Original Article

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2007.01810.x

Homocysteine-lowering therapy does not affect inflammatory markers of atherosclerosis in patients with stable coronary artery disease

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Abstract. Bleie Ø, Semb AG, Grundt H, Nordrehaug JE, Vollset SE, Ueland PM, Nilsen DWT, Bakken AM, Refsum H, Nygård OK (Haukeland University Hospital, Bergen; University of Bergen, Bergen; Diakonhjemmet Hospital, Oslo; Stavanger University Hospital, Stavanger; and Institute of Basic Medical Sciences, University of Oslo, Oslo; Norway; and University of Oxford, Oxford, UK). Homocysteine-lowering therapy does not affect inflammatory markers of atherosclerosis in patients with stable coronary artery disease. *J Intern Med* 2007; **262:** 244–253.

Objectives. A high level of total homocysteine (tHcy) is a risk marker for cardiovascular disease (CVD), and is related to inflammation. We wanted to test the effect of homocysteine-lowering B-vitamin therapy, as used in the Western Norway B-vitamin Intervention Trial (WENBIT), on inflammatory markers associated with atherosclerosis.

Design. Single centre, prospective double-blind clinical interventional study, randomised in a 2×2 factorial design.

Subjects and methods. Ninety patients (21 female) with suspected coronary artery disease (CAD), aged 38–80 years, were blindly randomised into one of four groups of daily oral treatment with (A) folic acid (0.8 mg)/vitamin B12 (0.4 mg)/vitamin B6 (40 mg), (B) folic acid/vitamin B12, (C) vitamin B6 alone or (D) placebo. Blood samples were collected before and after 6 months of treatment.

Results. Before intervention, median levels of the analytes were: tHcy 11.0 μmol L^{-1}, neopterin 8.1 nmol L^{-1}, soluble CD40 ligand (sCD40L) 3.9 ng mL^{-1}, interleukin (IL)-6 1.9 pg mL^{-1}, C-reactive protein (CRP) 1.9 mg L^{-1} and low-density lipoprotein (LDL) cholesterol 3.3 mmol L^{-1}. tHcy was significantly associated with neopterin ($r = 0.49$, $P < 0.001$) and with IL-6 ($r = 0.29$, $P = 0.01$), but not with CRP or sCD40L. Neither treatment with folic acid/B12 nor with B6 induced significant changes in any of these inflammatory biomarkers ($P \geq 0.14$). In patients receiving folic acid/B12 (groups A and B), tHcy was reduced with 33% ($P < 0.001$).

Conclusions. In patients with stable CAD, homocysteine-lowering therapy with B-vitamins does not affect levels of inflammatory markers associated with atherogenesis. Failure to reverse inflammatory processes, may partly explain the negative results in clinical secondary B-vitamin intervention trials.

Keywords: atherosclerosis, B-vitamins, homocysteine, inflammation, neopterin, soluble CD40 ligand.

Introduction

Numerous studies demonstrate that hyperhomocysteinaemia is related to an increased risk of the existence, extent and progression and of prognosis of cardiovascular disease (CVD) [1, 2]. Recent data also indicate that homocysteine is associated with several important components of atherogenesis, including endothelial dysfunction, platelet and immune activation and inflammation [3–5].

Based on these observations, several randomised clinical trials with homocysteine-lowering B-vitamins have been initiated in order to investigate whether homocysteine is causally related to atherosclerosis [6]. In published trials, no significant effect on the primary outcome was documented in patients with stroke (VISP) [7], acute myocardial infarction (NORVIT) [8], CVD (HOPE-2) [9] or chronic renal failure (ASFAST) [10]. A nearly significant increase of myocardial infarction was demonstrated in the NORVIT trial (in the group receiving both folic acid and vitamin B6) [8], whereas a significant reduction in stroke was demonstrated in HOPE-2 [9]. Both the VISP and the ASFAST trials were underpowered to detect clinical effects of the intervention of a magnitude similar to what might have been expected from the epidemiological data [7, 10]. In addition, two randomized trials evaluating restenosis after PCI showed conflicting results [11, 12]. Thus, the results from these clinical trials still leave uncertainty of the possible role of homocysteine in the progression of atherosclerotic disease. It is therefore important to investigate the metabolic effects of B-vitamin supplementation to understand why intervention may fail despite of epidemiological evidence.

