Dr. Nissen characterizes the censoring of event data after 14 days as "unusual." Our impression is that such follow-up is actually common and is usually conservative, since it avoids the dilution of a "toxicity signal" that may occur when an active drug is discontinued.[1,2]

Dr. Furberg implies that our analysis was not an intention-to-treat analysis. This is true, in the sense that we did not follow patients more than 14 days after they discontinued treatment. However, all cardiovascular events observed during the study follow-up were assigned to treatment groups according to the original randomized assignments, according to the intention-to-treat principle. As noted above, the data for an analysis incorporating longer monitoring after the discontinuation of treatment were not available until April 28, 2006. Dr. Furberg also asks for information regarding a test for proportionality of hazards on the "three-year event data." We presented such a test in the original report, noting that the modeling for the test for proportionality of hazards contained a treatment-by-log(time) term, with a P value of 0.014. That P value was actually derived from a model that used a treatment-by-time term. The P value derived from the treatment-by-log(time) term was 0.07 (a correction notice appears in this issue of the *Journal*[3]).

Clearly, an in-depth analysis of the extended experience of the patients in the APPROVe Trial is indicated, and it is under way. It will include an independent statistical analysis of the cardiovascular data. Until that is completed and a formal report is peer-reviewed, speculations regarding what will be found are premature and may be misleading. However, it is clear that the main conclusion of the article — that "among patients with a history of colorectal adenomas, the use of rofecoxib was associated with an increased cardiovascular risk" — is unaffected.

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These letters were published at www.nejm.org on June 26, 2006.

Homocysteine, B Vitamins, and Cardiovascular Disease

TO THE EDITOR: In light of the numerous observational studies that have found a positive association between plasma homocysteine levels and the risk of cardiovascular disease, the results of two homocysteine-lowering trials — the Heart Outcomes Prevention Evaluation 2 (HOPE-2) and the Norwegian Vitamin (NORVIT) trials (April 13 issue)[1,2] — are disappointing. The relationship between homocysteine and dementia offers a similar paradox. Observational studies have shown positive associations, whereas homocysteine lowering with folic acid and B vitamins has revealed no cognitive benefit.[3]

However, the negative outcomes of these trials may not come as a complete surprise. Studies of genetic association (the so-called mendelian randomization studies) have not provided evidence of a causal relationship between functional variants of the homocysteine gene and the risk of coronary heart disease.[4] Therefore, unlike patients with familial hyperhomocysteinemia (for whom a higher level of homocysteine is a causal risk factor), patients with such increased levels in the population at large may already have vascular disease or cognitive impairment.[5] Thus, the aggregated data suggest that higher homocysteine levels may be a consequence rather than a cause of disease.

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TO THE EDITOR: The investigators in the HOPE-2 and NORVIT trials recommend against the use of folic acid, vitamin B_6, and vitamin B_{12} as preventive treatment. However, mean levels of homocysteine, folic acid, vitamin B_6, and vitamin B_{12} were in the normal range in both studies. If patients with hyperhomocysteinemia were included in these trials, would the results have been different? Furthermore, can vitamin supplementation impart benefits to patients with normal levels of these nutrients?

Asian Indians, who have a reduced intake of vitamin B_{12} and folate, are predominantly vegetarian and have higher homocysteine levels and lower levels of folate and vitamin B_{12} than do whites.[1,2] A study assessing Asian Indians reported a mean plasma homocysteine level of 19.8 mmol per liter, with 77 percent of the subjects having hyperhomocysteinemia and more than 50 percent having a deficiency of vitamin B_{12}.[3] Hyperhomocysteinemia is an independent risk factor for coronary heart disease in India and may account for the fact that twice as many Asian Indians die from the disease as do Europeans.[1] Furthermore, unlike Asian Indians, approximately 70 percent of the patients in the HOPE-2 study were exposed to folate-fortified food. Whether the study results would be applicable to Asian Indians cannot be answered until prospective, randomized trials are conducted in this population.

