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Homocysteine lowering with b vitamins for stroke prevention—a history

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Homocysteine Lowering with B Vitamins for Stroke Prevention—A History

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Early trials of B vitamin therapy to lower plasma total homocysteine (tHcy) reported no reduction of stroke with high doses of folate/B6 and cyanocobalamin 400–1,000 µg daily. In patients with diabetic nephropathy, folate/B6 and cyanocobalamin 1,000 µg daily accelerated the decline of renal function and doubled cardiovascular events. Patients with renal failure have high cyanide levels. The French SUpplementation with FOlate, vitamin B6 and B12 and/or OMega-3 fatty acids (Su.Fol.OM3) trial—with the best renal function of the early trials and the lowest dose of cyanocobalamin (20 µg daily)—reported a 43% reduction of stroke. Then the China Stroke Primary Prevention Trial (CSPPT) reported that folic acid alone reduced stroke and was beneficial even in patients with impaired renal function. Patient-level data from the Vitamin Intervention to Prevent Stroke (VISP) and VITAmins TO Prevent Stroke (VITATOPS) trials and meta-analyses stratified by renal function and dose of cyanocobalamin confirmed that harm from cyanocobalamin among participants with renal impairment obscured the benefit of B vitamins in the early trials. It does seem that B vitamins reduce the risk of stroke. In the era of folate fortification, B12 is the main nutritional determinant of tHcy, and metabolic B12 deficiency is very common and usually missed. Therefore, folate alone is not the optimal way to lower tHcy: the use of folate (and possibly B6) with methylcobalamin or oxocobalamin should be considered.

Keywords

Homocysteine, B vitamins, cyanocobalamin, renal function, stroke

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Despite substantial evidence to the contrary, there is still a widespread belief that B vitamins to lower plasma total homocysteine (tHcy) do not prevent stroke. This belief is based on the failure of early clinical trials to show a reduction of stroke with B vitamin therapy. However, it is increasingly clear that harm from cyanocobalamin among participants with impaired renal function obscured the benefit of B vitamin therapy in these early trials. *Table 1* shows the timeline of the history of B vitamin therapy for stroke prevention by lowering of tHcy.[1–15]

The biological plausibility of the hypothesis that lowering tHcy with vitamin therapy should reduce the risk of stroke was overwhelming.[16] McCully first suggested in 1969[1] that high levels of tHcy may accelerate atherosclerosis based on findings in patients with severe hyperhomocysteinemia in patients homozygous for cystathionine synthase deficiency. In 1986, Boers et al.[2] reported that patients with premature coronary disease were more likely to have high tHcy. By 1997 it was clear that there was a strong, graded increased risk of cardiovascular disease with higher levels of tHcy.[3,17] There are several mechanisms by which elevated tHcy aggravates cardiovascular disease, and stroke in particular. High levels of homocysteine increase oxidative stress, impair endothelial function and increase thrombosis.

There are several B vitamins that are cofactors in the metabolism of homocysteine, as shown in *Figure 1*.[15] It was hypothesized, therefore, that a combination of folic acid and vitamin B12, which catalyze remethylation of homocysteine to methionine, and vitamin B6, which catalyzes transsulfuration to cystathionine, should lower levels of tHcy and thereby reduce cardiovascular events.[18] A series of clinical trials ensued to test that hypothesis.

The first large trial that failed to show benefit of B vitamins was the Vitamin Intervention to Prevent Stroke (VISP) trial,[4] published in 2004. There were several reasons why this trial did not show benefits of B vitamins. Firstly, folate fortification of the grain supply in North America coincided with the 1989 initiation of the trial, thereby negating the benefit of folic acid. Secondly, participants were not randomized to placebo versus active vitamins; they received high-dose B vitamins versus low dose B vitamins, and the low-dose regimen contained the recommended daily intake of vitamin B12 (6 µg daily). Thirdly, participants with a serum B12 below the reference range were given monthly injections of B12, regardless of which arm of the study to which they were randomized, thus preventing the benefit of B12 in the very participants who would have benefited most.

