

Effect of Homocysteine-Lowering Therapy With Folic Acid, Vitamin B₁₂, and Vitamin B₆ on Clinical Outcome After Percutaneous Coronary Intervention

The Swiss Heart Study: A Randomized Controlled Trial

Guido Schnyder, MD

Marco Roffi, MD

Yvonne Flammer, MD

Riccardo Pin, MD

Otto Martin Hess, MD

DESPITE TECHNICAL IMPROVEMENTS, restenosis and overall adverse events after percutaneous coronary interventions remain important limitations of this procedure.¹ Epidemiological evidence suggests that total plasma homocysteine level is an independent cardiovascular risk factor,^{2,3} correlates with the severity of coronary artery disease,^{4,5} predicts mortality in patients with established coronary atherosclerosis,^{6,7} and may have a potential role with regard to outcome after coronary interventions. Studies on the pathogenesis of homocysteine-induced vascular damage have suggested adverse interaction with vascular smooth muscle cells,^{8,9} endothelium function,^{10,11} plasma lipoproteins,¹² and coagulation cascade,¹³⁻¹⁶ which may contribute to homocysteine-induced atherogenesis, restenosis, and overall adverse events after coronary interventions, such as angioplasty.

Previous reports have documented that plasma homocysteine levels predict outcome after coronary angioplasty^{17,18} and our group has shown that homocysteine-lowering therapy significantly decreases restenosis rate after coronary angioplasty.¹⁹ Based on those results, we now report in an extension

Context Plasma homocysteine level has been recognized as an important cardiovascular risk factor that predicts adverse cardiac events in patients with established coronary atherosclerosis and influences restenosis rate after percutaneous coronary intervention.

Objective To evaluate the effect of homocysteine-lowering therapy on clinical outcome after percutaneous coronary intervention.

Design, Setting, and Participants Randomized, double-blind placebo-controlled trial involving 553 patients referred to the University Hospital in Bern, Switzerland, from May 1998 to April 1999 and enrolled after successful angioplasty of at least 1 significant coronary stenosis ($\geq 50\%$).

Intervention Participants were randomly assigned to receive a combination of folic acid (1 mg/d), vitamin B₁₂ (cyanocobalamin, 400 μ g/d), and vitamin B₆ (pyridoxine hydrochloride, 10 mg/d) (n=272) or placebo (n=281) for 6 months.

Main Outcome Measure Composite end point of major adverse events defined as death, nonfatal myocardial infarction, and need for repeat revascularization, evaluated at 6 months and 1 year.

Results After a mean (SD) follow-up of 11 (3) months, the composite end point was significantly lower at 1 year in patients treated with homocysteine-lowering therapy (15.4% vs 22.8%; relative risk [RR], 0.68; 95% confidence interval [CI], 0.48-0.96; $P=.03$), primarily due to a reduced rate of target lesion revascularization (9.9% vs 16.0%; RR, 0.62; 95% CI, 0.40-0.97; $P=.03$). A nonsignificant trend was seen toward fewer deaths (1.5% vs 2.8%; RR, 0.54; 95% CI, 0.16-1.70; $P=.27$) and nonfatal myocardial infarctions (2.6% vs 4.3%; RR, 0.60; 95% CI, 0.24-1.51; $P=.27$) with homocysteine-lowering therapy. These findings remained unchanged after adjustment for potential confounders.

Conclusion Homocysteine-lowering therapy with folic acid, vitamin B₁₂, and vitamin B₆ significantly decreases the incidence of major adverse events after percutaneous coronary intervention.

