Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

Vol. 40, No. 4, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)02016-8

Coronary Endothelial Function in Hyperhomocysteinemia: Improvement After Treatment With Folic Acid and Cobalamin in Patients With Coronary Artery Disease

Frank F. Willems, MD,* Wim R. M. Aengevaeren, MD, PHD,† Godfried H. J. Boers, MD, PHD,‡ Henk J. Blom, PHD,§ Freek W. A. Verheugt, MD, PHD, FACC†

Arnhem and Nijmegen, the Netherlands

OBJECTIVES We evaluated the effect of therapy with folic acid and cobalamin on coronary endothelial function, expressed as a change in volumetric coronary blood flow (CBF), in hyperhomocysteinemic patients with coronary artery disease (CAD).

BACKGROUND Hyperhomocysteinemia is an independent risk factor for CAD. The mechanism responsible for this increased risk is unclear, but it is generally assumed that hyperhomocysteinemia causes endothelial dysfunction. It is unknown whether lowering plasma homocysteine levels with folic acid and cobalamin improves coronary endothelial function in patients with hyperhomocysteinemia and symptomatic CAD.

METHODS Fifteen patients scheduled for elective percutaneous transluminal coronary angioplasty (PTCA) with plasma homocysteine levels of ≥16 μmol/l were randomized for six months of treatment with folic acid 5 mg and cobalamin 400 μg daily or placebo. Coronary endothelial function was evaluated in a non-PTCA vessel using acetylcholine infusion in dosages of 10^{-8} M, 10^{-7} M, and 10^{-6} M. Endothelium- dependent CBF is determined using intracoronary Doppler velocity and quantitative coronary angiography at baseline and after six months.

RESULTS In the folic acid/cobalamin treated group, CBF increased after acetylcholine infusion with 96% (standard deviation 54; 95% confidence interval [CI]: 44% to 154%) compared with a decrease of 16% (standard deviation 35; 95% CI: −20% to +30%) of the CBF in the placebo-treated group ($p < 0.005$).

CONCLUSIONS This is the first prospective randomized placebo-controlled intervention study evaluating coronary endothelial function in hyperhomocysteinemic patients with CAD. Our results suggest that coronary endothelial function improves after treatment with folic acid and cobalamin. (J Am Coll Cardiol 2002;40:766–72) © 2002 by the American College of Cardiology Foundation

Hyperhomocysteinemia is an important risk factor for premature cardiovascular disease. To date, more than 80 studies have been published about the association between homocysteine and vascular disease (1). Homocysteine levels can be reduced 25% to 30% using folic acid, and supplementation of vitamin B12 provides an additional 7% reduction. This reduction is higher in patients with hyperhomocysteinemia and patients with a low pretreatment folate status (2). The pathophysiologic mechanisms responsible for the increased risk remain unclear. Generally, it is assumed that homocysteine is toxic to the vascular wall and causes endothelial dysfunction (3). Endothelial dysfunction is a key mechanism in the current hypothesis of atherothrombosis, and it is shown that functional impairment of the endothelial function, defined as an impairment of endothelium-dependent coronary blood flow, precedes significant arterial vessel disease (4). Until now, it was unknown whether homocysteine-lowering therapy would improve prognosis in patients with elevated homocysteine levels and coronary artery disease (CAD). Some investigators have shown that folic acid improves endothelial function in the forearm model in patients with CAD (5). However, until recently, it has been unclear whether homocysteine-lowering therapy improves coronary endothelial function. The aim of this study was to evaluate the effect of six months of therapy with folic acid 5 mg in combination with cobalamin 400 μg on coronary endothelial function in patients with hyperhomocysteinemia with symptomatic CAD.

METHODS

Patient selection. This study is a double-blind, randomized, placebo-controlled study with a follow-up of six months investigating the effect of folic acid 5 mg and cobalamin on coronary endothelial function. Cobalamin 400 μg was added to avoid unopposed folic acid treatment in undiagnosed vitamin B12 deficiency. End points were acetylcholine (ACH)-induced changes in endothelium-dependent coronary blood flow at baseline and after six months of therapy. Other end points were change in mean

From the *Department of Cardiology, Rijnstate Hospital, Arnhem, the Netherlands; †Heart Center, ‡Department of Internal Medicine, and the §Laboratory of Pediatrics and Neurology, University Medical Center, Nijmegen, the Netherlands. Supported by a grant from the Netherlands Heart Foundation (Grant no. 96.071). Dr. H. J. Blom is an established investigator of the Netherlands Heart Foundation (D 97.021).

Manuscript received November 30, 2001; revised manuscript received April 16, 2002, accepted May 15, 2002.