Table 1: History of B vitamin therapy to lower homocysteine for stroke prevention

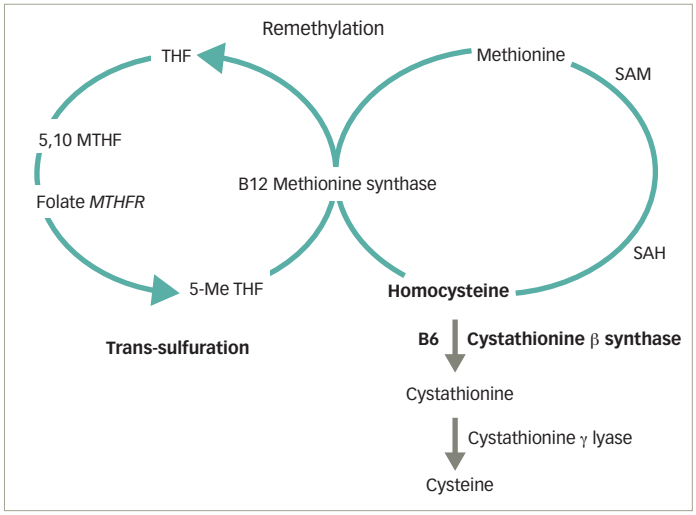
Year	Development
1969	McCully described thrombosis, atherosclerosis and strokes in children with homocystinuria.[1]
1986	Boers et al. reported that 40% of patients with premature atherosclerosis had high tHcy.[2]
1990s	High tHcy is demonstrated to be a clear graded risk factor for cardiovascular disease and interacts with smoking.[3]
2004	The VISP trial showed no benefit of high-dose versus low-dose folate/B6/B12 including cyanocobalamin 400 µg daily in the high-dose arm.[4]
2005	An analysis of a subgroup of the VISP trial, which excluded participants who received B12 injections and those in the lowest decile of eGFR (48 mL/min/1.73 m^2), showed a 34% reduction of stroke, MI and vascular death when comparing participants with good B12 absorption who received high dose vitamins versus those with lower B12 at baseline who received low-dose vitamins.[5]
2006	The NORVIT trial showed no benefit of B vitamins, and harm in the arm with cyanocobalamin 1,000 µg daily.[6]
2006	The HOPE-2 trial, with better renal function than the earlier trials, showed a 25% reduction of stroke with B folate/B6 and 1,000 µg daily of cyanocobalamin, but reported this as a chance finding because there was no reduction of MI.[7]
2006	Loscalzo hypothesized that the reason for the null results of the trials was high doses of unmetabolized folic acid.[8]
2010	The DIVINe study investigating high dose folate/B6 and 1,000 µg of cyanocobalamin showed harm in patients with diabetic nephropathy, accelerated decline of renal function and a doubling of cardiovascular events; all these events occurred in patients with a GFR <50 mL/min/1.73 m^2.[9]
2010	VITATOPS reported no benefit of folate/B6 with cyanocobalamin 500 µg daily.[10]
2010	The French Su.Fol.OM3 study, with the best renal function of the studies and folate/B6 with only 20 µg of cyanocobalamin showed a 43% reduction of stroke; the report focused on the failure to reduce MI, so this was not much noticed.[11]
2011	Spence and Stampfer hypothesized that harm from cyanocobalamin among study participants with impaired renal function obscured the benefit of B vitamins in the earlier trials.[13]
2012	The VITATOPS subgroup analysis, excluding patients who received antiplatelet agents, reported a 24% reduction of stroke with folate/B6 and cyanocobalamin 500 µg daily.[14]
2015–16	The CSPPT showed that folic acid reduced the risk of stroke by 25% in the main study, reduced stroke by 30% in participants with LDL-C >2 mmol/L (76 mg/dL) and was beneficial in participants with eGFR <60 mL/min/1.73 m^2.[16]
2017	Patient-level data from VISP and VITATOPS, and meta-analyses stratified by renal function and dose of cyanocobalamin, confirmed that harm from cyanocobalamina among participants with impaired renal function obscured the benefit of B vitamins. In patients with good renal function and low doses or no cyanocobalamin, it was demonstrated that B vitamins reduced the risk of stroke. The authors recommended the use of methylcobalamin or oxocobalamin instead of cyanocobalamin.[17]

