Paper IV

Folic Acid Intervention Increases Coronary Blood Flow in Patients with Stable Coronary Artery Disease

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Abstract

Purpose. We examined the effects of B-vitamin therapy on coronary flow and vascular function in patients with established coronary artery disease (CAD).

Methods and results. Forty patients with CAD and on statin treatment, recruited into the Western Norway B-Vitamin Intervention Trial (WENBIT), were randomly assigned to daily oral treatment with 0.8 mg folic acid and 0.4 mg vitamin B12 or placebo, and 40 mg vitamin B6 or placebo, using a 2 x 2 factorial design. At baseline, and after 9 and 24 months, coronary blood flow were assessed by coronary angiography and doppler flow-wire measurements during intra-coronary infusion of saline (basal), incremental (0.72 μg/min, 7.2 μg/min and 36.0 μg/min) doses of acetylcholine, 2.4 mg/min adenosine and nitroglycerin.

At baseline, mean (SD) age was 57.8 (9.0) years (eight females), serum LDL-cholesterol 2.9 (0.7) mmol/L, creatinine 88 (9.9) μmol/L, folate 12.2 (6.5) nmol/L and plasma total homocysteine 10.7 (2.9) μmol/L ($p \geq 0.4$ between treatment groups). We found a significant increase in basal ($p < 0.02$) and adenosine-induced ($p < 0.05$) coronary blood flow in subjects who received folic acid/vitamin B12 compared to placebo or vitamin B6 alone for 24 months. Folic acid/vitamin B12 or vitamin B6 treatment did not change endothelial-dependent response following acetylcholine infusion or flow-dependent proximal dilatation in response to adenosine-induced maximal hyperemia ($p \geq 0.45$).

Conclusion. Treatment with a combination of folic acid and vitamin B12 increased basal and adenosine-induced maximal coronary blood flow, reflecting improved vascular function. The potential beneficial effect of folic acid therapy on vascular function should be furthered studied.

INTRODUCTION

Numerous studies suggest that the serum or plasma concentration of total homocysteine (tHcy) is an independent risk factor for cardiovascular disease (CVD) [1,2]. The mechanisms of this association are not fully understood [3,4]. To date, randomized trials on secondary prevention of CVD have shown no benefit on risk of cardiac events or mortality by homocysteine-lowering folic acid supplementation [5]. Still, high levels of tHcy is associated with arterial endothelial dysfunction [6] and even postprandial increments in tHcy are found to impair endothelial function [7].

Intact endothelium is important for the maintenance of vascular integrity and regulates vasomotor tone through release of nitric oxide to meet increased blood flow demands during physical strain. Endothelial dysfunction is an early marker of atherosclerotic disease, it is involved in the pathogenesis, and is associated with future cardiac events [8,9]. Various treatment strategies have been shown to improve endothelial function and cardiovascular prognosis, including statins, angiotensin converting enzyme inhibitors and exercise [8].

High doses of folic acid given to patients with CVD improve endothelial function measured as flow-mediated dilatation (FMD) in forearm arteries in some, but not in all studies [10]. However, low dose (0.4 mg/d) folate therapy seems to be ineffective [11,12]. Typically, the duration of the studies was for a few weeks to months [10].

So far, only one study has evaluated the effect of folic acid and vitamin B12 on coronary endothelial function in patients with coronary artery disease (CAD) [13], and no study investigated vitamin B6 alone. The objective of this substudy of the Western Norway B-vitamin Intervention Trial (WENBIT) was to evaluate the long-term effect of homocysteine-lowering B-vitamin therapy 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.

SUBJECTS AND METHODS

Patients, recruitment and study design

The current study is a single center substudy of WENBIT, a prospective randomized double-blind study on the clinical effects of homocysteine-lowering therapy in 3090 adult patients undergoing coronary angiography for suspected CAD. Recruitment to WENBIT was started in 1999 and completed in March 2004. Using a 2 x 2 factorial design, we could simultaneously assess the effect of the combination of folic acid/vitamin B12 versus no folic acid/vitamin B12 and separately vitamin B6 versus no vitamin B6. 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 two weeks, group A and group B received an additional loading dose of folic acid (5 mg/day). 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 to 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 blocker therapy at the time of inclusion. All patients were treated with statins for at least two months prior to inclusion. Long-acting nitrates were not allowed the last week before the testing procedures.