Inflammation and persistent immune activation are hallmarks of the atherosclerotic process [13]. The markers C-reactive protein (CRP), CD40 ligand (CD40L) and neopterin represent different inflammatory pathways related to atherogenesis. CRP is an acute phase reactant; the production of CRP is stimulated by interleukin (IL)-6 effects on the liver. CRP represents an overall marker of the inflammation and is extensively investigated and characterized [14]. CD40L is produced by many cell types, but is excreted mainly from platelets and is related to unstable coronary artery disease (CAD) [15]. Soluble CD40L (sCD40L) may therefore reflect the role of activated platelets in inflammation [16]. Neopterin is a pteridine which reflects activation of the cellular part of inflammation, and is released from activated monocytes through stimulation by interferon (IFN)-γ from T lymphocytes [17]. Neopterin has been related to the extent [18], complexity [19] and progression [20] of the atherosclerotic disease. Furthermore, neopterin is strongly related to the homocysteine homeostasis [21].

The current investigation was designed to assess the effects of homocysteine-lowering B-vitamins on inflammatory markers associated with atherosclerosis. The investigation is a substudy of the Western Norway B-vitamin Intervention Trial (WENBIT), a randomised placebo-controlled clinical trial in patients with CAD.

Subjects and methods

Patients, recruitment and study design

Western Norway B-vitamin Intervention Trial is a prospective randomised double-blind study on the clinical effects of homocysteine-lowering therapy in 3098 adult patients undergoing coronary angiography for suspected CAD. Using a 2×2 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 accordingly randomised 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 or Group D: placebo. For the first 2 weeks, Group A and Group B received an additional loading dose of folic acid (5 mg day^{-1}). Packages of trial capsules were prepared and randomised in blocks of 20 by Alpharma A/S (Copenhagen, Denmark).

The current study is confined to a subgroup of 90 consecutive patients who were recruited at start of

the WENBIT study, in the period of April 1999 to September 1999. These patients underwent more extensive follow up during the initial 6 months of the intervention, a follow up designed to investigate mechanisms and biochemical effects of provided treatment. One patient died, two patients withdrew their consent. A total of 83 patients attended the visit at 6 months. This subpopulation of WENBIT has been characterized in detail previously [22]. The study protocol was approved by the Regional ethics committee and by the Norwegian Medicines Agency. Written informed consent was obtained from all patients.

Blood collection and biochemical analyses

In the present study, we used nonfasting blood samples collected at baseline and after 6 months of B-vitamin intervention. EDTA blood samples for analysis of B-vitamins, total homocysteine (tHcy) and metabolites were immediately placed on ice and centrifuged within 30 min. Plasma and serum were stored at −80 °C until further analysis.

Routine blood analyses, including haematological parameters, renal function markers and lipid-related factors, were analysed at Laboratory of Clinical Biochemistry, Haukeland University Hospital, using Technicon Chem 1® (Bayer, Leverkusen, Germany) and CELL-DYN® 4000 (Abbot, Abbott Park, IL, USA) platforms. tHcy and MMA were analysed using a modification of a gas chromatography method based on ethylchloroformate derivatization [23]. Plasma folate [24] and cobalamin [25] were analysed by published methods. Results on vitamin B6 (pyridoxal phosphate, PLP) have previously been presented [26].

Commercially available enzyme immunoassays were used to determine serum levels of sCD40L [intra-assay coefficient of variation (CV) 6.8%; Bender Medsystems, Vienna, Austria] and serum neopterin (CV 3.6–6.8%; IBL, Hamburg, Germany). Analyses were performed according to the manufacturer's instructions and in duplicates on the same microtitre plate. IL-6 was analysed in duplicates by ELISA technique (CV 6.9–7.8%; R&D Systems, Abingdon, UK). CRP was determined in serum by an ultra sensitive immunoassay applying the Behring nephelometer II system (CV 8.1–11.4%; N Latex CRP mono, Behring Diagnostics, Germany).