Abbreviations and Acronyms

ACH = acetylcholine
CAD = coronary artery disease
CBF = volumetric coronary blood flow
CI = confidence interval
CVR = coronary velocity range
LAD = left anterior descending artery
MOD = minimal obstruction diameter
MSD = mean segment diameter
PTCA = percutaneous transluminal coronary angioplasty
RCA = right coronary artery
RCX = ramus circumflexus
SD = standard deviation
tHcy = total plasma homocysteine

segment diameter (MSD) and in minimal obstruction diameter (MOD) of a predefined segment of a coronary vessel after infusion of ACH.

Patients with objectively confirmed ischemic heart disease who were referred for elective percutaneous transluminal coronary angioplasty (PTCA) between April 1997 and June 1999 to our catheterization laboratory were eligible for this study. Inclusion criteria were a plasma homocysteine level of 15.5 μmol/l or more, age between 18 and 70 years, and at least one coronary vessel without a significant stenosis (stenosis <50%). Patients who used medication or vitamins involved in homocysteine metabolism were excluded from this study as were patients with diabetes mellitus and patients with uncontrolled hypertension. During follow-up, patients were asked to make no changes in their diet or exercise levels. Patients in whom a coronary stent was placed received 160 mg of acetylsalicylic acid instead of 80 mg during the first month. Otherwise, the medication was kept unchanged when possible. Patients with kidney failure reflected as a creatinine level of more than 150 μg/l or serious liver failure were also excluded. Patients returned after 2, 8, 12, and 23 weeks for reassessment of lipid profile, vitamin levels, homocysteine levels, and clinical signs of recurrent angina pectoris. The Ethical Committee of our institution approved the protocol. Written informed consent was obtained in all patients.

Coronary angiography. Long-acting nitrates were withdrawn 24 h before the procedure and replaced by short-acting nitrates if necessary. All procedures were performed under the same conditions in time and location. After the performance of the elective PTCA, a new guiding catheter 7F (Medtronic Inc., Minneapolis, Minnesota) was placed in a nondilated coronary artery without a stenosis (≤50%). A Doppler guidewire (0.014 in. diameter) (Flowire, EndoSonics Inc., Pleasanton, California) within a 2.2F coronary infusion catheter (Medtronic) was advanced in the vessel of interest just distal to a landmark of a proximal epicardial segment, defined as an important side-branch of a coronary vessel, with a diameter of at least 2.0 mm. After achieving a stable velocity signal, adenosine 18 μg was administered through the guiding catheter for maximal hyperemia, and endothelium-independent coronary flow velocity was measured. Assessment of endothelium-dependent vasodilation and coronary flow was performed by selective infusions of ACH with a total amount of 10^{-8} M, 10^{-7} M, and 10^{-6}M. Acetylcholine was dissolved in NaCl 0.9% and infused with a Terumo pump (Terumo Medical Corp., Tokyo, Japan) at a rate of 2 ml/min during 3 min. Nitroglycerin 200 μg was then injected as a bolus.

Before infusion of ACH, baseline angiography was performed using Hexabrix (Guerbet, Roissy Cedez, France) with biplane technique. Then ACH infusion started. After each infusion of ACH, a coronary angiography followed. This procedure was performed at baseline and after six months of treatment.

Assessment of coronary blood flow. Doppler flow velocity spectra were analyzed to determine time-averaged peak velocity. Volumetric coronary blood flow (CBF) is the cross-sectional area × average peak velocity × 0.5 (6). The cross-sectional area is the mean area over a distance of 5 mm in the segment 5 to 10 mm distal to the tip of the Flowire. Endothelium-dependent coronary flow reserve is calculated as percent change in CBF in response to ACH. Normal coronary endothelium-dependent function is defined as an increase of CBF of 50% or more (7). The endothelium-independent coronary flow velocity reserve (CVR) was calculated by dividing the average peak velocity by the baseline average peak velocity after 18 μg adenosine injection.

Quantitative coronary angiography. Analysis of baseline and follow-up angiographies was performed using the CAAS II QCA software (Pie Medical, Maastricht, the Netherlands). Analysis of MOD and MSD started at an important side-branch of the epicardial vessel and continued until the second important side-branch of the segment. Care was taken to ensure that the same segment of the coronary vessel was examined at baseline and follow-up. Therefore, the films were examined in the same session to ensure analysis of the identical portion of the vessel. The investigators were blinded for the treatment regimen during analysis of the coronary angiography.

Laboratory measurements. After an overnight fasting, a sample of venous blood was drawn on EDTA tube from the patients and put on ice immediately. Samples were stored at −20°C and analyzed within one week. Plasma total homocysteine was measured by high-performance liquid chromatography. After six months a second homocysteine sample was taken to investigate the effects of folic acid and cobalamin on homocysteine levels. Also, samples for vitamin analysis and lipids were analyzed at baseline and follow-up.