After successful treatment of at least one significant coronary stenosis, a non-intervened coronary artery (belonging to the left anterior descending artery or circumflex

artery) was used for baseline coronary function testing (study vessel). Forty patients were followed with repeated testing after nine months, and 35 patients returned for a third testing after two years of vitamin treatment. Four patients did not wish to follow the two year per protocol catheterization, and in one patient, the vascular function testing was not successfully performed at the two year visit 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. 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 reflects endothelial function [14]. Adenosine provokes hyperemia mainly by stimulating endothelium-independent dilatation in the microcirculation [15] and was used for assessment of maximum hyperemic flow in coronary arteries. Intracoronary nitroglycerin is a direct precursor of NO, and was used to measure endothelial-independent function.

Procedures

All patients were given heparin at start of the procedure to obtain an activated clotting time of 300 s. Guiding catheters 6-French (Launcher, Medtronic, Minnespolis, MN) 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, CA) was placed in a non-branching segment of the study vessel through the inner lumen of a 2.9-French coronary infusion catheter (UltraFuse-X, Boston Scientific, Maple Grove, MN) 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/hour (1 mL/min) with a pump delivering high-pressure output (Asena, Alaris, Basingstoke, UK). Infusions were done as follows in seven steps: 1) Saline 0.9% for three minutes, 2-4) incremental dosage (0.72 μg/min, 7.2 μg/min and 36.0 μg/min) of acetylcholine (Miochol-E 10 mg/mL, Novartis Healthcare, Copenhagen, Denmark) for three minutes and 20 seconds each (estimated transit time of 20 seconds), 5) saline 0.9% for approximately five minutes until return to basal flow (see below), 6) adenosine (Adenocor, Sanofi-Synthelabo, Bromma, Sweden) at a dose rate of 2.4 mg/min for 3 min and 20 sec., and finally the infusion line was flushed with saline, and 7) a 0.2 mg bolus of nitroglycerin was given. Average peak flow velocity (APV) was continuously recorded (FloMap, Cardiometrics (Volcano), Rancho Cordova, CA). 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 (QCA) (Quantcor QCA V5.0, Pie Medical Imaging, Maastricht, Netherlands) with the contrast-filled catheter as reference for calibration. Coronary blood flow (CBF) was calculated by use of APV and vessel diameter ($CBF = \pi r^2(½APV)$)[16]. FMD was calculated comparing mean diameter in a 10 mm segment (or at least 5 mm in shorter available segment lengths) of the study vessel proximal to the infusion catheter tip during 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) and nitroglycerin (CBF-ntg), and proximal coronary FMD at maximum hyperemic flow (FMD-hyperemia). Also, as a measure of endothelial function, response to acetylcholine infusion was calculated as percent increase in CBF-ach at each dose of acetylcholine compared to CBF-saline. A maximum increase in CBF-ach < 50% is considered representing endothelial dysfunction [9].

At follow-up nine months and two years after inclusion, the same protocol and target segments were used. All invasive studies were performed 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 -80º C until processing. Folate and cobalamin were measured in serum samples, otherwise, sample handling and analyses were done as previously described [17]. Glomerular filtration rate (GFR) was calculated according to the four-variable Modification of Diet in Renal Disease Equation [18].

Statistical analyses

Continuous variables are reported as means (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. 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/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 x 2 study design. Statistical package SPSS 13.0 (SPSS Inc., Chicago, IL) was used. 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, WA). A two-tailed $P < 0.05$ was considered statistical significant.

RESULTS

Subject and baseline characteristics

A total of 40 patients (8 female and 32 male) with median age 57 (range 39-74) years 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/B12 vs non-folic acid/B12 groups or between vitamin B6 vs non-B6 groups were found with respect to these characteristics.