Glomerular filtration rate (GFR) was calculated according to the 4-variable Modification of Diet in Renal Disease Equation [27], estimating GFR from creatinine, age, gender and ethnicity.

Statistical analysis

Because of the skewed distribution of vitamins and metabolites, continuous variables are reported as medians with interquartile range or geometric mean with reference range. Categorical variables are presented as numbers and proportions. Associations were assessed by Spearman rank correlation and linear regression. Kruskal–Wallis test was used for comparison of continuous variables between treatment groups. Wilcoxon signed rank test was used for comparison within groups over time. Effect of folic acid/B12 or vitamin B6 over time was also studied by repeated-measures ANOVA according to the 2×2 study design, using log-transformed numbers. Power calculations by PASS 2005 (NCSS, Kaysville, UT, USA) and NQUERY ADVISOR 6.0 (Statistical Solutions, Cork, Ireland) were based on a sample volume of 90 patients, a loss to follow up <10% and a significance level of 0.05. We calculated a power of 95% to detect a change of 20% in neopterin level by treatment (patients divided into two groups; folate and non folate or vitamin B6 and non-B6) using Wilcoxon signed rank test. In the univariate two-group repeated measure analysis, a power of 99% to detect a change in neopterin level of 20% by treatment during 6 months was calculated. Data were analysed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics at baseline

A total of 90 patients, all Caucasian, 21 female and 69 male, with median age 62 years (range: 38–80) were included. Major characteristics of the patients are given in Table 1. Except for tHcy ($P = 0.05$),

no significant differences for these variables were found between the four groups. Additional demographic data, including smoking, diabetes, prior myocardial infarction, prior revascularization, body mass index, blood pressure, details about cholesterol, creatinine and vitamin indices, have been presented earlier [22].

Median levels (25–75th percentiles) of key biomarkers was: tHcy 11.0 (9.3–12.9) μmol L^{-1}, neopterin 8.1 (5.4–10.6) nmol L^{-1}, sCD40L 3.9 (2.3–5.6) ng mL^{-1}, IL-6 1.9 (1.1–3.2) pg mL^{-1}, CRP 1.9 (0.9–3.7) mg L^{-1} and LDL cholesterol 3.3 (2.7–4.0) mmol L^{-1}. Baseline levels of inflammatory markers, except for IL-6, did not differ significantly between treatment groups (Table 2).

Association between tHcy or vitamins and inflammatory biomarkers at baseline

The univariate association between tHcy or vitamins and biomarkers at baseline are shown in Table 3. In addition to its well-known relations with age, creatinine, folate and cobalamin, baseline tHcy was positively associated with IL-6, and neopterin. No association was found between tHcy and LDL cholesterol. Amongst the B-vitamins, plasma folate was not associated with any of the biomarkers. Plasma cobalamin was positively related with sCD40L, whereas vitamin B6 (PLP) showed an inverse relation with IL-6. As expected, we observed significant associations between the different inflammatory markers. Both tHcy and neopterin were significantly associated with creatinine, age and gender. After adjustment for GFR, tHcy was still significantly correlated with neopterin ($r = 0.38$, $P < 0.001$), but not IL-6 ($r = 0.13$, $P = 0.23$).

In order to evaluate the association, we carried out multiple linear regression analyses with each of the various inflammatory markers as dependent variables, and tHcy, GFR, LDL cholesterol, folate, vitamin B6 and vitamin B12 as independent variables (all independent variables in each of the analyses). Predictors of neopterin were levels of tHcy ($\beta = 0.35$, $P < 0.001$), GFR ($\beta = -0.37$, $P < 0.001$) and LDL cholesterol ($\beta = 0.34$, $P < 0.001$). sCD40L was borderline associated with GFR ($\beta = 0.25$, $P = 0.05$). No such associations with CRP or IL-6 were found in this model.