Statistics. Data are expressed as mean ± SD. Analysis of baseline data was performed using chi-square test or Mann-Whitney *U* test where appropriate. Response differences in CBF, MSD, and MOD, after two subsequent amounts of ACH infusion (10^{-7} M and 10^{-6} M), were compared in two conditions (before treatment and after treatment) using

JACC Vol. 40, No. 4, 2002
August 21, 2002:766–72

Table 1. Baseline Characteristics

	Folic Acid/ Cobalamin Group (n = 7)	Placebo Group (n = 8)	p Value*
Age (mean) (yrs)	53.4 (range 40–63)	51.3 (range 42–66)	0.54
Male/female patients	7/0	7/1	0.73
Smoking	2	1	0.44
Hypertension	1	2	0.61
Hyperlipidemia	6	6	0.61
Family history	5	2	0.07
Beta-blockers	6	8	0.27
Calcium antagonist	4	3	0.45
Nitrates	3	3	0.83
Acetylsalicylic	7	8	1.0
ACE inhibitors	1	2	0.61
Statins	4	5	0.19
Homocysteine, μmol/l	17.1 (0.9)†	18.7 (2.0)†	0.15
Total cholesterol, mmol/l	6.07 (1.3)	5.56 (0.9)	0.40
HDL cholesterol, mmol/l	1.06 (0.11)	0.93 (0.19)	0.23
LDL cholesterol, mmol/l	4.29 (1.1)	3.96 (0.5)	0.61
Triglycerides, mmol/l	1.7 (0.8)	1.7 (0.5)	1.0
Folic acid, nmol/l	11.2 (1.3)	10.3 (1.4)	0.09
Vitamin B12, pmol/l	415 (95)	345 (38)	0.12
Creatinine, μmol/l	93 (10)	92 (13)	0.54

*p value of chi-square test or Mann-Whitney *U* test was appropriate; †between brackets: SD.

ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

a double multivariate repeated measures model (General Linear Model, SPSS 10.1, Chicago, Illinois).

RESULTS

In the period of two years, 18 patients were recruited to the study; 3 patients were excluded during follow-up because of symptomatic restenosis in the PTCA vessel, 2 patients in the folic acid group, 1 patient in the placebo group. Thus, follow-up coronary angiography could be obtained in 15 patients, 7 patients in the folic acid group and 8 patients in the placebo group. Two women and 13 men completed the study. Mean age was 52.2 years (range 40 to 66 years). Of the study group, baseline homocysteine levels were 17.9 μmol/l (range 15.6 to 21.2 μmol/l). Other baseline characteristics of the treatment and placebo groups are listed in Table 1. In the folic acid/cobalamin group, the right coronary artery (RCA) was used for analysis in four patients, the ramus circumflexus (RCX) was used in two patients, and the left anterior descending artery (LAD) was used in one patient. In the placebo group, the RCA was used for analysis in four patients, the RCX was used in three patients, and the LAD was used in one patient. No significant differences between groups were found. In the folic acid/cobalamin group, total plasma homocysteine (tHcy) decreased 31.5% from 17.1μmol/l (standard deviation [SD] 0.91) to 11.7 μmol/l (SD 1.59) ($p < 0.0001$) as compared with no change in the placebo-treated group. In the folic acid/cobalamin group, plasma folic acid improved from 11.2 nmol/l (SD 1.3) to 26.9 nmol/l (SD 3.4) ($p < 0.0001$), and plasma vitamin B12 improved from 415 pmol/l (SD 95) to 463 pmol/l (SD 119). No changes occurred in the placebo-treated group. All other biochemical parameters remained unchanged (Table 2). In all patients with complete follow-up, medication during follow-up was not changed. No significant differences in changes in CBF, MSD, and MOD were seen at baseline (Table 3). Changes in CBF, MSD and MOD are calculated after the administration of the highest dosage of ACH. Changes in CBF, MSD, and MOD after infusion of ACH at baseline were abnormal in all patients. At follow-up, after infusion of ACH, CBF increased in the folic acid/cobalamin group from 39.5 ml/min (SD 15) to 77.5 ml/min (SD 33) ($p < 0.01$). In the placebo-treated group, CBF decreased from 53.2 ml/min (SD 18) to 40 ml/min (SD 16) ($p = 0.38$). Volumetric coronary blood flow in the folic acid/cobalamin-treated group improved 96% (SD 58) (95% confidence interval [CI]: 44% to 154%) ($p < 0.05$) as compared with a decrease of 16% (SD 35) (95% CI: −20% to +30%) ($p = 0.15$) in the placebo-treated group. The general linear model determined a value of $p < 0.005$ for the change in CBF at baseline and follow-up between the folic acid/cobalamin group and the placebo group (Fig. 1). Mean segment diameter decreased from 2.19 mm (SD 0.22) to 1.83 mm (SD 0.14) in the placebo-treated group and increased from 1.99 mm (SD 0.15) to 2.12 mm (SD 0.14) in the folic acid/cobalamin group. Mean segment diameter decreased 16.4% in the placebo-treated group and increased 6.5% in the folic acid/cobalamin group ($p = 0.15$) (Fig. 2). Minimal obstruction diameter decreased from 1.76 mm (SD 0.13) to 1.43 mm (SD 0.09) in the placebo group and increased from 1.41 mm (SD 0.16) to 1.60 mm (SD 0.14) in the folic acid/cobalamin group. Minimal obstruction diameter increased 13.4% in the folic acid/cobalamin group and decreased 18.7% in the placebo-treated group ($p = 0.14$) (Fig. 3). Endothelium-independent vasodilation as presented in CVR was 2.78 (SD 0.29) at baseline in the placebo group and 2.91 (SD 0.32) in the folic acid/cobalamin group. In both groups, CVR did not change significantly at follow-up.

