

Impact of Homocysteine-Lowering Vitamin Therapy on Long-Term Outcome of Patients With Coronary Artery Disease

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Elevated homocysteine levels are associated with increased risk for mortality in patients with coronary artery disease (CAD). However, the benefit of homocysteine-lowering therapy remains controversial. The aim of this study was to examine the impact of homocysteine-lowering therapy on the long-term outcomes of patients with CAD and its interaction with the methylenetetrahydrofolate reductase genotype. The study sample included 492 patients with early-onset CAD who were genotyped for the C677T mutation in the methylenetetrahydrofolate reductase gene or screened for elevated homocysteine from January 1997 to December 2002. Folic acid ≥ 400 $\mu\text{g}/\text{day}$ with or without additional B vitamins was administered at the attending physicians' discretion. There was no difference between treated ($n = 140$) and untreated patients in age, gender, or prevalence of coronary risk factors. Forty-six patients (9%) died during a median follow-up period of 115 months. Treatment was associated with significantly lower all-cause mortality in patients with homocysteine levels >15 $\mu\text{mol}/\text{L}$ (4% vs 32%, $p < 0.001$) but not in patients with lower levels (5% vs 7%, $p > 0.05$). On Cox regression analysis, the following factors were independently associated with all-cause mortality: vitamin therapy (hazard ratio 0.33, 95% confidence interval 0.11 to 0.98, $p = 0.046$), elevated homocysteine level (hazard ratio 3.5, 95% confidence interval 1.31 to 9.43, $p = 0.013$), and older age (hazard ratio 1.1, 95% confidence interval 1.04 to 1.14, $p < 0.0001$ for an increment of 5 years). The methylenetetrahydrofolate reductase genotype was not associated with outcomes. In conclusion, long-term folate-based vitamin therapy was independently associated with lower all-cause mortality in patients with CAD and elevated homocysteine levels. This association was not observed in patients with lower homocysteine levels. © 2009 Elsevier Inc. All rights reserved. (Am J Cardiol 2009;104:745–749)

In 1969, McCully¹ reported autopsy evidence of arterial occlusive disease in homocystinuria, a rare genetic disease characterized by high plasma homocysteine levels and the development of myocardial infarction very early in life. Even less severely elevated plasma homocysteine levels are associated with coronary artery disease (CAD),^{2,3} carotid artery disease,⁴ stroke,^{2,3} and mortality in patients with CAD.⁵ The atherogenic and prothrombotic effects of elevated plasma homocysteine support its causative role in CAD development.⁶ The C677T mutation in the gene encoding for methylenetetrahydrofolate reductase (MTHFR), a key enzyme in homocysteine metabolism, is a common cause of hyperhomocysteinemia and may confer increased risk for CAD in certain ethnic groups^{7–11} but not in others.^{12,13} Homocysteine-lowering therapy prevents the vascular complications of homocystinuria.^{14,15} How-

ever, conflicting results were reported in studies investigating the benefits of treatment with folic acid and B vitamins or high folate intake on CAD^{16–24} and on the risk for restenosis after percutaneous coronary intervention^{25–27} or stroke.^{19,20,28} Furthermore, the role played by the MTHFR genotype in this context is still unclear. In the present study, we examined the long-term effect of folate-based homocysteine-lowering therapy on the outcomes of patients with early-onset CAD by baseline homocysteine level and MTHFR genotype.

Methods

The study group consisted of patients registered in the Rabin Medical Center's MTHFR and homocysteine database from January 1, 1997, to December 31, 2002. The criteria for inclusion in the registry were well-documented CAD, onset of CAD symptoms before 65 years of age, and genotyping for the C677T mutation in the MTHFR gene or screening for elevated homocysteine levels as part of different research protocols or during risk factor evaluation. Patients with recent myocardial infarctions (<1 month), renal insufficiency (serum creatinine >1.2 mg/dl), hypothyroidism, monogenic familial hypercholesterolemia, or severe systemic disease were excluded, as were patients tak-

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ing folic acid, vitamin B₁₂, vitamin B₆, or medications known to affect plasma homocysteine levels.

All patients were interviewed, and their records were reviewed for data on smoking habits, body height and weight, use of medications including vitamins, age at onset of CAD symptoms, previous myocardial infarction, hypertension (defined as blood pressure >140/90 mm Hg or intake of antihypertensive medications), diabetes mellitus, dyslipidemia (defined as low-density lipoprotein cholesterol >130 mg/dl or triglycerides >300 mg/dl or use of lipid-lowering medications), and co-morbidities. The diagnosis of CAD was based on a well-documented history of acute myocardial infarction or an angiographic finding of >50% stenosis in ≥ 1 epicardial coronary artery. Data on mortality were obtained from the hospital database.