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 at 9 months ($p<0.001$) and serum cobalamin increased to 622 (215) pmol/L ($p<0.001$). 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 (non-folic acid, 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 non-significantly higher (mean (SD) 31.8 (11.8) mL/min) in patients allocated to folic acid /B12 compared with patients not randomized to folic acid /B12 (25.7 (7.4) mL/min, p=0.06) (**Figure 1**). During the two years of follow-up, CBF-basal increased among patients treated with folic acid /B12, as apposed to a decrease in patients not receiving folic acid /B12, with a significant difference in time-trends of CBF-basal between the treatment groups (p=0.016) (Figure 1). After two years, CBF-basal was 36.3 (15.0) mL/min and 21.6 (9.2) mL/min in patients treated and not treated with folic acid/B12, respectively (p=0.002). Vitamin B6 treatment was not associated with significant changes in CBF-basal after follow-up.

Coronary blood flow and response to acetylcholine infusion

Maximum achieved coronary blood flow 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 percent change in CBF-ach compared to CBF-basal, was median (25 – 75th percentile) 90 (35-162) %. A total of 13 patients (33%) achieved increase in CBF-ach less than 50%. Flow profile during infusion with incremental dosages of acetylcholine did not change after 9 months and two years of treatment with either folic acid/B12 (p=0.9) (**Figure 2**) or vitamin B6 (p=0.7). Likewise, flow response during acetylcholine infusion, as a measure of endothelial-dependent response, did not change significantly after B-vitamin intervention for two years (**Table 2**, all p-values ≥ 0.45).

Maximum hyperemia and flow-dependent response following adenosine infusion

Adenosine infusion was used to achieve maximum hyperemic coronary flow (CBF-ado), mainly due to effects on microcirculation[15]. At study start, there was no difference in CBF-ado between the folic acid/B12 group, mean (SD) 96.8 (39.1) mL/min, and the non-folic acid/B12 group, 90.0 (32.9) mL/min (p=0.6) (Figure 1). Similar to observations with CBF-basal, treatment with folic acid /B12 was associated with a gradual increase in CBF-ado, whereas a decrease in CBF-ado was seen among patients not receiving folic acid /B12. As a result, there was a significant difference in time-trends of CBF-ado according to folic acid/B12 therapy (p=0.049) (Figure 1). At two years, mean (SD) CBF-ado was 121.8 (77.5) mL/min in patients allocated to folic acid/B12 compared to 76.0 (33.1) mL/min in patients not receiving folic acid /B12 (p=0.04). Vitamin B6 had no effect on CBF-ado ($p \geq 0.3$).

FMD-hyperemia, a measurement of endothelial function, 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/B12 or vitamin B6 during follow-up (repeated measure ANOVA; p=0.16 and p=0.84 respectively).

Endothelial-independent flow response following nitroglycerin infusion

Coronary blood flow after nitoglycerin administration (CBF-ntg) was 109.1 (34.3) mL/min in the folic acid/B12 group and 94.1 (34.5) mL/min in non-folic acid group at randomization (p=0.18). Although we observed a significant difference in CBF-ntg at two years between patients treated with folic acid/B12 compared to those not treated with folic acid/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 statistically significance (p=0.10) (Figure 1). CBF-ntg was not influenced by vitamin B6 treatment ($p \geq 0.5$).

DISCUSSION

In this randomized controlled trial, we have shown that two years of treatment with folic acid/vitamin B12 improved basal coronary blood flow and blood flow at maximum hyperaemia induced by adenosine. In contrast, folic acid/vitamin B12 did not improve coronary endothelial function as measured by flow-induced change in coronary vessel diameter or change in flow following stimulation with acetylcholine. Vitamin B6 treatment did not change any of the vascular function variables.

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 [10]. 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 [19], other studies find no such relation [20,21]. Therefore, caution must be taken when comparing results obtained in different target organs.

Strengths and weaknesses

Intra-coronary measurements in a population with no folate fortification, combined with long-term follow-up, are major strengths of our study. The factorial design allowed us to examine separate effects of folic acid/B12 and vitamin B6 treatment. Although the number of patients may seem limited, this is the largest study on folate therapy and coronary vascular function.

Earlier studies on coronary vascular function

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/d) and

vitamin B12 (0.4 mg/d) or placebo for six months. B-vitamin treatment was associated with a significant improvement in acetylcholine-induced coronary blood flow. 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 coronary blood flow, but no effect on acetylcholine stimulated flow.