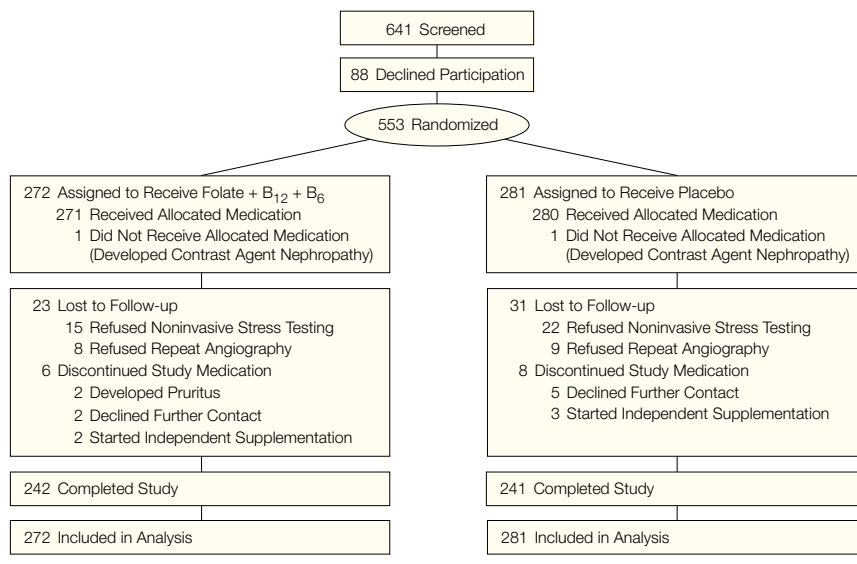
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of our original study, the effect of homocysteine-lowering therapy with folic acid, vitamin B₁₂ (cyanocobalamin), and vitamin B₆ (pyridoxine hydrochloride) on clinical outcome after successful coronary angioplasty and, in particular, whether the previously described 6 months' benefit is maintained at 1 year despite cessation of ho-

Author Affiliations: Division of Cardiology, Swiss Cardiovascular Center Bern, University Hospital, Bern, Switzerland (Drs Flammer, Pin, and Hess); Department of Cardiovascular Medicine/F25, The Cleveland Clinic Foundation, Cleveland, Ohio (Dr Roffi); and the Division of Cardiology, UCSD Medical Center, University of California, San Diego (Dr Schnyder).

Corresponding Author and Reprints: Guido Schnyder, MD, Division of Cardiology, UCSD Medical Center, University of California, San Diego, 200 West Arbor Dr, San Diego, CA 92103-8784 (e-mail: g.schnyder@lycos.com).

Figure 1. Flowchart of Study Patients

homocysteine-lowering therapy at 6 months.

METHODS

The protocol was approved by the Institutional Research Ethics Committee of the University Hospital in Bern, Switzerland. Each patient gave written informed consent. This was a prospective study enrolling 553 consecutive patients from May 1998 to April 1999 who had undergone angioplasty of at least 1 significant coronary stenosis ($\geq 50\%$) (FIGURE 1). After successful coronary angioplasty, patients were randomly assigned in double-blind fashion to receive folic acid (1 mg/d), vitamin B₁₂ (400 µg/d), and vitamin B₆ (10 mg/d) or placebo daily for 6 months. The study medication was formulated to obtain a maximal homocysteine-lowering effect with a minimal risk of adverse effects.²⁰ The study population included a subgroup of 205 patients independently randomized and scheduled for follow-up angiography at 6 months; the quantitative angiography results of this subgroup have been published.¹⁹

Patients with unstable angina, subacute myocardial infarction (<2 weeks), renal insufficiency (serum creatinine level >1.8 mg/dL [160 µmol/L]),

or taking vitamin supplements were not included. Patients were asked to withhold any multivitamin intake for the entire study duration. Fasting total plasma homocysteine levels were measured on admission and at 6 months follow-up using a rapid high-performance liquid chromatographic assay.²¹ Coronary angioplasty was performed according to standard clinical practice, with success defined as residual diameter stenosis less than 35% with normal flow pattern (Thrombolysis in Myocardial Ischemia [TIMI] III trial criteria).²²

Angiographic Evaluation

Quantitative evaluation was carried out in monoplane projection after predilatation with nitrates. Two orthogonal views were averaged for biplane assessment. Data analysis was performed using an automated edge-detection system (Philips Integris-BH-3000, Version 2 [if online] or Philips View-Station-CDM-3500, Version 2 [if offline]; Philips, Best, the Netherlands) with an institutional intraobserver variability of 0.15 mm for minimal luminal diameter and 7% for stenosis severity.¹⁹ The tip of the diagnostic or guiding catheter (positioned at the coronary ostium) was used for calibration purposes. The same views and calibration

techniques were used for target lesion revascularization. End-diastolic frames in the 2 orthogonal views that demonstrated maximal stenosis severity were used for luminal diameter measurement. Reference vessel diameter, minimal luminal diameter, diameter stenosis, and lesion length were calculated as the average value of the 2 views. Angiograms were reviewed by an experienced interventional cardiologist blinded to patients' homocysteine level and treatment assignments.

