Effect of B-Vitamin Therapy on Progression of Diabetic Nephropathy

A Randomized Controlled Trial

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Context Hyperhomocysteinemia is frequently observed in patients with diabetic nephropathy. B-vitamin therapy (folic acid, vitamin B_6, and vitamin B_{12}) has been shown to lower the plasma concentration of homocysteine.

Objective To determine whether B-vitamin therapy can slow progression of diabetic nephropathy and prevent vascular complications.

Design, Setting, and Participants A multicenter, randomized, double-blind, placebo-controlled trial (Diabetic Intervention with Vitamins to Improve Nephropathy [DIVINe]) at 5 university medical centers in Canada conducted between May 2001 and July 2007 of 238 participants who had type 1 or 2 diabetes and a clinical diagnosis of diabetic nephropathy.

Intervention Single tablet of B vitamins containing folic acid (2.5 mg/d), vitamin B_6 (25 mg/d), and vitamin B_{12} (1 mg/d), or matching placebo.

Main Outcome Measures Change in radionuclide glomerular filtration rate (GFR) between baseline and 36 months. Secondary outcomes were dialysis and a composite of myocardial infarction, stroke, revascularization, and all-cause mortality. Plasma total homocysteine was also measured.

Results The mean (SD) follow-up during the trial was 31.9 (14.4) months. At 36 months, radionuclide GFR decreased by a mean (SE) of 16.5 (1.7) mL/min/1.73 m^2 in the B-vitamin group compared with 10.7 (1.7) mL/min/1.73 m^2 in the placebo group (mean difference, −5.8; 95% confidence interval [CI], −10.6 to −1.1; $P=.02$). There was no difference in requirement of dialysis (hazard ratio [HR], 1.1; 95% CI, 0.4-2.6; $P=.88$). The composite outcome occurred more often in the B-vitamin group (HR, 2.0; 95% CI, 1.0-4.0; $P=.04$). Plasma total homocysteine decreased by a mean (SE) of 2.2 (0.4) μmol/L at 36 months in the B-vitamin group compared with a mean (SE) increase of 2.6 (0.4) μmol/L in the placebo group (mean difference, −4.8; 95% CI, −6.1 to −3.7; $P<.001$, in favor of B vitamins).

Conclusion Among patients with diabetic nephropathy, high doses of B vitamins compared with placebo resulted in a greater decrease in GFR and an increase in vascular events.

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DIABETIC NEPHROPATHY IS AN important cause of chronic kidney disease.[1,2] In the United States, diabetes mellitus accounts for more than 44% of patients with end stage renal failure.[3] In addition to the personal burden, the societal burden of diabetic nephropathy is enormous, exceeding US $10 billion in annual medical expenditures.[4] Despite effective therapies to slow disease progression,[5] approximately 40% of the estimated 21 million patients with diabetes in the United States develop overt nephropathy.[4] New treatment approaches to this problem are needed.

Several observational studies have shown a significant association between high concentrations of plasma total homocysteine and the risk of developing diabetic nephropathy, retinopathy, and vascular diseases, including myocardial infarction (MI) and stroke.[6-9] B-vitamin therapy has been shown to lower the plasma concentration of homocysteine and improve endothelial function.[10,11]

We hypothesized that B-vitamin therapy would slow the progression of diabetic nephropathy and prevent vascular events. To test this hypothesis, we conducted a multicenter, randomized, double-blind, placebo-controlled trial of participants with type 1 or 2 diabetes.

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METHODS

Participants

The Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe) trial was conducted between May 2001 and July 2007 at 5 university medical centers in Canada. Participants were recruited from nephrology and diabetes clinics. Eligible participants had type 1 or 2 diabetes and a clinical diagnosis of diabetic nephropathy, with at least 300 mg/d of urinary albumin excretion (or ≥500 mg/d of proteinuria). All participants were 18 years or older. Race/ethnicity was self-reported at the time of enrollment and included because of a very high prevalence of diabetic nephropathy among Canadian aboriginal people. Potential participants who were expected to survive fewer than 3 years, those with advanced renal failure (defined as stage 4 or 5 chronic kidney disease[12] with creatinine clearance of <30 mL/min[13] or on dialysis), those awaiting imminent dialysis, and women who were pregnant or unwilling to practice effective contraception were not eligible for the trial. The trial protocol was approved by the institutional review board at each center and all participants gave written informed consent.