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TO THE EDITOR: Despite the claim by Loscalzo in the editorial[1] accompanying the reports on the HOPE-2 and NORVIT trials, the lack of benefit of lowering homocysteine concentrations with folic acid is not an "unequivocal conclusion." Relatively little is known about how homocysteine affects cardiovascular disease. Unless perturbed nutritionally or pharmacologically, homocysteine concentrations change relatively little over a five-year period[2] and presumably over a longer term. The elevated homocysteine concentration found at diagnosis in patients with cardiovascular disease was probably a chronic condition. Consequently, the cardiovascular insult may have occurred over a period of many years. There is no indication as to how long it would take to reverse such damage. All three intervention studies cited by Loscalzo were of moderate duration (2, 3.5, and 5 years) and may not reflect the benefit of long-term intervention (e.g., prolonged supplementation or universal fortification, particularly for primary prevention). A case in point is the benefit of quitting smoking, since it takes more than five years after smoking cessation for the risks of cardiovascular disease,[3] laryngeal cancer,[4] and (in heavy smokers) lung cancer[5] to diminish.

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TO THE EDITOR: The HOPE-2 investigators show a significant, 24 percent reduction in the relative risk of stroke among patients treated with folic acid and vitamins B_6 and B_{12}. They downplay this result by relegating a striking figure on the effect of this treatment on stroke (Fig. 1) to their online Supplementary Appendix. The authors suggest that the result may be spurious, but it agrees closely with the predictions of two large meta-analyses, which suggested that the same change in homocysteine levels achieved in the HOPE-2 trial would result in a reduction in stroke of 19 to 24 percent.[1,2] Their view — that a treatment benefit restricted to stroke is biologically implausible — is surprising, given the etiologic differences in coronary disease and stroke. They claim that the findings of the Vitamin Intervention for Stroke Prevention (VISP)[3] and NORVIT studies support their conclusion. However, they did not refer to the reanalysis of the VISP trial, which revealed a significant effect on stroke and coronary events.[4] They also did not mention that the NORVIT study was smaller, with a total of 98 strokes, as compared with 258 strokes in their own trial.

The message of the HOPE-2 trial should be one of cautious optimism that B vitamins may protect against stroke. Consistent with this view is a reduction in the rate of death from stroke in the United States and Canada after the introduction of folic acid fortification of food.[5]

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Dr. Refsum reports having received lecture fees from Diatomics, Nycomed, and Recip AB, as well as grant support from Axis-Shield and Nycomed; and Dr. Smith, lecture fees from Nycomed and Recip AB.

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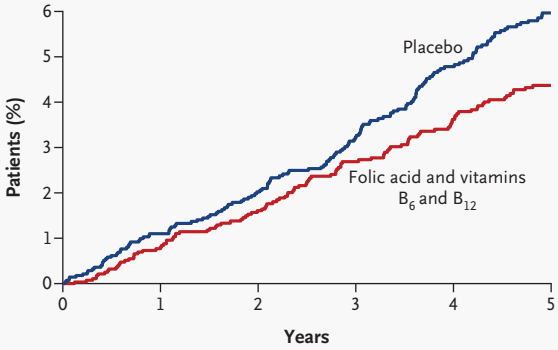

No. at Risk						
Placebo	2758	2685	2558	2441	2324	1026
Folate	2764	2670	2561	2439	2312	1044

Figure 1. Kaplan–Meier Estimates of the Percentage of Patients with Stroke during a Trial of Homocysteine Lowering.

The relative risk of stroke among patients in the active-treatment group (who received folic acid and vitamins B_6 and B_{12}), as compared with patients in the placebo group, was 0.75 (95 percent confidence interval, 0.59 to 0.97; P=0.03 by the log-rank test). Data are from the HOPE-2 trial.

TO THE EDITOR: As compared with the HOPE-2 and VISP trials, the NORVIT trial was unique in that it used a two-by-two factorial design, which allowed for an examination of the effect of each of the three treatments — folic acid plus vitamin B_{12}, vitamin B_6 alone, and folic acid plus both vitamin B_6 and vitamin B_{12}. However, the NORVIT trial did not report the analyses that we believe are important for a comprehensive assessment of the effects of B vitamins: a comparison of folic acid plus vitamin B_{12} with placebo, a comparison of vitamin B_6 with placebo, and a test of the interaction between folic acid plus vitamin B_6 and vitamin B_{12} in relation to the clinical outcome.