DISCUSSION

This is the first study to observe the changes in endothelium-dependent coronary flow in hyperhomocysteinemic patients with symptomatic CAD six months after treatment with folic acid and cobalamin. This regime significantly improved CBF, MSD, and MOD. Because clinical trials evaluating the effect of homocysteine-lowering therapy on clinical end points in patients with mild hyperhomocysteinemia are not yet available, our research focused on endothelial dysfunction as a key mechanism in homocysteine-induced vascular disease.

Homocysteine and endothelial function. To date, angiographic studies on the association between plasma homocysteine levels and coronary endothelial function have not been published, but some human studies used the reactive hyperemia model of the brachial artery, demonstrating that

Table 2. Changes in Risk Factors

	Folic Acid/ Cobalamin Group (n = 7)	p Value*	Placebo Group (n = 8)	p Value*
Homocysteine, μmol/l				
Baseline	17.1 (0.91)†	<0.0001	18.7 (1.97)	0.92
Follow-up	11.7 (1.59)		18.8 (2.21)	
Serum folate, nmol/l				
Baseline	11.2 (1.3)	<0.0001	10.3 (1.4)	0.90
Follow-up	26.9 (3.4)		10.4 (1.3)	
Vitamin B12, pmol/l				
Baseline	415 (95)	0.42	346 (38)	0.98
Follow-up	463 (119)		348 (41)	
Total cholesterol, mmol/l				
Baseline	6.07 (1.3)	0.63	5.66 (0.9)	0.97
Follow-up	5.77 (0.9)		5.55 (0.6)	
HDL cholesterol, mmol/l				
Baseline	1.06 (0.12)	0.87	0.93 (0.19)	0.68
Follow-up	1.04 (0.13)		0.99 (0.19)	
LDL cholesterol, mmol/l				
Baseline	4.24 (1.15)	0.62	3.87 (0.69)	0.98
Follow-up	3.97 (0.81)		3.85 (0.46)	
Triglycerides, mmol/l				
Baseline	1.69 (0.85)	0.94	1.67 (0.34)	0.60
Follow-up	1.66 (0.72)		1.54 (0.38)	
Mean arterial pressure, mm Hg				
Baseline	105 (16)	0.92	111 (14)	0.68
Follow-up	107 (9)		108 (8)	

*p value for difference between baseline and follow up with Mann-Whitney *U* test; †between brackets: standard deviation.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

severe hyperhomocysteinemia is associated with reduced flow-mediated vasodilation (8). Also, in patients with mild hyperhomocysteinemia, the plasma homocysteine level was a significant predictor of reduced flow-mediated vasodilation (9,10). In the forearm model, folic acid prevented the impairment of endothelium-dependent vasodilation after methionine loading (11) and ameliorated endothelium-dependent vasodilation in patients with familial hypercholesterolemia and normal tHcy (12,13). However, the effect of folic acid on endothelial function correlated with tHcy suggesting that folic acid is more effective in patients with high tHcy (14).

Endothelial function in epicardial vessels and resistance vessels. Acetylcholine is widely used as a standard substance to test endothelial function in human coronary arteries (15). In subjects with normal coronary vessels, ACH

Table 3. Baseline Coronary Flow Parameters After Infusion of Acetylcholine

	Folic Acid/ Cobalamin Group (n = 7)	Placebo Group (n = 8)	p Value
Mean change in CBF (%)			
10^{-8} M	38.6 (29)*	10.8 (15)	0.051
10^{-7} M	39.2 (46)	33.1 (42)	0.79
10^{-6} M	15.6 (36)	16.3 (35)	0.96
NTG	204.3 (133)	228.7 (137)	0.73
Mean change in MSD (%)			
10^{-8} M	−3.5 (1.7)	−1.7 (4.5)	0.64
10^{-7} M	−7.6 (3.8)	−11.0 (7.9)	0.06
10^{-6} M	−13.9 (6.2)	−12.1 (8.9)	0.08
NTG	6.1 (7.2)	8.0 (9.7)	0.39
Mean change in MOD (%)			
10^{-8} M	−6.7 (7.4)	−8.6 (9.8)	0.40
10^{-7} M	−13.4 (6.5)	−13.6 (10.6)	0.28
10^{-6} M	−16.2 (9.4)	−18.9 (18.3)	0.37
NTG	7.0 (5.2)	4.9 (6.0)	0.02

*Standard deviation between brackets.
CBF = volumetric coronary blood flow; MOD = minimal obstruction diameter; MSD = mean segment diameter; NTG = nitroglycerin.

