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Vitamin Intervention for Stroke Prevention Trial

An Efficacy Analysis

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Background and Purpose—The Vitamin Intervention for Stroke Prevention trial (VISP) intention-to-treat analysis did not show efficacy of combined vitamin therapy for recurrent vascular events in patients with nondisabling stroke. Reasons for lack of efficacy may have included folate fortification of grain products, inclusion of the recommended daily intake for B₁₂ in the low-dose arm, treatment with parenteral B₁₂ in patients with low B₁₂ levels in both study arms, a dose of B₁₂ too low for patients with malabsorption, supplementation with nonstudy vitamins, and failure of patients with significant renal impairment to respond to vitamin therapy. We conducted an efficacy analysis limited to patients most likely to benefit from the treatment, based on hypotheses arising from evidence developed since VISP was initiated. The criteria for this subgroup were defined before any data analysis.

Methods—For this analysis, we excluded patients with low and very high B₁₂ levels at baseline (<250 and >637 pmol/L, representing the 25th and 95th percentiles), to exclude those likely to have B₁₂ malabsorption or to be taking B₁₂ supplements outside the study and patients with significant renal impairment (glomerular filtration rate <46.18; the 10th percentile).

Results—This subgroup represents 2155 patients (37% female), with a mean age of 66±10.7 years. For the combined end point of ischemic stroke, coronary disease, or death, there was a 21% reduction in the risk of events in the high-dose group compared with the low-dose group (unadjusted *P*=0.049; adjusted for age, sex, blood pressure, smoking, and B₁₂ level *P*=0.056). In Kaplan–Meier survival analysis comparing 4 groups, patients with a baseline B₁₂ level at the median or higher randomized to high-dose vitamin had the best overall outcome, and those with B₁₂ less than the median assigned to low-dose vitamin had the worst (*P*=0.02 for combined stroke, death, and coronary events; *P*=0.03 for stroke and coronary events).

Conclusions—In the era of folate fortification, B₁₂ plays a key role in vitamin therapy for total homocysteine. Higher doses of B₁₂, and other treatments to lower total homocysteine may be needed for some patients. (*Stroke*. 2005;36:2404-2409.)

Key Words: homocysteine ■ stroke prevention ■ stroke ■ vitamins

Elevated plasma total homocysteine (tHcy) is a strong, graded, independent risk factor for stroke, myocardial infarction, and other vascular events. In prospective studies, tHcy >10.2 μmol/L is associated with a doubling of vascular risk,¹ and levels >20 μmol/L are associated with an 8.9-fold increase in vascular risk.² Mechanisms by which tHcy may cause vascular disease include propensity for thrombosis and impaired thrombolysis,^{3–6} increased production of hydrogen peroxide,⁷ endothelial dysfunction,^{8–11} and increased oxidation of low-density lipoprotein and lipoprotein(a).¹² Vitamin therapy with folate, pyridoxine (B₆), and cobalamin (B₁₂) reduces tHcy¹³ and reverses endothelial dysfunction induced by high tHcy.^{8,10}

Therefore, we conducted the Vitamin Intervention for Stroke Prevention (VISP) trial to determine whether treatment with high-dose vitamin therapy (2.5 mg folate, 25 mg B₆, and 400 mcg B₁₂ daily) significantly reduced stroke and the combined end point of stroke, death, and myocardial infarction compared with low-dose vitamin therapy (20 mcg folate, 200 mcg B₆, and 6 mcg B₁₂). In the main analysis, which was an intention-to-treat analysis, the difference in outcomes was very small, and the study was stopped because of futility.¹⁴

We considered several possible explanations for the lack of efficacy of vitamin therapy in VISP as alternatives to the possibility that vitamin therapy may not reduce vascular risk.