Follow-up and Study End Points

Clinical follow-up, including noninvasive stress test and resting electrocardiogram, was performed at 6 months and 1 year, or earlier if symptoms recurred. Adverse events were defined prospectively as (1) death; (2) cardiac death, defined as sudden, unexplained death or death related to myocardial infarction; (3) nonfatal myocardial infarction, defined as new Q waves (>40 ms; >0.2 mV) in 2 or more contiguous electrocardiographic leads; (4) need for repeat revascularization for proven ischemia demonstrated by either follow-up cardiac events or a positive noninvasive stress test with significant angiographic stenosis of at least 50%; and (5) a composite of major adverse events defined as any of the above events. Patients with more than 1 event had only the first occurring event computed for overall major adverse events determination.

Statistical Analysis

The target sample size of 555 patients was based on the assumption that the rate of major adverse events would be 25% or more in the placebo-treated group and less than 15% in the group treated with folate+B₁₂+B₆.^{17,19} Assuming a 10% dropout rate, the planned sample size would yield 500 patients with complete follow-up and give the study a statistical power of 80% at a significance level of .05.²³ All analyses were performed with the intent-to-treat principle, and patients lost to follow-up were censored at the time clinical data became no longer available.

Plasma homocysteine levels were positively skewed and therefore log-transformed prior to analysis. Results are shown in natural units. Categorical variables are reported as counts (percentages) and continuous variables as mean (SD). Categorical variables were examined by χ^2 test. Continuous variables were examined by a 2-tailed *t* test or by the Mann-Whitney U test if skewed. The Spearman rank correlation coefficient was used to estimate the correlation between homocysteine levels and different continuous variables.

Kaplan-Meier survival curves were used to evaluate freedom from major adverse events, and treatment effect differences were assessed with the Mantel-Cox log-rank test. Cox proportional hazards regression models were used to examine the relation between treatment groups and the different end points, after adjustment for multiple clinical and angiographic covariates including age, sex, use or nonuse of stent, treatment of restenotic or de novo lesions, vessel size, postprocedural minimal luminal diameter, target lesion location, and use or nonuse of glycoprotein IIb/IIIa inhibitors. Selected variables were those that were associated with at least 1 of the end points in unadjusted analysis. Cardiovascular risk factors (diabetes mellitus, hypertension, hypercholesterolemia, smoking status) and statin use were not associated with the different end points in unadjusted analysis. Furthermore, adjustment for those variables did not significantly modify the Cox proportional hazards regression analysis and were thus not included in the model. Patients with a history of renal failure (serum creatinine level, >1.8 mg/dL [160 μ mol/L]) were not included to avoid elevated creatinine values as confounders for increased plasma homocysteine levels. $P < .05$ was considered statistically significant. Data were prospectively collected and analyzed using StatView Version 4.5 (SAS Institute, Cary, NC).

RESULTS

Five hundred fifty-three patients were randomly assigned either to receive

folate+B₁₂+B₆ (n=272) or placebo (n=281), with a total of 741 successfully treated lesions (Figure 1). Seventy patients (110 lesions) were lost to follow-up or did not comply with the study protocol: 14 (6 in the folate+B₁₂+B₆ group) discontinued study medication, 37 (15 in the folate+B₁₂+B₆ group) refused noninvasive stress testing, 17 (8 in the folate+B₁₂+B₆ group) with proven ischemia refused reangiography, and 2 (1 in the folate+B₁₂+B₆ group) developed reversible contrast agent nephropathy. Two patients randomized to receive folate+B₁₂+B₆ discontinued study medication because of pruritus. No other adverse effect was reported. The baseline clinical, laboratory, and angiographic characteristics

of the 70 patients without complete follow-up did not significantly differ from the remaining study population. Given that clinical outcomes were the primary end points in this study, all analyses were performed with the intent-to-treat principle.

