LESS IS MORE

Effects of Lowering Homocysteine Levels With B Vitamins on Cardiovascular Disease, Cancer, and Cause-Specific Mortality

Meta-analysis of 8 Randomized Trials Involving 37 485 Individuals

Robert Clarke, FRCP; Jim Halsey, BSc; Sarah Lewington, DPhil; Eva Lonn, MD; Jane Armitage, FRCP; JoAnn E. Manson, MD, DrPH; Kaare H. Bønaa, MD; J. David Spence, MD; Ottar Nygård, MD; Rex Jamison, MD; J. Michael Gaziano, MD; Peter Guarino, PhD; Derrick Bennett, PhD; Fraz Mir, MD; Richard Peto, FRS; Rory Collins, FRCP; for the B-Vitamin Treatment Trialists' Collaboration

Elevated plasma homocysteine levels have been associated with higher risks of cardiovascular disease, but the effects on disease rates of supplementation with folic acid to lower plasma homocysteine levels are uncertain. Individual participant data were obtained for a meta-analysis of 8 large, randomized, placebo-controlled trials of folic acid supplementation involving 37 485 individuals at increased risk of cardiovascular disease. The analyses involved intention-to-treat comparisons of first events during the scheduled treatment period. There were 9326 major vascular events (3990 major coronary events, 1528 strokes, and 5068 revascularizations), 3010 cancers, and 5125 deaths. Folic acid allocation yielded an average 25% reduction in homocysteine levels. During a median follow-up of 5 years, folic acid allocation had no significant effects on vascular outcomes, with rate ratios (95% confidence intervals) of 1.01 (0.97-1.05) for major vascular events, 1.03 (0.97-1.10) for major coronary events, and 0.96 (0.87-1.06) for stroke. Likewise, there were no significant effects on vascular outcomes in any of the subgroups studied or on overall vascular mortality. There was no significant effect on the rate ratios (95% confidence intervals) for overall cancer incidence (1.05 [0.98-1.13]), cancer mortality (1.00 [0.85-1.18]) or all-cause mortality (1.02 [0.97-1.08]) during the whole scheduled treatment period or during the later years of it. Dietary supplementation with folic acid to lower homocysteine levels had no significant effects within 5 years on cardiovascular events or on overall cancer or mortality in the populations studied. *Arch Intern Med. 2010;170(18):1622-1631*

Elevated plasma total homocysteine has been suggested as a potentially modifiable risk factor for coronary heart disease (CHD), stroke, and other occlusive vascular conditions.[1-4] High rates of cardiovascular disease (CVD) in untreated children with homocystinuria (a rare autosomal recessive condition with plasma homocysteine levels greater than 100 µmol/L [to convert to milligrams per liter, divide by 7.397]) prompted the homocysteine hypothesis, which states that moderate elevations of homocysteine levels may be relevant to CVD in the general population.[5] Early observational studies reported that patients with CHD or stroke have 3- to 5-µmol/L higher measured homocysteine levels than age- and sex-matched controls,[1-3] and a 1995 meta-analysis of such studies reported that a 5-µmol/L higher homocysteine level (eg, 15 vs 10 µmol/L) was associated with a 70% higher risk of CHD.[2] Subsequently, however, prospective cohort studies of homocysteine reported more modest associations.[3,4] In 2002, a collabo-

See Invited Commentary at end of article

Author Affiliations are listed at the end of this article.
Group Information: Investigators in the B-Vitamin Treatment Trialists' Collaboration are listed at the end of this article.

rative meta-analysis involving individual data from prospective studies reported that, after adjustment for known cardiovascular risk factors, a 25%-lower usual plasma total homocysteine level was associated with an 11% (95% confidence interval [CI], 4%-17%) lower risk of CHD and 19% (5%-31%) lower risk of stroke.[4]

In patients with homocystinuria, supplementation with B vitamins has been shown to lower homocysteine levels and the risk of CVD.[5,6] A meta-analysis of randomized trials found that, in populations without folic acid fortification, supplementation with folic acid lowered homocysteine levels by 23% or, if given in combination with vitamin B_{12} (cyanocobalamin), by 30%.[7] The effects of B-vitamin supplementation were somewhat less pronounced in populations with preexisting mandatory folic acid fortification, but, even there, the combination therapy typically lowered homocysteine levels by 20%.[7]

Many large randomized trials of B-vitamin supplementation in patients at high risk of or with established CVD have been conducted to test the homocysteine hypothesis.[8-19] Several of those trials (guided by reviews of the early observational studies[2,3]) were designed to detect reductions in CHD risk of more than 30%, so they lacked statistical power to detect more modest, but still potentially important, effects.[20] Consequently, a collaboration between their investigators was established in 2004 to conduct a meta-analysis based on individual participant data from all large randomized trials of folic acid–based B-vitamin supplementation intended to lower plasma homocysteine levels for the prevention of CVD.[20,21] The present report describes the effects on cause-specific major morbidity and mortality from the 8 such trials that had been completed by the end of 2009.[8-15]

METHODS

TRIAL ELIGIBILITY

Randomized trials were eligible if (1) they involved a double-blind randomized comparison of B-vitamin supplements containing folic acid vs placebo for the prevention of vascular disease (irrespective of whether any other treatment was administered factorially); (2) the relevant treatment arms differed only with respect to the intervention to lower homocysteine levels (ie, they were unconfounded); and (3) the trial involved at least 1000 participants for a scheduled treatment duration of at least 1 year. Unpublished trials were sought through electronic searches and discussions with other experts in the field, but none was found. Individual participant data were obtained from 37 485 participants from all 8 available trials completed by the end of 2009. Data are not yet available from 3 unpublished trials involving almost 15 000 participants with prior CVD or renal disease, and these are not expected to report their results before late 2010.[16-18] Another trial that intended to involve 15 000 participants with hypertension only started enrollment in 2008.[19]

BASELINE AND FOLLOW-UP DATA

For each randomized participant, information was sought on characteristics recorded before randomization, the randomly allocated treatment, and the type and date (or time from randomization) of any systematically recorded outcomes occurring during the scheduled treatment period. Information on adverse events, including hospitalizations and cancer incidence, was collected in each trial at typically 3- to 6-month intervals during the scheduled treatment period. In addition to self-reported cancer, additional data on cancer incidence were obtained from national cancer registries in some trials.[10,14,15] Trial coordinators sought verification of all study-related outcomes from hospital electronic records or by writing to hospital or family physicians. Analyses of the individual patient data were checked for consistency with any published reports (and with the trialists) to help ensure that the data were incorporated correctly into the meta-analysis. Investigators were also asked to confirm summary data for each treatment group on the number of randomized patients; on plasma levels of total homocysteine, folate, and vitamin B_{12} before and after starting treatment; and on the numbers of patients who developed each of the predefined outcomes.

All events were prespecified and defined using standard criteria in the protocol for this meta-analysis.[21] The main outcomes were major vascular events (defined prospectively as major coronary events, strokes, or coronary and noncoronary artery revascularizations), cancer incidence, and total and cause-specific mortality.

