Coronary blood flow in patients with stable coronary artery disease treated long term with folic acid and vitamin B12

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Background **Plasma concentration of total homocysteine is associated with risk of cardiovascular disease in epidemiological studies. We wanted to examine the effects of B-vitamin therapy, which may lower total homocysteine, on coronary flow and vascular function in patients with established coronary artery disease (CAD).**

Methods **Forty patients with stable CAD, mean (standard deviation) aged 57.8 (9.0) years, recruited into the Western Norway B-Vitamin Intervention Trial, were randomly assigned to daily oral treatment with 0.8 mg of folic acid and 0.4 mg of vitamin B12 or placebo, and 40 mg of vitamin B6 or placebo, using a 2 × 2 factorial design. At baseline, and after 9 and 24 months, coronary blood flow was assessed by coronary angiography and Doppler flow-wire measurements during intracoronary infusion of saline (basal), incremental doses of acetylcholine, adenosine, and nitroglycerin.**

Results **We found a significant increase in basal ($P < 0.02$) and adenosine-induced ($P < 0.05$) coronary blood flow in patients who received folic acid/vitamin B12 for 24 months, compared with placebo or vitamin B6 alone. Folic acid/vitamin B12 or vitamin B6 treatment did not change endothelial-dependent response after acetylcholine infusion or flow-dependent proximal dilatation in response to adenosine-induced maximal hyperemia ($P \geq 0.45$).**

Conclusion **Long-term treatment with a combination of folic acid and vitamin B12 increase basal and adenosine-induced maximal coronary blood flow. This may reflect improved microvascular function in patients with stable CAD.** ***Coron Artery Dis*** **22:270–278 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Plasma concentration of total homocysteine (tHcy) is an independent risk factor for cardiovascular disease (CVD) [1]. The mechanisms behind this association are not fully understood [2]. To date, randomized trials on secondary prevention of CVD have shown no benefit on risk of cardiac events or mortality by homocysteine-lowering B-vitamin supplementation [3–5]. In contrast, a reduction in stroke events was seen in the Heart Outcomes Prevention Evaluation-2 study [6], and a reduction in stroke mortality rate has been observed in North America after folic acid fortification [7], suggesting some beneficial effects of B vitamins on vascular disease.

High levels of tHcy are associated with arterial endothelial dysfunction [8] and postprandial increments in tHcy are found to impair vascular function [9]. Intact endothelium is important for the maintenance of vascular integrity and regulates vasomotor tone, partly through release of nitric oxide, to meet increased blood flow demands during physical strain. Endothelial dysfunction is an early marker of atherosclerotic disease and is associated with future cardiac events [10,11].

Folic acid administered to patients with CVD improves endothelial function measured as flow-mediated dilatation (FMD) in forearm arteries in some, but not in all studies [12]. Thus far, only one study has evaluated the effect of folic acid and vitamin B12 treatment on coronary endothelial function in patients with coronary artery disease (CAD) [13], and no study has investigated vitamin B6 alone. Typically, the duration of the studies was for a few weeks to some months, and folic acid was given in high doses, most often 5 mg/day [12]. Safety concerns have been raised about daily doses above 1 mg, which may cause unmetabolized folic acid in serum [14,15].

The objective of this substudy of the Western Norway B-vitamin Intervention Trial was to evaluate the long-term effect of a moderate dose (0.8 mg/day) folic acid combined with vitamin B12, and separately evaluate the effect of vitamin B6, on coronary vascular function. The study population was patients with established stable CAD receiving contemporary medical therapy, including statins, and with no selection according to tHcy levels at baseline.

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Methods

Patients, recruitment, and study design

This study is a single-center substudy of the Western Norway B-vitamin Intervention Trial, a prospective randomized double-blind study on the clinical effects of homocysteine-lowering therapy in patients undergoing coronary angiography for suspected CAD [5]. Patients were randomized into four groups: group A, folic acid (0.8 mg), vitamin B12 (cyanocobalamin, 0.4 mg), and vitamin B6 (pyridoxine, 40 mg); group B, folic acid and vitamin B12; group C, vitamin B6; group D, placebo. For the first 2 weeks, group A and group B received an additional loading dose of folic acid (5 mg/day). Using a 2×2 factorial design, we could simultaneously assess the effect of the combination of folic acid/vitamin B12 (group A + B) versus no folic acid/vitamin B12 (group C + D) and separately vitamin B6 (group A + C) versus no vitamin B6 (group B + D). Packages of trial capsules were prepared and randomized in blocks of 20 by Alpharma A/S (Copenhagen, Denmark).

In this substudy of coronary vascular function, patients with stable CAD scheduled for elective percutaneous coronary intervention were eligible. Exclusion criteria were malignant disease, alcohol abuse, mental illness, reluctance or incapability for long-term follow-up. Other exclusion criteria were predicted high risk for procedural complications, severe chronic obstructive pulmonary disease, pulmonary hypertension, significant valvular disease, glaucoma, poorly regulated diabetes, or use of systemic corticosteroids. Furthermore, blood pressure should be well regulated and there should be no indication for starting angiotensin-converting enzyme inhibitor or calcium channel blocker therapy at the time of inclusion. All patients were treated with statins for at least 2 months before inclusion. Long-acting nitrates were not allowed the last week before the testing procedures.