Assessment, Randomization, and Follow-up

Baseline assessments for trial participants included medical history, laboratory analyses, and current medications. Participants were randomized 1:1 to receive a single tablet of B vitamins that contained 2.5 mg/d of folic acid, 25 mg/d of vitamin B_6, and 1 mg/d of vitamin B_{12}, or a matching placebo. A computer-generated randomization list was generated by the trial biostatistician (M.E.) using permuted block sizes of 2 and 4, and was stratified by center, sex, and baseline Cockcroft-Gault formula creatinine clearance (<50 vs ≥50 mL/min/1.73 m^2; to convert to mL/s/m^2, multiply by 0.0167).

B vitamins and matching placebos were bottled with participant numbers by pharmacy staff. As trial participants were given the next appropriately labeled bottle, all participants, research coordinators, and treating physicians remained blinded to the treatment assignment throughout the trial.

Participants were followed up with clinic visits every 6 months for up to 3 years. Participants were permitted to take usual doses of vitamins E, C, and beta carotene, and multivitamins containing usual low doses of B vitamins, but were asked not to take additional doses of the trial B vitamins. All other treatments were left to the discretion of the participants' attending physicians. Adherence to therapy was assessed by pill counts at every clinic visit and by annual measurements of plasma total homocysteine, serum folate, and serum B_{12}. Central laboratory reports were made available to attending physicians, and blinding was maintained by deleting the values for plasma total homocysteine, serum folate, and serum B_{12}. Plasma total homocysteine was measured by Liquid Chromatography Tandem Mass Spectrometry (MDS Laboratories Inc, Toronto, Ontario, Canada). The same laboratory measured serum folate and serum B_{12}, as well as serum glycated hemoglobin, serum creatinine, and urinary protein.

Outcome Measures

The primary outcome measure was progression of nephropathy, which was assessed by change in glomerular filtration rate (GFR). A validated technique for measuring radionuclide GFR was performed at baseline, 18 months, and 36 months using ^{99}Technetium-DTPA (^{99}Tc-diethylenetriaminepentaacetic acid).[14] Glomerular filtration rate was also estimated every 6 months using a 3-hour timed creatinine clearance commencing 1 hour after a single dose of 800-mg cimetidine (to prevent tubular secretion of creatinine).[14,15] Three values for each method were obtained at 30-minute intervals per participant visit. All radionuclide GFRs and cimetidine creatinine clearances were adjudicated by a blinded nephrologist (A.A.H.). In cases of outlying values (ie, >10% difference among the 3 values), the nephrologist discarded those values believed to be biologically implausible, most commonly based on inadequate urine flow. For each method, the median of the 3 values was used in the analyses. Glomerular filtration rate was also estimated by the 4-variable Modification of Diet in Renal Disease (MDRD) formula.[16]

Secondary and tertiary outcomes included dialysis, occurrence of vascular events and all-cause mortality, cognitive decline (measured by the Mini-Mental State Examination score), and amputation. Ascertainment of possible events was sought during participants' clinic visits and from hospital records. A blinded adjudication committee reviewed all events to determine their validity and to classify the vascular events as MI, stroke, revascularization (peripheral, cardiac angioplasty, or cardiac bypass), cause of death, and amputation for peripheral vascular disease, using the definitions of the Vitamin Intervention for Stroke Prevention trial.[17] In addition, all other undesirable or harmful changes in health that occurred among participants in the trial were recorded and classified as adverse events. Adverse events that were incapacitating with inability to perform usual activity were classified as severe. Adverse events that caused death, permanent damage, birth defects, were life-threatening, or required hospitalization were classified as serious.

Sample Size and Statistical Analyses

A previous study had reported a mean (SD) decrease in GFR of 4.4 (2.9) mL/min/1.73 m^2 per year.[18] Given an observed constant rate of decline over time, we assumed a 3-year mean (SD) decrease of 12 (9) mL/min/1.73 m^2 in the placebo group. A total trial size of 286 participants provided 80% power to detect a 25% reduction in GFR decline between the placebo and B-vitamin groups (12 vs 9 mL/min/1.73 m^2), at a 2-tailed 5% level of significance. Interim efficacy analyses were not planned.