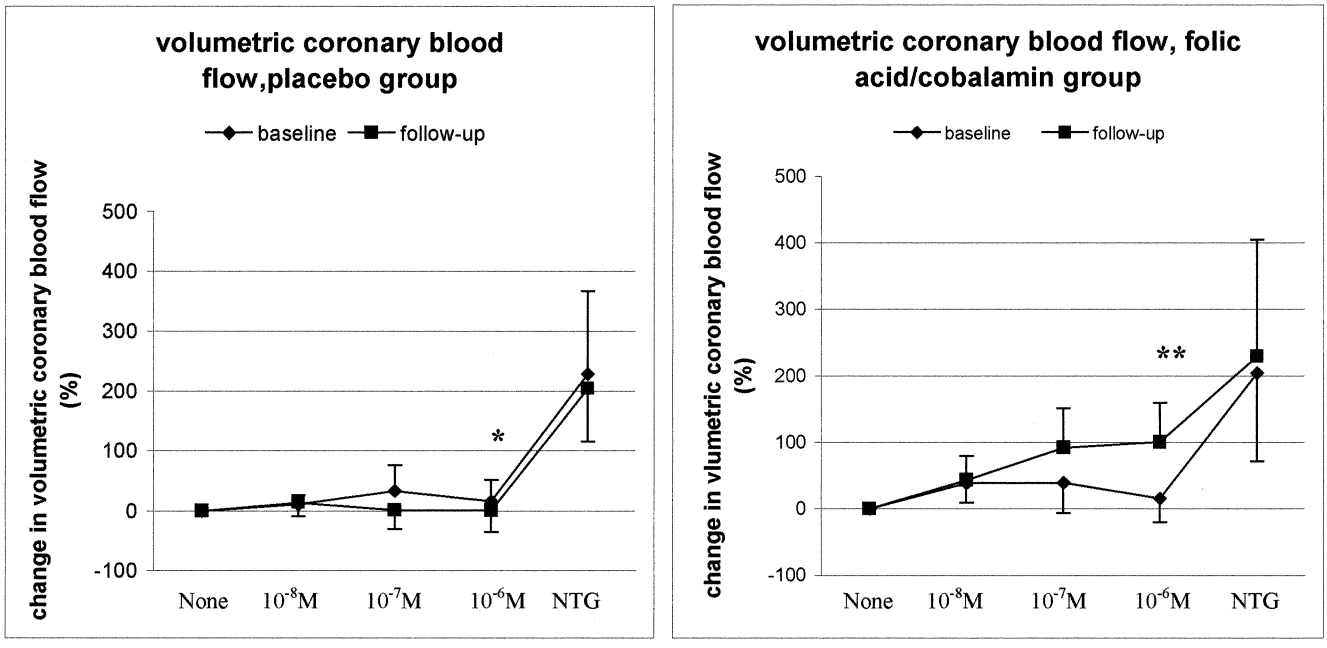

Figure 1. Effect of acetylcholine and nitroglycerin (NTG) on percent change in volumetric coronary blood flow in both groups. **Solid diamond** = baseline; **solid square** = follow-up. *p = 0.15 for the difference between baseline and follow-up at maximal dosage of acetylcholine (placebo group). **p < 0.05 for the difference between baseline and follow-up at the maximal dosage of acetylcholine (folic acid/cobalamin group). p < 0.005 for the difference at baseline and follow-up between the placebo group and the folic acid/cobalamin group with the general linear model.

induces vasodilation of coronary vessels. In patients with atherosclerosis, or in the presence of risk factors for vascular disease like hypercholesterolemia, hypertension, diabetes mellitus, and smoking, ACH has been shown to induce paradoxical vasoconstriction (16). All patients in the study group had severe coronary endothelial dysfunction after infusion of ACH at baseline. Changes in MSD after intracoronary infusion of ACH show a coronary endothelial dysfunction expressed as a decrease in MSD at baseline. The same is true for changes in MOD. Volumetric coronary