In Table 1, we present our analyses using data from the NORVIT trial. We calculated the rate ratios for each treatment using the number of observed cases and person-years and Poisson distribution and test-based methods to construct confidence intervals.[1] We also calculated the rate ratio for the interaction between folic acid plus vitamin B_6 and vitamin B_{12}, and we estimated the standard error of the rate ratio by the multivariate delta method.[2] We subsequently used this standard error to construct the confidence interval for the rate ratio of the interaction.

Our analyses showed that as compared with placebo, folic acid plus vitamin B_{12} has a slightly

Table 1. Rate Ratios for the Three Treatment Groups and Tests of Interactions between Folic Acid plus Vitamin B_6 and Vitamin B_{12} on Clinical Outcomes.*

Variable	Total No. of Patients	Folic Acid plus Vitamin B_{12} vs. Placebo		Vitamin B_6 vs. Placebo		Folic Acid plus Vitamin B_6 and Vitamin B_{12} vs. Placebo		Test of Interaction between Folic Acid plus Vitamin B_6 and Vitamin B_{12}†	
		Rate Ratio (95% CI)	P Value	Rate Ratio (95% CI)	P Value	Rate Ratio (95% CI)	P Value	Rate Ratio (95% CI)	P Value
Primary end point ‡	716	0.98 (0.79–1.22)	0.37	1.02 (0.82–1.26)	0.38	1.22 (1.00–1.50)	0.05	1.22 (1.12–1.32)	0.01
Myocardial infarction§	643	0.96 (0.76–1.21)	0.46	1.05 (0.84–1.32)	0.51	1.23 (0.99–1.52)	0.06	1.22 (1.10–1.34)	0.01
Stroke	98	1.04 (0.59–1.83)	0.73	0.81 (0.45–1.49)	0.28	0.83 (0.47–1.47)	0.52	0.99 (0.43–1.54)	0.49
Death from any cause	365	0.90 (0.66–1.23)	0.59	1.03 (0.76–1.40)	0.31	1.21 (0.91–1.61)	0.19	1.31 (1.06–1.55)	0.01
Hospitalization for unstable angina pectoris	488	0.95 (0.74–1.23)	0.44	0.80 (0.61–1.04)	0.12	0.93 (0.73–1.19)	0.57	1.22 (1.07–1.38)	0.01
Coronary-artery bypass surgery	584	0.89 (0.70–1.12)	0.16	0.96 (0.76–1.20)	0.40	0.89 (0.71–1.13)	0.34	1.04 (0.94–1.14)	0.07
Percutaneous coronary intervention	1096	0.93 (0.79–1.10)	0.14	0.96 (0.81–1.14)	0.37	0.86 (0.72–1.02)	0.08	0.96 (0.89–1.03)	0.06
Cancer	144	0.98 (0.61–1.55)	0.31	0.62 (0.37–1.06)	0.07	1.02 (0.65–1.58)	0.94	1.68 (1.12–2.24)	0.02

* CI denotes confidence interval.

† The interaction was calculated by dividing the rate ratio of the group that received folic acid plus vitamins B_6 and B_{12} by the product of the rate ratio of the group that received vitamin B_6 multiplied by the rate ratio of the group that received folic acid plus vitamin B_{12}.

‡ The primary end point was a composite of nonfatal or fatal myocardial infarction (including sudden death attributed to coronary heart disease) and nonfatal or fatal stroke. Only the first event was included in the composite primary end point.

§ If a participant first had a nonfatal myocardial infarction and then a fatal myocardial infarction, only the nonfatal myocardial infarction was included in the category of myocardial infarction.

but not significantly beneficial effect on most of the clinical outcomes; the same is true for vitamin B_6. We found a significant adverse interaction between folic acid plus vitamin B_6 and vitamin B_{12} on all clinical outcomes except stroke, coronary-artery bypass surgery, and percutaneous coronary intervention. If our findings can be independently replicated, one would conclude that it was the interaction between folic acid plus vitamin B_6 and vitamin B_{12} that led to significantly worse outcomes, whereas there was no evidence that treatment with folic acid plus vitamin B_{12} or with vitamin B_6 alone was harmful.