During the trial, the data and safety monitoring board committee noted that, although the rate of GFR decrease in the placebo group was comparable with that predicted, the rate of GFR decrease in the B-vitamin group

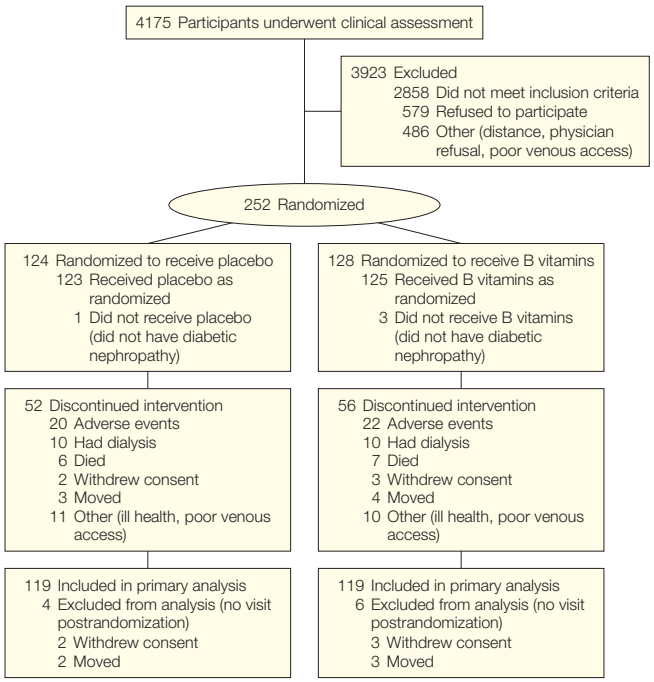
was higher than the predicted placebo rate. The data and safety monitoring board committee performed a conditional power calculation that indicated improbability of yielding a significant benefit in the primary outcome at the end of the trial, and therefore recommended stopping enrollment after 252 participants and stopping follow-up after the final radionuclide GFR was completed.

Trial data were collected by the center coordinators using standard 2-part carbonless copy paper, monitored for completeness and accuracy by the study monitor, and double-keyed into an Oracle Clinical database at Robarts Clinical Trials, London, Ontario, Canada. Biochemical results from the central laboratory were received electronically. Data were analyzed using a modified intention-to-treat approach, defined as participants who met all the inclusion criteria and none of the exclusion criteria, ingested their first tablet of medication, and had 1 or more postrandomization GFR efficacy measures. Statistical analyses were performed by using SAS software version 9.2 (SAS Institute Inc, Cary, North Carolina) and multiple imputation was performed by using SOLAS software version 3.0 (Statistical Solutions Ltd, Saugus, Massachusetts).

The primary analysis compared treatment groups on their mean change in radionuclide GFR between baseline and 36 months using a mixed linear model (PROC MIXED procedure in SAS [SAS Institute Inc]), analogous to an unbalanced repeated measures analysis of covariance. For the analysis, participant was considered as a random effect, treatment group and visit number as fixed effects, an interaction term between treatment and visit to assess for a differential treatment effect over time, and baseline GFR as the covariate. The results are summarized in terms of least square means with standard errors (SEs) and 95% confidence intervals (CIs) about treatment group differences. In cases of missing or unavailable radionuclide GFR values, GFR was estimated by cimetidine creatinine clearance. If neither was available, GFR was estimated by the MDRD formula. Substituting the estimated GFRs for the radionuclide GFRs was reasonable as the Pearson correlations were high (r=0.95 for cimetidine creatinine clearance and r=0.92 for the MDRD formula) and the relationships were linear. When all 3 values were not available, multiple imputation using the Predictive Model Based Method in SOLAS version 3.0 (Statistical Solutions Ltd) was used to impute GFR, with the exception that participants who commenced dialysis postrandomization were assigned a GFR value of 10 mL/min/1.73 m^2. Multiple imputation was used for all other missing laboratory and vital status variables that were collected longitudinally.

Kaplan-Meier event-free survival analysis was used to plot cumulative failure curves for the composite outcome (MI, stroke, revascularization, all-cause mortality) and to estimate 36-month risks of outcome for the 2 treatment groups. Treatment effectiveness was assessed using univariate Cox proportional hazards regression model, with only treatment group in the model. Hazard ratios (HRs) with 95% CIs were calculated. A 2-tailed $P<.05$ was considered statistically significant.

Figure 1. Flow of Participants in the Trial



RESULTS

Participants

FIGURE 1 shows the flow of participants in the trial. Four participants did not meet inclusion criteria and 10 did not return for their first postrandomization visit to provide their GFR efficacy measure. Therefore, 238 participants (119 in the B-vitamin group and 119 in the placebo group) were available for the modified intention-to-treat analyses. Participants were followed up for a mean (SD) of 31.9 (14.4) months and a median (interquartile range) of 32.2 (28.2) months.

