0022-3565/02/3031-158–162$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 303, No. 1
Copyright © 2002 by The American Society for Pharmacology and Experimental Therapeutics
36715/1006671
JPET 303:158–162, 2002
Printed in U.S.A.

Effects of Folate Treatment and Homocysteine Lowering on Resistance Vessel Reactivity in Atherosclerotic Subjects

OLAF STANGER, HANS-JUERGEN SEMMELROCK, WILLIBALD WONISCH, URSULA BÖS, EDMUND PABST, and THOMAS C. WASCHER

Department of Surgery, Division of Cardiac Surgery (O.S.); Department of Laboratory Medicine (H.-J.S., W.W.); and Department of Internal Medicine (U.B.), Division of Angiology (E.P.) and Diabetic Angiopathy Research Group (T.C.W.), Karl-Franzens University School of Medicine, Graz, Austria

Received April 9, 2002; accepted May 13, 2002

ABSTRACT

Hyperhomocysteinemia is associated with arterial hypertension and endothelial dysfunction in healthy humans. Placebo-controlled vitamin intervention studies cannot distinguish intrinsic actions of homocysteine (tHcy) and folate concentrations on the endothelium. The present two-period crossover study investigates the effects of tHcy lowering through oral folic acid on antioxidant status and resistance vessel reactivity in patients with established coronary artery disease (CAD). We investigated 27 male patients with angiographically documented multivessel CAD aged 50 (range 46–56) years. Resistance vessel reactivity was assessed by measurement of postischemic reactive hyperemia (RH) in the forearm using venous occlusion plethysmography at baseline, after 6 weeks of treatment with 5 mg of oral folic acid, and after a washout period of another 6 weeks. Plasma folate increased 3.49-fold with a mean tHcy reduction of 21.3%. Peak reactivity of resistance vessels improved significantly (18.97–23.60 ml/min^{-1} per 100 ml; P = 0.01) with unchanged total antioxidant status (TAS; 0.912–0.944 μM; P = 0.4). This effect was limited to subjects (n = 14) with a tHcy reduction >2 μM (median reduction, 14.4–9.6 μM, P < 0.001). In the 13 subjects with a below-median reduction, tHcy remained unaltered (9.7–9.6 μM, P = 0.88) and TAS increased significantly (0.923–1.055 μM, P = 0.006), whereas RH peak flow was not affected (20.22–22.99 ml/min^{-1} per 100 ml, P = 0.28). Homocysteine lowering >2 μM through folic acid supplementation improves resistance vessel reactivity in patients with CAD. Our data support the hypothesis that homocysteine lowering may have intrinsic vasoprotective effects largely independent of folate.

Elevated total plasma homocysteine (tHcy) is an independent risk factor for peripheral vascular, cerebrovascular, and coronary artery disease (CAD) (Boushey et al., 1995). Even moderate hyperhomocysteinemia (>9 μM) is prospectively associated with increased risk of mortality in CAD patients (Nygard et al., 1997; Anderson et al., 2000).

Homocysteine concentrations are determined by genetic and nutritional factors (Ueland and Refsum, 1989). Deficiencies of vitamins B_6, B_{12}, and folic acid in particular are associated with hyperhomocysteinemia (Ubbink et al., 1993). Consequently, vitamin supplementation, especially with folic acid, has been demonstrated to effectively lower elevated homocysteine levels (Clarke et al., 1998).

Homocysteine is a highly reactive amino acid derived from methionine metabolism and is known to produce endothelial cell injury in experimental animals (Harker et al., 1983), in cell culture (Wall et al., 1980), and in humans (Tawakol et al., 1997). The vascular endothelium plays a critical role in the control of vascular tone and regulation of blood flow. Vascular dysfunction is an established early step in the development of vascular disease and contributes to the pathogenesis of atherosclerosis (Celermajer et al., 1992) and CAD (Zeiher et al., 1991). Furthermore, systemic endothelial dysfunction as assessed in the brachial artery is closely related to the extent of CAD (Neunteufl et al., 1997).