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Folate fortification of the grain supply in North America coincided with the initiation of the study (it was mandated in the United States as of January 1998 but began in 1996 in Canada and the United States as grain producers prepared for the mandated fortification). Fortification has markedly reduced the proportion of the population with very low blood levels of folate (to $\approx 1\%$). Consequently, plasma levels of tHcy are also lower.¹⁵ Thus, the difference in mean tHcy levels between the high-dose and low-dose groups was only $2 \mu\text{mol/L}$ at the beginning of the study and decreased to only $1.5 \mu\text{mol/L}$ by the end of the study. The study vitamin in both arms included the recommended daily intake (RDI) of all vitamins other than folate/B₆/B₁₂. Because of concern about subacute combined degeneration and neuropathy, we included the RDI for B₁₂ in the low-dose arm. In both the high-dose and low-dose arms of the study, patients with low levels of B₁₂ ($<150 \text{ pmol/L}$) received B₁₂ injections to prevent neurological complications. Furthermore, we used in the high-dose arm a dose of B₁₂ that may have been too low for elderly patients with impaired absorption of B₁₂. Rajan et al¹⁶ showed that in elderly patients with B₁₂ $<221 \text{ pmol/L}$, a dose of $1000 \mu\text{g/day}$ was required for adequate absorption of B₁₂; in VISP, we used only 400 mcg/day .

Additional evidence that higher doses of B₁₂ may be required for patients with vascular disease is provided by comparing the results of vitamin therapy in patients undergoing coronary angioplasty. Treatment with folate, B₆, and a dose of B₁₂ equal to that used in VISP reduced restenosis¹⁷ and subsequent events,¹⁸ but in a study using only one-tenth the dose of B₁₂, there was no reduction of restenosis.¹⁹

Some of our patients had very high levels of B₁₂ ($\approx 1000 \text{ pmol/L}$) suggesting supplementation with nonstudy vitamins. In VISP, we excluded patients with severe renal failure requiring dialysis, because such patients have very high levels of tHcy that are known not to respond well to vitamin supplements.²⁰ However, we found that 10% of our patients had significant renal impairment, with a glomerular filtration rate (GFR) <47 calculated from the Cockcroft–Gault formula; these patients would be less responsive to vitamin therapy.

Finally, we found in patients in a stroke prevention clinic that, in the era since the folate fortification of grain products, serum B₁₂ is strongly related to plasma tHcy and to carotid plaque area.²¹ Therefore, we hypothesized that B₁₂ status may be a key determinant of response to vitamin therapy in VISP.

We therefore analyzed the relationship between serum B₁₂ and tHcy and conducted an efficacy analysis limited to participants who we considered a priori (before any data analysis) to be most likely to benefit from the treatment based on these hypotheses.

Methods

The methods for the VISP trial have been reported previously.^{14,22} In brief, it was a randomized, double-blind trial conducted September 1996 to May 2003 in 3680 adults with nondisabling cerebral infarction at 56 university-affiliated hospitals, community hospitals, private neurology practices, and VA Medical Centers across the United States, Canada, and 1 center in Scotland. We compared the best medical/surgical management plus a daily multivitamin with the Food and Drug Administration RDI of other vitamins; the high-dose formulation containing 25 mg of vitamin B₆, 0.4 mg of

vitamin B₁₂, and 2.5 mg of folic acid; and the low-dose formulation containing 200 μg of vitamin B₆, 6 μg of vitamin B₁₂, and 20 μg of folic acid. The main outcome measures were recurrent cerebral infarction (primary outcome), coronary heart disease (CHD), and death (secondary outcomes).

Subgroup Selected for This Analysis

Based on the hypotheses described above, we selected patients who would be more likely to respond to vitamin therapy, with GFR above the 10th percentile for calculated GFR (≥ 46.18) and with serum B₁₂ levels in the 25th to 95th percentiles (250 to 637 pmol/L). We hypothesized that those above the 95th percentile were likely taking nonstudy supplements and that those below the 25th percentile likely had some form of B₁₂ malabsorption, based on the findings of Rajan et al.¹⁶ Only a single subgroup was defined based on criteria that were set before any subgroup analyses were performed.

