Vitamin Intervention for Stroke Prevention Trial: An Efficacy Analysis

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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.
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 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 al\(^{16}\) showed that in elderly patients with B12 <221 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 restenosis\(^{17}\) 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 (>1000 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 \( \mu \text{g} \) of vitamin B6, 6 \( \mu \text{g} \) of vitamin B12, and 20 \( \mu \text{g} \) 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 method\(^{23}\) 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 plasma B12 are 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.
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. Figure 2 shows Kaplan–Meier plots for the 4 groups, divided by B12 levels, and randomized treatment assignment for survival free of stroke or 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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>High Dose</th>
<th>Low Dose</th>
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<tr>
<td></td>
<td>All Randomized</td>
<td>Subgroup</td>
</tr>
<tr>
<td></td>
<td>(n=1827)</td>
<td>(n=1047)</td>
</tr>
<tr>
<td>Age, y§</td>
<td>66.4 (10.8)</td>
<td>65.6 (10.6)</td>
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<tr>
<td>Women</td>
<td>37.7</td>
<td>38.2</td>
</tr>
<tr>
<td>Race, black</td>
<td>14.2</td>
<td>15.3</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>200.8 (46.9)</td>
<td>202.1 (45.9)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>45.2 (15.3)</td>
<td>45.7 (15.2)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL‡</td>
<td>121.6 (40.2)</td>
<td>124.0 (40.0)</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>141.1 (18.6)</td>
<td>140.3 (18.2)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78.0 (10.0)</td>
<td>78.1 (10.1)</td>
</tr>
<tr>
<td>B12, pmol/L§</td>
<td>362.4 (268.3)</td>
<td>371.1 (89.3)</td>
</tr>
<tr>
<td>tHcy at baseline, µmol/L§</td>
<td>13.4 (4.9)</td>
<td>12.5 (4.0)</td>
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<tr>
<td>GFR, mL/min/1.73 m²§</td>
<td>74.6 (44.1)</td>
<td>79.4 (41.0)</td>
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<tr>
<td>Folate, nmol/L</td>
<td>26.8 (18.7)</td>
<td>26.9 (17.8)</td>
</tr>
<tr>
<td>Smoking, ever</td>
<td>67.8%</td>
<td>67.9%</td>
</tr>
<tr>
<td>Present smoker</td>
<td>18.3%†</td>
<td>19%*</td>
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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; \( |P|<0.05; \)

**Figure 1.** Baseline plasma total homocysteine (mean±SE; µmol/L), by deciles of serum B12 (pmol/L) adjusted for age, sex, smoking, and GFR within our subgroup.
(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. 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, 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. 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. Dose-response studies have shown that the dose of folate achieved with fortification of grain products will approach maximal effects of folate supplementation. 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. 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 completed, showed that the benefit of high-dose vitamin therapy was greatest for those with the lowest baseline B12 levels.

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

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<th>Adjusted</th>
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<td></td>
<td>95% HRR</td>
<td>95% HRR</td>
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<tr>
<td>Death</td>
<td>0.79</td>
<td>0.54</td>
</tr>
<tr>
<td>Coronary</td>
<td>0.86</td>
<td>0.60</td>
</tr>
<tr>
<td>Stroke</td>
<td>0.92</td>
<td>0.68</td>
</tr>
<tr>
<td>Combined stroke and coronary</td>
<td>0.83</td>
<td>0.66</td>
</tr>
<tr>
<td>Combined stroke, death, coronary</td>
<td>0.79</td>
<td>0.63</td>
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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.

**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.
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. 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 al estimated that reducing tHcy by 3 μmol/L would be associated with a 24% decrease in stroke risk and a 16% decrease in CHD. For the 2-μmol/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 endpoints. Another meta-analysis 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 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.

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 fortification 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 betaine and thiols, 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.”
Acknowledgments

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References