In healthy humans, impaired endothelial function of conduit vessels is found in hyperhomocysteinemia (Woo et al., 1997) and is even induced through physiological increments of plasma homocysteine (Chambers et al., 1999b). Vitamin C prevents these effects, suggesting a role for oxidant stress in homocysteine-induced impairment of vascular endothelial function (Chambers et al., 1999a). Furthermore, folic acid was recently demonstrated to improve endothelial function in healthy adults with hyperhomocysteinemia (Woo et al., 1999).

The work was primarily performed at the Departments of Surgery, Division of Cardiac Surgery and the Department of Internal Medicine, Diabetic Angiopathy Research Group, both part of the Clinical Atherosclerosis Research Group, Karl-Franzens University School of Medicine, Graz, Austria.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.102.036715.

ABBREVIATIONS: tHcy, total plasma homocysteine; CAD, coronary artery disease; FMD, flow-mediated dilation; RH, reactive hyperemia; TAS, total antioxidant status; NO, nitric oxide.

All of these studies used flow-mediated vasodilation (FMD) of the brachial artery in healthy subjects to examine endothelial function of conduit vessels. Blood flow to target organs, in the absence of hemodynamically significant stenoses in conduit vessels, is regulated at the level of local resistance vessels (Chillian et al., 1986). Impaired reactivity of coronary resistance vessels has been shown to be associated with exercise-induced ischemia in subjects free of macrovascular coronary artery disease (Zeiher et al., 1995). Recently, endothelial dysfunction in patients with moderate CAD was found to be accompanied by myocardial perfusion defects (Hasdai et al., 1997). Both reports demonstrate the importance of resistance vessel reactivity in the regulation of myocardial blood flow.

At present, the role for homocysteine on resistance vessel function is less clear. In particular, the effect of folic acid supplementation on resistance vessel function in patients with atherosclerotic disease has not been investigated before. The extent of homocysteine lowering required for a beneficial effect on vascular dysfunction is unknown.

This study was designed to investigate the intrinsic effects of folate supplementation and homocysteine lowering on resistance vessel reactivity in patients with angiographically documented multivessel CAD.

Materials and Methods

Study Protocol and Subjects. Twenty-seven male patients with angiographically documented CAD, mean age 50.3 ± 6.2 (range 46.0–56.6) years, were enrolled in this two-period crossover study. CAD was defined as ≥50% stenosis of the lumen diameter in at least one of the major epicardial vessels. All subjects gave written informed consent, and the study was approved by the Ethics Board of the Karl-Franzens University. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Patients were excluded if they had myocardial infarction, unstable angina or coronary intervention within the past 6 months, diabetes mellitus, dysfunction of the thyroid gland, or impaired liver or kidney function. Patients with prior intake of nitrates, angiotensin-converting enzyme inhibitors, vitamins, and other medication known to interfere with homocysteine metabolism, or change of medication during the time of investigation, were also excluded. Blood pressure was recorded (after 15 min of supine rest) on each day of the study. Hypertension was defined as diastolic blood pressure >90 mm Hg and/or systolic blood pressure >140 mm Hg. Subjects were normotensive during the 12-week period of investigation. All participants received standard medication for CAD [low-dose acetylsalicylic acid (100 mg) and β-blockers] and were asked to refrain from intake of lipid-lowering drugs 6 weeks before the first measurement. This remained unchanged during the entire course of investigation.

After the baseline investigation, each patient received 5 mg of folic acid orally per day for a period of 6 weeks, followed by a washout period of another 6 weeks. At baseline (0) and after 6 and 12 weeks, fasting blood samples were taken and reactivity of resistance vessels was assessed as measured by postischemic reactive hyperemia (RH) by venous occlusion plethysmography. All subjects were tested on all three visits after an 8-h overnight fast and nonsmoking period.

