.09 (0.81-1.46)	.67
No	2105	2088	312 (14.8)	302 (14.5)	1.02 (0.87-1.19)	
Vitamin C						
Active	1349	1356	213 (15.8)	177 (13.1)	1.21 (0.99-1.48)	.03
Placebo	1372	1365	193 (14.1)	213 (15.6)	0.89 (0.73-1.08)	
Vitamin E						
Active	1364	1356	190 (13.9)	198 (14.6)	0.94 (0.77-1.15)	.22
Placebo	1357	1365	216 (15.9)	192 (14.1)	1.12 (0.93-1.37)	
Beta carotene						
Active	1358	1349	199 (14.7)	203 (15.1)	0.96 (0.79-1.17)	.32
Placebo	1363	1372	207 (15.2)	187 (13.6)	1.11 (0.91-1.35)	

[a] Numbers do not always sum to group totals due to missing information for some subgroup variables.
[b] Denotes reported history of myocardial infarction, stroke, coronary revascularization, angina pectoris, transient ischemic attack, carotid endarterectomy, or peripheral artery surgery.
[c] Denotes self-reported systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, self-reported physician-diagnosed hypertension, or reported treatment with medication for hypertension.
[d] Denotes self-reported high cholesterol, cholesterol level of 240 mg/dL or greater, self-reported physician-diagnosed high cholesterol levels, or reported treatment with cholesterol-lowering medication.
[e] Parental history of myocardial infarction in father younger than age 60 years or mother younger than age 65 years.
[f] Body mass index is calculated as weight in kilograms divided by height in meters squared.
[g] Denotes beer, wine, or liquor.
[h] Estimated from the semiquantitative food-frequency questionnaire.
[i] Aspirin use at least 4 times per month.
[j] Any multivitamin use in the past month.

Table 4. Distribution of Plasma Levels of Folate and Homocysteine in 300 Participants in the Blood Substudy at Baseline Prior to Randomization and at the End of Treatment and Follow-up

Characteristic	Baseline, No. (%)		Follow-up, No. (%)	
	Placebo (n = 150)	Active (n = 150)	Placebo (n = 150)	Active (n = 150)
Folate, ng/mL				
<7	52 (34.7)	49 (32.7)	2 (1.33)	0
7-<15	71 (47.3)	80 (53.3)	69 (46.0)	1 (0.67)
15-<25	22 (14.7)	19 (12.7)	54 (36.0)	21 (14.0)
25-40	4 (2.67)	2 (1.33)	18 (12.0)	54 (36.0)
>40	1 (0.67)	0	7 (4.67)	74 (49.3)
Homocysteine, μmol/L[b]				
<9	30 (20.0)	21 (14.0)	21 (14.0)	64 (42.7)
9-<12	40 (26.7)	52 (34.7)	57 (38.0)	43 (28.7)
12-<15	35 (23.3)	39 (26.0)	35 (23.3)	28 (18.7)
≥15	45 (30.0)	38 (25.3)	37 (24.7)	15 (10.0)

SI conversion: for folate, multiply by 2.266 for nmol/L units.

above or below the median of folate intake and among women with or without prior vascular disease. A possible interaction with randomized vitamin C and with nonrandomized angiotensin-converting enzyme inhibitor treatment on the primary outcome was observed; however, due to the large number of comparisons, these results could have been due to chance.

These null results for women are consistent with those previously reported in randomized trials composed primarily of men with preexisting vascular disease.[3] In the Heart Outcomes Prevention Evaluation Trial (HOPE) 2,[5] the same regimen of folic acid, vitamin B_6, and vitamin B_{12} failed to significantly lower the risk of a combined outcome of death from cardiovascular causes, MI, and stroke among 5522 patients with prior vascular disease over an average of 5 years. Although there was a significant reduction in the secondary end point of stroke in this trial (RR, 0.75; 95% CI, 0.59-0.97), a reduction in stroke was not found with a similar B-vitamin regimen in the Vitamins Intervention for Stroke Prevention (VISP) trial,[13] in which stroke was the primary end point. The HOPE-2 trial also reported an increased risk of hospitalization for unstable angina (RR, 1.24; 95% CI, 1.04-1.49) among participants randomized to active treatment.

In the Norwegian Vitamin (NORVIT) Trial,[14] a 2×2 factorial trial of vitamin B_6 and a combination pill of folic acid and vitamin B_{12} among recent post MI patients, a marginally significant 22% (95% CI, 0%-50%; $P = .05$) increased risk of recurrent MI, stroke, and sudden death was found among participants assigned to the combination of folic acid, B_{12} and vitamin B_6 over a median follow-up of 40 months. Smaller studies among patients postcoronary intervention have found both decreased[15] and increased[16] rates of restenosis among patients treated with B-vitamin regimens. In the present study, we found no evidence for a benefit on stroke or any evidence for harm regarding the primary composite end point or any of the individual secondary end points including coronary revascularization.