Possible mechanisms

The reported relations between folate or tHcy levels and brachial endothelial-dependent FMD are somewhat conflicting [22]. However, most studies suggest that a rapid increase in plasma tHcy levels, as observed during methionine or homocysteine loading [6,10,22], or after a protein rich meal [7], impairs brachial endothelial function, while high doses of folic acid improve endothelial function, possibly partly independent of its homocysteine-lowering effect[10,23]. The mechanism by which homocysteine impairs endothelial function may involve homocysteine-induced reduction of intracellular tetrahydrobiopterin, thereby causing eNOS-uncoupling [24]. 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 [25,26]. Recent data from an isolated rat heart model supports our findings of increased coronary flow by folic acid treatment, and suggests a mechanistic role of NO [27].

Whereas 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 [28]. In renal microcirculation of rats, EDHF-mediated vasodilatation is impaired during methionine loading and partly restored by 5-methyltetrahydrofolate [29]. This suggests an additional mechanism by which folic acid therapy in our patients has improved

vascular tone and microcirculation, since resting coronary flow (CBF-basal) and maximal hyperemia (CBF-ado) largely depend on microvascular dilatation in non obstructive coronary vessels [15]. A beneficial effect on microvascular flow, together with reduced arterial stiffness [30], may explain a reduced frequency of electrocardiographic changes at exercise tests [31] and reduction in blood pressure [32] observed after treatment with folic acid.

Dosage of folic acid

High doses of folic acid 5 – 10 mg/d improve endothelial function as measured by FMD [33]. Likewise, a short-term study (six weeks) in patients with CAD showed no effect on forearm FMD with 0.4 mg/d of folic acid, but a significant improvement with 5 mg daily [11]. In contrast, a study using magnetic resonance imaging revealed that both 0.4 mg/d and 5 mg/d of folic acid for seven weeks improved FMD in brachial artery [30]. The latter study also demonstrated that the low-dose folic acid significantly enhances folate status in the vascular endothelium, with no significant additional increase in vascular tissue 5-methyltetrahydrofolate level in the high-dose group [30]. Thus, our dose of 0.8 mg/d should be adequate to obtain optimal effect on endothelial-dependent response and vascular stiffness.

Clinical trials

If long-term treatment with folic acid improves vascular function, this may have important clinical implications. and explain a reduction in stroke mortality rate observed in North America following folic acid fortification [34] and a reduction in stroke events in the HOPE-2 study [35]. Prior studies have demonstrated that even minor reductions in blood pressure are associated with significant lower risk of stroke, but with less effect on ischemic heart disease [36]. Thus, potential treatment effects of folic acid related to a reduction in blood pressure, may act differently upon risk of stroke and CAD [1,36]. Notably, folic acid/B12 therapy has not been

associated with reduced risk of coronary events in any of the published randomized trials with B-vitamin intervention [37,38].

Vitamin B6

Data on vitamin B6 and vascular function are sparse [39,40], 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 [41,42], but supplementation studies with vitamin B6 have revealed no clinical benefits [37].

Conclusion

In patients with stable CAD, we demonstrate that treatment with moderate doses of folic acid in addition to vitamin B12 is associated with a significant increase in both basal and in adenosine-stimulated maximal coronary blood flow, reflecting improved vascular function. This treatment does however not change endothelial-dependent acetylcholine-induced response or flow-mediated coronary dilatation. Treatment with high doses of vitamin B6 shows no effect on coronary vascular function. The effect of folate therapy on vascular function should be furthered studied.

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Disclosures.

PM Ueland reports having received consulting fees from Nycomed and is a member of the steering board of both the non-profit Foundation to Promote Research into Functional Vitamin B12 Deficiency and Bevital, a company owned by the foundation. A PTC application (62924 [52365]) for a patent entitled “Determination of folate in fresh and stored serum or plasma as paraaminobenzoylglutamate” was filed on March 3, 2005; PM Ueland is listed as one of the inventors. The patent is owned by Bevital.