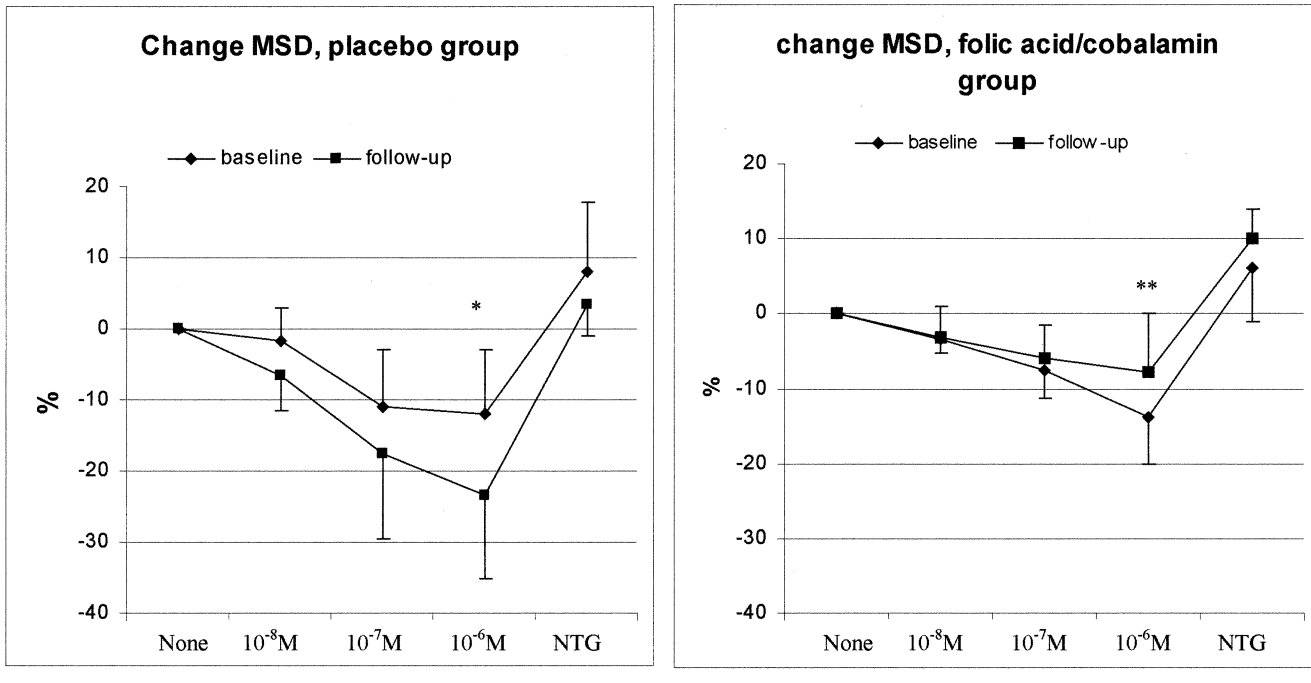

Figure 2. Effect of acetylcholine and nitroglycerin (NTG) on percent change in mean segment diameter (MSD) in both groups. **Solid diamond** = baseline; **solid square** = follow-up. *p = 0.09 for the difference between baseline and follow-up at maximal dosage of acetylcholine (placebo group). **p = 0.10 for the difference between baseline and follow-up at the maximal dosage of acetylcholine (folic acid/cobalamin group). p = 0.15 for the difference at baseline and follow-up between the placebo group and the folic acid/cobalamin group with the general linear model.