Laboratory Assays. Fasting tHcy, plasma folate, cholesterol, triglycerides, vitamins B_6 and B_{12}, lipoprotein (a), and total antioxidant status (TAS) were measured for each subject on each day of investigation.

All blood samples were taken from an antecubital vein between 7:00 and 8:00 AM after an overnight fasting period of at least 8 h. Samples were processed immediately, centrifuged at 4°C (3000*g* for 10 min) within 15 min, and stored at –70°C until analysis. All serological analyses were carried out without prior knowledge of clinical data. Measurements of plasma homocyst(e)ine in EDTA plasma were performed using high-performance liquid chromatography and fluorescence detection according to the method of Araki and Sako (1987) with modifications by Ubbink et al. (1991) and Vester and Rasmussen (1991). Briefly, after reduction with tri-*N*-butylphosphine, the free thiol groups were derivatized with SBD-F (7-fluorobenzofurazane-4-sulfonic acid). Separation was performed under isocratic conditions on a reversed phase column at pH 2.1 with mercaptopropionylglycine as internal standard. The intra-assay variability of the method was between 1.3% (27 μM) and 2.5% (10 μM).

Because this procedure involved a reducing step, the method did not distinguish between homocysteine and its oxidized analogs. Therefore, the measured moiety may be referred to as homocyst(e)ine.

Plasma folate and vitamin B_{12} concentrations were determined with an Abbott AxSYM analyzer by commercially available Ion Capture Assay and Microparticle Enzyme Immunoassay, respectively (Abbott Diagnostics, Wiesbaden, Germany). Standard methods were used for serum lipids.

TAS of plasma was measured with the TAS assay (Trolox equivalent antioxidant capacity) commercially available in a modified form (Randox Laboratories, Crumlin, Antrim, UK). With this assay, antioxidants in the plasma inhibit formation of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cations detected photometrically at a wavelength of 600 nm. Absorptions obtained 3 min after start of the reaction are compared with those of Trolox, and results are given in millimoles per liter Trolox equivalents.

Measurement of Forearm Blood Flow. For measurement of resistance vessel endothelial function, forearm blood flow was determined by venous strain-gauge plethysmography (EC5R; Hokanson Inc., Bellevue, WA) with electrically calibrated mercury-in-Silastic strain gauges (Wascher et al., 1998). In brief, the experiments were started at 8:00 AM after an overnight fast in a temperature-controlled room (22–23°C). Subjects were in a supine position for 30 min before the measurements of resting blood flow to assure stable baseline conditions. Measurements were performed on the right arm; the strain gauge was positioned at the widest part of the forearm. For occlusion of venous outflow, the upper arm cuff pressure was set at 50 mm Hg; for induction of ischemia a pressure of 200 mm Hg was chosen. During measurements of forearm perfusion, the hand was occluded from the circulation with a second cuff positioned at the wrist, inflated immediately before the measurements. Resting blood flow was determined by calculation of the mean of three measurements within 1 min. RH was induced by 5 min of ischemia. After release of the upper arm cuff, forearm blood flow was measured every 10 s for up to 60 s. Peak blood flow values, termed peak postischemic RH, are observed thereby 10 s after the onset of reperfusion.

Statistical Analysis. The MacLab package (AD Instruments Pty Ltd., Castle Hill, Australia) on an Apple Macintosh computer was used to acquire and analyze data from the measurements of resistance vessel endothelial function. The mean slope of the volume curve from the second to the fifth pulsewave at each measurement was taken as the respective blood flow. Blood flow data are given as milliliters of perfusion per minute in 100-ml forearm tissue.

Data were analyzed with use of SYSTAT Version 5.1 (SYSTAT Inc., Richmond, CA) on an Apple Macintosh computer. The effects of folic acid on resistance vessel function were compared with analysis of variance for two-way repeated measurements. An unpaired Student's *t* test was used for group comparison. All data are expressed as mean ± S.E., unless otherwise stated. The level of significance was $P < 0.05$.