No other potential conflict of interest relevant to this article was reported

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Figure 1. Coronary blood flow during intra coronary infusion of saline (CBF-basal), maximal hyperemia during infusion of adenosine 2.4 mg/min (CBF-ado) and endothelium-independent flow induced by nitroglycerin (CBF-ntg). The flow indices (mean, SEM) at inclusion, 9 months and 24 months for the folic acid/vitamin B12 group (n = 20, 20, 19) and non-folic acid group (n = 20, 20, 16) are shown.
* P-values for differences in time trends according to folic acid/vitamin B12 treatment and p-value between intervention groups at each study visit byANOVA.

Figure 2. Coronary blood flow during infusion of incremental doses of acetylcholine (CBF-ach) 0.72 µg/min, 7.2 µg/min and 36 µg/min. CBF-ach (mean, SEM) at inclusion, 9 months and 24 months for the folic acid/vitamin B12 group (n = 20, 20, 19) and non-folic acid group (n = 20, 20, 16) are shown. There was no significant treatment effect of folic acid/vitamin B12 during follow-up (p=0.85, repeated measures ANOVA, effect between treatment groups and dosage of Ach).

TABLE 1 Characteristics of the study population at baseline

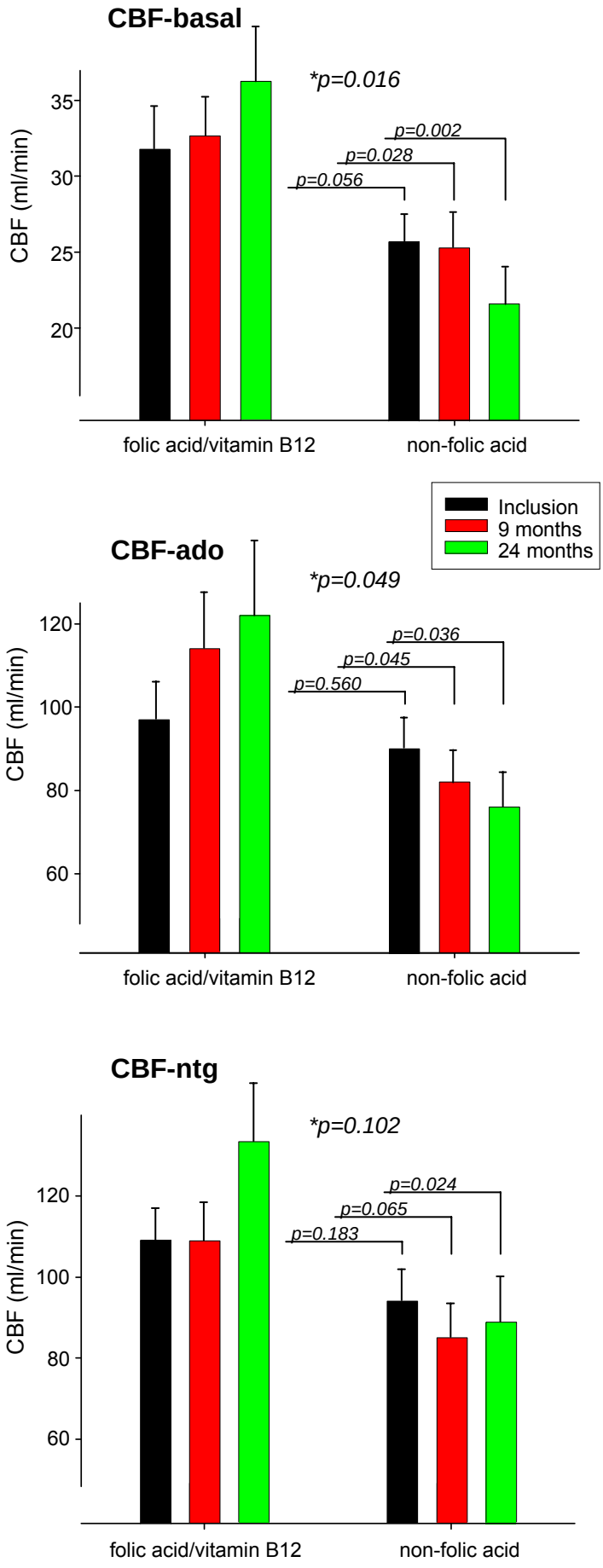
	Total group * (n=40)	*Treatment groups* *					
		Folic acid/B12 (n=20)	Non-folic acid/B12 (n=20)	*P-values*†	B6 (n=20)	Non-B6 (n=20)	*P-values*†
Age, years	57.8 (9.0)	57.4 (10.4)	58.2 (7.7)	0.78	56.3 (6.3)	59.4 (11.1)	0.28
Women, n (%)	8 (20)	3 (15)	5 (25)	0.44	3 (15)	5 (25)	0.44
Current smokers, n (%)	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
Prior PCI, n (%)	9 (23)	4 (20)	5 (25)	0.71	4 (20)	5 (25)	0.71
Prior AMI, n (%)	6 (15)	2 (10)	4 (20)	0.38	2 (10)	4 (20)	0.38
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
Waist, cm	94.2 (9.1)	93.8 (7.6)	94.7 (10.6)	0.79	91.7 (5.4)	96.9 (11.4)	0.08
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
Beta 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
Serum Creatinine, µmol/L	88 (9.9)	89 (11.1))	87 (8.9)	0.67	88 (11.2)	87 (8.7)	0.74
GFR, $mL/min/1.73m^2$	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