Baseline characteristics were similar between the placebo and B-vitamin groups (TABLE 1). Participants were predominantly men (178 [74.8%]) and white (198 [83.2%]), with type 2 diabetes (195 [81.9%]). Consistent with the presence of diabetic nephropathy,

Table 1. Baseline Characteristics of Participants in Placebo and B-Vitamin Groups[a]

Characteristics	Placebo Group (n = 119)	B-Vitamin Group (n = 119)
Demographics and risk factors		
Age, y	60.1 (10.8)	60.7 (11.6)
Men, No. (%)	88 (73.9)	90 (75.6)
White race, No. (%)	99 (83.2)	99 (83.2)
Weight, kg	93.0 (17.4)	94.1 (20.3)
BMI	32.4 (5.5)	32.6 (6.0)
Blood pressure, mm Hg		
Systolic	153 (26)	150 (24)
Diastolic	81 (12)	80 (11)
Current smoker	20 (16.8)	15 (12.6)
MMSE score[b]	28.7 (2.2)	28.8 (1.6)
Plasma total homocysteine, µmol/L	16.4 (5.4)	14.7 (4.9)
Lipid values, mg/dL		
Total cholesterol	182 (51)	183 (44)
HDL cholesterol	43 (13)	44 (15)
LDL cholesterol	99 (37)	97 (36)
Triglycerides	221 (151)	217 (136)
Vitamin levels		
Serum folate, ng/mL	15 (15)	16 (37)
Serum vitamin B_{12}, pg/mL	474 (286)	412 (193)
Diabetic features		
Type 2 diabetes, No. (%)	100 (84.0)	95 (79.8)
Duration of diabetes, median (IQR), y	18.0 (16.0)	19.0 (17.0)
Duration of diabetic nephropathy, median (IQR), y	2.0 (3.0)	2.0 (3.0)
Proteinuria, median (IQR), g/24 h	0.9 (1.5)	0.8 (1.2)
Glycated hemoglobin, %	8.1 (1.5)	8.1 (1.6)
History, No. (%)		
Hypertension	111 (93.3)	112 (94.1)
Hyperlipidemia	102 (85.7)	103 (86.6)
Myocardial infarction or angina	43 (36.1)	31 (26.1)
Stroke or transient ischemic attack	17 (14.3)	17 (14.3)
Peripheral vascular disease	17 (14.3)	24 (20.2)
Renal function		
Serum creatinine, median (IQR), mg/dL	1.6 (1.1)	1.4 (0.9)
Cockcroft-Gault creatinine clearance, mL/min/1.73 m^2	58.5 (27.5)	64.0 (32.1)
Radionuclide GFR, mL/min/1.73 m^2	51.7 (28.2)	57.6 (30.8)
GFR <60 mL/min/1.73 m^2, No. (%)	79 (66.4)	73 (61.3)
MDRD-4 variable GFR, mL/min/1.73 m^2	51.4 (26.6)	56.6 (27.5)
Cimetidine GFR, mL/min/1.73 m^2	54.7 (23.3)	59.4 (30.4)
Current medication use, No. (%)		
Insulin	81 (68.1)	65 (54.6)
Hypoglycemic medications	55 (46.2)	61 (51.3)
Hypoglycemic medications or insulin	114 (95.8)	109 (91.6)
Diuretic	87 (73.1)	72 (60.5)
β-Blocker	43 (36.1)	47 (39.5)
Calcium channel blocker	67 (56.3)	74 (62.2)
Angiotensin blockade	108 (90.8)	116 (97.5)
Antiplatelet or warfarin	74 (62.2)	74 (62.2)
Lipid-lowering medications	92 (77.3)	94 (79.0)
Other antihypertensive medications	18 (15.1)	13 (10.9)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); GFR, glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease; MMSE, Mini-Mental State Examination.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113; serum folate to nmol/L, multiply by 2.266; serum vitamin B_{12} to pmol/L, multiply by 0.7378; serum creatinine to µmol/L, multiply by 88.4; Cockcroft-Gault creatinine clearance to mL/s/m^2, multiply by 0.0167.

[a] Data are presented as mean (SD), unless otherwise indicated.

[b] Percentage of patients with a score of less than 25 for dementia (4.2% for placebo and 2.5% for B vitamins).

the mean (SD) baseline GFR was low at 54.7 (29.5) mL/min/1.73 m^2, with 152 participants (63.9%) having at least stage 3 chronic kidney disease (GFR <60 mL/min/1.73 m^2). The baseline mean plasma total homocysteine was increased at 15.5 (5.2) µmol/L. The prevalent use of hypoglycemic medications, insulin, angiotensin blockade, and other cardioprotective medications indicated that participants were well-treated by their attending physicians.