Major coronary events were defined as the first occurrence of nonfatal myocardial infarction or coronary death (including death from heart failure and sudden or unexpected deaths considered coronary in origin). Strokes were the first occurrence of ischemic, hemorrhagic, or unclassified stroke (but not just transient cerebral ischemia). Coronary revascularizations involved coronary artery bypass grafting or coronary angioplasty (with or without stent insertion), and noncoronary revascularization included carotid artery endarterectomy or angioplasty, repair of aortic aneurysm, peripheral arterial surgery, or noncoronary angioplasty. Incident cancers were the first occurrence after randomization of any new cancers excluding (where possible) nonfatal nonmelanoma skin cancers. Having individual participant data from each trial allowed outcomes to be recoded uniformly using these definitions.

STATISTICAL ANALYSIS

Comparisons were intention-to-treat, time-to-event analyses of first events of a particular type occurring during the scheduled treatment period among all patients allocated to folic acid vs all allocated to the control treatment. The log-rank observed minus expected ($o-e$) statistics and their variances (v) from each trial were summed to produce, respectively, a grand total observed minus expected statistic (G) and its variance (V).[22,23] The 1-step estimate of the logarithm of the event rate ratio is then G/V with variance $1/V$ (and 95% CI [G/V] ± [$1.96/V^{1/2}$]). For n trials, the χ^2 statistic for heterogeneity with $n-1$ degrees of freedom (χ^2_{n-1}) is $S-(G^2/V)$, where S is the sum over all the trials of $(o-e)^2/v$.[22,23] The effects on vascular outcomes were assessed in the following predefined subgroups[21]: sex, age, approximate thirds of pretreatment blood levels of folate (<4.4, 4.4-7.9, and >7.9 ng/mL [to convert to nanomoles per liter, multiply by 2.266]) or of homocysteine (<11, 11-14, and ≥15 μmol/L), mandatory folic acid fortification, years since randomization, baseline smoking (current/not), alcohol consumption (current/not), presence of diabetes mellitus, statin use, aspirin use, body mass index (calculated as weight in kilograms divided by height in meters squared) (<25.0, 25.0-29.9, and ≥30.0), and approximate thirds of serum creatinine levels (<0.90, 0.90-1.06, and ≥1.07 mg/dL [to convert to micromoles per liter, multiply by 88.4]). Heterogeneity of the rate ratios (RRs) among these prespecified subgroups was investigated by

Table 1. Design and Eligibility Criteria of Included Trials

Trial	No. Randomized	Prior Disease	Main Country	Mandatory Fortification	Median Duration of Treatment, y	Daily Dose of Vitamin B, mg		
						Folic Acid	Vitamin B_{12}	Vitamin B_6
CHAOS-2[8]	1882	CHD	UK	–	2.0	5.0	0	0
HOST[12]	2056	Renal	US	+	3.2	40.0	2.0	100
WENBIT[14]	3090	CHD	Norway	–	3.2	0.8	0.4	40
VISP[9]	3680	Stroke	US[a]	+/–	2.0	2.5	0.4	25
NORVIT[10]	3749	CHD	Norway	–	3.4	0.8	0.4	40
WAFACS[13]	5442	CVD	US	+	7.3	2.5	1.0	50
HOPE-2[11]	5522	CVD[b]	Canada[c]	+/–	5.0	2.5	1.0	50
SEARCH[15]	12 064	CHD	UK	–	7.0	2.0	1.0	0

Abbreviations: CHAOS-2, Cambridge Heart Antioxidant Study 2; CHD, coronary heart disease; CVD, cardiovascular disease or increased risk of cardiovascular disease; HOPE-2, Heart Outcomes Prevention Evaluation 2; HOST, Homocysteinemia in Kidney and End Stage Renal Disease; NORVIT, Norwegian Vitamin Trial; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; UK, United Kingdom; US, United States; VISP, Vitamin Intervention for Stroke Prevention; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study; WENBIT, Western Norway B Vitamin Intervention Trial; –, absent; +, present.

[a] Includes 3634 from the United States and 46 from the United Kingdom.

[b] Includes diabetes mellitus.

[c] Includes 3982 from Canada and the United States, 426 from Western Europe, 849 from Slovakia, and 265 from Brazil.

Table 2. Median Plasma Levels of Folate and Homocysteine Before and After Study Treatment

Trial	No. Randomized	Folate Level, ng/mL				Homocysteine Level, μmol/L				Homocysteine Reduction, %[b]
		Before		After[a]		Before		After[a]		
		Treated	Control	Treated	Control	Treated	Control	Treated	Control	
Folate Fortified										
VISP[9]	3634	10.1	10.2	27.8	10.0	12.3	12.3	9.8	11.7	17
HOST[12]	2056	6.9	6.8	890.9	7.3	22.5	22.2	16.5	21.6	25
HOPE-2[11]	3982	12.7	12.8	20.0	10.2	11.0	11.0	9.0	12.0	24
WAFACS[13]	5442	8.8	8.9	38.9	15.4	12.1	12.5	9.8	11.8	18
Subtotal	15 114	10.0	9.8	30.5	9.8	13.2	13.2	11.0	13.5	22[c]
Non–Folate Fortified										
VISP[9]	46	7.8	5.8	29.1	5.6	13.5	14.0	8.7	14.4	35
HOPE-2[11]	1540	6.4	5.9	20.0	6.2	13.0	12.7	9.0	13.1	29
CHAOS-2[8]	1882	6.4	6.3	20.0	7.2	9.3	9.9	8.2	9.2	11
WENBIT[14]	3090	4.3	4.5	27.2	3.8	10.0	10.1	7.7	10.2	26
NORVIT[10]	3749	3.6	3.5	27.5	3.2	12.1	12.1	8.9	12.4	28
SEARCH[15]	12 064	6.2	6.1	22.1	6.7	12.6	12.5	8.8	12.5	27
Subtotal	22 371	5.3	5.2	22.1	6.7	12.0	12.0	8.4	11.4	27[c]
All	**37 485**	6.1	6.0	22.1	6.7	12.3	12.3	9.3	12.2	25[c]

Abbreviations: For the study name expansions, see Table 1.

SI conversion factor: To convert folate to nanomoles per liter, multiply by 2.266.

Conventional conversion factor: To convert homocysteine to milligrams per liter, divide by 7.397.

[a] Indicates first available postrandomization values.

[b] The values shown are the weighted mean values (with weights equal to the variance of the log-rank statistic for major vascular events).

[c] For calculation of percentage of homocysteine reduction, the analysis used the average of the first 4 follow-up values (where available).

a global test to reduce the chance of misinterpreting false-positive results arising from multiple comparisons.[23] The CIs used were 99% for individual trials or subgroups and 95% for the overall estimates. In addition, the RR for major vascular events in each trial was plotted against the percentage homocysteine reduction achieved in that trial. The mean percentage homocysteine reduction in the aggregate of all trials was calculated as the weighted mean of the study-specific percentage reductions, with weights equal to the variances of the log-rank statistics for major vascular events. Analyses used commercially available software (SAS, version 9.1; SAS Institute Inc, Cary, North Carolina).