The three trials raise further questions to be addressed in future research. First, all three trials used a high-dose formulation, including 2 to 6 times the recommended daily allowance (RDA) of folic acid, 166 to 416 times the RDA of vitamin B_{12}, and 12 to 25 times the RDA of vitamin B_6. The interactions among high-dose treatments may lead to undesirable clinical outcomes. Future studies will need to determine the optimal dose and combination that maximize efficacy and minimize adverse effects. Second, all three trials were conducted in patients with existing cardiovascular disease. Studies are needed to assess the role of B vitamins in the primary prevention of cardiovascular disease, especially in persons with a low intake of B vitamins or with a genetic susceptibility to hyperhomocysteinemia. Third, all three trials reported the averages of the effects in all the treatment groups. However, individual responses to the treatments may vary greatly, depending on the person's homocysteine metabolism and genetic susceptibility, as well as on the presence of other known risk factors for cardiovascular disease. Future studies will need to find a better way to identify patients who will benefit the most from interventions with B vitamins.

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TO THE EDITOR: Though elevated levels of homocysteine are predictive of cardiovascular risk, the demonstration that homocysteine-lowering therapy was without benefit in the HOPE-2 and NORVIT trials provides further evidence that homocysteine represents an epiphenomenon in atherosclerosis. The pathogenesis of atherosclerosis is characterized by chronic inflammation,[1] and elevated plasma homocysteine concentrations have been correlated with inflammation in conditions such as chronic renal failure, rheumatoid arthritis,[2] and psoriasis and in the period after myocardial infarction.[3] However, clinical studies have demonstrated that endothelial dysfunction does not improve despite effective lowering of homocysteine levels with 400 μg of folic acid orally per day.[4] High-dose oral folic acid (5 to 20 mg per day) improves endothelial function in a manner largely independent of plasma homocysteine lowering, though the underlying mechanism has not been established.[5] It is our view, therefore, that high-dose folate therapy (exceeding 5 mg daily) has pleiotropic effects, the benefits of which have yet to be tested in large-scale secondary-prevention trials in appropriate subjects.

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DR. LONN REPLIES: De Craen et al. refer to a recent meta-analysis of mendelian randomization studies to suggest reverse causality as an explanation for the results of the HOPE-2 and NORVIT trials involving patients with coronary heart disease. Findings in patients with stroke may differ.[1] Additional plausible explanations include residual

confounding in epidemiologic studies and the possibility that any harmful effects of high-dose folate may offset the benefits of homocysteine lowering.

In response to the comments of Refsum and Smith, we do not dismiss the results of the HOPE-2 study in regard to stroke but caution against an overenthusiastic interpretation on the basis of multiple considerations: the overall neutral effect of treatment on the primary outcome and on most secondary and tertiary outcomes (including transient ischemic attacks), the wide confidence intervals around the estimated reduction in the risk of stroke, the apparent increase in the risk of unstable angina, and the neutral results regarding stroke in the VISP and NORVIT trials. The quoted reanalysis of the VISP trial was not prespecified and showed no reduction in stroke (the primary study outcome), death, or events associated with coronary heart disease. At best, the reanalysis showed a borderline effect on a composite cardiovascular outcome (unadjusted outcome, P=0.049; adjusted outcome, P=0.056), which is not a very convincing result to use to justify any clear treatment recommendations. A causal link between recent trends toward a lower rate of death from stroke in the United States and Canada and the fortification of food with folic acid remains speculative, since many other factors may have contributed to the decline.

With regard to the comments by Wang et al. and Tomlinson et al., the doses and combinations of B vitamins used in the large clinical trials are based on the ability of the drugs to reduce homocysteine levels in most people and on the perceived safety of the drugs.[2] Whether lower or higher doses or different combinations may be useful remains unproven. A reasonable approach is to encourage people to have balanced diets, since such diets provide adequate amounts of needed macronutrients and micronutrients in most people, and to reserve the use of vitamin therapy for those with proven deficiencies.

Khare et al. and Wang et al. note that the clinical trials studied primarily white, middle-aged patients with vascular disease. Trials of primary prevention and in populations with higher homocysteine levels that are related to genetic and dietary factors are of interest, and we strongly support the completion of ongoing studies in various populations. However, we are unaware of any cardiovascular therapies that are exclusively effective for primary prevention, and in our trial, even patients in the upper fifth of the baseline homocysteine distribution (≥19.7 μmol per liter) derived no benefit.