Baseline Characteristics

Patients in the 2 groups were well matched at baseline with regard to demographic variables and cardiovascular risk factors (TABLE 1). Severity of coronary artery disease (as measured by a history of previous myocardial infarction, previous revascularization, and the number of treated lesions per patient), baseline laboratory values, and discharge drug therapy were not sig-

Table 1. Baseline Characteristics*

Variable†	Folate+B ₁₂ +B ₆ (n = 272)	Placebo (n = 281)	P Value
Sex, %			
Male	79	82	.42
Female	21	18	
Age, mean (SD), y	63.4 (10.6)	61.8 (11.0)	.10
Smoker, No. (%)	110 (40)	116 (41)	.87
Diabetes mellitus, No. (%)	77 (28)	77 (27)	.83
Arterial hypertension, No. (%)	177 (65)	183 (65)	.86
Hypercholesterolemia, No. (%)	218 (80)	221 (79)	.66
Previous MI, No. (%)	136 (50)	155 (55)	.27
MI within last 6 mo, No. (%)	87 (32)	98 (35)	.35
Previous PTCA, No. (%)	83 (31)	92 (33)	.67
Previous CABG, No. (%)	35 (13)	34 (12)	.79
Laboratory findings, mean (SD)‡			
HbA _{1c} , %	6.0 (1.1)	6.0 (1.0)	.58
Creatinine, mg/dL	1.04 (0.20)	1.04 (0.19)	.70
Baseline homocysteine, mg/L	1.54 (0.64)	1.50 (0.62)	.68
6-month follow-up	1.01 (0.34)	1.36 (0.57)	<.001
Cholesterol, mg/dL	214 (43)	212 (46)	.29
HDL cholesterol, mg/dL	46 (13)	45 (13)	.37
LDL cholesterol, mg/dL	130 (37)	130 (40)	.74
Triglycerides, mg/dL	181 (113)	178 (113)	.86
Discharge therapy, No. (%)			
Statins	189 (69)	199 (71)	.71
β -Blockers	166 (61)	179 (64)	.52
ACE inhibitors	100 (37)	102 (36)	.94
Aspirin	252 (93)	265 (94)	.53
ADP inhibitors	150 (55)	162 (58)	.45

*MI indicates myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACE, angiotensin-converting enzyme; and ADP, adenosine diphosphate.

†Criteria for variables: (1) smoking: current or discontinued during the last 6 months; (2) diabetes mellitus: HbA_{1c} level at least 6.2% and current insulin or oral hypoglycemic therapy; (3) hypertension: blood pressure greater than 140/90 mm Hg or current antihypertensive therapy; (4) hypercholesterolemia: cholesterol level at least 200 mg/dL or current lipid-lowering drugs.

‡Conversion factors between conventional and SI units: creatinine: 88.4 \times mg/dL = μ mol/L; homocysteine: 7.397 \times mg/L = μ mol/L; cholesterol (total, HDL, LDL): 0.0259 \times mg/dL = mmol; triglycerides: 0.0113 \times mg/dL = mmol/L.

nificantly different between study groups. As expected, mean homocysteine levels (SD) at 6 months were significantly lower with folate+B₁₂+B₆ therapy compared with placebo (1.01 [0.34] mg/L [7.5 (2.5) μmol/L] vs 1.36 [0.57] mg/L [10.1 (4.2) μmol/L], *P*<.001). Mild to moderate elevation of homocysteine levels (>1.62 mg/L [12 μmol/L]) was present in 29% of patients at baseline. None of the patients had severe hyperhomocysteinemia (>13.5 mg/L [100 μmol/L]). Baseline homocysteine levels correlated with age (Spearman *r*=0.212, *P*<.001), serum creatinine levels (Spearman *r*=0.251,

P<.001), and high-density lipoprotein (HDL) cholesterol levels (Spearman *r*=−0.128, *P*=.004).