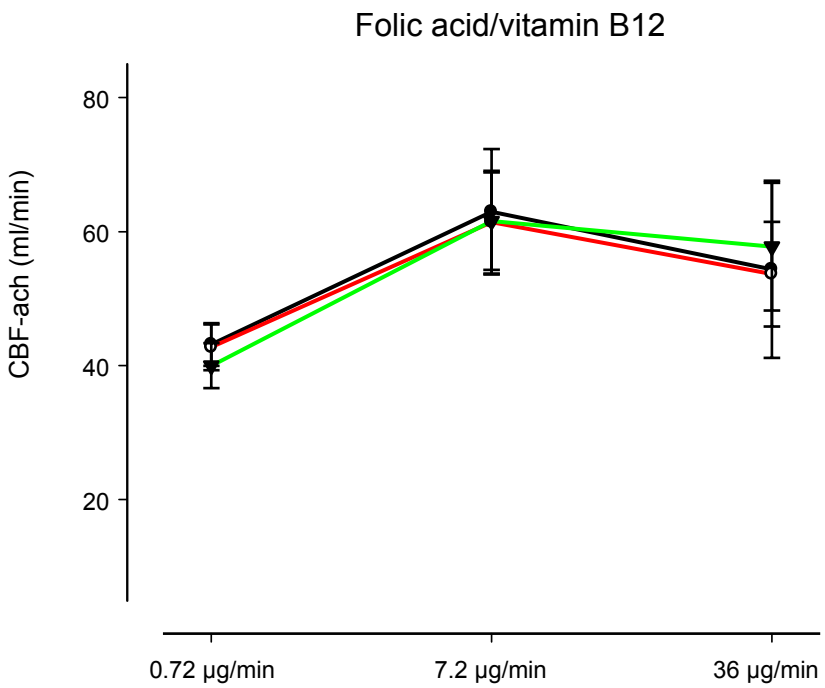
*Mean (SD) or numbers (%), except CRP which is given in median (25-75th percentile). †Comparison of continuous variables between groups by ANOVA, except for CRP in which is comparison is done by Kruskal-Wallis Test. Comparison of proportions between groups by Chi-Square Test. BMI = body mass index, ACEI = angiotensin converting enzyme inhibitor, GFR = estimated glomerular filtration ratio, LDL-cholesterol = low density lipoprotein cholesterol, CRP = C-reactive protein.