At baseline, percutaneous coronary intervention was done to at least one significant coronary stenosis. Then, a nonintervened, nonstenotic coronary artery (belonging to the left anterior descending artery or circumflex artery) was used for coronary function testing (study vessel). Forty patients were followed with repeated testing after 9 months, and 35 patients returned for a third testing after 2 years of vitamin treatment. Four patients did not wish to follow the 2-year per protocol catheterization, and in one patient, the vascular function testing was not successfully performed due to technical problems. No procedure related complications occurred.

The study protocol was approved by the regional ethics committee, and the medication was approved by the Norwegian Medicines Agency. The investigation conforms with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all patients.

Assessment of coronary vascular function

Measurements were done during consecutive intracoronary administration of saline, acetylcholine, adenosine, and nitroglycerin. Acetylcholine induces a vasodilatation mediated by release of nitric oxide (NO) from intact endothelium, counterbalancing its direct effect on smooth muscles in the vessel wall causing vasoconstriction. The response to acetylcholine infusion thereby is a measure of endothelial function [16]. Adenosine provokes hyperemia mainly by dilatation in the microcirculation mediated by release of NO [17] and other factors [18], and was used for assessment of maximum hyperemic flow in coronary arteries. Increase in coronary blood flow may result in vasodilatation induced by shear stress on vascular endothelium, reflecting local endothelial function measured as FMD [19]. Intracoronary nitroglycerin is a direct precursor of NO, and was used to measure endothelial-independent function.

Procedures

All patients were given heparin at the start of the procedure to obtain an activated clotting time of 300 s. Guiding catheters 6-French (Launcher, Medtronic, Minneapolis, Minnesota, USA) were used for cannulation of the left main coronary artery. Before vascular function testing, intracoronary nitrates were not permitted. A Doppler guide wire (0.014 inch, FloWire, Volcano, Rancho Cordova, California, USA) was placed in a nonbranching segment of the study vessel through the inner lumen of a 2.9-French coronary infusion catheter (UltraFuse-X, Boston Scientific, Maple Grove, Minnesota, USA) ending 1 cm distal to the catheter tip. The positions of the infusion catheter and Doppler wire were documented by angiography. Infusion through the UltraFuse-X catheter was done at 60 ml/h (1 ml/min) with a pump delivering high-pressure output (Asena, Alaris, Basingstoke, UK). Infusions were done as follows in seven steps: (i) saline 0.9% for 3 min; (ii–iv) incremental dosage (0.72, 7.2, and 36.0 μg/min) of acetylcholine (Miochol-E 10 mg/ml, Novartis Healthcare, Copenhagen, Denmark) for 3 min and 20 s each (estimated transit time of 20 s); (v) saline 0.9% for approximately 5 min until return to basal flow (see below); (vi) adenosine (Adenocor, Sanofi-Synthelabo, Bromma, Sweden) at a dose rate of 2.4 mg/min for 3 min and 20 s; and finally the infusion line was flushed with saline; and (vii) a 0.2 mg bolus of nitroglycerin was given. Average peak flow velocity (APV) was continuously recorded [FloMap, Cardiometrics (Volcano), Rancho Cordova, California, USA]. At the end of each infusion step, an angiogram was done in the same position and angle. A coronary artery segment of 10 mm, 2–3 mm distal to the Doppler wire, was used for mean diameter measurement by digitalized quantitative coronary angiography (Quantcor QCA V5.0, Pie Medical Imaging, Maastricht, the Netherlands) with the contrast-filled catheter as reference for calibration. Coronary blood flow

(CBF) was calculated by use of APV and vessel diameter [$CBF = \pi r^2(1/2APV)$] [20]. FMD was calculated comparing mean diameter in a 5–10 mm segment of the study vessel proximal to the infusion catheter tip during basal saline infusion and during hyperemia induced by adenosine infusion.

Measurements

Coronary vascular function was assessed by five indices: CBF at basal conditions during saline infusion (CBF-basal), infusion with acetylcholine (CBF-ach), adenosine (CBF-ado) or nitroglycerin (CBF-ntg), and proximal coronary FMD at maximum hyperemic flow (FMD-hyperemia). In addition, as a measure of endothelial function, response to acetylcholine infusion was calculated as percentage increase in CBF-ach at each dose of acetylcholine compared with CBF-basal. A maximum increase in CBF-ach less than 50% is considered representing endothelial dysfunction [11]. In addition, coronary blood flow reserve (CBFR) was calculated by CBF-ado divided by CBF-basal.

At 9 months and 2 years follow-up after inclusion, the same protocol and target segments were used. All invasive studies were carried out by the same operator. A dedicated technician was in charge of all off-line measurements.

Blood collection and analyses

Blood samples were collected at baseline, after 9 and 24 months and stored at $-80^{\circ}C$ until processing. Folate and cobalamin were measured in serum samples; otherwise, sample handling and analyses were done as described earlier [21]. Glomerular filtration rate was calculated according to the four-variable Modification of Diet in Renal Disease equation [22].