Results

All 27 male subjects (mean age 50.3 years) completed the crossover trial according to protocol. No side effects of the

vitamin medication were observed or reported by the participants. The baseline clinical and biochemical measurements are summarized in Table 1. In linear regression analysis, folate and homocyst(e)ine levels were not significantly related at baseline ($P = 0.1$, $r^2 = 0.11$).

At baseline, subjects with above median tHcy reduction had higher homocysteine levels (14.4 ± 1.2 versus 9.7 ± 0.7 μM; $P = 0.002$) and lower folate levels (5.1 ± 0.6 versus 8.5 ± 1.4 ng/ml; $P = 0.03$) than subjects responding with below median tHcy reduction. After 6 weeks without folic acid supplementation, plasma folate and tHcy reversed but not quite to baseline values, indicating a carryover effect (Table 2).

Biochemical Measurements. Oral folic acid (5 mg/day) induced a 3.49-fold increase of plasma folate (6.7 ± 0.8 to 23.4 ± 2.5 ng/ml; $P < 0.0001$) and lowered homocysteine levels 21.3% (12.2 ± 0.8 to 9.6 ± 0.5 μM; $P < 0.001$). TAS increased significantly after 6 weeks of folic acid supplementation and returned to baseline at day 12. Throughout the observation period, vitamin supplementation was not seen to have any effects on vitamin B_6 and B_{12} levels, plasma cholesterol, triglycerides, and lipoprotein(a).

After 6 weeks of folic acid supplementation, the median change of plasma homocysteine was 2 μM. Fourteen patients had responded with above median reduction (>2 μM) of plasma homocysteine levels (14.4 ± 1.1 versus 9.6 ± 0.8 μM; $P < 0.0001$), whereas homocysteine concentrations were not altered in 13 patients with individual reductions below median (9.7 ± 0.7 to 9.6 ± 0.8 μM; $P = 0.88$) (Table 2). TAS did not change in the 14 subjects with above median reduction of plasma homocysteine levels (0.912 ± 0.037 versus 0.944 ± 0.037 mM; $P = 0.4$) but increased significantly in the remaining 13 subjects (0.923 ± 0.045 versus 1.055 ± 0.045 mM; $P = 0.006$) between study days 0 and 6.

Resistance Vessel Reactivity. Resting blood flow remained almost unchanged in both groups over the entire 12 weeks (Table 2). Homocysteine lowering above median (>2 μM) significantly increased peak reactive hyperemic blood flow after 6 weeks (18.97 ± 1.12 versus 23.60 ± 1.41 ml/min^{-1} per 100 ml; $P = 0.01$), but subjects with below median reduction showed no improvement (20.22 ± 2.96 versus 22.99 ± 1.81 ml/min^{-1} per 100 ml; $P = 0.28$). At the 6-week follow-up visit, the change of total blood flow (1 min) was borderline significant in above median subjects (11.09 ± 0.98 versus 13.03 ± 1.09 ml/min^{-1} per 100 ml; $P = 0.058$) and insignificant in below median subjects (10.13 ± 1.32 versus 13.12 ± 1.37 ml/min^{-1} per 100 ml; $P = 0.09$). After 6 weeks of washout, resting flow, peak flow (10 s), and total flow (1 min) in responders were not significantly different from baseline.

Discussion

The major findings of this study are that lowering plasma homocysteine through supplementation with folic acid improves resistance vessel function in patients with documented CAD. Our results further suggest that the lower homocysteine concentration is responsible for this effect rather than the increase of folate itself.

There is experimental (Wall et al., 1980) and clinical (Bellamy et al., 1998) evidence suggesting that hyperhomocysteinemia may cause endothelial dysfunction as an early key event in homocysteine-related vascular disease, but the mechanisms are not fully understood. Damage to the vascular endothelium is considered to be a crucial step in the development and progression of atherosclerosis and precedes overt manifestation of disease (Ross,1993). Endothelial dysfunction affects conduit and resistance vessels and is a general phenomenon closely associated with CAD (Anderson et al., 1995; Neunteufl et al., 1997). The effect of homocysteine on resistance vessel function and the potential effect of lowering plasma homocysteine concentrations had been unclear.