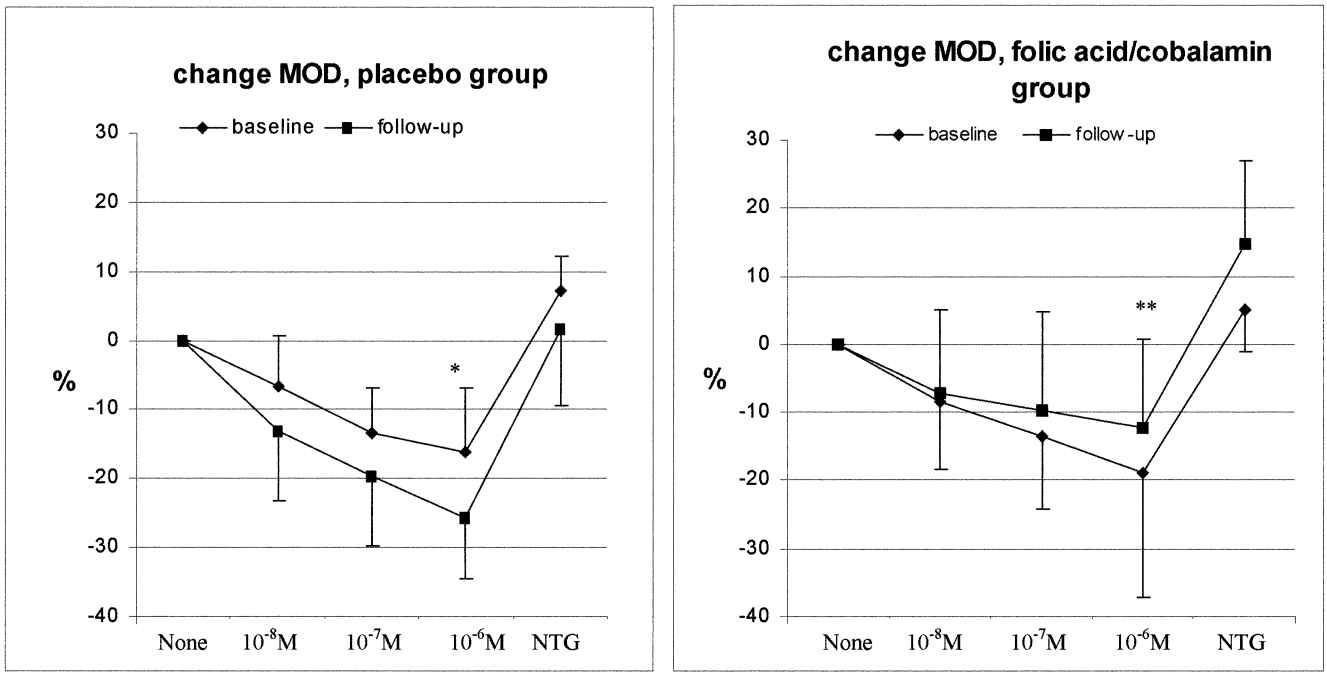

Figure 3. Effect of acetylcholine and nitroglycerin (NTG) on percent change in minimal obstruction diameter (MOD) in both groups. **Solid diamond** = baseline; **solid square** = follow-up. *p = 0.06 for the difference between baseline and follow-up at maximal dosage of acetylcholine (placebo group). **p = 0.26 for the difference between baseline and follow-up at the maximal dosage of acetylcholine (folic acid/cobalamin group). p = 0.14 for the difference at baseline and follow-up between the placebo group and the folic acid/cobalamin group with the general linear model.

blood flow in all patients was severely disturbed at baseline. Follow-up demonstrated a significant improvement of CBF in the patients who used folic acid and cobalamin. The changes in MSD improved after infusion of ACH at follow-up in the folic acid/cobalamin group as compared with the placebo group. Changes in MOD improved also, albeit not significantly.

Our patients were characterized by an abnormal response upon ACH infusion at baseline. Because improvement of ACH-induced vasoconstriction depends on the response at baseline, we could expect a stronger improvement of endothelial response on ACH infusion after intervention (17).

Suwaidi et al. (4) demonstrated that severe coronary endothelial dysfunction, defined as an increase of CBF <0% after infusion of ACH, in the absence of significant CAD, is associated with an increase in cardiac events in the follow-up. This supports the concept that coronary endothelial dysfunction is an important marker for future progression of coronary vascular disease. Assessment of CVR, demonstrated that, in our study, all patients at baseline and follow-up had a CVR within the normal range of 2.7 ± 0.6, testifying that the investigated coronary vessels at baseline and follow-up had no significant stenosis, which could influence changes in CBF, MSD, and MOD (18). Our results support the hypothesis that patients with hyperhomocysteinemia, by using folic acid and cobalamin, might improve their cardiovascular prognosis.

Mechanisms of endothelial dysfunction. Many observational, case-control and prospective studies have been published on the association of hyperhomocysteinemia and CAD (1). However, these studies cannot exclude the possibility that hyperhomocysteinemia is rather a marker of vascular disease than being causally related to vascular disease. Mechanisms responsible for endothelial dysfunction in hyperhomocysteinemia are poorly understood. Several investigators have shown that homocysteine reduces the bioavailability of nitric oxide and enhances smooth muscle cell proliferation, both of which are important markers of atherothrombotic disease (3). Recently, it was hypothesized that hyperhomocysteinemia may stimulate the formation of asymmetrical dimethylarginine, an endogenous inhibitor of nitric oxide synthase (19).

Folic acid and restoration of endothelial function. Folic acid reduced homocysteine levels 25%, and the addition of cobalamin led to a further reduction of 7% (2,20). In our study, plasma homocysteine levels reduced 31% after six months of treatment with folic acid 5 mg and cobalamin 400 μg compared with no change in the placebo-treated group. The mechanism responsible for the effect of folic acid is unclear. Apart from lowering of homocysteine, folic acid may influence endothelial function via other mechanisms. Folates are also involved in endogenous restoration of tetrahydrobiopterin, an essential cofactor for nitric oxide synthase (21). Tetrahydrobiopterin restores endothelial function in the forearm model in patients with hypercholesterolemia (22). Other studies suggest an antioxidant effect of folic acid (12). Improvement of endothelial function by antioxidant supplementation has been studied before, but, until now, the results are variable, and more extensive

studies are needed to resolve the question whether antioxidant therapy is useful in patients with CAD (14,23).

Also, the natural outcome of untreated homocystinuria patients, with fasting plasma homocysteine levels >100 μmol/l and a 50% risk of suffering from a vascular event before the age of 30 years, suggests a strong effect of homocysteine on vascular disease (24). In these patients with severe hyperhomocysteinemia, it was demonstrated that appropriate homocysteine-lowering therapy reduced the risk of vascular events significantly with more than 90% (25). Our study adds evidence to the concept that homocysteine-lowering therapy is clinically beneficial in patients with elevated homocysteine levels.