Statistical analyses

Continuous variables are reported as means [standard deviation (SD)] if not otherwise indicated. Skewed variables are presented as median and selected percentiles. Categorical variables are presented as numbers and proportions. Chi-Square test was used for comparison of proportions. Analysis of variance (ANOVA) was used to compare mean levels between groups, and paired-samples *t*-test was used for comparison within groups over time. Treatment effects of folic acid/vitamin B12 or vitamin B6 over time and interaction between treatment groups and acetylcholine dose during follow-up were studied by repeated-measures ANOVA according to the 2×2 study design. Statistical package SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA) was used. Taking into account possible baseline imbalances, differences in time trends of flow (change during follow-up) by treatment were analyzed by a linear mixed-effect model with random intercept on subject level (S-PLUS 7.0 for Windows, Insightful Corporation, Seattle, Washington, USA). A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Patient and baseline characteristics

A total of 40 patients were enrolled. Key baseline demographic and clinical characteristics and medical treatment are given in Table 1. Mean (SD) serum folate was 12.2 (6.5) nmol/l, cobalamin 381 (129) pmol/l, plasma pyridoxal phosphate (PLP) 43.3 (25.0) nmol/l, and tHcy 10.7 (2.9) μmol/l. No significant differences between folic acid/vitamin B12 versus nonfolic acid/vitamin B12 groups or between vitamin B6 versus nonvitamin B6 groups were found with respect to these characteristics.

Follow-up

During 2 years of follow-up, medication was not changed unless indicated. Blood pressure did increase slightly from baseline to follow-up [systolic blood pressure increased with 4 mmHg at 9 months ($P = 0.18$) and 6.6 mmHg at 24 months ($P = 0.06$) compared with baseline; diastolic blood pressure increased with 3.3 mmHg at 9 months ($P = 0.07$) and 6.3 mmHg at 24 months ($P = 0.001$) compared with baseline], but there were no significant differences between treatment groups ($P > 0.43$). Low-density lipoprotein cholesterol did not change during the first 9 months ($P = 0.8$).

B vitamins and total homocysteine after treatment

In patients randomized to folic acid and vitamin B12 treatment (groups A + B), serum folate concentrations increased significantly to 70.6 (18.6) nmol/l ($P < 0.001$) and serum cobalamin increased to 622 (215) pmol/l ($P < 0.001$) after 9 months. There was a concurrent 34% reduction in plasma tHcy to 7.3 (2.0) μmol/l ($P < 0.001$). At 24 months, tHcy remained at the same low level [7.4 (2.0) μmol/l, $P = 0.5$]. In the patients randomized to vitamin B6 alone or placebo (nonfolic acid/vitamin B12, groups C + D), no significant change in folate, vitamin B12, and tHcy were observed (all *P* values > 0.4).

In patients treated with vitamin B6 (groups A + C), plasma PLP increased significantly to 375 (154) nmol/l ($P < 0.001$) at 9 months. No significant change in PLP was observed in patients randomized to treatment with folic acid/vitamin B12 alone or placebo (groups B + D, $P = 0.8$). Vitamin B6 treatment did not change levels of tHcy, folate, or cobalamin (all *P* values > 0.6).

Basal coronary blood flow before and after B-vitamin treatment

Basal coronary blood flow during saline infusion (CBF-basal) at inclusion was nonsignificantly higher [mean (SD) 31.8 (11.8) ml/min] in patients allocated to folic acid/vitamin B12 compared with no folic acid/vitamin B12 [25.7 (7.4) ml/min, $P = 0.06$]. During the 2 years of follow-up, CBF-basal increased gradually among patients treated with folic acid/vitamin B12, as opposed to a decrease in patients not receiving folic acid/vitamin B12, with a significant difference in time trends of CBF-basal

Table 1 Characteristics of the study population at baseline

		Treatment groups[a]					
	Total group[a] ($N=40$)	Folic acid/vitamin B12 (group A+B) ($n=20$)	Nonfolic acid/vitamin B12 (group C+D) ($n=20$)	P values[b]	Vitamin B6 (group A+C) ($n=20$)	Nonvitamin B6 (group B+D) ($n=20$)	P values[b]
Age (years)	57.8 (9.0)	57.4 (10.4)	58.2 (7.7)	0.78	56.3 (6.3)	59.4 (11.1)	0.28
Women, *n* (%)	8 (20)	3 (15)	5 (25)	0.44	3 (15)	5 (25)	0.44
Current smokers, *n* (%)	13 (33)	7 (35)	6 (30)	0.50	9 (45)	4 (20)	0.04
Diabetes, *n* (%)	2 (5)	2 (10)	0 (0)	0.15	0 (0)	2 (10)	0.15
BMI (kg/m^2)	26.7 (2.8)	26.6 (2.5)	26.8 (3.1)	0.89	26.0 (2.1)	27.4 (3.2)	0.11
Pulse (beats/min)	64 (8.5)	64 (9.5)	64 (7.6)	0.99	64 (9.2)	65 (7.9)	0.75
Systolic blood pressure (mmHg)	146 (25.4)	144 (20.4)	147 (29.9)	0.67	143 (17.4)	149 (31.6)	0.46
Diastolic blood pressure (mmHg)	80 (11.8)	81 (10.7)	79 (13.0)	0.63	83 (9.3)	77 (13.4)	0.10
ACEI, *n* (%)	14 (35)	7 (35)	7 (35)	1.00	6 (30)	8 (40)	0.51
β blockers, *n* (%)	29 (73)	14 (70)	15 (75)	0.73	13 (65)	16 (80)	0.29
Ca-channel blockers, *n* (%)	7 (18)	3 (15)	4 (20)	0.68	5 (25)	2 (10)	0.22
GFR (ml/min/1.73 m^2)	76 (12.3)	76 (12.9)	77 (11.9)	0.95	79 (13.7)	74 (10.6)	0.26
Serum LDL cholesterol (mmol/l)	2.9 (0.7)	2.9 (0.5)	2.9 (0.9)	0.95	2.8 (0.6)	3.0 (0.9)	0.36
CRP (mg/l)	1.6 (0.9–2.7)	1.6 (0.9–2.7)	1.5 (0.9–2.7)	0.82	1.7 (1.0–2.5)	1.5 (0.7–2.8)	0.72
Diameter study vessel (mm)[c]	2.25 (0.43)	2.31 (0.49)	2.20 (0.36)	0.43	2.17 (0.46)	2.34 (0.39)	0.22