Most studies investigated endothelial function using FMD in the forearm and experimental hyperhomocysteinemia in healthy subjects. The results reflect endothelium-dependent vasodilatation and relate mainly to release of endothelial nitric oxide (NO) in response to shear stress (Joannides et al., 1995; Upchurch et al., 1997). Hyperhomocysteinemia induced through methionine loading using invasive administration of acetylcholine in the brachial artery was recently demonstrated to impair resistance vessel endothelial function in young healthy volunteers (Kanani et al., 1999). Noninvasive testing of resistance vessel function by measurement of postischemic reactive hyperemia in the human forearm represents an alternative to intra-arterial drug in-

TABLE 1
Baseline clinical and biochemical characteristics in patients with CAD ($n = 27$)
Data are mean ± S.E. and percentage of a group. P value compares reponders to nonresponders.

Parameter	All ($n = 27$)	tHcy Reduction above Median ($n = 14$)	tHcy Reduction below Median ($n = 13$)	P
Age, yr (range)	50.3 ± 6.2	48.5 ± 6.5	52.1 ± 5.5	N.S.
Body mass index, kg/m^2	27.0 ± 2.9	26.5 ± 3.5	27.4 ± 2.1	N.S.
Smoking				
Never	12 (44.4%)	6 (42.8%)	6 (46.1%)	N.S.
Former	15 (55.5%)	8 (57.1%)	7 (53.8%)	N.S.
Current				
Arterial hypertension	14 (51.8%)	6 (42.8%)	8 (61.5%)	N.S.
TAS, mM	0.918 ± 0.028	0.912 ± 0.037	0.923 ± 0.045	N.S.
Plasma homocyst(e)ine, μM	12.2 ± 0.8	14.4 ± 1.1	9.7 ± 0.7	0.002
Plasma folate, ng/ml	6.7 ± 0.8	5.1 ± 0.6	8.5 ± 1.4	0.03
Plasma vitamin B_{12}, ng/l	377 ± 26	377 ± 37	377 ± 35	N.S.
Serum vitamin B_6, ng/l	16.8 ± 4.5	18.8 ± 7.1	13.7 ± 3.7	N.S.
Plasma cholesterol, mM	205 ± 9	223 ± 13	184 ± 8	0.03
Plasma triglycerides, mM	161 ± 25	171 ± 47	150 ± 11	N.S.
Lipoprotein(a), mM	21.4 ± 3.8	17.4 ± 4.7	24.8 ± 5.8	N.S.

N.S., not significant.

TABLE 2
Vascular reactivity and target metabolic variables at baseline, after treatment (6 weeks) and after washout (12 weeks)
Data are shown for all patients as well as for subjects with a homocyst(e)ine reduction above median (>2 μM, "responders") and below median (<2 μM, "nonresponders").