Table 1 Characteristics of the study population at baseline

		Treatment groups[a]				
	Total group[a] ($n = 90$)	Folic acid/B12/B6 ($n = 22$)	Folic acid/B12 ($n = 23$)	B6 ($n = 21$)	Placebo ($n = 24$)	P-value[b]
Age (years)	62 (54–68)	64 (55–70)	59 (52–65)	61 (57–71)	64 (53–71)	0.4
Women, *n* (%)	21 (23)	6 (27)	3 (13)	7 (33)	5 (21)	0.4
Creatinine (μmol L^{-1})	94 (87–101)	91 (83–98)	94 (89–101)	90 (82–98)	98 (91–114)	0.07
GFR (mL min/1.73 m^2)	73 (64–79)	75 (65–82)	73 (67–80)	72 (64–80)	74 (58–76)	0.5
Total cholesterol (mmol L^{-1})	5.3 (4.8–6.1)	5.5 (4.7–6.2)	5.3 (4.9–5.7)	5.4 (4.8–6.4)	5.2 (4.2–7.0)	0.9
LDL cholesterol (mmol L^{-1})	3.3 (2.7–4.0)	3.6 (2.7–4.0)	3.3 (2.8–3.9)	3.4 (2.4–4.4)	3.3 (2.3–4.8)	0.9
Statin use, *n* (%)	65 (72)	14 (64)	20 (87)	13 (62)	18 (75)	0.2
tHcy (μmol L^{-1})	11.0 (9.3–12.9)	9.9 (9.0–11.9)	12.0 (10.3–13.1)	11.3 (8.8–12.1)	11.4 (9.9–15.8)	0.05
Plasma folate (nmol L^{-1})	8.2 (6.1–11.1)	8.3 (6.1–10.9)	7.7 (5.6–11.3)	8.2 (6.6–10.7)	9.0 (5.8–11.8)	0.9
Cobalamin (pmol L^{-1})	369 (311–431)	361 (321–402)	400 (316–469)	361 (313–463)	349 (270–400)	0.2
Vitamin B6 (PLP, nmol L^{-1})	23.0 (17.7–37.1)	21.5 (16.5–33.5)	27.9 (19.3–44.6)	21.8 (15.0–39.7)	24.3 (16.1–39.5)	0.6

[a]Data are presented as median (25–75th percentiles) or numbers (%).
[b]Kruskal–Wallis test between treatment groups.
GFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; B6, vitamin B6; PLP, pyridoxal 5-phosphate.

Table 2 Values of inflammatory markers at baseline and after 6 months

		Treatment groups				
	Total group	Folic acid/B12/B6 (A)	Folic acid/B12 (B)	B6 (C)	Placebo (D)	*P*-value[a]
Neopterin (nmol L^{-1})						
n	83	22	21	19	21	
Baseline	7.6 (7.0–8.2)	7.1 (6.1–8.2)	7.9 (6.8–9.3)	6.8 (5.8–8.0)	8.6 (7.1–10.4)	ns
6 months	7.7 (7.1–8.3)	8.1 (7.0–9.3)	7.3 (6.2–8.6)	7.0 (6.2–8.0)	8.3 (6.8–10.1)	ns
P-value[b]	ns	ns	ns	ns	ns	
sCD40L (ng mL^{-1})						
n	81	22	21	19	19	
Baseline	3.6 (3.1–4.1)	3.9 (2.9–5.3)	3.8 (3.0–4.8)	3.5 (2.5–5.0)	3.1 (2.5–3.9)	ns
6 months	3.2 (2.6–4.0)	3.3 (2.1–5.2)	3.6 (2.5–5.3)	3.7 (2.4–5.7)	2.4 (1.4–4.0)	ns
P-value[b]	ns	ns	ns	ns	ns	
IL-6 (pg mL^{-1})						
n	83	22	21	19	21	
Baseline	1.8 (1.5–2.1)	1.5 (1.0–2.0)	1.5 (1.1–2.1)	2.8 (2.1–3.8)	1.8 (1.4–2.5)	0.02
6 months	1.7 (1.5–1.9)	1.6 (1.2–2.2)	1.4 (1.1–1.7)	2.0 (1.5–2.6)	1.8 (1.4–2.3)	ns
P-value[b]	ns	ns	ns	ns	ns	
CRP (mg L^{-1})						
n	83	22	21	19	21	
Baseline	1.8 (1.4–2.3)	1.5 (1.0–2.2)	1.9 (1.3–2.8)	2.4 (1.4–4.1)	1.5 (0.8–2.8)	ns
6 months	1.9 (1.5–2.5)	1.5 (1.0–2.2)	2.4 (1.5–3.8)	2.0 (1.2–3.3)	1.9 (1.0–3.6)	ns
P-value[b]	ns	ns	ns	ns	ns	