ACEI, angiotensin-converting enzyme inhibitor; BMI, body mass index; CRP, C-reactive protein; GFR, estimated glomerular filtration ratio; LDL cholesterol, low-density lipoprotein cholesterol.
[a]Mean (standard deviation) or numbers (%), except CRP which is given in median (25–75th percentile).
[b]Comparison of continuous variables between groups by analysis of variance, except for CRP in which comparison is done by Kruskal–Wallis Test. Comparison of proportions between groups by χ^2 test.
[c]Measured distal to the Doppler wire.

between the treatment groups ($P = 0.016$) (Fig. 1a). After 2 years, CBF-basal was 36.3 (15.0) and 21.6 (9.2) ml/min in patients treated and not treated with folic acid/vitamin B12, respectively ($P = 0.002$). Heart rate during CBF-basal measurements at 24 months was not significantly changed compared with inclusion, 66.0 (14.3) and 65.6 (13.4), respectively ($P = 0.93$ between groups). Vitamin B6 treatment was not associated with significant changes in CBF-basal during follow-up ($P > 0.9$).

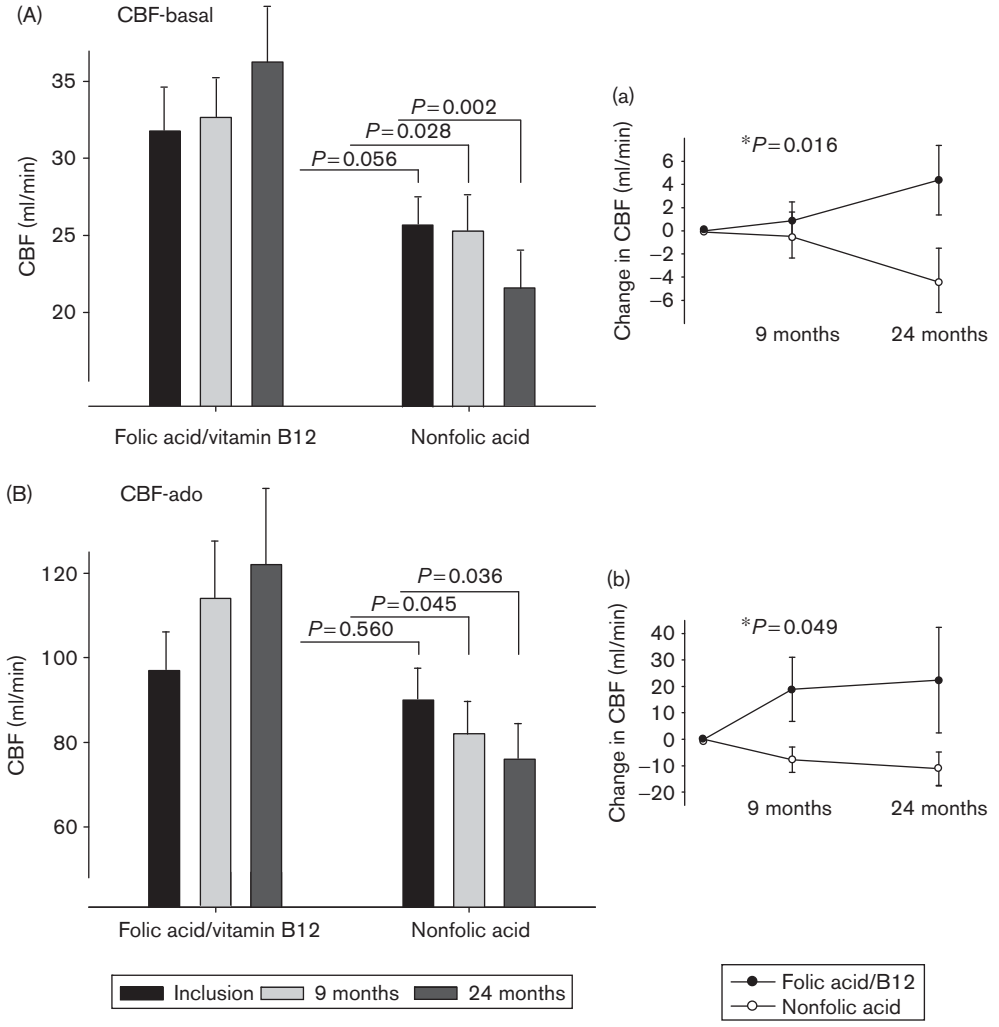
Coronary blood flow and response to acetylcholine infusion