		0 weeks	6 weeks	12 weeks
Folic acid, ng/ml	All	6.7 ± 0.8	23.4 ± 2.5***	10.8 ± 0.6***
	Responder	5.1 ± 0.6	23.1 ± 3.8***	9.3 ± 0.5**
	Nonresponder	8.5 ± 1.4	23.8 ± 3.3*	12.5 ± 0.8*
Homocyst(e)ine, μM	All	12.2 ± 0.8	9.6 ± 0.5***	10.9 ± 0.7***
	Responder	14.4 ± 1.1	9.6 ± 0.8*	11.4 ± 1.1***
	Nonresponder	9.7 ± 0.7	9.6 ± 0.8	10.3 ± 0.7
TAS, mM	All	0.918 ± 0.028	0.997 ± 0.031*	0.907 ± 0.024
	Responder	0.912 ± 0.037	0.944 ± 0.037	0.890 ± 0.022
	Nonresponder	0.923 ± 0.154	1.055 ± 0.045*	0.927 ± 0.047
Resting flow, ml/min^{-1}/100 ml	All	3.76 ± 0.38	3.71 ± 0.33	3.29 ± 0.25
	Responder	4.15 ± 0.58	3.45 ± 0.35	3.49 ± 0.35
	Nonresponder	3.31 ± 0.45	4.01 ± 0.59	3.06 ± 0.36
Peak flow (10 s), ml/min^{-1}/100 ml	All	19.46 ± 1.31	23.34 ± 1.10*	22.53 ± 1.50
	Responder	18.97 ± 1.12	23.60 ± 1.41*	20.61 ± 1.99
	Nonresponder	20.22 ± 2.96	22.99 ± 1.81	24.80 ± 2.16
Total flow (60 s) ml/min^{-1}/100 ml	All	10.70 ± 0.78	13.07 ± 0.83*	11.97 ± 0.81
	Responder	11.09 ± 0.98	13.03 ± 1.09(*)	11.97 ± 0.93
	Nonresponder	10.13 ± 1.32	13.12 ± 1.37	11.96 ± 1.39

* $P < 0.05$.
** $P = 0.05$.
*** $P < 0.001$.

fusion and reflects the vasodilatatory capacity of a vascular bed upon a predefined stimulus (Iwatsubo et al., 1997; Wascher et al., 1998).

Impairment of FMD through methionine loading was prevented by oral administration of antioxidant vitamin C, suggesting a role for oxidative stress in homocysteine-induced vascular (endothelial) dysfunction (Chambers et al., 1999a).

In our study, plasma folate increased 349% in all subjects, but TAS increased only in subjects with no change in tHcy levels. Infusion of 5-methyltetrahydrofolate, the biologically active form of folic acid, improved endothelial function in patients with familial hypercholesterinemia but normal homocysteine concentrations (Verhaar et al., 1998). The response was immediate and did not affect homocysteine levels, suggesting a scavenging capacity of folate with an effect on endothelial function. This is supported by the finding that pretreatment with oral folic acid prevents the lipid-induced decrease in FMD as well as the lipid-induced increase in urinary radical-damage end products in healthy volunteers (Wilmink et al., 2000). Folic acid may therefore increase TAS by adding to antioxidative capacity, especially when homocysteine concentrations are unchanged. Furthermore, unchanged TAS in subjects with above median lowering of homocysteine may reflect a decrease in SH-(thiol) groups with simultaneous decrease of antioxidative capacity.

Homocysteine has been demonstrated to disturb NO bioavailability (Upchurch et al., 1997) and increase oxidative stress through autoxidation of homocysteine yielding hydrogen peroxide (Loscalzo, 1996; Kanani et al., 1999). Under these conditions, folic acid may improve NO bioavailability indirectly through decrease of homocysteine concentration leading to decreased oxidative stress and less impairment of NO-induced vasodilatation, with possibly additional antioxidative effect, however, consumed during homocysteine lowering. This is supported by our observation that in CAD patients nonresponders showed no improvement in endothelial function, although there was an increase in folate (and TAS). Because folate increased in both groups, but peak flow reactivity improvement was limited to subjects responding with a homocysteine-lowering effect of at least 2 μM, this effect is therefore most likely to be due to lower homocysteine concentrations and not to folate itself.

It must be noted that this effect has been found in CAD patients with presumably impaired NO metabolism. The findings are important because they demonstrate a vasoprotective effect through lowering homocysteine in atherosclerotic patients.

Potential Study Limitations. Potential study limitations comprise intake of medication that may influence endothelial function measurements. All participants refrained from intake of lipid-lowering drugs but received the same standard medication for CAD (only acetylsalicylic acid and β-blockers), as discontinuation would have been considered unethical.