CSPPT = China Stroke Primary Prevention Trial; DIVINe = Diabetic Intervention with Vitamin in Nephropathy; eGFR = estimated glomerular filtration rate; GFR = glomerular filtration rate; HOPE-2 = Heart Outcomes Prevention Evaluation 2; LDL-C = low density lipoprotein cholestrol, MI = myocardial infarction; NORVIT = Norwegian Vitamin Trial; tHcy = total homocysteine; Su.Fol.OM3 = SUpplementation with FOlate, vitamin B6 and B12 and/or OMega-3 fatty acids; VISP = Vitamin Intervention to Prevent Stroke; VITATOPS = VITAmins TO Prevent Stroke.

In 2006, the Norwegian Vitamin Trial (NORVIT)[6] and the Heart Outcomes Prevention Evaluation 2 (HOPE-2) trial[7] were published in the same edition of the *New England Journal of Medicine*. The NORVIT trial showed no benefit of B vitamin therapy, and even showed harm among participants receiving cyanocobalamin. The HOPE-2 trial actually showed a 25% reduction of stroke with B vitamins that was arguably misinterpreted by the authors because they found no reduction of myocardial infarction. Being cardiologists, and perhaps unaware of the cerebral circulation, they may not have conceived a biological difference between myocardial infarction and stroke, so concluded that the reduction of stroke was a chance finding. However, stroke and myocardial infarction are not the same. Myocardial infarctions are virtually all caused by a plaque rupture with occlusion of a coronary artery; most strokes are due to small vessel disease, or to embolization from the carotid arteries, the aorta, or the heart. High levels of tHcy quadruple the risk of stroke in atrial fibrillation,[19] increase venous thrombosis, and are associated with microemboli in patients with carotid stenosis.

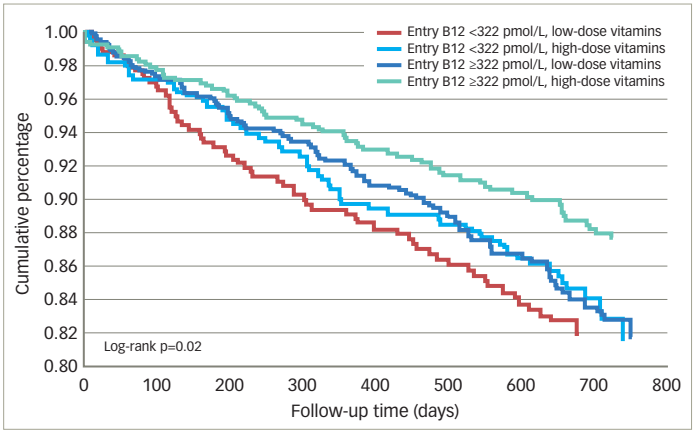
In the same edition of the *New England Journal of Medicine*, an editorial by Loscalzo[8] hypothesized that the failure to show benefit of B vitamins was due to toxicity from high doses of folic acid. All of this led to the conclusion by most physicians that "homocysteine is dead".

Figure 1: Homocysteine metabolism



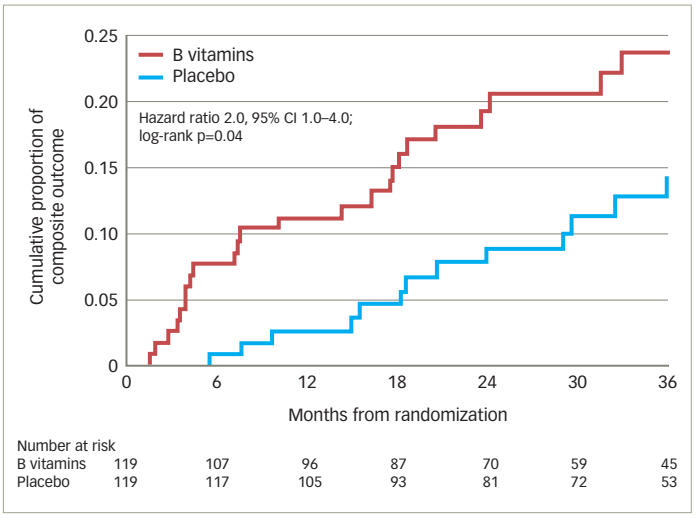
5-Me THF = 5-methyl tetrahydrofolate; B12 = cobalamin; B6=pyridoxine; MTHF = methylenetetrahydrofolate; MTHFR = methylenetetrahydrofolate reductase; SAH = S-adenosylhomocysteine; SAM = S-adenosylmethionine; THF = tetrahydrofolate. Reproduced with permission from Spence et al. 2017.[15]