Renal Outcomes

Among the 238 participants at baseline, 205 and 118 remained in the analyses at 18 and 36 months, respectively. Only 25 (12.2%) and 16 (13.6%) GFRs were unavailable at 18 and 36 months, respectively, and had to be imputed. Participants assigned to the B-vitamin group had a much greater decrease in radionuclide GFR compared with the placebo group ($P = .045$) (TABLE 2). Over 36 months, the GFR decreased by a mean (SE) of 16.5 (1.7) mL/min/1.73 m^2 in the B-vitamin group compared with 10.7 (1.7) mL/min/1.73 m^2 in the placebo group, a significant mean difference of −5.8 (95% CI, −10.6 to −1.1; $P = .02$). This result was similar to the MDRD formula results, which had estimated GFR measurements at 12 and 24 months.

Despite the unexpected reverse findings for GFR, the plasma total homocysteine results were as expected. At 36 months, participants in the B-vitamin group had a mean (SE) decrease of 2.2 (0.4) µmol/L and participants in the placebo group had a mean (SE) increase of 2.6 (0.4) µmol/L, resulting in a significant mean difference of −4.8 (95% CI, −6.1 to −3.7; $P < .001$, in favor of B vitamins). This result was congruent with the significantly large increases ($P < .001$) in mean serum folate and serum vitamin B_{12} at all time points in the B-vitamin group (eTable, available at http://www.jama.com). Proteinuria did not significantly change during the follow-up period (Table 2), and there was no difference in the proportion of participants who required di-

Table 2. Least Squares Mean Change From Baseline at Prespecified Follow-up Visits by Treatment Group From Mixed Linear Models[a]

	Overall Baseline Mean (SE) Score (n = 238)	LS Mean (SE) Change				*P* Value			Difference in LS Mean Change at 36 mo	
		12 mo (n = 111) (n = 107)	18 mo (n = 104) (n = 101)	24 mo (n = 88) (n = 83)	36 mo (n = 61) (n = 57)	Between Treatment Groups	Among Visits	Treatment × Visit Interaction	Between Groups (95% CI)	*P* Value
Radionuclide GFR										
Placebo	54.7 (1.9)		−8.1 (1.4)		−10.7 (1.7)	.045	<.001	.09	−5.8 (−10.6 to −1.1)	.02
B vitamins			−10.2 (1.4)		−16.5 (1.7)					
MDRD GFR										
Placebo	54.0 (1.8)	−5.4 (1.0)	−8.5 (1.0)	−8.7 (1.1)	−9.1 (1.2)	.26	<.001	.02	−4.4 (−7.8 to −1.0)	.01
B vitamins		−5.5 (1.0)	−8.7 (1.0)	−9.9 (1.1)	−13.5 (1.2)					
Plasma total homocysteine										
Placebo	15.5 (0.3)	1.1 (0.4)		2.1 (0.4)	2.6 (0.4)	<.001	<.001	.16	−4.8 (−6.1 to −3.7)	<.001
B vitamins		−2.7 (0.4)		−2.1 (0.4)	−2.2 (0.4)					
Proteinuria										
Placebo	1.47 (0.11)	0.25 (0.13)	0.33 (0.13)	0.21 (0.14)	0.17 (0.16)	.46	.88	.66	0.05 (−0.39 to 0.50)	.82
B vitamins		0.08 (0.13)	0.11 (0.13)	0.08 (0.14)	0.22 (0.16)					
MMSE score[b]										
Placebo	28.7 (0.12)	−0.08 (0.11)		−0.49 (0.12)	−0.12 (0.14)	.82	.38	<.001	−0.28 (−0.67 to 0.11)	.15
B vitamins		−0.20 (0.11)		0.01 (0.12)	−0.40 (0.14)					

Abbreviations: CI, confidence interval; GFR, glomerular filtration rate; LS, least squares; MDRD, Modification of Diet in Renal Disease; MMSE, Mini-Mental State Examination.
[a] Units for radionuclide GFR and MDRD GFR are mL/min/1.73 m^2; for plasma total homocysteine, μmol/L; and for proteinuria, g/24 hours. Stacked numbers of participants for LS mean (SE) change at 12 through 36 months indicate numbers of participants receiving placebo or B vitamins, respectively, for each outcome.
[b] Percentage of patients with score of less than 25 (dementia) at 36 months (3.3% for placebo and 0% for B vitamins).

alysis (HR, 1.1; 95% CI, 0.4-2.6; *P* = .88) (TABLE 3).