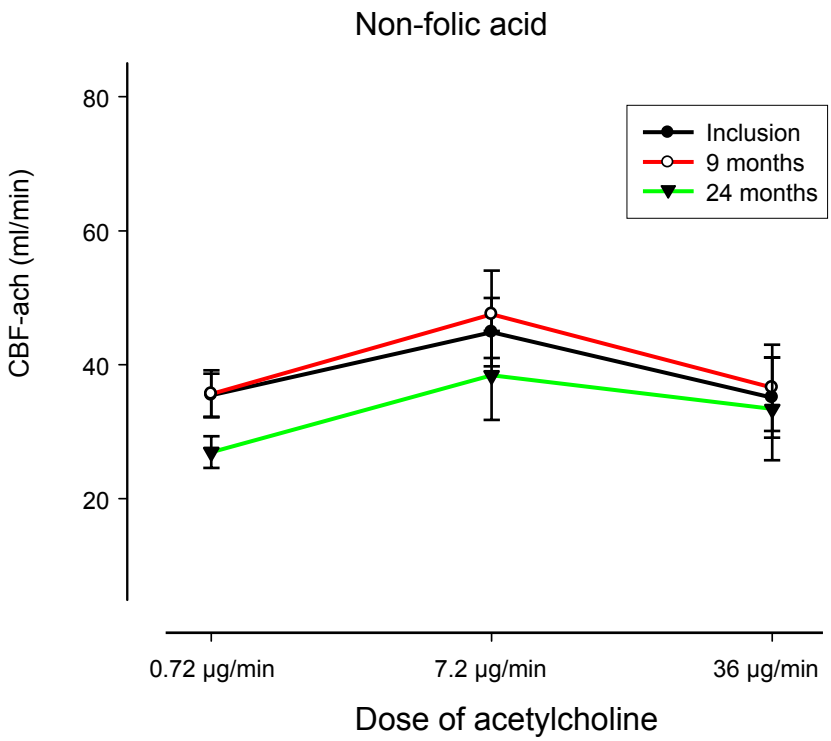
TABLE 2 Coronary blood flow response to acetylcholine infusion at inclusion and after 9 and 24 months of B-vitamin treatment.

			% change in flow compared to baseline (NaCl infusion)*			
Acetylcholine dosage			*Folate*	*Non-Folate*	*B6*	*Non-B6*
0.72 µg/min	Inclusion	(n=40)	39.8 (8.7)	38.1 (9.5)	43.7 (9.8)	34.2 (8.3)
	9 months	(n=40)	34.8 (9.2)	39.5 (8.5)	43.9 (9.7)	30.4 (7.6)
	24 months	(n=35)	14.5 (6.8)	35.5 (13.3)	33.5 (9.5)	14.2 (10.7)
	P-values†		p=0.51		p=0.91	
7.2 µg/min	Inclusion	(n=40)	93.4 (20.1)	76.8 (18.4)	105.6 (21.7)	64.7 (16.0)
	9 months	(n=39)	87.3 (19.9)	83.5 (21.8)	108.7 (23.8)	60.9 (15.0)
	24 months	(n=34)	71.8 (14.1)	80.6 (26.8)	99.2 (19.6)	49.8 (20.0)
	P-values†		p=0.60		p=0.60	
36.0 µg/min	Inclusion	(n=38)	54.7 (27.4)	34.1 (21.4)	55.2 (30.1)	32.5 (16.4)
	9 months	(n=35)	77.3 (26.5)	35.3 (22.6)	74.4 (28.7)	33.6 (16.8)
	24 months	(n=32)	53.5 (18.8)	42.0 (23.5)	54.7 (21.6)	40.4 (19.0)
	P-values†		p=0.58		p=0.45	
	P-values overall‡		p=0.66		p=0.77	

*Values in %, mean (SEM). †Repeated measures ANOVA, effect between groups during 24 months. ‡Repeated measures ANOVA, effect between groups and dosage of Ach during 24 months


CBF-basal
*p=0.016
p=0.002
p=0.028
p=0.056
CBF (ml/min)
35
30
25
20
folic acid/vitamin B12
non-folic acid
Inclusion
9 months
24 months
CBF-ado
*p=0.049
p=0.036
p=0.045
p=0.560
120
100
80
60
folic acid/vitamin B12
non-folic acid
CBF-ntg
*p=0.102
p=0.024
p=0.065
p=0.183
120
100
80
60
folic acid/vitamin B12
non-folic acid


Folic acid/vitamin B12
CBF-ach (ml/min)
80
60
40
20
0.72 µg/min
7.2 µg/min
36 µg/min


Non-folic acid
CBF-ach (ml/min)
80
60
40
20
Inclusion
9 months
24 months
0.72 µg/min
7.2 µg/min
36 µg/min
Dose of acetylcholine