[a]Kruskal–Wallis test between treatment groups.
[b]Wilcoxon signed rank test between baseline and 6 months. Data are presented as geometric mean in natural units and geometric reference range in parentheses (antilog: mean log ± 1.96 SEM).
B12, vitamin B12; B6, vitamin B6; sCD40L, soluble CD40 ligand; IL-6, interleukin 6; CRP, C-reactive protein.

Table 3 Spearman correlations at baseline

	Age	Creatinine	GFR	LDL-chol	Neopterin	sCD40L	IL-6	CRP	Folate	Cobalamin	Vitamin 6
tHcy	0.50†	0.44†	−0.34†	−0.09	0.49†	−0.10	0.29†	0.16	−0.21*	−0.21*	−0.02
LDL-chol	−0.13	0.00	−0.03		0.11	0.01	−0.10	0.01	−0.04	0.08	0.09
Neopterin	0.36†	0.41†	−0.45†			0.08	0.30†	0.23*	−0.09	0.07	−0.05
sCD40L	−0.09	−0.15	0.20				0.07	0.01	0.03	0.29†	0.05
IL-6	0.32†	0.07	−0.13					0.41†	−0.09	−0.13	−0.24*
CRP	0.11	0.05	−0.01						−0.09	−0.07	−0.05

*$P < 0.05$; † $P < 0.01$.
GFR, estimated glomerular filtration rate; LDL-chol, low-density lipoprotein cholesterol; sCD40L, soluble CD40 ligand; IL-6, interleukin 6; CRP, C-reactive protein; tHcy, total homocysteine.

Effect of treatment

Combination of folic acid and vitamin B12 caused a significant increase in plasma folate and plasma cobalamin, and a 33% reduction in plasma tHcy (from median 11.0 μmol L^{-1} to median 7.4 μmol L^{-1}, $P < 0.001$; Fig. 1). Neither folic acid/vitamin B12 nor vitamin B6 significantly influenced the levels of the inflammatory markers (Table 2 and Fig. 1). Analysing the effect of folic acid/B12 (combined Groups A + B)