Lesion location was independent of study group: 40% of all lesions were located in the left anterior descending coronary artery and about 30% each in the circumflex coronary artery and the right coronary artery (TABLE 2).²⁴ Lesion severity (lesion complexity, lesion length, vessel size, minimal luminal diameter, and diameter stenosis) before and after coronary angioplasty was comparable between study groups. The use of stents and glycoprotein IIb/IIIa inhibitors was also identical between study groups.

Study End Points

After a mean (SD) follow-up of 11 (3) months, 14.0% of patients treated with folate+B₁₂+B₆ underwent repeat revascularization vs 19.9% of control patients (relative risk [RR], 0.70; 95% confidence interval [CI], 0.49-1.01; *P*=.06) (TABLE 3). This difference was primarily due to the number of patients with repeat target lesion revascularization, as 4.0% of patients in the folate+B₁₂+B₆ group and 3.9% in the placebo group had revascularization of a lesion other than a target lesion (RR, 1.03; 95% CI, 0.45-2.34; *P*=.94). Among patients who received folate+B₁₂+B₆, 9.9% had repeat target lesion revascularization vs 16.0% in the placebo group, a relative reduction of 38% (RR, 0.62; 95% CI, 0.40-0.97; *P*=.03). The need for target lesion revascularization was also significantly associated with smaller vessel size (SD) (2.91 [0.78] mm vs 3.16 [0.79] mm, *P*=.02), smaller postprocedural minimal luminal diameter (SD) (2.22 [0.53] mm vs 2.45 [0.78] mm, *P*=.03), and the restenotic nature of previously treated lesions (RR, 3.36; 95% CI, 1.67-6.76; *P*=.002). Adjustment for multiple risk factors including age, sex, and variables known to influence the need for target lesion revascularization after coronary angioplasty (use of stents, treatment of restenotic lesions, vessel size, postprocedural minimal luminal diameter, target lesion location, use of IIb/IIIa inhibitors) did not significantly change the association between homocysteine-lowering therapy and the need for repeat target lesion revascularization. In Cox proportional

Table 2. Lesion Characteristics and Treatment Options*

	Folate+B ₁₂ +B ₆ (n = 369)	Placebo (n = 372)	P Value
Lesion location, No. (%)			
LAD	146 (40)	151 (41)	.78
Circumflex	98 (27)	110 (30)	.36
Right coronary artery	125 (34)	111 (30)	.24
Restenotic lesions, No. (%)	16 (4.4)	20 (5.5)	.50
Complex lesions, No. (%)†	241 (65)	231 (62)	.41
No. of treated lesions per patient, mean (SD)	1.35 (0.57)	1.34 (0.58)	.76
Treatment options, No. (%)			
Stents	199 (54)	197 (53)	.79
Glycoprotein IIb/IIIa inhibitors	42 (11)	42 (11)	.97
Reference vessel diameter, mean (SD), mm			
Before angioplasty	2.81 (0.67)	2.75 (0.68)	.21
After angioplasty	3.06 (0.64)	3.01 (0.81)	.35
Minimal luminal diameter, mean (SD), mm			
Before angioplasty	0.94 (0.48)	0.88 (0.45)	.16
After angioplasty	2.35 (0.61)	2.31 (0.77)	.52
Diameter stenosis, mean (SD), %			
Before angioplasty	66.6 (14.7)	68.2 (16.4)	.15
After angioplasty	23.5 (10.2)	23.4 (10.9)	.89
Lesion length, mean (SD), mm	12.5 (7.8)	12.2 (6.9)	.29

*LAD indicates left anterior descending coronary artery.