Statistical Analyses

Unadjusted survival probabilities within each treatment arm were estimated by the Kaplan–Meier method²³ in this subgroup. Log-rank tests are reported for the test of equality of survival curves between groups. To control for potential confounding, the Cox proportional hazard regression model was used after adjusting for important covariates at baseline, including age, sex, blood pressure, smoking, and B₁₂ level.²⁴ Adjusted tHcy values (along with confidence limits) were estimated using the analysis of covariance. SAS software, version 8 (SAS Institute Inc.), was used for all of the analyses, and 2-sided hypotheses were adopted for statistical inference. To explore the role of baseline B₁₂ status on response to therapy, we also studied the survival probabilities within strata of baseline B₁₂ using a median split. We made no adjustment for multiple testing.

Results

After the exclusions of original participants because of baseline vitamin B₁₂ levels below the 25th percentile or above the 95th percentile and those with GFR below the 10th percentile, 2155 patients remained in the analysis. Table 1 shows the characteristics of the participants in this subgroup compared with the entire study group according to the randomized assignment. The characteristics were very similar to those of the overall trial participants, except that, by definition, the mean GFR was slightly higher (78.6 versus 74.7), and the mean tHcy levels were slightly lower (12.6 versus 13.4). The subgroup was also slightly younger and had a slightly higher mean low-density lipoprotein cholesterol level. As in the intention-to-treat analysis, there were significantly more smokers in the high-dose vitamin group. Otherwise, the values were virtually identical across the randomized treatment assignment in the subgroup.

Figure 1 shows the relationship of tHcy to deciles of serum B₁₂ in our subgroup. Plasma tHcy rises significantly as serum B₁₂ falls, from levels that are above the median (322 pmol/L). This suggests that B₁₂ levels that are sufficient to maintain low tHcy are higher than those usually regarded as normal or adequate and supports the hypothesis that higher doses of B₁₂ may be needed in many patients.

Table 2 shows the results for the comparison of the high-dose vitamin versus low-dose vitamin treatment groups in the subgroup of patients who met the criteria for this efficacy analysis. For the combined end point of ischemic stroke, coronary disease, or death, there was a 21% reduction in the risk of end point in the high-dose group compared with the low-dose group ($P=0.049$). There was little change when adjusting for age, sex, blood pressure, and smoking

TABLE 1. Baseline Characteristics of Our Subgroup Versus All Randomized Mean (SD) for Continuous Variables and Percentage for Binary Variables

| Variables | High Dose | | Low Dose | |
|-----------------------------------|----------------------------|----------------------|----------------------------|----------------------|
| | All Randomized (n=1827) | Subgroup (n=1047) | All Randomized (n=1853) | Subgroup (n=1108) |
| Age, y§ | 66.4 (10.8) | 65.6 (10.6) | 66.2 (10.8) | 65.8 (10.7) |
| Women | 37.7 | 38.2 | 37.2 | 36.6 |
| Race, black | 14.2 | 15.3 | 15.4 | 16.5 |
| Total cholesterol, mg/dL | 200.8 (46.9) | 202.1 (45.9) | 203.0 (46.5) | 203.0 (46.0) |
| HDL cholesterol, mg/dL | 45.2 (15.3) | 45.7 (15.2) | 45.6 (15.7) | 45.9 (15.8) |
| LDL cholesterol, mg/dL‡ | 121.6 (40.2) | 124.0 (40.0) | 123.3 (40.1) | 123.3 (40.4) |
| Systolic blood pressure, mm Hg | 141.1 (18.6) | 140.3 (18.2) | 140.6 (18.9) | 140.4 (19.1) |
| Diastolic blood pressure, mm Hg | 78.0 (10.0) | 78.1 (10.1) | 77.8 (10.1) | 77.9 (9.9) |
| B ₁₂ , pmol/L§ | 362.4 (268.3) | 371.1 (89.3) | 360.6 (180.7) | 373.9 (90.5) |
| tHcy at baseline, μ mol/L§ | 13.4 (4.9) | 12.5 (4.0) | 13.4 (5.1) | 12.6 (3.9) |
| GFR, mL/min/1.73 m ² § | 74.6 (44.1) | 79.4 (41.0) | 74.8 (26.1) | 77.9 (22.9) |
| Folate, nmol/L | 26.8 (18.7) | 26.9 (17.8) | 26.7 (18.1) | 26.9 (17.3) |
| Smoking, ever | 67.8% | 67.9% | 65.1% | 66.0% |
| Present smoker | 18.3% | 19%* | 15.5% | 15.6% |