Figure 2: Benefit of B vitamins in the VISP trial



In this post-hoc *analysis of the VISP trial the following participants were excluded: participants with serum cyanocobalamin below the 25th percentile (250 pmol/L; to exclude those who received cyanocobalamin injections); participants with serum cyanocobalamin above the 95th percentile (950 pmol/L; to exclude those receiving cyanocobalamin supplements outside the study); and participants with the lowest 10% of eGFRs, as calculated by the Cockcroft-Gault equations (eGFR <46.18 mL/min/1.73 m²). Survival free of stroke or myocardial infarction, and cardiovascular death was stratified by treatment arm (high-dose versus low-dose B vitamins) and by baseline serum cyanocobalamin, cut off at the median (322 pmol/L) to define groups more able and less able to absorb the vitamin. A 34% reduction in the number of events was observed in the participants with a baseline serum cyanocobalamin above the median who received high-dose B vitamins including cyanocobalamin (400 µg daily) compared with those with a baseline serum cyanocobalamin below the median who received low-dose study vitamins. eGFR=estimated glomerular filtration rate; VISP = Vitamin Intervention to Prevent Stroke. Reproduced with permission from Spence et al., 2017[15] and Spence et al., 2005.[5]*

Figure 3: Effect of B vitamins on the risk of a composite cardiovascular outcome in patients with diabetic nephropathy in the DIVINe trial



Cumulative proportion of myocardial infarction, stroke, revascularization and all-cause mortality in the DIVINe trial. Following treatment with B vitamins (2.5 mg folic acid, 25 mg pyridoxine and 1,000 µg cyanocobalamin daily), the 36-month risk of the composite outcome was 23.5% in the treatment group and 14.4% in the placebo group (log-rank p=0·04). All the events occurred in participants with a glomerular filtration rate of less than 50 mL/min/1.73 m². CI = confidence interval; DIVINe = Diabetic Intervention with Vitamin in Nephropathy. Reproduced with permission from Spence et al., 2017.[15] and House et al., 2010.[9]

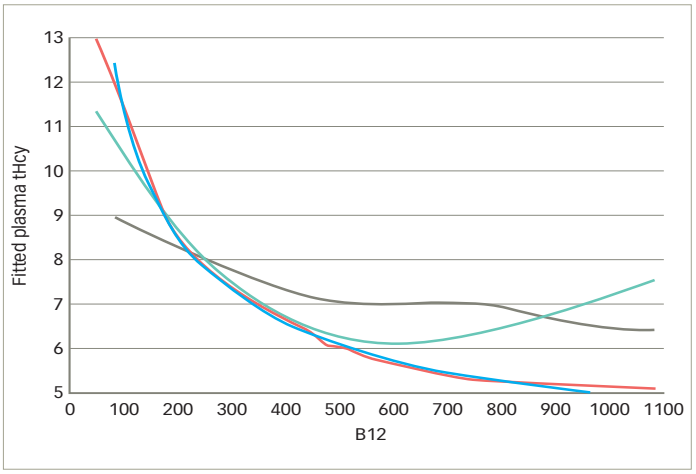
In 1999, I had reported with colleagues that among dialysis patients, 5 mg daily of folic acid did not reduce tHcy compared with 1 mg daily.[20] I thought, therefore, that B vitamins would not be effective in patients with renal impairment. Taken with the effect of B12 injections among patients with low B12 levels at baseline, this may have obscured the benefit of B vitamins in the VISP trial. We therefore carried out a subgroup analysis in the VISP study population, excluding 1,525/3,680 participants who received B12 injections, and/or had impaired renal function with an estimated glomerular filtration rate in the lowest decile (below 48 mL/min/1.73 m²).[5] In the remaining subgroup of 2,155 patients, the median baseline serum B12 was 322 pmol/L.