†Complex lesions were defined as lesions of type B2 and C according to the modified American College of Cardiology/American Heart Association classification.²⁴

Table 3. Clinical Events at 1 Year Follow-up

Events	No. (%)		Unadjusted		Adjusted for Multiple Risk Factors*	
	Folate+B ₁₂ +B ₆ (n = 272)	Placebo (n = 281)	Hazard Ratio (95% Confidence Interval)	P Value	Hazard Ratio (95% Confidence Interval)	P Value
Target lesion revascularization	27 (9.9)	45 (16.0)	0.62 (0.40-0.97)	.03	0.61 (0.41-0.95)	.02
Any revascularization	38 (14.0)	56 (19.9)	0.70 (0.49-1.01)	.06	0.69 (0.51-0.98)	.04
Nonfatal myocardial infarction	7 (2.6)	12 (4.3)	0.60 (0.24-1.51)	.27	0.57 (0.27-1.42)	.17
Cardiac death	3 (1.1)	6 (2.1)	0.52 (0.13-2.04)	.34	0.51 (0.15-2.00)	.23
Any death	4 (1.5)	8 (2.8)	0.54 (0.16-1.70)	.27	0.52 (0.21-1.56)	.17
Any event	42 (15.4)	64 (22.8)	0.68 (0.48-0.96)	.03	0.66 (0.47-0.94)	.01

*Hazard ratio and *P* values were determined by Cox proportional hazards regression analysis and were adjusted for age, sex, use or nonuse of stent, treatment of restenotic or de novo lesions, vessel size, postprocedural minimal luminal diameter, target lesion location, and use or nonuse of glycoprotein IIb/IIIa inhibitors.

hazards regression analysis, only folate+B₁₂+B₆ therapy ($P=.02$), the restenotic nature of previously treated lesions ($P=.005$), and postprocedural minimal luminal diameter ($P=.01$) retained statistical significance.

The need for target lesion revascularization was independent of cholesterol levels, but the benefit of folate+B₁₂+B₆ therapy was most apparent for patients in the highest cholesterol tertile. Compared with controls, patients treated with folate+B₁₂+B₆ with cholesterol levels in the highest (>228 mg/dL [5.90 mmol/L]) tertile had the largest risk reduction in terms of target lesion revascularization (RR, 0.44; 95% CI, 0.21-0.92; $P=.04$). This benefit was not significant among patients treated with folate+B₁₂+B₆ in the middle (189-228 mg/dL [4.89-5.90 mmol/L]) tertile (RR, 0.55; 95% CI, 0.25-1.23; $P=.20$) and was smallest in the lowest (<189 mg/dL [4.89 mmol/L]) tertile (RR, 0.72; 95% CI, 0.33-1.55; $P=.53$). A similar trend was seen for low-density lipoprotein (LDL) cholesterol levels ([highest tertile: >145 mg/dL (3.75 mmol/L); RR, 0.50; 95% CI, 0.26-0.91; $P=.03$] [middle tertile: 108-145 mg/dL (2.80-3.75 mmol/L); RR, 0.58; 95% CI, 0.32-1.14; $P=.29$] [lowest tertile: <108 mg/dL (2.80 mmol/L); RR, 0.66; 95% CI, 0.25-1.74; $P=.39$], respectively). Adjustment for statin use did not significantly change those associations.

There was a nonsignificant trend for a lower incidence of nonfatal myocardial infarction (RR, 0.60; 95% CI, 0.24-1.51; $P=.27$), cardiac deaths (RR, 0.52; 95% CI, 0.13-2.04; $P=.34$), and overall deaths (RR, 0.54; 95% CI, 0.16-1.70; $P=.27$) in patients receiving folate+B₁₂+B₆ therapy. Older age (SD) was the only variable significantly associated with mortality (65.4 [11.5] years vs 61.2 [10.8] years, $P=.002$).

The incidence of major adverse events was significantly lower in patients receiving folate+B₁₂+B₆ therapy at 6 months (11.4% vs 18.9%; RR, 0.60; 95% CI, 0.40-0.91; $P=.02$) and at 1 year follow-up (15.4% vs 22.8%; RR, 0.68; 95% CI, 0.48-0.96; $P=.03$) (FIGURE 2). Adjustment for the previously men-

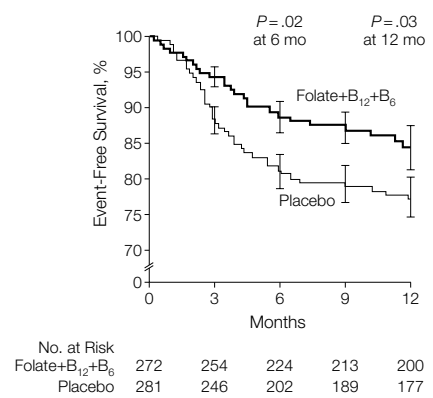
tioned variables did not significantly change this association ($P=.01$). These findings were reproduced in subgroups of patients stratified according to the traditional cardiovascular risk factors (sex, diabetes mellitus, hypertension, hypercholesterolemia, and smoking) (FIGURE 3). The only other variable independently associated with the incidence of major adverse events was the restenotic nature of previously treated lesions ($P=.008$).