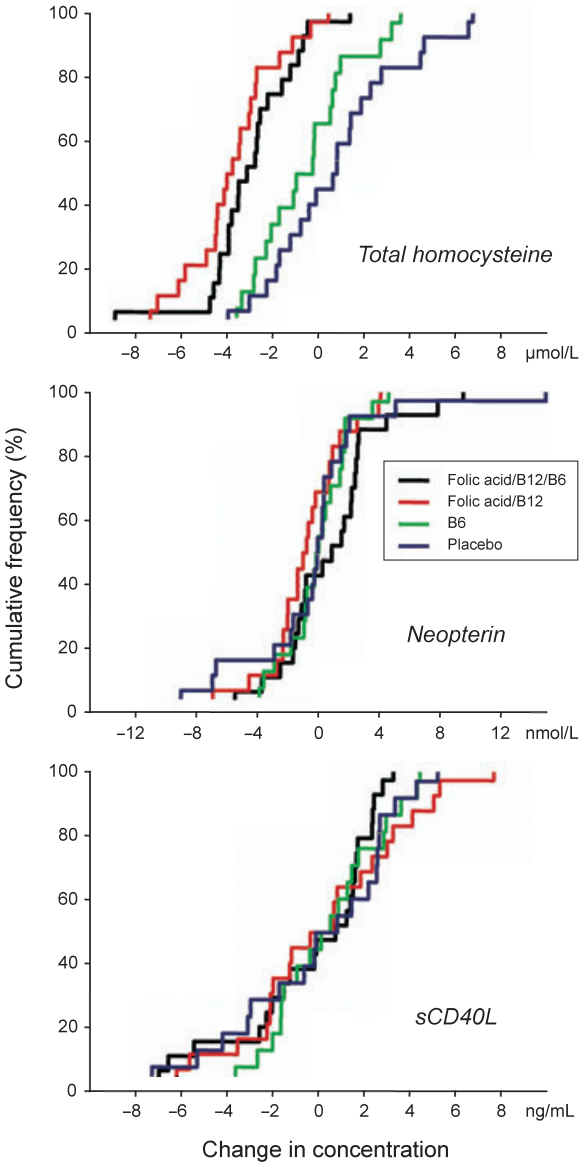

Fig. 1 Cumulative frequency plots of change in concentration between baseline and 6 months of plasma total homocysteine (tHcy; $n = 83$; upper panel), serum neopterin ($n = 83$; middle panel) and serum soluble CD40 ligand (sCD40L; $n = 81$; bottom panel), presented in four treatment groups.

between baseline and 6 months, reveal no significant effect on neopterin ($P = 0.78$), sCD40L ($P = 0.67$), IL-6 ($P = 0.96$) or on CRP ($P = 0.83$). Testing the effect of vitamin B6 (A + C), show a similar negative result; neopterin ($P = 0.25$), sCD40L ($P = 0.45$), IL-6 ($P = 0.50$) and CRP ($P = 0.33$). Tested by repeated measures ANOVA, no effect of folic acid/B12 (Groups A + B versus C + D) was observed on neopterin ($P = 0.76$), sCD40L ($P = 0.99$), IL-6 ($P = 0.34$) or on CRP ($P = 0.83$). Likewise, no effect of vitamin B6 (Groups A + C versus B + D) was observed on neopterin ($P = 0.07$), sCD40L ($P = 0.77$), IL-6 ($P = 0.79$) or on CRP ($P = 0.22$).

The strong relation seen at baseline between tHcy and neopterin in multiple linear regression analyses, was relatively unchanged after 6 months in those subjects receiving vitamin B6 alone or placebo ($\beta = 0.43$, $P = 0.003$). In patients receiving folic acid and vitamin B12, this relation was no longer present ($\beta = -0.05$, $P = 0.8$).

During the observation period, 19 patients started or intensified statin therapy (five patients in Group A, five in Group B, four in Group C and five in Group D) and two patients reduced the statin dose (in Groups C and D). Mean increase in statin dose was equally distributed between Groups A–D (2.8, 2.7, 2.5 and 3.3 mg, respectively). As a result, median LDL cholesterol was reduced by 0.4 mmol L^{-1} ($P = 0.001$) without significant difference in LDL cholesterol between treatment groups ($P > 0.9$). In a multiple linear regression model adjusting for tHcy, GFR, folate, vitamin B6 and vitamin B12, inflammatory markers neopterin, sCD40L, IL-6 or CRP at 6 months were not associated with level of LDL cholesterol ($P \geq 0.1$) or change in LDL cholesterol ($P > 0.3$).

Discussion

In the current substudy of WENBIT, we evaluated the effect of B-vitamin intervention on important biomarkers of atherogenesis. Prior investigations have not only indicated that tHcy is significantly related to many of these processes [5, 28], but also indicate that folate and vitamin B6 are associated with CVD, independent from their relation with tHcy [29]. However, despite the fact that plasma tHcy was lowered by 33% by folate 0.8 mg combined with vitamin B12 0.4 mg, and that a high dose of vitamin B6 (40 mg)

was given, no significant changes in the evaluated biomarkers neopterin, sCD40L, IL-6 or CRP were observed.

Statins is known to have immunomodulatory effects [16], and 72% of patients were on statin therapy at baseline (Table 1). Statin therapy was intensified during the study period, but no differences were observed in LDL cholesterol between groups at follow up. In theory, change in statin therapy and change in level of cholesterol may interfere with the inflammatory markers and even mask an effect of B-vitamin therapy on inflammation. This is, however unlikely, because the LDL reduction was modest and uniform between groups. Moreover, LDL cholesterol at 6 months was not associated with any of the inflammatory markers.