Maximum CBF achieved during incremental dosages of acetylcholine (CBF-ach) at inclusion was 59.5 (39.2) ml/min. At the same time, maximum flow response to acetylcholine measured as percentage change in CBF-ach compared with CBF-basal, reflecting epicardial endothelial function, was median (25–75th percentile) 90 (35–162)%, of which 13 patients (33%) achieved increase in CBF-ach less than 50%. CBF-ach profile during infusion with incremental dosages of acetylcholine did not change significantly after 9 months and 2 years of treatment with either folic acid/vitamin B12 ($P = 0.9$) (Fig. 2) or vitamin B6 ($P = 0.7$). Similarly, flow response (CBF-ach compared with CBF-basal) during acetylcholine infusion (0.72, 7.2, and 36.0 μg/min) did not change significantly during 2 years according to folic acid/vitamin B12 (Table 2) or vitamin B6 treatment ($P = 0.66$ and 0.77, respectively). Although the coronary luminal diameter reduction at the highest dose acetylcholine may seem less after 2 years of folic acid/vitamin B12 treatment compared with no folic acid/vitamin B12 (Fig. 3), change in coronary luminal diameter did not differ significantly between groups.

Maximum hyperemia and flow-dependent response after adenosine infusion

Adenosine infusion was used to achieve maximum hyperemic coronary flow (CBF-ado), mainly due to effects on microcirculation [17]. At the start of the study, there was no difference in CBF-ado between the folic acid/vitamin B12 group, mean (SD) 96.8 (39.1) ml/min, and the nonfolic acid/vitamin B12 group, 90.0 (32.9) ml/min ($P = 0.6$). Similar to observations with CBF-basal, treatment with folic acid/vitamin B12 was associated with a gradual increase in CBF-ado, whereas a decrease in CBF-ado was seen among patients not receiving folic acid/vitamin B12. As a result, there was a significant difference in time trends of CBF-ado according to folic acid/vitamin B12 therapy ($P = 0.049$) (Fig. 1b). At 2 years, mean (SD) CBF-ado was 121.8 (77.5) ml/min in patients allocated to folic acid/vitamin B12 compared with 76.0 (33.1) ml/min in patients not receiving folic acid/vitamin B12 ($P = 0.04$), with no significant differences in epicardial luminal diameter response to adenosine infusion ($P = 0.72$) (Fig. 3). Heart rates during CBF-ado measurements

Fig. 1



Coronary blood flow (CBF) (ml/min) during intracoronary infusion of saline (CBF-basal) (A and a), maximal hyperemia during intracoronary infusion of adenosine 2.4 mg/min (CBF-ado) (B and b). The flow indices (mean, standard error of the mean) at inclusion, 9 and 24 months for the folic acid/vitamin B12 group (A + B) ($n = 20$, 20,19) and nonfolic acid group (C + D) ($n = 20$, 20,16) are shown. *P* values between intervention groups at each study visit (panels A and B) and **P* values for differences in time trends of flow (change during follow-up) (a and b) according to folic acid/vitamin B12 treatment.

at 24 months were comparable between intervention groups, 69.8 (15.7) and 63.6 (7.2), respectively ($P = 0.18$ between groups). Vitamin B6 had no effect on CBF-ado ($P \geq 0.3$). CBFR was 3.3 (1.4) in the folic acid/vitamin B12 group and 3.6 (1.3) in the nonfolic acid/vitamin B12 group at baseline ($P = 0.47$). At 24 months, there was still no difference between groups [3.6 (2.0) and 3.7 (1.5), respectively, $P = 0.80$].