COMMENT

This study provides evidence that homocysteine-lowering therapy with folic acid, vitamin B₁₂, and vitamin B₆ improves outcome after percutaneous coronary intervention by reducing the need for repeat revascularization and decreasing the overall incidence of major adverse events 1 year after successful coronary angioplasty. This benefit is primarily related to a decrease in target lesion revascularization, as the need for revascularization of lesions other than a target lesion was almost identical between study groups. Furthermore, these findings were reproduced in subgroups of patients stratified according to the traditional cardiovascular risk factors. Vessel size, postprocedural minimal luminal diameter, and treatment of restenotic lesions are known to influence the need for target lesion revascularization.^{25,26} These parameters were equally distributed between study groups and the benefit of folate+B₁₂+B₆ therapy on the outcome after coronary angioplasty remained unaltered after adjustment for those risk factors. These results are consistent with those of recent randomized trials with homocysteine-lowering therapy showing decreased risk of atherosclerotic coronary events among healthy patients,²⁷ halting in the progression of carotid plaque,²⁸ improved arterial endothelial function,²⁹⁻³¹ and significant benefit on restenosis rate after coronary angioplasty.¹⁹

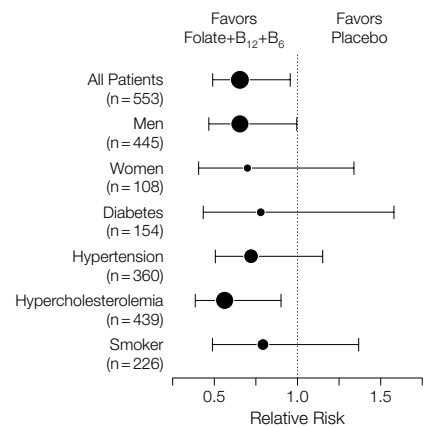
This study further suggests that the benefit obtained with homocysteine-lowering therapy at 6 months is maintained at 1 year despite cessation of

Figure 2. Kaplan-Meier Survival Curves for Freedom From Major Adverse Events in 553 Patients



The rate of event-free survival was significantly higher among patients assigned to receive folate+B₁₂+B₆ therapy than among control patients. The relative risk of a major adverse event with folate+B₁₂+B₆ therapy was 0.60 (95% confidence interval [CI], 0.40-0.91; log-rank $P=.02$) at 6 months and 0.68 (95% CI, 0.48-0.96; log-rank $P=.03$) at 1 year (mean [SD] follow-up, 11 [3] months).

Figure 3. Risk of Major Adverse Events With Folate+Vitamin B₁₂+Vitamin B₆ Therapy Among Total Study Population and Subgroups of Patients Stratified According to Traditional Cardiovascular Risk Factors



The size of each data marker is proportional to the number of patients; horizontal bars represent 95% confidence intervals.

folate+B₁₂+B₆ therapy at 6 months. Our previously reported significant decrease in restenosis rate after coronary angioplasty¹⁹ could have been questioned as a temporary benefit triggered by a homocysteine-lowering therapy-related delay of the restenosis process.