Figure 2 shows the result of that study.[5,15] Participants with a baseline serum B12 above the median (meaning they could absorb B12 well) and who had received high-dose B vitamins had a 34% reduction of stroke, myocardial infarction, and vascular death compared with those whose baseline B12 was below the median and received low-dose vitamins.

In 2010, the Diabetic Intervention with Vitamin in Nephropathy (DIVINe) study was published. Patients with diabetic nephropathy were randomized to high-dose B vitamins, containing folic acid 5 mg, B6 25 mg and cyanocobalamin 1,000 µg daily versus placebo. We found that B vitamins were harmful, accelerating the decline of renal function, and doubling cardiovascular events (*Figure 3*).[9] All the events occurred in participants with a glomerular filtration rate of <50 mL/min/1.73 m².[21]

After this study, I received emails pointing out that Koyama et al. had reported that patients with renal failure had high levels of thiocyanate.[22] They had also reported that in patients with renal failure, methylcobalamin

Figure 4: Polynomial and inverse regression, spline, loess fits for plasma total homocysteine with B12



Unadjusted analysis. Red, green, blue and grey lines represent loess, cubic polynomials, cubic polynomials of the inverse covariate and smooth splines respectively. tHcy = total homocysteine. Reproduced with permission from Bang et al., 2006.[32]

lowered levels of both tHcy and asymmetric dimethylarginine (ADMA; a nitric oxide antagonist that is an evil companion of homocysteine),[23] whereas in a Norwegian study, cyanocobalamin did not lower levels of ADMA.[24]

In 2010, the Australian VITAmins TO Prevent Stroke (VITATOPS) trial reported no benefit of folate/B6 and cyanocobalamin 500 µg daily in the main study;[10] later they reported that B vitamins reduced stroke by 24% in a subgroup that did not receive antiplatelet agents.[13]

Table 2: Dose of cyanocobalamin, renal function and reduction of stroke or cardiovascular events

Trial	Dose of cyanocobalamin	Serum creatinine (µmol/L*) (SD or 95% CI**)		HR of stroke (95% CI)	p-value
		Active	Control		
DIVINe[9]	1,000 µg	141.4 (97.2)	123.8 (79.6)	6.6 (0.8–54.4)	0.08
VISP[4]	400 µg	99.9 (55.7)	97.2 (47.7)	1.0 (0.8–1.3)	0.80
SEARCH[25]	1,000 µg	N/A	N/A	1.02 (0.86–1.21)	N/A
VITATOPS[10]	500 µg	92.4 (40.3)	91.4 (34.6)	0.92 (0.81–1.06)	0.25
NORVIT[6]	400 µg	91.0 (27.0)	91.0 (24.0)	0.83 (0.47–1.47)	0.52
HOPE-2[7]	1,000 µg	88.4 (26.5)	88.4 (26.5)	0.75 (0.59–0.97)	0.03
SU.Fol.OM3[26]	20 µg	78.0 (70.0–88.0)**	78.0 (69.0–88.0)**	0.57 (0.33–0.97)	0.04
CSPPT (all)[14]	0 µg	65.95 (19.0)	65.95 (19.0)	0.79 (0.68–0.93)	0.003
CSPPT with eGFR <60 mL/min/1.73 m^2	0 µg	126.6 (72.7)	130.6 (68.6)	0.88 (0.33–2.36)	0.81
CSPPT excluding eGFR <60 mL/min/1.73 m^2	0 µg	64.7 (13.8)	64.6 (13.6)	0.79 (0.67–0.92)	0.003
		eGFR (mL/min/1.73 m^2) (SD)		HR for MI, stroke and/or death (95% CI)	
VISP[4]					
eGFR <50 mL/min/1.73 m^2	400 µg	38.7 (9.2)	39.3 (8.6)	1.01 (0.74–1.37)	0.977
eGFR ≥50 mL/min/1.73 m^2	400 µg	89.2 (44.6)	87.3 (31.1)	0.92 (0.77–1.11)	0.382
VITATOPS[10]					
eGFR <50 mL/min/1.73 m^2	500 µg	38.6 (10.0)	38.3 (9.5)	0.88 (0.59–1.32)	0.54
eGFR >50 mL/min/1.73 m^2	500 µg	80.2 (18.1)	80.9 (18.3)	0.82 (0.68–0.98)	0.03