Using postischemic RH in the human forearm to measure resistance vessel function does not allow discrimination between such mechanisms involved as NO, adenosine, prostaglandins, or myogene relaxation.

It was recently suggested that reduced concentrations of free homocysteine may be responsible for improved vascular endothelial function of conduit vessels (Chambers et al., 2000). Most investigators measure total plasma homocysteine concentrations, but this does not permit discrimination between free and protein-bound homocysteine. For technical reasons, determination of free homocysteine is limited to experimental investigations. Measurement of total plasma homocysteine is most likely to continue universally, and our study indicates that it is suitable for clinical use.

Implications. In our study, supplementation with 5 mg of folic acid was associated with 21% lowering of homocysteine concentrations, which confirms previous reports that found similar lowering effects using dosages between 0.4 and 10 mg (Clarke et al., 1998). Importantly, Chambers et al. (1999b) have recently shown that a physiologic homocysteine increment of only 2 to 3 μM may induce endothelial dysfunction in healthy humans.

These data are in agreement with our finding that a reduction of >2 μM is required to produce a beneficial effect on endothelial function. Furthermore, a mean difference of ~2 to 3 μM generally discriminates cohorts of vascular patients

from age- and sex-matched healthy subjects in case-control studies (Kang et al., 1992). It appears that a deviation of as little as 2 to 3 μM homocysteine may produce a measurable effect on human vascular endothelium and distinguishes cases from healthy subjects. In meta-analyses, increased risk for CAD is found above 8 μM without threshold (Boushey et al., 1995), suggesting that this is the lower limit that should be targeted for maximum risk reduction. Currently, most references use a homocysteine plasma concentration of ~12 to 15 μM to define hyperhomocysteinemia. Our findings suggest that it is not the absolute value that is important for maximum vasoprotection, but the ability to further decrease homocysteine concentrations by >2 μM, which can be achieved through low-dose vitamin supplementation. Whether long-term homocysteine lowering through vitamin supplementation will not only improve endothelial function but also prevent cardiovascular complications must be determined in prospective studies currently in progress.

In conclusion, our findings demonstrate that lowering of total plasma homocysteine concentration improves resistance vessel reactivity in patients with established CAD.

Our results support the hypothesis that intake of folic acid is vasoprotective through lowering plasma homocysteine.

References

Anderson JL, Muhlestein JB, Horne BD, Carlquist JF, Bair TL, Madsen TE, and Pearsson RR (2000) Plasma homocysteine predicts mortality independently of traditional risk factors and C-reactive protein in patients with angiographically defined coronary artery disease. *Circulation* **102:**1227–1232.

Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, and Yeung AC (1995) Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* **26:**1235–1241.

Araki A and Sako Y (1987) Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* **422:**43–52.

Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Bones C, Newcombe RG, and Lewis MJ (1998) Hyperhomocysteinemia after an oral methionine load acutely impairs endothelial function in healthy adults. *Circulation* **98:**1848–1852.

Boushey CJ, Beresford SAA, Omenn GS, and Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *J Am Med Assoc* **274:**1049–1057.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, and Deanfield JE (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk for atherosclerosis. *Lancet* **340:**1111–1115.

Chambers JC, McGregor A, Jean-Marie J, Obeid OA, and Kooner JS (1999a) Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia. An effect reversible with vitamin C therapy. *Circulation* **99:**1156–1160.

Chambers JC, Obeid OA, and Kooner JS (1999b) Physiological increments in plasma homocysteine induce vascular endothelial dysfunction in normal human subjects. *Arterioscler Thromb Vasc Biol* **19:**2922–2927.

Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, and Kooner JS (2000) Improved vascular endothelial function after oral B vitamins: an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation* **102:** 2479–2483.

Chillian W, Eastham C, and Marcus M (1986) Microvascular distribution of coronary vascular resistance in beating left ventricle. *Am J Physiol* **251:**779–786.