The current study confirms that a 6-month course of this inexpensive treatment has minimal adverse effects and helps to control excessive restenosis mechanisms. Nevertheless, it is unclear whether a longer treatment course (ie, up to 12 months) would have benefited the other end points, such as death or myocardial infarction, for which only a trend in favor of homocysteine-lowering therapy was found. These issues should be answered by several ongoing clinical trials: the Norwegian Vitamin Interventional Trial (NORVIT) and the Western Norway B-vitamin Intervention Trial (WENBIT) will assess the effects of homocysteine-lowering therapy in patients with coronary artery disease; the Vitamin Intervention for Stroke Prevention (VISP) study in the United States will report the effect of B vitamins on stroke recurrence in patients with cardiovascular disease; and the Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC) study in Australia and the Study of Effectiveness of Additional Reduction in Cholesterol and Homocysteine (SEARCH) in the United Kingdom will address similar issues.³²

The mechanisms by which elevated homocysteine levels impair vascular function and possibly influence outcome after percutaneous coronary intervention are not clearly understood, although several hypotheses have been suggested. Elevated homocysteine levels stimulate vascular smooth muscle cell growth^{8,9} and collagen synthesis,³³ which promote intimal-medial thickening.³⁴ Elevated homocysteine levels may also have a procoagulant effect through interaction with coagulation factor V,¹³ protein C,¹⁴ tissue plasminogen activator,¹⁵ and tissue factor activity.¹⁶ However, increasing evidence suggests that the primary mechanism may be oxidative-endothelial injury and dysfunction.^{10,11} Elevated homocysteine levels decrease the release of nitric oxide^{35,36} and promote the generation and accumulation of hydrogen peroxide, thus rendering nitric oxide more susceptible to oxidative inactivation.³⁴ Furthermore, elevated plasma homocysteine levels pro-

mote lipid peroxidation,³⁷ which alters growth factor production and influences smooth muscle cell proliferation.³⁸ Oxidized LDL cholesterol has been shown to increase smooth muscle cells proliferation and chemoattraction^{39,40} and enhance platelet-derived growth factor gene expression and receptor formation in vascular smooth muscle cell.⁴¹ Therefore, homocysteine-induced endothelial dysfunction and lipid peroxidation may promote smooth muscle cell proliferation, extracellular matrix formation, and ultimately increase the need for repeat target lesion revascularization. Our findings that the benefit of homocysteine-lowering therapy increases with higher levels of LDL cholesterol supports this possible mechanism.

A critical question is whether the benefit of homocysteine-lowering therapy on the outcome after coronary intervention reflects causality. In the current study, the treatment of restenotic lesions, the treatment of lesions in smaller vessels, and smaller postprocedural minimal luminal diameter were all significantly associated with a worse outcome after coronary angioplasty. Adjustment for these factors did not weaken the benefit of homocysteine-lowering therapy, suggesting an independent association.

A limitation of the study design was that it precluded assessment of the separate effects of folic acid, vitamin B₁₂, and vitamin B₆, and the effect of different doses of these vitamins. Furthermore, we cannot exclude the possibility that the benefit seen was not also influenced by other homocysteine-independent treatment effects. Specifically, folic acid likely improves nitric oxide availability independently of its homocysteine-lowering effect,⁴² and vitamin B₆ deficiency appears to be an independent predictor of coronary artery disease⁴³ and further has been shown to alter platelet function.⁴⁴ Therefore, and despite the findings of the Homocysteine Lowering Trialists' Collaboration group that vitamin B₆ does not significantly lower homocysteine levels,²⁰ the inclusion of vitamin B₆ in the homocysteine-

lowering therapy or possibly another homocysteine-unrelated effect of folic acid or vitamin B₁₂ could have contributed to the improvement seen in the patients treated with folate+B₁₂+B₆. In conclusion, the findings in this study, in conjunction with our previously described association between homocysteine levels and restenosis rate,¹⁷ support the conclusion that the combination of folic acid, vitamin B₁₂, and vitamin B₆, at least partially by lowering of homocysteine levels, is an effective therapy for improving outcome in patients undergoing coronary angioplasty.

Author Contributions: *Study concept and design:* Schnyder, Hess.

Acquisition of data: Schnyder, Roffi, Flammer, Pin. *Analysis and interpretation of data:* Schnyder, Roffi, Flammer, Pin, Hess.

Drafting of the manuscript: Schnyder.

Critical revision of the manuscript for important intellectual content: Roffi, Flammer, Pin, Hess.

Statistical expertise: Schnyder, Hess.

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Study supervision: Hess.

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