Other Outcomes

Participants randomized to receive B vitamins had a significantly greater number of cardiovascular and cerebrovascular events (Table 3). The 36-month risk of a composite outcome, including MI, stroke, revascularization, and all-cause mortality, in the B-vitamin group was double that in the placebo group (HR, 2.0; 95% CI, 1.0-4.0; *P* = .04) (Table 3), and the corresponding cumulative failure curves are shown in FIGURE 2. The 36-month risks of the composite end point was 23.5% (95% CI, 15.0%-32.0%) in the B-vitamin group and 14.4% (95% CI, 6.9%-21.8%) in the placebo group. All-cause mortality and amputation (Table 3) and cognitive decline based on the Mini-Mental State Examination score (Table 2) did not significantly differ between the treatment groups. There were no significant differences in mean glycated hemoglobin between the 2 groups during the course of the trial (eTable). Mean systolic and diastolic blood pressures significantly decreased over time but did not differ between treatment groups, and most elements of the lipid profile also did not differ between treatment groups (eTable).

Table 3. Kaplan-Meier 36-Month Risk of Outcomes and Hazard Ratios From Cox Proportional Hazards Regression Model[a]

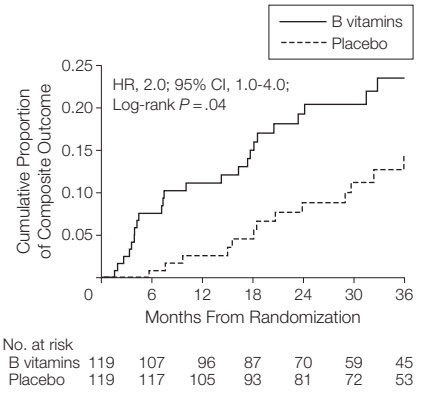
Outcomes	Outcomes, No. (%)		Hazard Ratio (95% CI)	*P* Value
	Placebo Group (n = 119)	B-Vitamin Group (n = 119)		
Secondary outcomes				
Dialysis	10 (11.7)	10 (12.3)	1.1 (0.4-2.6)	.88
MI	4 (4.6)	8 (7.8)	2.1 (0.6-6.9)	.23
Stroke	1 (1.3)	6 (7.2)	6.6 (0.8-54.4)	.08
Revascularization	5 (6.1)	7 (6.3)	1.5 (0.5-4.6)	.51
All-cause mortality	6 (6.6)	7 (6.7)	1.2 (0.4-3.6)	.72
MI, stroke, revascularization, all-cause mortality	13 (14.4)	24 (23.5)	2.0 (1.0-4.0)	.04
Tertiary outcomes				
MI, stroke, all-cause mortality	10 (10.8)	20 (20.1)	2.2 (1.0-4.6)	.046
Amputation	1 (1.6)	2 (2.1)	2.1 (0.2-23.2)	.54

Abbreviations: CI, confidence interval; MI, myocardial infarction.
[a] Revascularization indicates peripheral and cardiac angioplasty and cardiac bypass procedures. Amputation indicates amputation for peripheral vascular disease.

Adverse Events Other Than Outcomes

A total of 1060 adverse events were reported (535 in the placebo group and 525 in the B-vitamin group). These included many transient minor complaints, such as dizziness, nausea, or headache. None was deemed by the attending physician to be related to trial medication. Counting participants only once, 1 or more adverse events were reported in 107 participants (89.9%) in the placebo group and 105 (88.2%) in the B-vitamin group (*P* = .68). One or more severe adverse events were reported in 38 participants (31.9%) in the placebo group and 39 (32.8%) in the B-vitamin group (*P* = .89). One or more serious adverse events were

Figure 2. Cumulative Proportion of Myocardial Infarction, Stroke, Revascularization, and All-Cause Mortality



HR indicates hazard ratio; CI, confidence interval. The 36-month risk of the composite outcome was 23.5% in the B-vitamin group and 14.4% in the placebo group (log-rank $P = .04$).

reported in 48 participants (40.3%) in the placebo group and 40 (33.6%) in the B-vitamin group ($P = .28$).

COMMENT

In this multicenter, randomized, double-blind, placebo-controlled trial, we found that high doses of combined B vitamins (folic acid, vitamin B_6, and vitamin B_{12}) significantly lowered plasma total homocysteine in participants with diabetic nephropathy compared with matching placebo. However, the group of participants assigned to the B-vitamin therapy had a more rapid decrease in renal function, measured by radionuclide GFR, and had a higher rate of MI and stroke.