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

* $P < 0.05$; ‡ $P < 0.05$; § $P < 0.01$ for comparisons between subgroup and all randomized treatment groups combined;

|| $P < 0.05$;

($P = 0.056$), implying that no serious confounding exists. The differences for the other end points were in the same direction although not statistically significant in either unadjusted or adjusted analysis.

We found no significant diurnal variation during the daytime hours of the VISP visits nor any significant relation to fasting versus fed state and tHcy. There was no significant association between outcome and change in tHcy between the baseline and 1-month visit controlling for treatment group, B₁₂, age, sex, smoking status, and baseline homocysteine.

To additionally examine the impact of baseline levels of vitamin B₁₂ (reflecting ability to absorb B₁₂) as a potential modifier of the effect of vitamin therapy, we analyzed the

data by dichotomizing the participants at the median vitamin B₁₂ level (322 pmol/L), representing groups with and without adequate ability to absorb B₁₂.¹⁶ Figure 2 shows Kaplan–Meier plots for the 4 groups, divided by B₁₂ levels, and randomized treatment assignment for survival free of stroke and coronary event, and the combined end point of stroke, CHD, or death. Inspection of the graphs reveals that those in the top half of the distribution for the baseline B₁₂ level who were randomized to the high-dose vitamin intervention had the best overall outcome, and those with lower B₁₂ levels at baseline, assigned to the low-dose group, had the worst. The analysis for the 4-group comparison showed a significant difference in survival free from stroke or coronary event

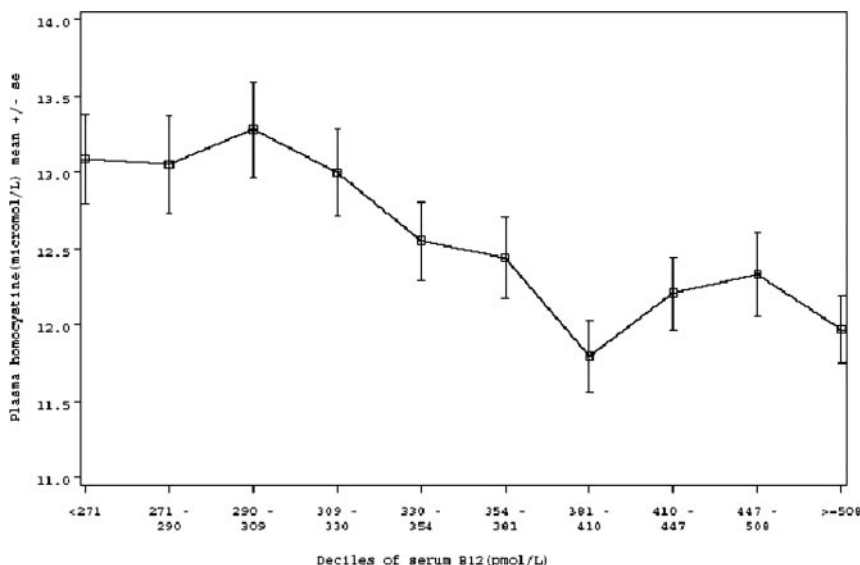


Figure 1. Baseline plasma total homocysteine (mean \pm SE; μ mol/L), by deciles of serum B₁₂ (pmol/L) adjusted for age, sex, smoking, and GFR within our subgroup.