RESULTS

CHARACTERISTICS OF THE PARTICIPATING TRIALS

Selected characteristics of the 8 randomized trials are shown in **Table 1**. Four trials recruited individuals with prior CHD,[8,10,14,15] 1 with prior stroke,[9] 2 with prior CVD or increased risk of CVD,[11,13] and 1 with advanced renal disease (in which plasma homocysteine levels were particularly high).[12] Six trials[8-11,14,15] recruited partly or entirely from nonfortified populations (22 371 individuals; 6311 major vascular events), and 4[9,11-13] recruited partly or entirely from fortified populations (15 114 individuals; 3015 major vascular events). Morbidity and mortality follow-up were more than 99% complete in 4 trials[10,11,14,15] but ranged from 93% to 97% in the others.[8,9,12,13] The me-

Table 3. Number of Serious Events and Event Rates by Trial

Trial	No. Randomized	Major Vascular Events		Cancer Events		Total Mortality	
		No. of Events	Rate[a]	No. of Events	Rate[a]	No. of Events	Rate[a]
CHAOS-2[8]	1882	206	70	NA	NA	144	48
HOST[12]	2056	471	89	137	24	884	143
WENBIT[14]	3090	642	76	144	17	131	15
VISP[9]	3680	613	101	187	33	216	32
NORVIT[10]	3749	2000	321	149	15	365	30
WAFACS[13]	5442	778	24	414	12	506	14
HOPE-2[11]	5522	1586	71	662	28	945	34
SEARCH[15]	12 064	3030	42	1317	18	1934	25
All	**37 485**	**9326**	NA	**3010**	NA	**5125**	28

Abbreviations: NA, not available. For the study name expansions, see Table 1.
[a]Standardized for age and sex and reported as number of events per 1000 per year.

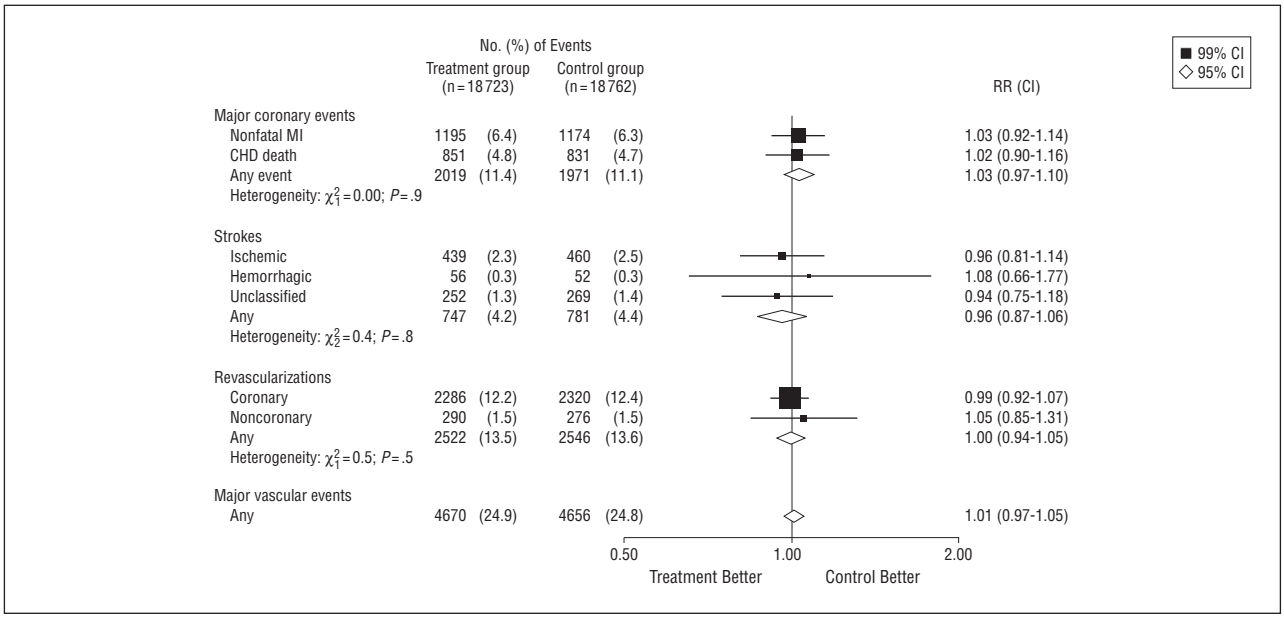

Figure 1. Effects of folic acid on first major vascular events. (Some people had more than 1 type of major vascular event.) The black squares denote the rate ratios (RRs) and horizontal lines the 99% confidence intervals (CIs). Each square has an area inversely proportional to the variance of the logarithm of the RR. The diamonds represent the summary estimates and their corresponding 95% CIs. CHD indicates coronary heart disease; MI, myocardial infarction.

dian duration of treatment in the different trials varied from 2.0 to 7.3 years, with an overall median of 5.0 years. All trials compared the effects of folic acid with placebo, except 1 trial[9] that compared 2.5 mg with 20.0 µg of folic acid (which is approximately equivalent to a placebo because this dose is less than one-tenth of the daily dietary intake of folic acid). The doses of folic acid ranged from 0.8 to 5.0 mg/d, except in 1 trial that used 40.0 mg/d.[12] All but 1 trial[8] added vitamin B_{12} (dose range, 0.4 to 2.0 mg) to the folic acid, and all but 2[8,15] also added vitamin B_6 (pyridoxine hydrochloride). Two-thirds of the participants were men, the mean (SD) age at entry was 65 (10) years, 18% were current smokers, 20% had diabetes mellitus, and 30% were obese (defined as a body mass index of ≥30.0) (eTable 1; http://www.archinternmed.com).

EFFECTS ON PLASMA HOMOCYSTEINE LEVELS

Table 2 shows the effect of folic acid allocation on the median postrandomization plasma levels of folate and total homocysteine. Overall, there was a 25% reduction in homocysteine levels, which was maintained on average for 5 years. As expected, allocation to folic acid was associated with slightly smaller relative and absolute reductions of homocysteine levels in folate-fortified compared with non–folate-fortified populations (22% vs 27% relative reductions; $P < .001$ for differences between these percentages).