There is a strong biological rationale for homocysteine to be a risk factor for vascular disease, as shown in animal models,[19,20] in vitro scientific work,[21-23] and observational studies.[6,7,24] However, most large homocysteine-lowering B-vitamin clinical trials have failed to show a benefit for the prevention of stroke[17] and cardiovascular outcomes.[25-28] One interpretation of the conflicting results is that the mechanisms by which homocysteine influences vascular outcomes are not primarily related to the pathogenesis of coronary artery disease, which is largely due to plaque formation and rupture, although an imaging substudy showed that patients receiving B-vitamin therapy had less plaque progression of 3-dimensional vessel wall volume using ultrasound.[29] Any putative role of high homocysteine levels might, therefore, be at the level of the microvasculature promoting endothelial dysfunction and lacunar infarction, and through increased thrombosis.[30]

Our trial is the first study to our knowledge to show significant detrimental effects from pharmacological doses of B vitamins (folic acid, vitamin B_6, and vitamin B_{12}). Other studies have suggested potential harm, or at best a neutral effect of B vitamins. Our results are consistent with a recent small study[31] showing increased vascular events in patients with unstable angina and non–ST-elevation MI, particularly in the subgroup with diabetes. Another recent publication has raised concerns about increased cancer risk and all-cause mortality in patients with ischemic heart disease receiving B vitamins.[32] Although our study was limited by its sample size to detect differences in cancer and other infrequent outcomes, it was adequately powered to test our hypothesis regarding renal function decline and did show a significant difference in predefined cardiovascular and cerebrovascular events. Whether these results can be generalized to other populations beyond those similar to the participants enrolled into the trial or dosing strategies that differ from the combination chosen herein is speculative. Patient discontinuation over time did not affect our results, because there were no differences in baseline characteristics between participants who were and were not available for the primary 36-month analyses.

One interpretation of our completed trial is that these results are simply the play of chance. This explanation seems unlikely, given that renal function decline was mirrored by the occurrence of vascular outcomes, suggesting that B vitamins were in some way associated with both renal and vascular toxicity. An alternative explanation is that homocysteine lowering may be protective, but offset by toxicity associated with pharmacological doses of B vitamins. At the beginning of the trial, serum levels of B vitamins were rather high among participants randomized to the B-vitamin group and by the end of the trial, they were very high. Mandatory folic acid fortification likely contributed to the observation of relatively high folate levels in all participants, limiting generalizability of our results to an unsupplemented population. Because these vitamins are water-soluble and renally excreted, vitamin toxicity may be more of a concern in patients with impaired renal function. One or more of 3 potential mechanisms[33] may explain the renal and vascular toxicity observed in our trial. First, folic acid may promote cell proliferation through its role in thymidine synthesis. Second, the use of folic acid and vitamin B_{12} might alter the methylation potential in vascular cells. Finally, B-vitamin therapy could potentially increase the methylation of L-arginine to the nitric oxide synthase inhibitor asymmetric dimethylarginine.

If the homocysteine theory of atherosclerosis is to be proved or disproved with certainty, it may be necessary to find alternative, nonvitamin strategies to lower homocysteine, such as enhancing the conversion of homocysteine to cysteine in the liver or enhancing urinary excretion.[33] To this end, investigators have begun to examine the use of agents capable of thiol exchange, liberating homocysteine bound by disulfide bonds to proteins; therefore, facilitating its urinary excretion and lowering plasma levels significantly without the use of vitamins.[34]

In conclusion, in our randomized trial of participants with diabetic nephropathy and stages 1 to 3 chronic kidney disease, the use of high doses of B vitamins (containing 2.5 mg/d of folic acid, 25 mg/d of vitamin B_6, and 1 mg/d of vitamin B_{12}) compared with placebo resulted in a greater decrease in GFR and an increase in MI and stroke. Given the recent large-scale clinical

trials showing no treatment benefit, and our trial demonstrating harm, it would be prudent to discourage the use of high-dose B vitamins as a homocysteine-lowering strategy outside the framework of properly conducted clinical research.