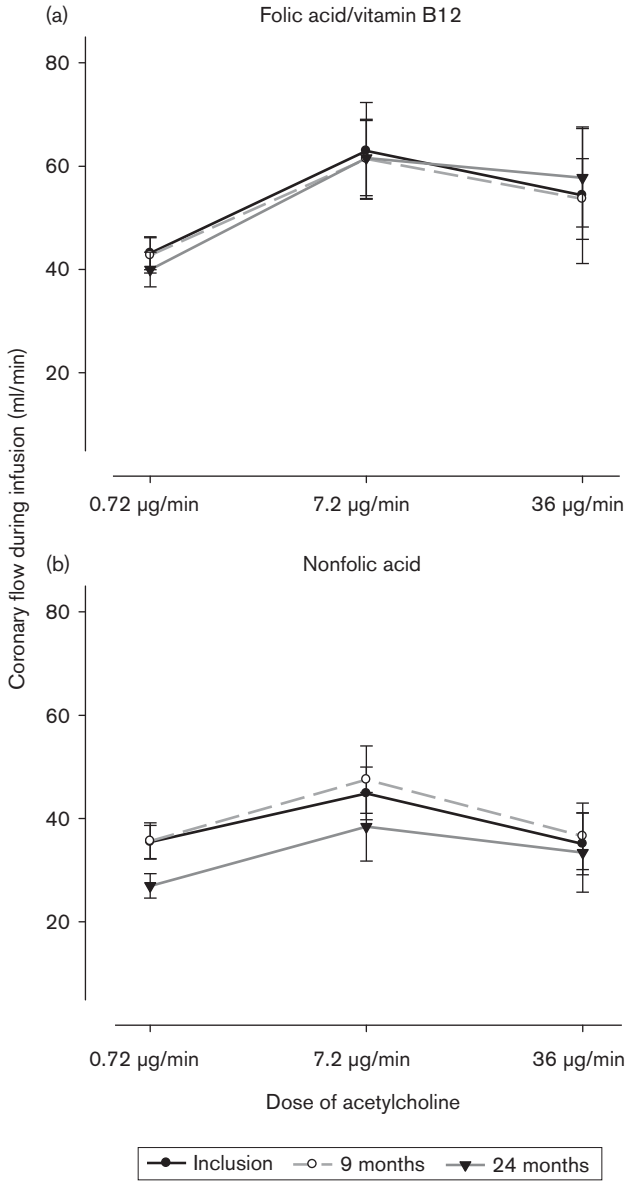
FMD-hyperemia, a measurement of endothelial function in the epicardial artery, was 0.10 (0.23) mm at baseline, with no overall change at 9 months [0.10 (0.21) mm, $P = 0.9$] or 2 years [0.13 (0.39) mm, $P = 0.6$]. There was no significant difference in FMD-hyperemia according to folic acid/vitamin B12 or B6 during follow-up (repeated measure ANOVA; $P = 0.16$ and 0.84 respectively).

Endothelial-independent response after nitroglycerin infusion

CBF after nitroglycerin administration (CBF-ntg) was 109.1 (34.3) ml/min in the folic acid/vitamin B12 group and 94.1 (34.5) ml/min in the nonfolic acid group/vitamin B12 at randomization ($P = 0.18$). Although we observed a significant difference in CBF-ntg at 2 years between patients treated with folic acid/vitamin B12 compared with those not treated with folic acid/vitamin B12 (133.5 (62.8) and 88.9 (45.0) ml/min, respectively ($P = 0.02$), the difference in time trends of CBF-ntg did not reach statistical significance ($P = 0.10$). CBF-ntg was not influenced by vitamin B6 treatment ($P \geq 0.5$).

At baseline, epicardial luminal diameter response to nitroglycerin was more pronounced in patients assigned

Fig. 2



Coronary blood flow (CBF) (ml/min) during intracoronary infusion of incremental doses of acetylcholine (CBF-ach) 0.72, 7.2, and 36 µg/min. CBF-ach (mean, standard error of the mean) at inclusion, 9 and 24 months for the folic acid/vitamin B12 group (A + B) (n = 20, 20,19) (a) and nonfolic acid group (C + D) (n = 20, 20,16) (b) are shown. There was no significant treatment effect of folic acid/vitamin B12 during follow-up (P = 0.85, repeated measures analysis of variance, effect between treatment groups and dosage of acetylcholine).

to treatment with folic acid/vitamin B12 [14.2 (10.2)%] compared with the nonfolic acid group [7.3 (10.1)%] (P = 0.036). This difference was attenuated and no longer significant at 2 years [11.1 (12.4)% and 10.5 (10.6)%, respectively] (P = 0.88) (Fig. 3). However, we observed no significant treatment effect of folic acid/vitamin B12 on luminal diameter response to nitroglycerin during follow-up (P = 0.44, repeated measures ANOVA).

Table 2 Coronary blood flow response to acetylcholine infusion at inclusion and after 9 and 24 months of B-vitamin treatment

	Percentage change in flow[a]		
Acetylcholine dosage	Folic acid/vitamin B12 (group A + B)		Nonfolic acid/vitamin B12 (group C + D)
0.72 µg/min			
Inclusion (n = 40)	39.8 (8.7)		38.1 (9.5)
9 months (n = 40)	34.8 (9.2)		39.5 (8.5)
24 months (n = 35)	14.5 (6.8)		35.5 (13.3)
P values[b]		P = 0.51	
7.2 µg/min			
Inclusion (n = 40)	93.4 (20.1)		76.8 (18.4)
9 months (n = 39)	87.3 (19.9)		83.5 (21.8)
24 months (n = 34)	71.8 (14.1)		80.6 (26.8)
P values[b]		P = 0.60	
36.0 µg/min			
Inclusion (n = 38)	54.7 (27.4)		34.1 (21.4)
9 months (n = 35)	77.3 (26.5)		35.3 (22.6)
24 months (n = 32)	53.5 (18.8)		42.0 (23.5)
P values[b]		P = 0.58	
P values overall[c]		P = 0.66	

[a]Values in %, mean (standard error of the mean), coronary blood flow during acetylcholine infusion compared with coronary blood flow during saline infusion.
[b]Repeated measures analysis of variance, effect between groups during 24 months.
[c]Repeated measures analysis of variance, effect between groups and dosage of acetylcholine during 24 months.

Discussion

In this randomized controlled trial, we have shown that 2 years of treatment with a moderate dose of folic acid and vitamin B12 improved basal CBF and blood flow at maximum hyperemia induced by adenosine. In contrast, folic acid/vitamin B12 did not improve flow-induced change in coronary vessel diameter (FMD) or change in flow after stimulation with acetylcholine.