EFFECTS ON MAJOR VASCULAR EVENTS

Among all 37 485 participants in all 8 trials, 9326 had a major vascular event during the scheduled treatment period (**Table 3** and eTable 2). Allocation to folic acid treatment had no significant effect on major vascular events, with 4670 (24.9%) first events among the 18 723 participants allocated to folic acid vs 4656 (24.8%) among the 18 762 allocated to placebo (RR, 1.01; 95% CI, 0.97-1.05; $P = .6$) (**Figure 1**). There was no signifi-

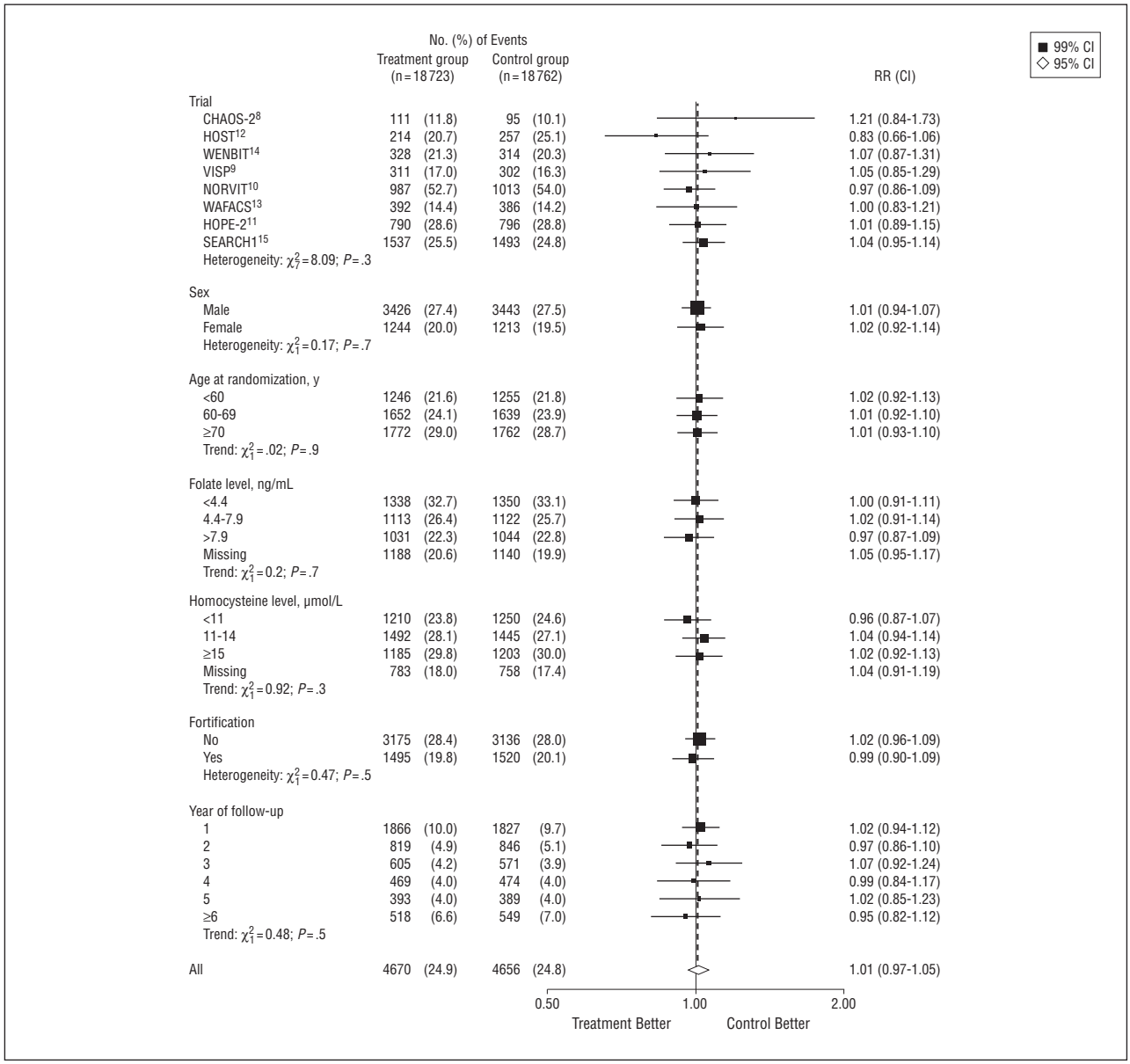

Figure 2. Effects of folic acid on major vascular events in prespecified subgroups (global test for heterogeneity, χ^2_5=1.78; *P*=.9). Symbols and conventions are given in Figure 1. To convert folate to nanomoles per liter, multiply by 2.266; homocysteine to milligrams per liter, divide by 7.397. CHAOS-2 indicates Cambridge Heart Antioxidant Study 2; HOPE-2, Heart Outcomes Prevention Evaluation 2; HOST, Homocysteinemia in Kidney and End Stage Renal Disease; NORVIT, Norwegian Vitamin Trial; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; VISP, Vitamin Intervention for Stroke Prevention; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study; and WENBIT, Western Norway B Vitamin Intervention Trial.

cant effect on the numbers of participants having major coronary events, with 2019 (11.4%) vs 1971 (11.1%) first MCEs (RR, 1.03; 95% CI, 0.97-1.10; *P*=.3). Moreover, there was no significant effect in these trials on the risk of stroke, with 747 (4.2%) vs 781 (4.4%) first events (RR, 0.96; 95% CI, 0.87-1.06; *P*=.4). There was no significant effect on ischemic stroke (RR, 0.96; 99% CI, 0.81-1.14), hemorrhagic stroke (RR, 1.08; 99% CI, 0.66-1.77), or unclassified stroke (RR, 0.94; 99% CI, 0.75-1.18). Finally, there was no significant effect on arterial revascularization (RR, 1.00; 95% CI, 0.94-1.05) or on coronary revascularization or MCE (RR, 1.01; 95% CI, 0.96-1.06).

Despite substantial differences between the trials in pretreatment plasma folate status and folic acid doses, there was no significant difference between the proportional effects on major vascular events in the individual trials (χ^2_7 for heterogeneity, 8.09; *P*=.3) (**Figure 2**). There was no suggestion of beneficial effects even in those trials that achieved larger homocysteine level reductions (**Figure 3**). No significant effect was observed in any of the prespecified subgroups, including those defined by sex, age, pretreatment levels of folate or homocysteine, or population-level fortification status (global χ^2_5 for heterogeneity, 1.78; *P*=.9) (Figure 2).The one-third of participants with the highest baseline homocysteine levels (mean, 21 µmol/L) experienced the greatest reduction in homocysteine levels (about 6 µmol/L), but even among these, folic acid allocation was not

associated with any significant effect. There was no trend of increasing benefit with increasing duration of treatment (χ^2_1, 0.48; P=.5). Likewise, no significant effect was found on major vascular events in further exploratory subgroup analyses (global χ^2_7 for heterogeneity, 7.41; P=.4) (eFigure 1). Finally, analyses of major coronary events only (eFigure 2) or of stroke only (eFigure 3) found no apparent effects in any of the prespecified subgroups.

EFFECTS ON CANCER INCIDENCE

Data were available on 3010 people with incident cancers that occurred after randomization among the 35 603 individuals included in the 7 randomized trials that collected such data.[9-15] Allocation to folic acid was not associated with any significant difference in the overall incidence of cancer, with 1541 new cases (8.7%) among the 17 783 participants allocated to folic acid vs 1469 (8.2%) among the 17 820 allocated to placebo (RR, 1.05; 95% CI, 0.98-1.13; P=.14) (**Figure 4**). There was no significant difference between the proportional effects on cancer in any of the individual trials (χ^2_6 for heterogeneity, 4.68; P=.6), despite the doses of folic acid ranging from 0.8 to 40.0 mg/d. Even among patients who received folic acid treatment for more than 5 years, there was no suggestion of any effect on cancer rates beginning to emerge with longer duration of treatment (χ^2_1 for trend, 0.01; P=.9), in the other prespecified subgroups (global χ^2_5 for heterogeneity, 5.90; P=.3) (Figure 4), or in further exploratory subgroups (global χ^2_7 for heterogeneity, 7.84; P=.4) (eFigure 4).