Author Contributions: Dr Eliasziw had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: House, Eliasziw, Cattran, Churchill, Dresser, Spence.
Acquisition of data: House, Cattran, Churchill, Oliver, Fine, Spence.
Analysis and interpretation of data: House, Eliasziw, Cattran, Oliver, Dresser, Spence.
Drafting of the manuscript: House, Eliasziw, Spence.
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Statistical analysis: House, Eliasziw, Oliver.
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Study supervision: House, Cattran, Churchill, Oliver, Spence.
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REFERENCES

1. Chaturvedi N. The burden of diabetes and its complications: trends and implications for intervention. *Diabetes Res Clin Pract*. 2007;76(suppl 1):S3-S12.
2. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med*. 1997; 14(suppl 5):S1-S85.
3. US Renal Data System. USRDS 2009 Annual Data Report. http://www.USRDS.org. Accessed March 8, 2010.
4. Palmer AJ, Valentine WJ, Chen R, et al. A health economic analysis of screening and optimal treatment of nephropathy in patients with type 2 diabetes and hypertension in the USA. *Nephrol Dial Transplant*. 2008;23(4):1216-1223.
5. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. nephropathy in patients with type 2 diabetes. *N Engl J Med*. 2002;346(15):1145-1151.
6. Bostom AG, Silbershatz H, Rosenberg IH, et al. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. *Arch Intern Med*. 1999; 159(10):1077-1080.
7. Refsum H, Nurk E, Smith AD, et al. The Hordaland homocysteine study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr*. 2006;136(6)(suppl):1731S-1740S.
8. Becker A, Smulders YM, van Guldener C, Stehouwer CD. Epidemiology of homocysteine as a risk factor in diabetes. *Metab Syndr Relat Disord*. 2003;1(2): 105-120.
9. Looker HC, Fagot-Campagna A, Gunter EW, et al. Homocysteine as a risk factor for nephropathy and retinopathy in type 2 diabetes. *Diabetologia*. 2003; 46(6):766-772.
10. Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *Circulation*. 1999;99(9):1156-1160.
11. Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins: an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation*. 2000;102(20):2479-2483.
12. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002; 39(2)(suppl 1):S1-S266.
13. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976; 16(1):31-41.
14. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int*. 1985;28(5):830-838.
15. Zaltzman JS, Whiteside C, Cattran DC, Lopez FM, Logan AG. Accurate measurement of impaired glomerular filtration using single-dose oral cimetidine. *Am J Kidney Dis*. 1996;27(4):504-511.
16. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130(6): 461-470.
17. 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.
18. Parving HH, Rossing P, Hommel E, Smidt UM. Angiotensin-converting enzyme inhibition in diabetic nephropathy: ten years' experience. *Am J Kidney Dis*. 1995;26(1):99-107.
19. Dayal S, Lentz SR. Murine models of hyperhomocysteinemia and their vascular phenotypes. *Arterioscler Thromb Vasc Biol*. 2008;28(9):1596-1605.
20. McCully KS. Hyperhomocysteinemia and arteriosclerosis: historical perspectives. *Clin Chem Lab Med*. 2005;43(10):980-986.
21. Majors AK, Sengupta S, Willard B, Kinter MT, Pyeritz RE, Jacobsen DW. Homocysteine binds to human plasma fibronectin and inhibits its interaction with fibrin. *Arterioscler Thromb Vasc Biol*. 2002;22 (8):1354-1359.
22. Hajjar KA, Mauri L, Jacovina AT, et al. Tissue plasminogen activator binding to the annexin II tail domain: direct modulation by homocysteine. *J Biol Chem*. 1998;273(16):9987-9993.
23. Poddar R, Sivasubramanian N, DiBello PM, Robinson K, Jacobsen DW. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. *Circulation*. 2001;103(22):2717-2723.
24. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*. 2002;288(16):2015-2022.
25. Bønaa KH, Njolstad 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.
26. Ebbing M, Bleie O, 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.
27. 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. *N Engl J Med*. 2006;354 (15):1567-1577.
28. 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.
29. Mallett C, House AA, Spence JD, Fenster A, Parraga G. Longitudinal ultrasound evaluation of carotid atherosclerosis in one, two and three dimensions. *Ultrasound Med Biol*. 2009;35(3):367-375.
30. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol*. 2007;6(9): 830-838.
31. Imasa MS, Gomez NT, Nevado JB Jr. Folic acid-based intervention in non-ST elevation acute coronary syndromes. *Asian Cardiovasc Thorac Ann*. 2009; 17(1):13-21.
32. Ebbing M, Bonaa KH, Nygard O, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B_{12}. *JAMA*. 2009;302(19):2119-2126.
33. Loscalzo J. Homocysteine trials: clear outcomes for complex reasons. *N Engl J Med*. 2006;354(15): 1629-1632.
34. Urquhart BL, Freeman DJ, Spence JD, House AA. Mesna as a nonvitamin intervention to lower plasma total homocysteine concentration: implications for assessment of the homocysteine theory of atherosclerosis. *J Clin Pharmacol*. 2007;47(8): 991-997.