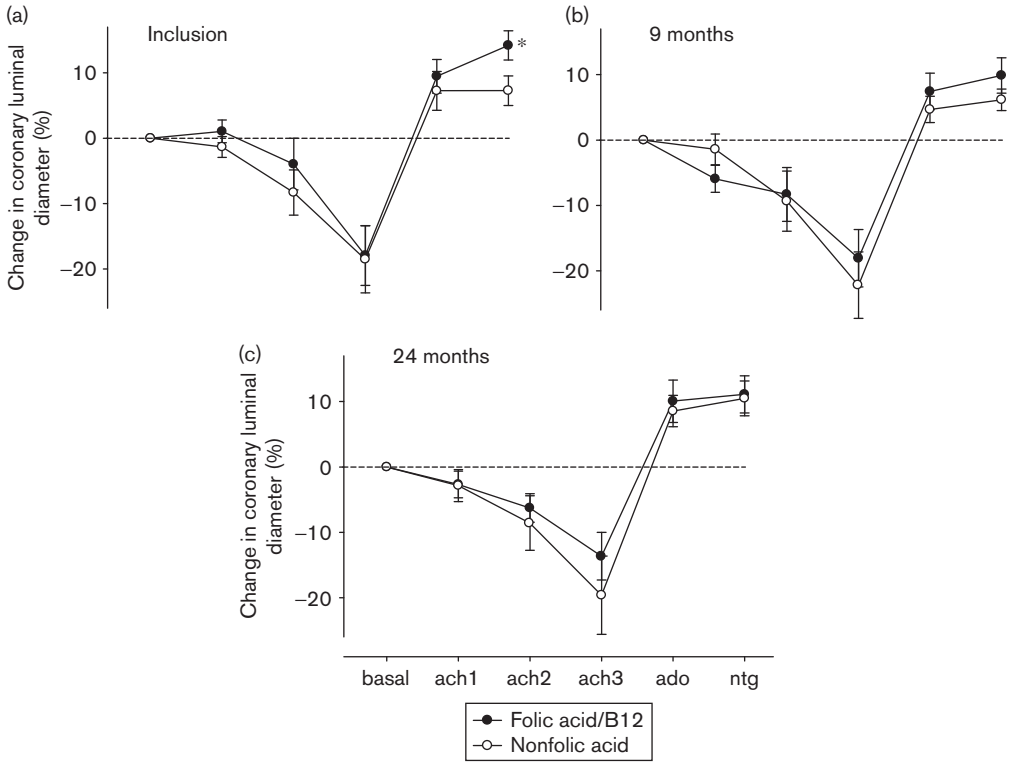
Measurements in different target organs

We have investigated parameters of vascular function in coronary vessels. This is in contrast to most other studies on endothelial function and B vitamins, which have measured brachial FMD [12]. The relation between coronary endothelial function and brachial FMD is uncertain, and, although one study found a significant but weak relation between brachial FMD and coronary response to acetylcholine [23], other studies find no such relation [24,25]. Therefore, caution must be taken when comparing results obtained in different target organs.

Strengths and weaknesses

Major strengths of our study include intracoronary measurements, a population without folate fortification, long-term follow-up, and a factorial design allowing us to examine separate effects of folic acid/vitamin B12 and vitamin B6 treatment. Our moderate dose of folic acid, although reducing tHcy, may be too low to exhibit full effect on endothelial function [12]. The design of our study limits the number of patients and may conceal minor effects of the intervention. Still, this is the largest study on B-vitamin therapy and coronary vascular function. All patients were on medical treatment, including

Fig. 3



Change (%) in distal epicardial coronary luminal diameter in response to intracoronary infusion of incremental doses of acetylcholine (ach1, 0.72 μg/min; ach2, 7.2 μg/min and ach3, 36 μg/min), adenosine (ado) 2.4 mg/min, and nitroglycerin (ntg) 0.2 mg, relative to saline (basal) infusion (mean, standard error of the mean), at inclusion (a), 9 months (b), and 24 months (c). No significant differences are seen between the folic acid/vitamin B12 group (A + B) and nonfolic acid group (C + D) at any measurement ($P > 0.15$), except for nitroglycerin at inclusion *($P = 0.036$).

statins, before entering the study. This may have attenuated potential effects of our intervention. At baseline, data show a nonsignificant higher CBF-basal together with more a pronounced diameter response to nitroglycerin in patients assigned to treatment with folic acid/vitamin B12 compared with the nonfolic acid group. Such baseline imbalance warranted careful considerations regarding statistical methods.

Earlier studies

There is only one published study on the effect of B vitamins on coronary flow in patients with CAD [13]. A total of 15 patients were randomized to treatment with folic acid (5 mg/day) and vitamin B12 (0.4 mg/day) or placebo for 6 months. B vitamin treatment was associated with a significant improvement in acetylcholine-induced CBF. It is noteworthy that these patients had relatively high baseline tHcy (17.9 μmol/l), few patients received statin therapy, and a high dose of folic acid was used. In contrast, we found an effect of folic acid/vitamin B12 on basal and maximal hyperemic CBF, but no effect on endothelial function as measured by change in flow after acetylcholine.