EFFECTS ON MORTALITY

Data were available on 5125 deaths among the 37 485 randomized participants. Allocation to folic acid was not associated with any significant differences in total mortality, with 2578 deaths (13.8%) among the 18 723 participants allocated to folic acid vs 2547 deaths (13.6%) among the 18 762 allocated to placebo (RR, 1.02; 95% CI, 0.97-1.08; P=.46) (**Figure 5**). Consistent with the lack of any apparent effect on major vascular events, there were no significant effects on mortality from CHD (RR, 1.02; 99% CI, 0.90-1.16; P=.65), stroke (RR, 0.92; 99% CI, 0.67-1.25; P=.47), or other vascular causes (RR, 0.99; 99% CI, 0.83-1.18; P=.85). Similarly, consistent with the lack of any apparent effect on cancer incidence, there was no significant effect on cancer mortality (RR, 1.00; 99% CI, 0.85-1.18; P=.99).

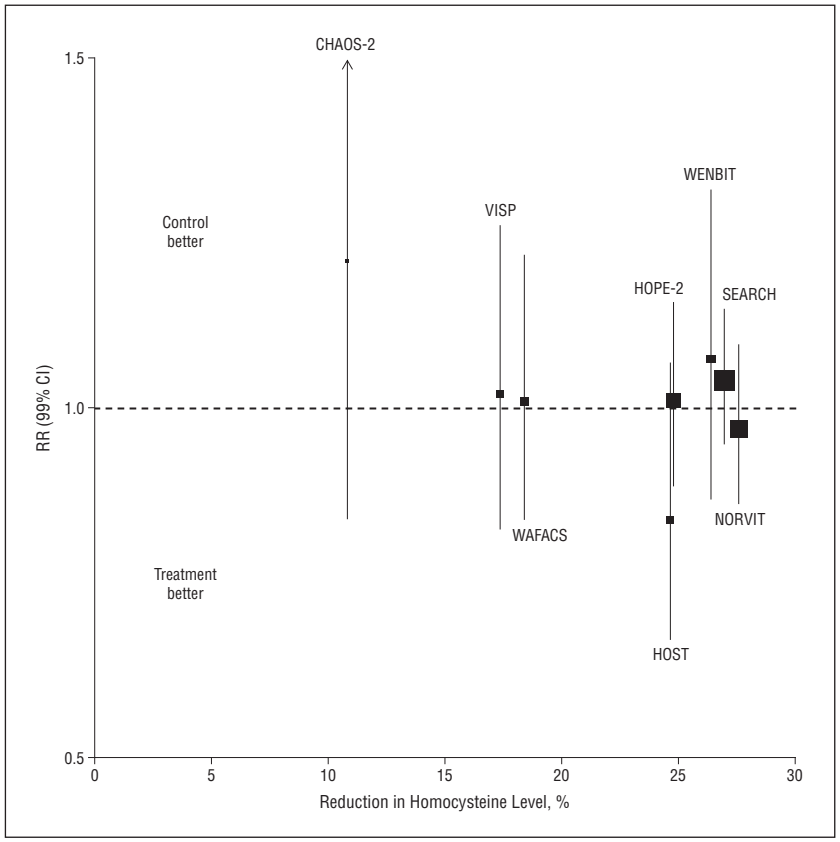

Figure 3. Effects of folic acid on major vascular events by percentage reduction in plasma total homocysteine levels. The black squares denote the rate ratios (RRs) and vertical lines the 99% confidence intervals (CIs). Each square has an area inversely proportional to the variance of the logarithm of the RRs. Other abbreviations are given in Figure 2.

COMMENT

The present meta-analysis has demonstrated that lowering homocysteine levels by an average of 25% (about 3 µmol/L) for an average of 5 years has no significant effect on the incidence of major vascular events during the scheduled treatment period. With 9326 such events among 37 485 individuals, this collaboration had more than 99% power to detect the 10% reduction that might plausibly have been anticipated from the observational studies if the association of homocysteine with major vascular events seen in those studies was causal and if protection emerges within just a few years.[4,20]

Observational studies have found stronger associations of homocysteine with stroke than with CHD[4] and in women than in men.[4] Based on secular trends in the United States and the United Kingdom, the greater reduction in stroke mortality from 1990 through 2002 in the United States had been tentatively attributed to the introduction of folic acid fortification,[24] although this is not supported by reexamination of the trends in stroke mortality in middle age (35-69 years; trends at older ages may be less reliable).[25] A previous meta-analysis of published results from some of the folic acid trials involving a total of 778 first strokes reported that folic acid could reduce the risk of stroke.[26] By contrast, this meta-analysis of individual patient data involving 1528 first strokes