Quinlivan and Gregory point out that the trials of B vitamin supplementation are of intermediate duration (two to five years). Most proven preventive therapies, such as the lowering of levels of cholesterol and blood pressure, reduce risk within months to a few years, and in several studies, such as the British Doctors Study, excess risk was halved within two to three years after the cessation of smoking.[3]

In summary, completed clinical trials do not provide evidence to support the preventive use of B vitamin supplements. Ongoing large trials and the planned meta-analysis of all trials[4] will answer remaining relevant clinical questions.

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DR. BØNAA AND COLLEAGUES REPLY: The data from the HOPE-2, NORVIT, and VISP trials are quite consistent in showing that homocysteine lowering with folic acid and vitamin B_{12} (with or without vitamin B_6) has no clinical benefit in patients with established vascular disease. The negative results may be interpreted in three ways.

First, homocysteine may not be a causative agent in vascular disease. High homocysteine levels may be an indicator of an unhealthy lifestyle, an epiphenomenon reflecting atherogenic processes, or a consequence of vascular disease itself, as suggested by de Craen et al. and Tomlinson et al.

Second, homocysteine-lowering therapy may still be beneficial in populations other than those

studied — for example, in patients with hyperhomocysteinemia, as suggested by Khare et al. However, in the NORVIT trial, this therapy had no benefit in the 40 percent of patients with a baseline homocysteine level above 13 μmol per liter (in this subgroup, the mean homocysteine level was 17.4 μmol per liter). As pointed out by Quinlivan and Gregory and Wang et al., the trials may have been too short (mean duration, 2.5 to 5 years), and the results could possibly be different for primary prevention. However, most conventional treatments (including smoking cessation) show effects on vascular disease within five years. The results of the NORVIT trial do not preclude a protective effect of more physiologic doses of B vitamins or in primary prevention. However, it is difficult to explain biologically how a therapy that does not work in patients who have had a clinical vascular event would work well in those without a clinical event (many of whom have subclinical atherosclerosis).

Finally, as suggested by Loscalzo in his editorial, B vitamin therapy could have harmful effects that offset the homocysteine-lowering benefit. Wang et al. suggest that the trend toward a harmful effect in the combined B vitamin treatment group that was observed in the NORVIT trial was due to a significant interaction between folate plus vitamin B_6 and vitamin B_{12}. However, we believe the confidence intervals they present for the test of interaction are too narrow, and they cannot precisely estimate the rate ratios adjusted for study center from the numbers given in our article. Using rate ratios adjusted for study center estimated from Cox proportional-hazard regression and the method described by Altman and Bland,[1] we obtained a test statistic (ratio of ratios) of 1.18 (95 percent confidence interval, 0.88 to 1.59) for the primary end point, indicating that there was no significant interaction.

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The Challenge of Subgroup Analyses

TO THE EDITOR: The Perspective article by Lagakos (April 20 issue)[1] was a welcome explication of a contentious topic. Although the article focused on the role of chance and false positive results, it did not discuss another, more pernicious problem — bias. Whenever a subgroup analysis is performed, the randomization of patient characteristics between the treatment group and the control group is no longer necessarily maintained. Consider a subgroup analysis according to sex. The randomization process should ensure, if the sample is large enough, that the treatment and control groups are balanced according to sex. But randomization does not ensure that the two groups are balanced within the sex strata. If the men who received placebo are older and more severely ill than those in the treatment group, then the treatment may appear to be more beneficial among men, when in fact the result is due to the confounding effect of age and severity of illness. Specifying subgroups before the trial is conducted does not mitigate this bias; mitigation would require stratification according to the subgroup variable before randomization, so that patient characteristics would be balanced in the two groups within each subgroup stratum.

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1. Lagakos SW. The challenge of subgroup analyses — reporting without distorting. N Engl J Med 2006;354:1667-9.

DR. LAGAKOS REPLIES: When performing ordinary randomization, we expect treatment groups to be balanced with respect to important patient characteristics, both in the entire sample and in any specific subgroup. However, randomization does not guarantee such balance, and when multiple subgroup analyses are conducted, the chances are