TABLE 2. Hazard Rate Ratios Comparing High-Dose With Low-Dose Vitamin Groups in Selected Analysis Subgroup (Unadjusted or Adjusted*) With P Values

| Variables | Unadjusted | | | | Adjusted | | | |
|----------------------------------|------------|------------|------------|----------|----------|------------|------------|----------|
| | HRR | 95% HRR LL | 95% HRR UL | P Values | HRR | 95% HRR LL | 95% HRR UL | P Values |
| Death | 0.79 | 0.54 | 1.16 | 0.23 | 0.81 | 0.55 | 1.18 | 0.28 |
| Coronary | 0.86 | 0.60 | 1.24 | 0.43 | 0.88 | 0.61 | 1.27 | 0.49 |
| Stroke | 0.92 | 0.68 | 1.25 | 0.60 | 0.91 | 0.67 | 1.24 | 0.56 |
| Combined stroke and coronary | 0.83 | 0.66 | 1.06 | 0.14 | 0.84 | 0.66 | 1.06 | 0.14 |
| Combined stroke, death, coronary | 0.79 | 0.63 | 1.00 | 0.049 | 0.80 | 0.63 | 1.01 | 0.056 |

HRR indicates hazard rate ratio; LL, lower limit; UL, upper limit (of the 95% CI).

*From the Cox proportional hazard regression model, adjusted for age, sex, blood pressure, smoking, and B₁₂ level.

(*P*=0.03; Figure 2) and for stroke, death, or coronary event (*P*=0.02; Figure 3). Event-free survival was not significantly different for the lone events of stroke (*P*=0.31), death (*P*=0.52), or coronary event (*P*=0.41).

Discussion

The VISP trial showed no effect of high-dose vitamin therapy on the outcome measures of stroke, CHD events, or death among individuals with a nondisabling stroke.¹⁴ However, in the present analyses, based on a priori hypotheses, we identified a substantial subgroup that appeared to benefit from the intervention.

Folate fortification of the grain products was mandated in the United States as of January 1998,²⁵ but its implementation in the United States and Canada began in 1996, the time that the VISP trial was initiated. Fortification increased folate intake and substantially reduced the prevalence of high homocysteine and low folate. For example, in the Framingham Study, the proportion with folate deficiency declined from 22% before fortification to 1.7% afterward.¹⁵ As noted earlier, the distribution of tHcy levels changed during the course of our trial, with a decline of the 25th percentile. Fortification probably reduced the number of those with high tHcy who might be most likely to benefit.²⁶ Dose-response

studies have shown that the dose of folate achieved with fortification of grain products will approach maximal effects of folate supplementation.²⁷ Furthermore, the correction of low serum B₁₂ levels may have blunted the effect of the high-dose vitamin treatment.

To address these issues, we identified, based on the hypotheses described above, a subgroup within the VISP trial participants who were most likely to benefit from the intervention. We excluded those within the bottom 10% of the distribution of renal function, based on the estimated GFR. As noted, although patients with renal failure tend to have elevated tHcy levels, they are relatively insensitive to vitamin therapy.²⁸ We additionally excluded those above the 95th percentile for vitamin B₁₂ levels, suspecting that they may have already been taking B₁₂ supplements and would be unlikely to benefit from the intervention. Finally, we excluded those below the 25th percentile for B₁₂ levels. This exclusion may appear paradoxical, as one might expect those with the lowest B₁₂ levels to benefit most from supplements. However, patients in both treatment groups in VISP with serum B₁₂ <150 pmol/L were treated with parenteral B₁₂; furthermore, except in vegetarians, low-circulating levels of B₁₂ largely reflect some form of B₁₂ malabsorption. A dose-response study in elderly patients, performed after VISP was