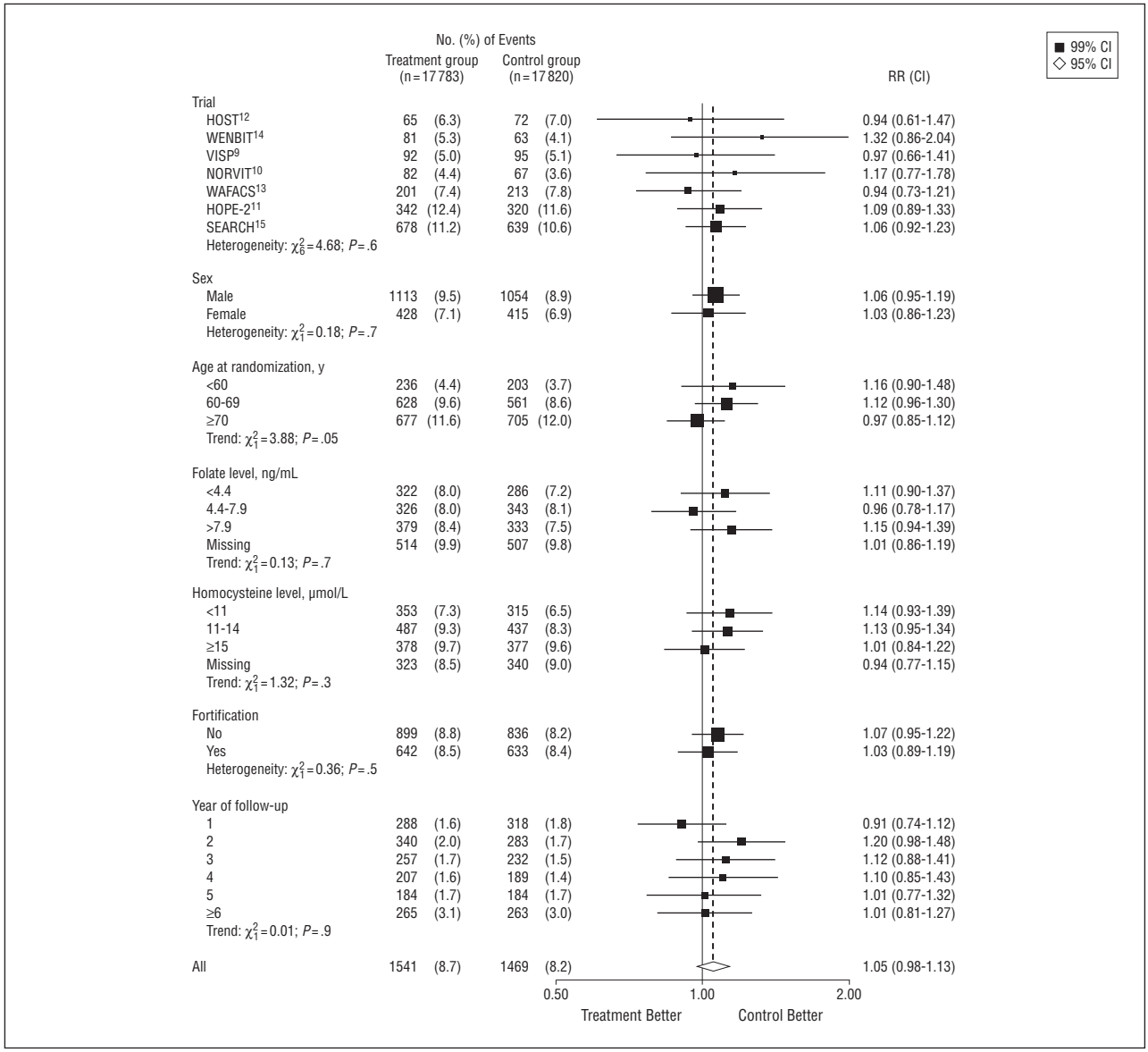

Figure 4. Effects of folic acid on cancer incidence in prespecified subgroups (global test for heterogeneity, $\chi^2_5 = 5.90$; $P = .3$). Symbols and conventions are given in Figure 1. Other abbreviations and SI conversion factors are given in Figure 2.

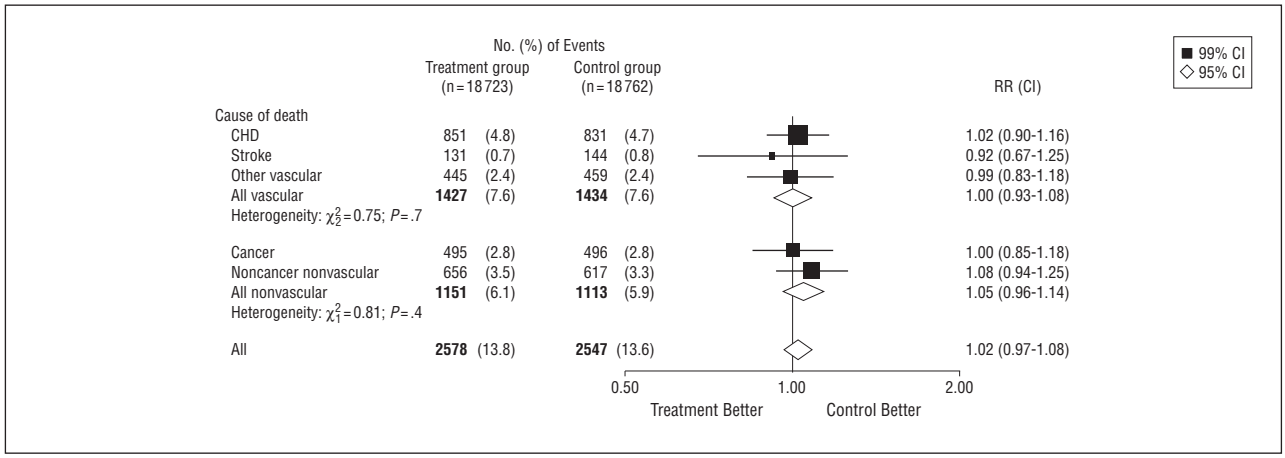

Figure 5. Effects of folic acid on cause-specific mortality. Symbols and conventions are given in Figure 1. CHD indicates coronary heart disease.

found no significant effects on stroke. Folic acid did not produce significant effects on major vascular or coronary events in those presenting with low blood folate levels or with high blood homocysteine levels.

The doses of folic acid used in all the trials included in this meta-analysis exceeded those required for near-maximal reduction in homocysteine levels.[7] In addition, all except 1 trial[8] used vitamin B_{12}, which further reduces homocysteine levels (and, with a dose of at least 1.0 mg/d in 4 trials,[11-13,15] should also correct for any undetected vitamin B_{12} deficiency[27]). The randomized trials in the present meta-analysis found no evidence of benefit with treatment continued for more than 5 years. Although some benefit might emerge with even longer treatment and follow-up, the trial results give no reason to expect this (particularly because cardiovascular benefits tend to emerge within just a few years with other cardioprotective treatments, such as antihypertensives[28] or statins[29]). Thus, the meta-analysis indicates that supplementation with folic acid has little or no beneficial effect on coronary disease, stroke, or mortality during the 5 years of supplementation. In contrast with previous reviews[30,31] based on summary results of these trials (but excluding the largest[15]), the use of individual participant data from these trials allows the present report to provide results for a broader range of vascular and nonvascular outcomes and to explore the effects of B vitamins in relevant subgroups reliably. It is unlikely that findings from ongoing trials will differ much from those of this meta-analysis; so, the addition of their results is likely only to improve the precision of the estimates around the lack of benefit.[16-19] Although trials of folic acid supplementation suggest no reduction in CHD or stroke associated with an average 25% reduction in homocysteine levels maintained for a median duration of 5 years, the lower confidence limits are compatible with a 3% reduction in CHD risk and a 13% reduction in stroke risk—and, if there is any real effect, the effects of lifelong differences could be somewhat larger.

Some observational studies have reported that folate status is inversely related to the risk of colorectal cancer[32] and breast cancer.[33] Conversely, it has also been suggested that increasing folic acid intake might increase the rate of transformation of adenomas into cancers or of small cancers into larger ones.[34] An analysis of trends in colorectal cancer incidence in the United States and Canada from 1986 through 2002 indicated a transient reversal in the downward trends that coincided with the introduction of folic acid fortification in 1996, and it was suggested that this might be causal[35]; again, however, this is not confirmed by reexamination of the national mortality trends at ages 35 to 69 years.[25] Three trials of folic acid supplementation involving a total of 2652 participants with a history of colorectal adenoma (and so not eligible for the present meta-analysis) have reported conflicting results on the recurrence of adenomas and on cancer incidence.[36-39] An analysis of the 6-year follow-up of 6837 participants with a history of CHD in 2 Norwegian trials included in the meta-analysis also suggested that folic acid plus vitamin B_{12} might increase overall of cancer incidence and mortality.[40] By contrast, based on 3010 incident cancers among 35 603 individuals, the present meta-analysis did not find any significant adverse effect of folic acid on cancer incidence overall or in any prespecified subgroup. There was no heterogeneity in the effects on incident cancer by dose of folic acid, which ranged from 0.8 to 40.0 mg/d. For example, the 40.0-mg/d folic acid dose studied in the Homocysteinemia in Kidney and End Stage Renal Disease trial produced a 100-fold increase in plasma folate level but had no apparent adverse effect on cancer incidence.[12] Even with more than 5 years of treatment, there was no evidence of any emerging effect on overall cancer incidence. Moreover, the absence of heterogeneity in the effects of folic acid on cancer incidence in the different trials refutes concerns about any differential effects of folic acid in trials that relied on self-reported cancer events compared with those that also included surveillance by cancer registries. (The present meta-analyses do not address the possibility of effects on some particular types of cancer, which will be considered in a separate report.)