After an 8-hour fast, blood was drawn for the determination of plasma homocysteine, folate, and vitamin B₁₂ levels; lipid profile; and creatinine. Deoxyribonucleic acid was isolated from peripheral leukocytes with the DNA Isolation Kit for Mammalian Blood (Boehringer GmbH, Mannheim, Germany). Screening for the C677T substitution was performed by polymerase chain reaction of genomic deoxyribonucleic acid, followed by Hinf I digestion and agarose gel electrophoresis, as described by Frosst et al.²⁹ For the determination of plasma homocysteine, the fresh blood samples were immediately cooled on ice and protected from light. The plasma was separated shortly thereafter by cold centrifugation and stored at -20°C until assayed with the IMx analyzer (Abbott Laboratories, Abbott Park, Illinois). A plasma total homocysteine level above the normal range ($>15 \mu\text{mol/L}$) was defined as hyperhomocysteinemia. Plasma cobalamin (vitamin B₁₂) level was measured by microparticle enzyme immunoassay using a commercial kit (IMx B12; Abbott Laboratories), and folate levels were measured by ion capture assay using a commercial kit (AxSYM folate; Abbott Laboratories).

Folate-based vitamin therapy, defined as folic acid ≥ 0.4 mg/day (≥ 2.8 mg/week), with or without additional B vitamins, was given at the discretion of the attending physician. Data on therapy were analyzed on an intention-to-treat basis. During the study period, folic acid was usually available in 5-mg tablets. Therefore, the dose typically consisted of folic acid 5 mg by mouth 3 to 7 times weekly. Vitamin B₁₂ was given sublingually at 1 mg/week or orally at 0.25 to 0.4 mg/day. Vitamin B₆ was added at the physician's discretion. Patients who were not given folate-based therapy or in whom information on vitamin therapy was lacking were categorized as untreated.

Differences in mean parameters between groups were analyzed using Student's *t* test and differences in percentages using Pearson's chi-square test. Cox regression analysis was used to examine the impact of therapy, age, and elevated homocysteine on mortality. A *p* value <0.05 was considered statistically significant. Biochemical parameters (plasma levels of folate, homocysteine, and vitamin B₁₂) were analyzed as continuous or dichotomous variables as appropriate. On multivariate analysis, homocysteine was entered as a discrete variable (elevated vs nonelevated). Survival functions for patients with high versus normal homocysteine levels and for treatment versus no treatment were estimated using a Cox proportional-hazards model.

Table 1
Patients' characteristics by treatment status

Variable	Vitamin Therapy		p Value
	Yes (n = 140)	No (n = 352)	
Men	89 (64%)	246 (70%)	0.175
Age (years)	50.9 \pm 9.6	50.4 \pm 8.7	0.581
Diabetes mellitus	29 (29%)	56 (26%)	0.563
Dyslipidemia*	65 (64%)	71 (72%)	0.170
Hypertension†	31 (33%)	91 (42%)	0.154
Smokers	64 (63%)	137 (54%)	0.130
T/T genotype	43 (35%)	32 (10%)	<0.001
Hyperhomocysteinemia	70 (55%)	23 (10%)	<0.001
Homocysteine ($\mu\text{mol/L}$)	19.2 \pm 17.3	11.0 \pm 4.3	<0.001
Folate (nmol/L)	19.0 \pm 10.6	19.7 \pm 8.5	0.479
Vitamin B ₁₂ (pmol/L)	264 \pm 166	265 \pm 117	0.912

Data are expressed as mean \pm SD or as number (percentage).

* Low-density lipoprotein cholesterol >130 mg/dl, non-high-density lipoprotein cholesterol >160 mg/dl, triglycerides >300 mg/dl, or use of lipid-lowering medications.

† Blood pressure >140/90 mm Hg or use of antihypertensive medications.

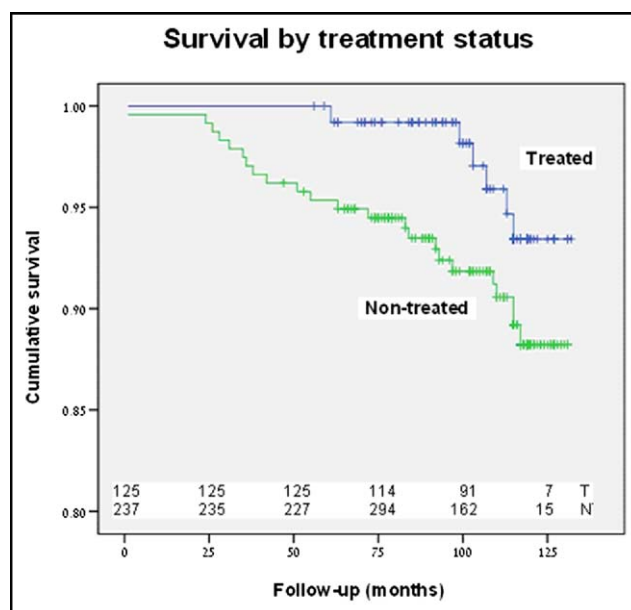


Figure 1. Impact of treatment on survival. Data on the number of patients at risk appear in the box. N = untreated; T = treated.