Clarke RC and the Homocysteine Lowering Trialists' Collaboration (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomized trials. *Br Med J* **316:**894–898.

Harker LA, Harlan JM, and Ross R (1983) Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. *Circ Res* **53:**731–739.

Hasdai D, Gibbons RJ, Holmes DR, Higano ST, and Lerman A (1997) Coronary endothelial function in humans is associated with myocardial perfusion defects. *Circulation* **96:**3390–3395.

Iwatsubo H, Nagano M, Sakai T, Kumamoto K, Morita R, Higaki J, Ogihara T, and Hata T (1997) Converting enzyme inhibitor improves forearm reactive hyperaemia in essential hypertension. *Hypertension* **29:**286–290.

Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, and Luscher TF (1995) Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* **91:**1314–1319.

Kanani PM, Sinkey CA, Browning RL, Allaman M, Knapp HR, and Haynes WG (1999) Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocyst(e)inemia in humans. *Circulation* **100:**1161–1168.

Kang SS, Wong PWK, and Malinow MR (1992) Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Annu Rev Nutr* **12:**279–298.

Loscalzo J (1996) The oxidant stress of hyperhomocyst(e)inemia. *J Clin Investig* **98:**5–7.

Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, and Weidinger F (1997) Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* **129:**111–118.

Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, and Vollset SE (1997) Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* **337:**230–236.

Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature (Lond)* **362:**801–809.

Tawakol A, Omland T, Gerhard M, Wu JT, and Creager MA (1997) Hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilation in humans. *Circulation* **95:**1119–1121.

Ubbink JB, Hayward-Vermaak WJ, and Bissbort S (1991) Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chromatogr* **565:**441–446.

Ubbink JB, Vermaak WJH, van der Merwe A, and Becker PJ (1993) Vitamin B-12, vitamin B-6 and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* **57:**47–53.

Ueland PM and Refsum H (1989) Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease and drug therapy. *J Lab Clin Med* **114:**473–501.

Upchurch GR, Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF, and Loscalzo J (1997) Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* **272:**17012–17017.

Verhaar MC, Wever R, Kastelein J, van Dam T, Koomans HA, and Rabelink TJ (1998) 5-Methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation* **97:**237–241.

Vester B and Rasmussen K (1991) High-performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Clin Biochem* **29:**549–554.

Wall RT, Harlan JM, Harker LA, and Striker GE (1980) Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb Res* **18:**113–121.

Wascher TC, Bammer R, Stollberger R, Bahadori B, Wallner S, and Toplak H (1998) Forearm composition contributes to differences in reactive hyperaemia between healthy men and women. *Eur J Clin Investig* **28:**243–248.

Wilmink HW, Stroes ES, Erkelens WD, Gerritsen WB, Wever R, Banga JD, and Rabelink TJ (2000) Influence of folic acid on postprandial endothelial dysfunction. *Arterioscler Thromb Vasc Biol* **20:**185–188.

Woo KS, Chook P, Lolin YI, Cheung AS, Chan LT, Sun YY, Sanderson JE, Metreweli C, and Celermajer DS (1997) Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* **96:**2542–2544.

Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, and Celermajer DS (1999) Folic acid improves arterial endothelial function in adults with hyperhomocysteinemia. *J Am Coll Cardiol* **34:**2002–2006.

Zeiher A, Drexler H, Wolletschläger H, and Just H (1991) Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different stages of early coronary atherosclerosis. *Circulation* **83:**391–401.

Zeiher AM, Krause T, Schächinger V, Minners J, and Moser E (1995) Impaired endothelium-dependent vasodilation of coronary resistance vessels is associated with exercise-induced myocardial ischemia. *Circulation* **91:**2345–2352.

Address correspondence to: Dr. Olaf Stanger, Karl-Franzens University School of Medicine, Department of Surgery, Division of Cardiac Surgery, Clinical Atherosclerosis Research Group, Auenbruggerplatz 29, A-8036 Graz, Austria. E-mail: o.stanger@lks.at