One-third of adults in the United States[41] and one-quarter of those in the United Kingdom[42] report taking daily multivitamin supplements containing folic acid. The present meta-analysis, however, found no evidence that routine use of folic acid for 5 years has any material effect on cardiovascular or noncardiovascular events in the North American and European populations studied. The doses of folic acid used in these trials (0.8-40.0 mg/d) were substantially greater than the mandatory folic acid fortification adopted in the United States (140 µg per 100 g of cereal grain products; approximately equivalent to 0.1 mg/d of folic acid) for the prevention of neural tube defects.[43,44] Although the lack of any other benefits is disappointing (albeit faily definitive), the lack of any significant adverse effects on vascular events, cancer incidence, cancer mortality, and overall mortality provides reassurance about the safety of population-wide folic acid fortification.

Accepted for Publication: June 7, 2010.

Author Affiliations: Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, England (Drs Clarke, Lewington, Armitage, Bennett, Peto, and Collins and Mr Halsey); Population Health Research Institute and Division of Cardiology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada (Dr Lonn); Department of Medicine, Harvard Medical School, Brigham and Women's Hospital (Dr Manson), and Massachusetts Veterans Epidemiology Research and Information Centre, Veterans Affairs Boston Healthcare System (Dr Gaziano), Boston, Massachusetts; Department of Heart Disease, University Hospital of Northern Norway, Tromsø (Dr Bønaa); Department of Neurology, Robarts Research Institute, University of Western Ontario, London, Ontario (Dr Spence); Department of Heart Disease, University of Bergen, Bergen, Norway (Dr Nygård); Department of Medicine, Veterans Affairs

Palo Alto Healthcare System and Stanford University School of Medicine, Palo Alto, California (Dr Jamison); Cooperative Studies Program, Department of Veterans Affairs, Connecticut Veterans Affairs Healthcare System, West Haven (Dr Guarino); and Department of Clinical Pharmacology, Addenbrookes' Hospital, Cambridge, England (Dr Mir).

Correspondence: Robert Clarke, FRCP, Clinical Trial Service Unit and Epidemiological Studies Unit, Richard Doll Building, University of Oxford, Old Road Campus, Oxford OX3 7LF, England (robert.clarke@ctsu.ox.ac.uk).

Author Contributions: The authors accept full responsibility for the content of this article. *Study concept and design:* Clarke, Lonn, Armitage, Manson, Bønaa, Spence, Nygård, Jamison, Gaziano, Guarino, Mir, and Collins. *Acquisition of data:* Clarke, Lonn, Armitage, Manson, Bønaa, Spence, Nygård, Jamison, and Gaziano. *Analysis and interpretation of data:* Clarke, Halsey, Lewington, Lonn, Manson, Armitage, Bønaa, Nygård, Jamison, Bennett, Peto, and Collins. *Drafting of the manuscript:* Clarke, Lewington, Mir, Peto, and Collins. *Critical revision of the manuscript for important intellectual content*: Halsey, Lewington, Lonn, Manson, Bønaa, Spence, Nygård, Jamison, Gaziano, Guarino, Bennett, and Collins. *Statistical analysis:* Halsey, Lewington, Guarino, Bennett, Peto, and Collins. *Obtained funding:* Clarke, Lonn, Bønaa, Spence, Nygård, Jamison, and Gaziano. *Administrative, technical, and material support:* Clarke, Halsey, Armitage, Manson, Bønaa, Nygård, Jamison, Gaziano, Bennett, and Collins. *Study supervision:* Clarke, Lonn, Bønaa, Jamison, Mir, and Collins.

B-Vitamin Treatment Trialists' (BVTT) Collaboration: Fraz Mir, MD, and Morris Brown, MD (Cambridge Heart Antioxidant Study 2); Eva Lonn, MD, and Salim Yusuf, MD (Heart Outcomes Prevention Evaluation 2); Kaare H. Bønaa, MD, and Inger Njolstad, MD (Norwegian Vitamin Trial); Jane Armitage, FRCP, Louise Bowman, MD, Robert Clarke, FRCP, Sarah Parish, DPhil, Richard Peto, FRS, and Rory Collins, FRCP (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine); Rex Jamison, MD, J. Michael Gaziano, MD, and Peter Guarino, PhD (Homocysteinemia in Kidney and End Stage Renal Disease); James Toole, MD, M. Rene Malinow, MD (deceased), L. Creed Pettigrew, MD, J. David Spence, MD, Virginia J. Howard, MD, Elizabeth G. Sides, MEd, Chin H. Wang, PhD, and Meir Stampfer, MD (Vitamin Intervention for Stroke Prevention); JoAnn E. Manson, MD, DrPH, Robert Glynn, PhD, Francine Grodstein, MD, Christine M. Albert, MD, and Nancy R. Cook, ScD (Women's Antioxidant and Folic Acid Cardiovascular Study); Ottar Nygård, MD, Marta Ebbing, MD, Jan E. Nordrehaug, MD, Dennis W. T. Nilsen, MD, Hegla Refsum, MD, and Stein E. Vollset, MD (Western Norway B Vitamin Intervention Trial); BVTT secretariat: Robert Clarke, FRCP, Jim Halsey, BSc, Sarah Lewington, DPhil, Derrick Bennett, PhD, and Rory Collins, FRCP.

Financial Disclosure: Dr Gaziano reports receiving investigator-initiated research funding from the National Institutes of Health, the US Department of Veterans Affairs, Veroscience, and Amgen and pills and packaging for a research study from BASF, DSM, and Wyeth. He reports serving as a consultant to Bayer and having served as a medical expert for Merck.

Funding/Support: Sources of funding for the individual trials are described in their separate publications. The Clinical Trial Service Unit and Epidemiological Studies Unit, where the BVTT Secretariat is located, has a policy of not accepting fees, honoraria, or paid consultancies directly or indirectly from industry. It receives funding from the British Heart Foundation, UK Medical Research Council, and Cancer Research UK. Support for this project was also provided by a grant from the UK Food Standards Agency.

Online-Only Material: The eFigures and eTables are available at http://www.archinternmed.com.