Data were analyzed using SPSS version 16.0 (SPSS, Inc., Chicago, Illinois). All analyses were univariate unless stated otherwise.

Results

The study sample comprised all 492 patients (335 men) included in the database. Data on MTHFR genotype were available for 430 patients, and data on homocysteine level were available for 365 patients. Table 1 lists the patients' characteristics according to vitamin therapy status. Compared to untreated patients, the treated patients (n = 140) were more often T/T homozygotes, had higher homocysteine levels, and more often had hyperhomocysteinemia.

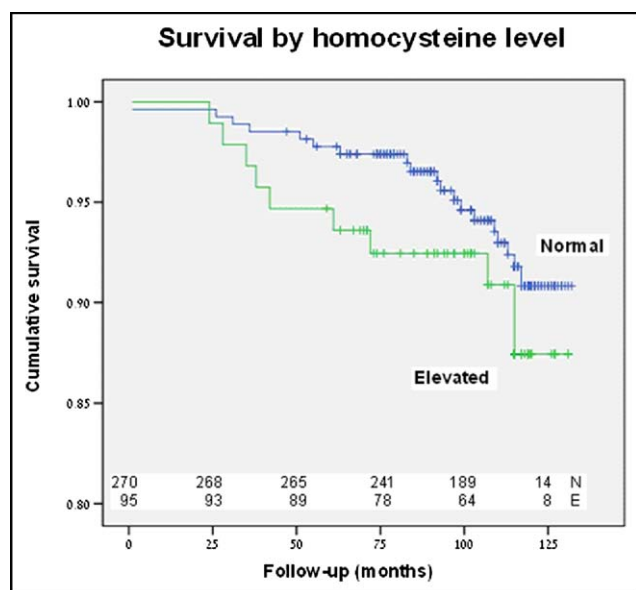


Figure 2. Overall survival according to baseline homocysteine level. Data on the number of patients at risk appear in the box. E = elevated homocysteine level; N = normal homocysteine level.

Table 2
Baseline characteristics of survivors and nonsurvivors

Variable	Nonsurvivors (n = 46)	Survivors (n = 446)	p Value
Men	36 (78%)	299 (67%)	0.120
Age (years)	55.2 ± 10.1	50.1 ± 8.9	<0.001
Diabetes mellitus	10 (39%)	75 (26%)	0.176
Dyslipidemia	20 (69%)	216 (70%)	0.936
Hypertension	11 (39%)	111 (39%)	0.936
Smokers	19 (63%)	182 (56%)	0.428
T/T genotype	5 (14%)	70 (18%)	0.788
Vitamin therapy	7 (15%)	133 (30%)	0.037
Hyperhomocysteinemia	10 (35%)	84 (26%)	0.263
Homocysteine (μmol/L)	13.8 ± 6.7	13.9 ± 11.8	0.960
Folate (nmol/L)	20.7 ± 10.5	19.3 ± 9.0	0.407
Vitamin B ₁₂ (pmol/L)	361 ± 241	255 ± 115	0.012

Data are expressed as mean ± SD or as number (percentage).

There were no differences between these 2 groups in age, gender, or prevalence of traditional coronary risk factors. The patients were followed for a median of 115 months (range 1 to 132). Forty-six patients (9%) died during follow-up. Multivariate Cox regression analysis showed that survival was significantly higher in the treated than in the untreated patients ($p = 0.029$). In addition, it showed that a normal homocysteine level was associated with better survival, as expected. Figure 1 shows the Kaplan-Meier survival curves according to treatment status. Figure 2 shows the Kaplan-Meier survival curves for untreated patients with elevated and normal baseline homocysteine levels.

Table 2 lists the patients' characteristics by outcome. The survivors were younger, had lower vitamin B₁₂ levels, and were more often given vitamin therapy. There were no differences between survivors and nonsurvivors in traditional coronary risk factors, homocysteine level, or preva-

Table 3
Hazard ratios for death by treatment status

	Treated	Not Treated	95% CI	p Value
Mortality rate	7 (5%)	39 (11%)		0.037
Hazard ratio	1.0	2.4	1.07–5.34	0.029
Hazard ratio*	1.0	4.0	1.38–11.39	0.011
Hazard ratio†	1.0	3.07	1.02–9.23	0.046

* Adjusted for age, gender, and elevated homocysteine.

† Adjusted for age, gender, homocysteine level, and vitamin B₁₂ level.

lence of hyperhomocysteinemia or the MTHFR homozygous mutant (T/T) genotype.