Possible mechanisms

Most studies suggest that a rapid increase in plasma tHcy levels, as observed during methionine or homocysteine loading [8,12], or after a protein rich meal [9], impairs brachial FMD, whereas high doses of folic acid improve endothelial function, possibly partly independent of its homocysteine-lowering effect [12,26]. The mechanism by which homocysteine impairs endothelial function may involve homocysteine-induced reduction of intracellular tetrahydrobiopterin, thereby causing eNOS-uncoupling [27]. Folic acid, through its circulating form, 5-methyltetrahydrofolate, is believed to enhance regeneration of tetrahydrobiopterin and improve eNOS-coupling and thereby improve endothelial function independently of homocysteine [28,29]. Recent data from an isolated rat heart model support our findings of increased coronary flow by folic acid treatment, most likely mediated by reduced peripheral resistance, and suggests a mechanistic role of NO [30].

Elevated tHcy is also associated with reduced endogenous 0w?>plasma and tissue adenosine [31] and diminished vasodilating effect of infused adenosine, probably

due to enhanced cellular uptake [32]. We speculate that homocysteine-lowering by folate may reverse these effects and promote arterial flow mediated by adenosine.

Although NO is an established regulator of vascular tone, there is some evidence that endothelium-derived hyperpolarizing factor (EDHF) plays a major role in regulating microcirculation [33]. In renal microcirculation of rats, EDHF-mediated vasodilatation is impaired during methionine loading and partly restored by 5-methyltetrahydrofolate [18]. This suggests an additional mechanism by which folic acid therapy in our patients may have improved vascular tone and microcirculation. Owing to the design of our study, we cannot differentiate whether these effects are mediated by NO, EDHF, or other mediators.

Our study did not show any effect of folic acid/vitamin B12 on FMD, acetylcholine infusion induced flow, or on epicardial luminal diameter response to acetylcholine. On the contrary, the more pronounced epicardial diameter response to nitroglycerine at randomization was attenuated during 2 years. Thus, methods mainly testing response in large conduit arteries did not show any beneficial effect of B-vitamin therapy. We used a moderate dose of folic acid 0.8 mg/day in combination with vitamin B12, which reduced tHcy by 34%. Even folic acid at 0.4 mg/day has been shown to improve vascular function quantified by MRI [34], although a higher dose seems to be more effective in restoring endothelial function [12]. The dose of folic acid may have been too low to effectively ameliorate endothelial function in diseased epicardial coronary vessels.

Resting coronary flow (CBF-basal) and maximal hyperemia (CBF-ado) largely depend on regulation of microvascular resistance in nonobstructive coronary vessels [17]. Our data thus indicate that long-term moderate dose of folic acid/vitamin B12 affect endothelial function in large epicardial coronary vessels to a lesser degree, but rather improve microvascular function in patients with stable CAD. A nonsignificant trend toward increased CBF-ntg in patients treated with folic acid/vitamin B12, together with attenuated epicardial diameter response to nitroglycerine, may support this interpretation. Folic acid may protect against homocysteine-induced proliferation of vascular smooth muscle cells [35]. Such effects on vascular smooth muscle cells in the microvascular bed could be a possible explanatory mechanism of observed changes in coronary flow patterns. Our data complement findings of increased adenosine-stimulated myocardial blood flow in diseased coronary artery segments measured by PET acutely after high doses of folic acid, presumably due to increased NO bioavailability [36]. As both CBF-basal and CBF-ado change in a parallel manner (Fig. 1), the widely used index, CBFR, as a measure of microvascular disease, is not influenced by folic acid/vitamin B12 treatment. The same observation is reported earlier [13], as opposed to a recent study in elderly patients with vitamin B12 deficiency [37].

A beneficial effect on microvascular flow, together with reduced arterial stiffness [34], may account for reduced frequency of electrocardiographic changes at exercise tests [38] and reduction in blood pressure [39] observed after treatment with folic acid. Improved vascular function, together with reduction in blood pressure [39], may explain a possible modest reduction in stroke observed after folic acid fortification [7] and B-vitamin treatment [6,40]. Earlier studies have shown that even minor reductions in blood pressure are associated with significant lower risk of stroke, but with less effect on ischemic heart disease [41]. Thus, folic acid may have effect on risk of stroke without overt effect on recurrent CAD [3–5,41,42].

Vitamin B6

Data on vitamin B6 and vascular function are sparse [43], and our study is the first to examine the effect of B6 in the coronary circulation. We observed no effect of vitamin B6 treatment on the vascular indices despite marked increases in plasma concentrations of PLP. Vitamin B6 status is associated with CVD in some observational studies [44], but supplementation studies with vitamin B6 have shown no clinical benefits [4,5].

Conclusion

In patients with stable CAD, we showed that treatment with moderate doses of folic acid in addition to vitamin B12 is associated with a significant increase in both basal and adenosine-stimulated maximal CBF. This observation may reflect improved microvascular function. Our treatment does not, however, change acetylcholine-induced response or flow-mediated epicardial coronary dilatation. Treatment with high doses of vitamin B6 has no effect on coronary vascular function. In the era of widespread folate fortification of foods, the possible beneficial effects of folate therapy on vascular function should be further studied, despite negative results of secondary prevention studies in patients with established CAD.

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The authors have no conflicts of interest to declare.

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