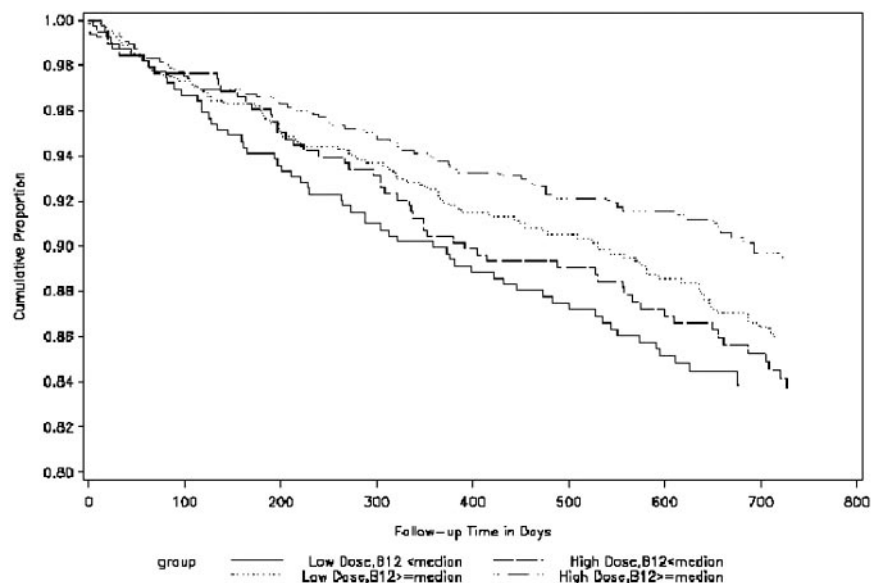


Figure 2. Kaplan–Meier survival free of combined stroke and coronary events by baseline B₁₂ stratum (above or below the median of 322 pmol/L) and treatment group (high-dose versus low-dose vitamin therapy); *P*=0.03 from log-rank test comparing all 4 groups.

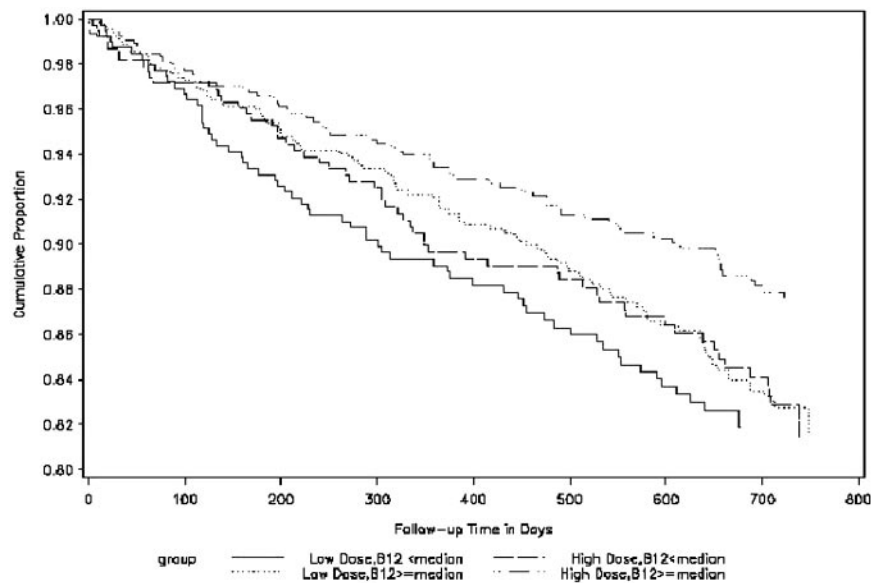


Figure 3. Kaplan-Meier survival free of combined stroke, death, and coronary events by baseline B₁₂ stratum (above or below the median level of 322 pmol/L) and treatment group (high-dose versus low-dose vitamin therapy); $P=0.02$ from log-rank test comparing all 4 groups.