In patients with hyperhomocysteinemia, the mortality rate was significantly lower for treated than for untreated patients (4% vs 32%, $p < 0.001$). In patients with homocysteine levels $< 15 \mu\text{mol/L}$, the difference in mortality rate between treated and untreated patients was not significant (5% vs 7%, $p = 0.57$).

On Cox multivariate regression analysis, the following variables were independently associated with all-cause mortality: vitamin therapy (hazard ratio 0.33, 95% confidence interval 0.11 to 0.98, $p = 0.046$), elevated homocysteine level (hazard ratio 3.5, 95% confidence interval 1.31 to 9.43, $p = 0.013$), and older age (hazard ratio 1.1, 95% confidence interval 1.04 to 1.14, $p < 0.0001$ for an increment of 5 years). The MTHFR genotype was not associated with outcome.

Table 3 lists the effect of therapy on unadjusted and adjusted hazard ratios for death according to the Cox multivariate regression analysis. The hazard ratio for death associated with lack of therapy was markedly higher after adjustment for age, baseline homocysteine, and vitamin B₁₂ level.

Discussion

The present study shows that folate-based homocysteine-lowering therapy is associated with lower long-term total mortality rate in patients with CAD and elevated plasma homocysteine levels. In contrast, it has no apparent effect on mortality in patients with normal plasma homocysteine levels.

Our findings in patients with elevated homocysteine levels add to the growing body of evidence of a beneficial effect of folate-based therapy on outcomes. Our results of long-term follow-up of a cohort of patients with CAD are consistent with the 14-year follow-up results of Rimm et al¹⁶ in a cohort of 80,082 women participating in the Nurses' Health Study (NHS), which showed a significantly lower risk for CAD in multiple-vitamin users as well as in subjects with high intakes of folate from food. The beneficial effect of folate was similar for fatal and nonfatal CAD. A beneficial effect of folate-based homocysteine-lowering therapy on risk for cardiovascular events was also reported by Righetti et al²³ in hemodialysis patients. Others have found that folate therapy reduced mortality in heart transplant recipients²⁴ and decreased the incidence of major adverse events after percutaneous coronary intervention.^{25,26} In contrast, several prospective studies have reported no beneficial effect of folate-based therapy on mortality, when administered for either primary or secondary prevention.^{17–21}

The reason for the discrepancy between these prospective multicenter studies and our findings is not clear, although there are several potential explanations. First, their follow-up was shorter. The only prospective study with long follow-up (14 years) was the NHS,¹⁶ which had similar results to ours. Second, patients in earlier studies were given fixed daily doses of folic acid,^{17–21} whereas in our patients, it was often given on an alternate-day basis. The total weekly dose in our patients was relatively high, although the alternate-day regimen could have led to fluctuations in plasma folate and homocysteine levels. Third, there are apparent differences in patient selection: we included only patients with early-onset CAD and excluded patients with acquired conditions known to predispose to hyperhomocysteinemia, for example, renal insufficiency and hypothyroidism. Thus, the hyperhomocysteinemia in our patients was more likely than the hyperhomocysteinemia in the earlier studies to be genetic or due to other long-standing causes. Fourth, we studied patients with a specific ethnic background who were not represented in previous prospective studies. Differences among populations in the relation between homocysteine and risk for CAD are well documented.^{2,3} They may be attributed to differences in the prevalence of underlying genetic factors,³⁰ and they may cause differences in the effect of therapy.

We did not find a significant influence of the C677T mutation on outcomes or on the effect of therapy. However, other polymorphisms that predispose individuals to hyperhomocysteinemia may play a role in this context. Importantly, the baseline homocysteine levels in our treated patients were 57% higher in than the treated patients in the Heart Outcomes Prevention Evaluation 2 (HOPE-2) trial¹⁹ and 47% higher than in the treated patients in the Norwegian Vitamin Trial (NORVIT).¹⁸ Their baseline vitamin B₁₂ levels were 18% lower and 32% lower, respectively, whereas their folate levels were 31% lower than in treated HOPE-2 participants but 45% higher than in treated NORVIT participants who were given the triple vitamin therapy. Thus, the higher homocysteine levels and lower vitamin B₁₂ levels in our patients might explain the greater benefit they derived from treatment.

Of note, plasma folate is affected by vitamin B₁₂, and both folate and homocysteine levels are affected by certain coronary risk factors, for example, obesity and hypertension, which vary in prevalence among populations. Finally, there may be certain differences between our population and others, in nutrition as well as the timing and use of folate fortification.

Our findings imply that patients with CAD should be screened for elevated fasting plasma homocysteine and that those with hyperhomocysteinemia may benefit from homocysteine-lowering vitamin therapy. Because we studied only patients with CAD, we cannot comment on the potential value of homocysteine-lowering therapy in all subjects with hyperhomocysteinemia. However, further research is necessary.

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