REFERENCES

1. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med*. 1991;324(17):1149-1155.
2. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA*. 1995;274(13):1049-1057.
3. Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *J Cardiovasc Risk*. 1998;5(4):229-232.
4. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*. 2002;288(16):2015-2022.
5. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol*. 1969;56(1):111-128.
6. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta-synthase deficiency: effects of homocysteine-lowering therapy. *Semin Thromb Hemost*. 2000;26(3):335-340.
7. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr*. 2005;82(4):806-812.
8. Baker F, Picton D, Blackwood S, et al. Blinded comparison of folic acid and placebo in patients with ischaemic heart disease: an outcome trial [abstract]. *Circulation*. 2002;106(suppl 2):741S.
9. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA*. 2004;291(5):565-575.
10. Bønaa KH, Njølstad I, Ueland PM, et al; NORVIT Trial Investigators. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med*. 2006;354(15):1578-1588.
11. Lonn E, Yusuf S, Arnold MJ, et al; Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering with folic acid and B-vitamins in vascular disease [published correction appears in *N Engl J Med*. 2006;355(7):746]. *N Engl J Med*. 2006;354(15):1567-1577.
12. Jamison RL, Hartigan P, Kaufman JS, et al; Veterans Affairs Site Investigators. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial [published correction appears in *JAMA*. 2008;300(2)170]. *JAMA*. 2007;298(10):1163-1170.
13. Albert CM, Cook NR, Gaziano JM, et al. 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. *JAMA*. 2008;299(17):2027-2036.
14. Ebbing M, Bleie Ø, Ueland PM, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA*. 2008;300(7):795-804.
15. Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group. Effects of homocysteine-lowering with folic acid plus vitamin B_{12} vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. *JAMA*. 2010;303(24):2486-2494.
16. VITATOPS Trial Study Group; Hankey GJ, Algra A, Chen C, et al. VITATOPS, the Vitamins to Prevent Stroke Trial: rationale and design of a ran-

domised trial of B-vitamin therapy in patients with recent transient ischaemic attack or stroke (NCT00097669) (ISRCTN74743444). *Int J Stroke*. 2007;2(2):144-150.
17. Bostom AG, Carpenter MA, Hunsicker L, et al; FAVORIT Study Investigators. Baseline characteristics of participants in the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) trial. *Am J Kidney Dis*. 2009;53(1):121-128.
18. Galan P, Briancon S, Blacher J, Czernichow S, Hercberg S. The SU.FOL.OM3 Study: a secondary prevention trial testing the impact of supplementation with folate and B-vitamins and/or omega-3 PUFA on fatal and non fatal cardiovascular events, design, methods and participants characteristics. *Trials*. 2008;9:35. doi:10.1186/1745-6215-9-35.
19. China Stroke Primary Prevention Trial. http://clinicaltrials.gov/ct2/results?term=NCT00794885. Accessed June 1, 2010.
20. B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials. *Am Heart J*. 2006;151(2):282-287.
21. Clarke R, Armitage J, Lewington S, Collins R; B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of vascular disease: protocol for a collaborative meta-analysis. *Clin Chem Lab Med*. 2007;45(12):1575-1581.
22. Early Breast Cancer Trialists' Collaborative Group. *Worldwide Evidence 1985-1990*. Oxford, England: Oxford University Press; 1990. *Treatment of Early Breast Cancer*, vol 1.
23. Yusuf S, Collins R, Peto R, et al. Intravenous and intracoronary fibrinolytic therapy in acute myocardial infarction: overview of results on mortality, reinfarction and side-effects from 33 randomized controlled trials. *Eur Heart J*. 1985;6(7):556-585.
24. Yang Q, Botto LD, Erickson JD, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation*. 2006;113(10):1335-1343.
25. Whitlock G. Trends in national mortality rates. http://www.mortality-trends.org. Accessed June 1, 2010.
26. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*. 2007;369(9576):1876-1882.
27. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol*. 2007;6(9):830-838.
28. Turnbull F; Blood Pressure Lowering Treatment Trialists' Collaboration. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet*. 2003;362(9395):1527-1535.
29. Baigent C, Keech A, Kearney PM, et al; Cholesterol Treatment Trialists' Collaboration. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins [published corrections appear in Lancet. 2005;366(9494):1358 and 2008;371(9630)2084]. *Lancet*. 2006;366(9493):1267-1278.
30. Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *JAMA*. 2006;296(22):2720-2726.
31. Bazzano LA. Folic acid supplementation and cardiovascular disease: the state of the art. *Am J Med Sci*. 2009;338(1):48-49.
32. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. *J Nutr*. 2002;132(8)(suppl):2350S-2355S.
33. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst*. 2007;99(1):64-76.
34. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr*. 2004;80(5):1123-1128.
35. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev*. 2007;16(7):1325-1329.
36. Cole BF, Baron JA, Sandler RS, et al; Polyp Prevention Study Group. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA*. 2007;297(21):2351-2359.
37. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst*. 2009;101(6):432-435.
38. Logan RFA, Grainge MJ, Shepherd VC, Armitage NC, Muir KR; ukCAP Trial Group. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology*. 2008;134(1):29-38.
39. Wu K, Plarz EA, Willett WC, et al. A randomized trial of folic acid supplementation and risk of recurrent colorectal adenoma. *Am J Clin Nutr*. 2009;90(6):1623-1631.
40. Ebbing M, Bønaa KH, Nygård O, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B_{12}. *JAMA*. 2009;302(19):2119-2126.
41. Bailey RL, Dodd KW, Gahche JJ, et al. Total folate and folic acid intake from foods and dietary supplements in the United States: 2003-2006. *Am J Clin Nutr*. 2010;91(1):231-237.
42. Bates B, Lennox A, Swan G, eds. National diet and nutrition survey: headline results for year 1 of the rolling programme (2008/2009). London, England: Food Standards Agency; 2010. http://www.food.gov.uk/multimedia/pdfs/publication/ndnsreport0809year1results.pdf Accessed June 1, 2010.
43. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA*. 2001;285(23):2981-2986.
44. Ray JG. Folic acid food fortification in Canada. *Nutr Rev*. 2004;62(6, pt 2):S35-S39.

INVITED COMMENTARY

The Vital Amines

Too Much of a Good Thing?

In 1747, Joseph Lind demonstrated that giving citrus fruit to sailors on long voyages cured them of scurvy, a debilitating illness. Scurvy was the first disease for which the proven cause was a nutritional deficiency, although the missing nutrient, vitamin C (ascorbic acid), was not isolated until 1928.[1] In 1912, Casmir Funk proposed that inadequate consumption of "vital amines" (vitamins) in food could cause disease.[1] He had isolated vitamin B_1 (thiamine), the nutrient that, when deficient in the diet, will result in beriberi. Based on a growing recognition of diseases caused by insufficient vitamins in the diet, the US government began to require fortification of commonly consumed foods.[2,3] Examples include milk fortification with vitamin D and flour enriched with thiamine, folate, riboflavin, and niacin.[2] The goal of fortification is to prevent diseases of vitamin deficiency.

Americans, however, now take vitamins far in excess of the doses required to prevent diseases of deficiency. The number of Americans who take supplemental vitamins in hopes of optimizing their health has increased dramatically during the past 40 years. The 1999 to 2000 National Health and Nutrition Examination Survey found that 52% of adult Americans reported taking a dietary supplement.[3] This percentage has increased steadily from 23% in the first National Health and Nutrition Examination Survey performed in the early 1970s.[3] Multivitamins are the most common vitamin supplement used by Americans, followed by vitamin E (tocopherol compounds), vitamin C (ascorbic acid), and B-complex vitamins.[3] Ironically, patients with the poor-