initiated, showed that individuals with B₁₂ levels below the median for the population require higher doses of oral vitamin B₁₂ than used in VISP (1000 versus 400 μg daily) to achieve adequate B₁₂ levels.¹⁶ Those in the middle range (after exclusion of individuals already treated with B₁₂ outside the randomized treatment and those probably taking supplements outside the study) were, therefore, most likely to benefit from the intervention. It seems likely that the reason patients with baseline B₁₂ levels above the median responded best to the VISP high-dose vitamin (which contained only 400 mcg of B₁₂) was because they were able to absorb enough B₁₂ from the dose we used; it also seems likely that higher doses of B₁₂ would be effective in a larger proportion of patients. (Parenteral administration is seldom required if the dose of oral B₁₂ is high; exceptions might include patients with short bowel syndrome after removal of the small intestine.)

Wald et al²⁹ estimated that reducing tHcy by 3 $\mu\text{mol/L}$ would be associated with a 24% decrease in stroke risk and a 16% decrease in CHD. For the 2- $\mu\text{mol/L}$ difference observed in the overall VISP trial, this would translate to a power of 31% for the trial to detect a significant reduction in clinical end points. Another meta-analysis³⁰ observed similar overall findings from observational studies. The VISP trial also observed a relation between higher baseline tHcy levels and risk of recurrent stroke; this effect was blunted in the intervention group ($P=0.24$ versus 0.02 in the low-dose group), suggesting a potential benefit concentrated among those amenable to being affected by the treatment.

Our results are somewhat surprising in that we found a greater benefit of vitamin therapy in this subgroup that had lower baseline tHcy than in the main study and no significant relationship between the magnitude of reduction of tHcy between baseline and 1 month and subsequent cardiovascular events. It is possible that patients with vascular disease may benefit from effective lowering of homocysteine, regardless of their baseline tHcy, in a way analogous to the greater response to more intensive treatment with statin drugs regardless of baseline levels of low-density lipoprotein.^{31,32}

Post hoc subgroup analyses of randomized trials must be interpreted with considerable caution. Because the subgroup definition is based solely on baseline characteristics without regard to any postrandomization events, the potential to introduce bias or confounding is essentially eliminated with a large number of participants. The chief danger in such analyses is the potential to try many different definitions for subgroups and present selected findings based on the results of the analyses. In the present analysis, we defined the subgroup only once, based on the hypotheses described above, and before data analysis; only 1 analysis was carried out.

Several trials of vitamin supplementation are being conducted in Australia, Asia, and other countries that, in general, do not have folate fortification³³ and where mean levels of tHcy are higher than are now prevalent in North America. In such trials, it would be worthwhile to define a priori subgroups of patients based on their B₁₂ status. In postfortification North America, much of the variation in tHcy levels will be driven by vitamin B₁₂ status. Higher doses of oral B₁₂ and other treatments, such as betaine³⁴ and thiol compounds³⁵ to lower tHcy, may be needed for some patients.

Conclusions

Survival free of cardiovascular events was improved by vitamin therapy among a subgroup of patients in the VISP trial who were more likely to respond. The subgroup was selected by excluding those likely to have B₁₂ malabsorption, those who received parenteral B₁₂ and other B₁₂ supplements, and those with renal failure. Subdividing the patients by baseline B₁₂ levels, thus identifying those with or without adequate absorption of B₁₂, accentuated the differences between the 2 treatment groups. Thus, in the era after folate fortification of the grain supply, response to vitamin therapy to lower tHcy is largely dependent on B₁₂ status, and higher doses of B₁₂, in addition to other therapies such as betaine and thiols, will be required to achieve optimal reduction of tHcy. Treatment to lower tHcy should no longer be called "folate therapy."

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