**Conversions from mg/dL performed using www.endmemo.com/medical/unitconvert/Creatinine.php (accessed March 28, 2018).*
***Ranges between parentheses denote 95% confidence intervals; single numbers denote standard deviations.*
CI = confidence interval; CSPPT = China Stroke Primary Prevention Trial; DIVINe = Diabetic Intervention with Vitamin in Nephropathy; eGFR = estimated glomerular filtration rate; HOPE-2 = Heart Outcomes Prevention Evaluation 2; HR = hazard ratio; MI = myocardial infarction; N/A = not applicable; NORVIT = Norwegian Vitamin Trial; SD = standard deviation; SEARCH = Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; Su.Fol.OM3 = SUpplementation with FOlate, vitamin B6 and B12 and/or OMega-3 fatty acids; VISP = Vitamin Intervention to Prevent Stroke; VITATOPS = VITAmins TO Prevent Stroke. Reproduced with permission from Spence et al., 2017.[15]

Also in 2010, the French vitamin study, SUpplementation with FOlate, vitamin B6 and B12 and/or OMega-3 fatty acids (Su.Fol.OM3) was published.[11] In this study the dose of cyanocobalamin was only 20 µg daily, and the renal function was much better than in the other studies (*Table 2*).[4,6,7,9,10,14,25,26] The reduction of stroke was 43%.

In 2011, I hypothesized with Stampfer that harm from cyanocobalamin among study participants with renal impairment had obscured the benefit of B vitamins in the earlier trials.[12]

Then in 2015, the final piece of the puzzle fell into place. The China Stroke Primary Prevention Trial (CSPPT) reported that in China, where folate fortification does not exist, folic acid reduced stroke in primary prevention by 25%.[14] In higher-risk patients with low density lipoprotein cholesterol (LDL-C) of >2 mmol/L, folic acid reduced stroke by 30%.[27] Crucially, folic acid was beneficial among study participants with impaired renal function.[28]

In 2017, with Yi and Hankey, we performed a meta-analysis of the trials stratified by dose of cynanocobalamin and by renal function. In addition, we analyzed patient-level data from the VISP trial and the VITAPOPS trial. What emerged was that harm from high-dose cyanocobalamin among study participants obscured the benefit of B vitamins among study participants with good renal function.[15]

In line with this analysis, it turns out that besides folic acid and possibly vitamin B6, we should perhaps be using methylcobalamin or oxocobalamin, not cyanocobalamin, to prevent stroke.

This is important because in the era of folate fortification, B12 is the major determinant of elevated tHcy.[29,30] Unrecognized metabolic B12 deficiency is very common, and usually missed.[31] Measuring total serum B12 does not accurately assess metabolic adequacy of B12 because a small and variable fraction is active (~6–20%). Thus, among patients with a total serum B12 in the reference range (~60–600 pmol/L), many patients have metabolic B12 deficiency. In order to assess metabolic adequacy of serum B12 it is necessary to measure holotranscobalamin, or one of the metabolites that are elevated in B12 deficiency: methylmalonic acid (MMA), or tHcy.[31] The serum B12 level below which MMA or tHcy begins to rise is 400 pmol/L (*Figure 4*).[31,32] So among patients referred to a stroke prevention clinic, 10% of patients aged below 50 years had metabolic B12 deficiency; above the age of 71 years it was 30%.[33] In our community this appears to be declining as physicians become more aware of the issue.

Conclusion

Based on recent findings, it is now clear that B vitamins to lower homocysteine do prevent stroke. It is possible that this was obscured in the early trials by harm from cyanocobalamin among participants with

impaired renal function. Metabolic B12 deficiency is very common, often missed, and raises levels of tHcy. In the era of folate fortification, B12 is the major nutritional determinant of elevated tHcy. Folate alone is therefore probably not the optimal therapy to lower tHcy. We should probably be using folate (and possibly B6) with methylcobalamin or oxocobalamin instead of cyanocobalamin. ❑

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