Study limitations. Despite the relatively small number of patients, significant differences in coronary endothelial function after intervention were observed in the same range as seen in studies with cholesterol-lowering therapy. However, although this study may add evidence to the fact that treatment of hyperhomocysteinemia may be useful in vascular disease, ongoing intervention trials with clinical end points are needed to answer the question whether treatment of hyperhomocysteinemia indeed reduces cardiovascular events.

Conclusions. We conclude that CBF as an expression of coronary endothelial function improves significantly after six months of treatment with folic acid and cobalamin in hyperhomocysteinemic patients with symptomatic CAD.

Reprint requests and correspondence: Dr. Frank F. Willems, Department of Cardiology, Rijnstate Hospital, P.O. Box 9555, Arnhem, The Netherlands. E-mail: willemsf@xs4all.nl.

REFERENCES

1. Boers GH. Mild hyperhomocysteinemia is an independent risk factor for arterial vascular disease. Semin Thromb Hemost 2000;26:291–5.
2. Clarke R, Armitage J. Vitamin supplements and cardiovascular risk: review of the randomized trials of homocysteine-lowering vitamin supplements. Semin Thromb Hemost 2000;26:341–8.
3. van Guldener C, Stehouwer CD. Hyperhomocysteinemia, vascular pathology and endothelial function. Semin Thromb Hemost 2000;26: 281–9.
4. Suwaidi JAL, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–54.
5. Thambyrajah J, Landray MJ, Jones HJ, McGlynn FJ, Wheeler DC, Townend JN. A randomized double blind placebo-controlled trial of the effect of homocysteine-lowering therapy with folic acid on endothelial function in patients with coronary artery disease. J Am Coll Cardiol 2001;37:1858–63.
6. Doucette JW, Corl PD, Payne HN, et al. Validation of a Doppler guide wire for intravascular measurement of coronary artery flow velocity. Circulation 1992;85:1899–911.
7. Hasdai D, Gibbons RJ, Holmes DR, Jr., Higano ST, Lerman A. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. Circulation 1997;96:3390–5.
8. Celermajer DS, Sorensen K, Ryalls M, et al. Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. J Am Coll Cardiol 1993;22:854–8.
9. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilatation in humans. Circulation 1997;95:1119–21.
10. Woo KS, Chook P, Lolin YI, et al. Hyperhomocysteinemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 1997;96:2542–4.
11. Usui M, Mutsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T. Endothelial dysfunction by acute hyperhomocysteinemia: restoration by folic acid. Clin Sci 1999;96:235–9.
12. Verhaar MC, Wever RM, Kastelein JJ, van Dam T, Koomans HA, Rabelink TJ. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolaemia. Circulation 1998;97:237–41.
13. Verhaar MC, Wever RM, Kastelein JJ, et al. Effect of folic acid supplementation on endothelial function in familial hypercholesterolaemia: a randomized placebo-controlled trial. Circulation 1999;100: 335–8.
14. Title LM, Cummings PM, Giddens K, Genest JJ, Jr., Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. J Am Coll Cardiol 2000;36: 758–65.
15. Zeiher AM, Drexler H, Saurbier B, Just HB. Endothelium mediated coronary blood-flow modulation in humans: effects of age, atherosclerosis, hypercholesterolaemia and hypertension. J Clin Invest 1993;92: 652–62.
16. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;20:299–302.
17. Egashira K, Hirooka Y, Kai H, et al. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolaemia. Circulation 1994;89: 2519–24.
18. Kern MJ. Intracoronary Doppler flow velocity. In: Geschwind HJ, Kern MJ, editors. Guidebook to Endovascular Coronary Diagnostic Techniques. New York, NY: Futura Publishing, 1997:351–4.
19. Boger RH, Lentz SR, Bode-Boger SM, Knapp HR, Haynes WG. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inemia in humans. Clin Sci 2001;100:159–60.
20. Homocysteine-lowering Trialist Collaboration. Lowering blood homocysteine with folic acid based supplements. Br Med J 1998;316: 894–8.
21. Matthews RG, Kaufman S. Characterization of the dihydropterin reductase activity of pig liver methylenetetrahydrofolate reductase. J Biol Chem 1980;255:6014–7.
22. Stroes E, Kastelein J, Cosentino F, et al. Tetrahydrobiopterin restores endothelial function in hypercholesterolaemia. Clin Invest 1997;99: 41–6.
23. Raitakari OT, Adams MR, McCredie RJ, Griffiths KA, Stocker R, Celermajer DS. Oral vitamin C and endothelial function in smokers: short-term improvement but no sustained beneficial effect. J Am Coll Cardiol 2000;35:1616–21.
24. Mudd SH, Skovby F, Levy HL, et al. The natural history of homocystinuria due to cysthationine β-synthase deficiency. Am J Hum Genet 1985;37:1–31.
25. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine B-synthase deficiency: effects of homocysteine-lowering therapy. Semin Thromb Hemost 2000;26: 335–40.