Concerns have been raised regarding the power of this trial and other current trials to adequately test the homocysteine hypothesis[17,18]; especially in countries where folic acid fortification of the food supply has taken place.[19] Observational studies suggested that fortification of grain products with 140 µg of folic acid per 100 g, which began in 1996 and became mandatory in the United States and Canada by 1998, significantly reduced mean plasma homocysteine concentrations among middle-aged individuals, and decreased the prevalence of high homocysteine levels (>13 µmol/L) from 29.8% to 18.7%.[20] Based on these data, it has been estimated that additional B-vitamin supplementation in such a fortified population would only lower homocysteine levels by about 10%.[21] In the present trial, a greater but still somewhat modest lowering of homocysteine (18.5% reduction) was observed. Although almost complete elimination of low folate concentrations was observed (<7 ng/mL) in the placebo group after fortification, there was no apparent reduction over time on homocysteine concentrations or on the prevalence of elevated homocysteine levels (≥15 µmol/L) in this population. Although homocysteine levels were unchanged in the placebo group, folic acid fortification likely prevented further elevations in homocysteine levels that would have otherwise taken place due to the aging of the population.

Initially, epidemiologic studies, which were primarily retrospective and cross-sectional, suggested that reducing plasma homocysteine by 5 µmol/L would decrease vascular risk by one-third.[22] However, a more recent meta-analysis of prospective observational studies suggested that risk reductions associated with homocysteine lowering would be much more modest.[1] In these studies, a 25%-lower homocysteine level, approximately 3 µmol/L, was associated with 11% reduction in coronary heart disease risk and a 19% lower stroke risk.[1] The expected reductions in cardiovascular events may have been even lower in this trial where the reduction in homocysteine levels was only 18.5% (2.3 µmol/L) among adherent patients. Although this trial was not initially powered to detect such modest reductions in cardiovascular events, the 95% CIs for the primary end point excludes with reasonable certainty reductions as low as 10% in the combined end point of total cardiovascular events. However, such modest plausible reductions in the individual secondary end points of stroke, MI, and cardiovascular death cannot be excluded even in a trial of this size.

There are several caveats and/or limitations to this study, which warrant consideration. First, the study was conducted in a population of health professionals, who were at a relatively low risk of folate deficiency. These participants were allowed to take the RDA of folic acid and B-vitamins and were also exposed to folic acid–fortified grain products during the course of the trial. Although the blood study suggests that a significant proportion of the participants were folate deficient at the beginning of the trial, this was virtually eliminated over the course of study. Therefore, we cannot rule out the possibility that this same regimen may have resulted in an even greater reduction in homocysteine levels in a more folate-deficient population, which might have translated into an observable benefit on cardiovascular events. Alternatively, the optimal dose of these vitamins may actually be lower than the dose tested in this and other trials, and the potential for harm at higher doses has been raised by other studies.[14] Also, since homocysteine levels were only measured in 5% of the sample, we were unable to determine whether women with high homocysteine levels at baseline may have benefited to a greater extent both with respect to homocysteine lowering and cardiovascular events.

Although this trial was the first to include a significant number of participants without prior cardiovascular disease (n=1950), power is still insufficient to exclude moderate treatment effects in primary prevention. Also, morbidity and mortality follow-up rates in this high-risk population were lower than in other trials of primary prevention using similar methodology[23]; and it is thus plausible that a larger primary prevention population with higher rates of follow-up might have demonstrated a benefit. However, since the lost to follow-up rates did not differ between the 2 treatment groups in this study, they are unlikely to account for the null findings observed. These remaining issues, along with the hypotheses regarding possible novel drug interactions with vitamin C and angiotensin enzyme-converting inhibitors raised by the subgroup analyses of this study, warrant further investigation in future studies.

In summary, in the WAFACS trial, a combination pill of 2.5 mg of folic acid, 50 mg of vitamin B_6, and 1 mg vitamin B_{12} had no beneficial or adverse effects on a combined outcome of total major cardiovascular events in a high-risk population of women with prior cardiovascular disease or 3 or more coronary risk factors over 7.3 years of follow-up. Our results are consistent with prior randomized trials performed primarily among men with established vascular disease[3] and do not support the use of folic acid and B vitamin supplements as preventive interventions for CVD in these high-risk–fortified populations.

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Study concept and design: Albert, Gaziano, Manson.
Acquisition of data: Albert, Gaziano, Zaharris, MacFadyen, Danielson, Buring, Manson.
Analysis and interpretation of data: Albert, Cook, Manson.
Drafting of the manuscript: Albert.
Critical revision of the manuscript for important intellectual content: Albert, Cook, Gaziano, Zaharris, MacFadyen, Danielson, Buring, Manson.
Statistical expertise: Cook.
Obtained funding: Albert, Gaziano, Manson.
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Study supervision: Albert, MacFadyen, Danielson, Manson.
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