Because a moderate dose of folic acid was provided, we may not exclude that higher doses might have provoked different effects. However, folic acid/vitamin B12 therapy was associated with a marked 33% reduction in tHcy after 6 months. A similar reduction (27%) was found in NORVIT applying an identical regimen [8]. Despite higher doses of folic acid, less tHcy reductions (18–19%) were achieved in other clinical trials. This may partly be due to voluntary intake of B-vitamin supplements [10] or mandatory [7, 9] folate fortification programmes. It is noteworthy that our study is performed in a nonfortified population.

Our observation of a particular strong relation between tHcy and the marker of cellular immune response, neopterin, corroborates results from several previous reports [30, 31]. Neopterin is produced by activated macrophages and dendritic cells in response to stimulation by IFN-γ released from T lymphocytes, which are present at all stages of atherogenesis [17]. IFN-γ also inhibits collagen synthesis in vascular smooth muscle cells and increases collagen degradation by stimulating release of metalloproteinases from macrophages [13]. Elevated levels of neopterin (8.8 nmol L^{-1} compared with 6.9 nmol L^{-1}) [20] have been related to atherosclerotic progression and clinical events, possibly reflecting plaque destabilization associated with cell-mediated (Th1) immune response [5].

It has been suggested that stimulation and proliferation of immune cells may facilitate the production of reactive oxygen species (ROS), which may increase the demand of B-vitamins [5]. Although this was supported by the observation of a significant association between folate status and neopterin in patients with vascular disease [32], folate therapy caused only a minor and nonsignificant reduction of neopterin in the same study [32]. Stimulated human peripheral blood mononuclear cells (PBMCs) may respond with a parallel increase in both neopterin and homocysteine production, indicating that an increased homocysteine level is a direct cause of the Th1 immune response [5].

The most important finding from our study is the lack of effect of B-vitamins on the inflammatory markers, including neopterin, measured at follow up, indicating that vitamin B treatment may not reverse cellular-mediated inflammation involved in atherosclerosis. This is supported by observations on neopterin after B-vitamin treatment in demented patients [33]. Moreover, we found the association between neopterin and homocysteine at baseline was only modestly attenuated when we controlled for GFR, indicating mechanisms for elevated levels of both neopterin and homocysteine independent of renal function. In patients treated with folic acid/vitamin B12, there were no longer an association between homocysteine and neopterin. This suggests that an optimal folate status override the influence of immunostimulation on tHcy plasma levels. Our data further indicate that the utility of homocysteine as a predictor of CVD is limited to subjects not taking folic acid/vitamin B12 supplement.

CD40 ligand is structurally related to tumour necrosis factor (TNF)-α, and the activity of the TNF-α system has recently been shown to be significantly associated with homocysteine concentration [15, 34]. Both transmembrane bound and soluble CD40L, predominantly released from activated platelets, may interact with CD40 resulting in various immunomodulatory or inflammatory responses involved in arthrosclerosis development, progression and plaque destabilization [15, 16]. We found no association between tHcy and

sCD40L in this study, and treatment with B-vitamins did not significantly influence concentrations of sCD40L. Similar results have been observed in patients with cerebrovascular disease [35], although these patients had lower levels of sCD40L compared with our patients (sCD40L 0.55 ng mL^{-1} vs. 3.9 ng mL^{-1}, respectively). Thus, tHcy or B-vitamins may not be an important determinants of platelet activation in CAD patients treated with established medical therapy.

Previous investigations have shown that increasing levels of homocysteine in cell cultures may affect monocytes [36] and endothelial cells [37] to promote IL-6 production. IL-6 is the principal procoagulant cytokine, stimulating increased blood levels of fibrinogen, plasminogen activator inhibitor type 1 and CRP [38], and may promote the atherogenesis [13]. A significant association between tHcy and IL-6 has previously been reported in elderly with diabetes [39], but not in patients with CAD or peripheral artery disease [30]. We found that IL-6 was significantly related to tHcy at baseline, but CRP was not related to tHcy or the B-vitamins. IL-6 and CRP were both unaffected by the B-vitamin treatment, in line with previous results in patients with cerebrovascular disease [35]. Our population had relatively low baseline levels of CRP, possibly reflecting that this cohort comprised medically well treated patients with stable CAD.

Conclusions

Elevation of plasma tHcy is related to cardiovascular events and linked to inflammation in CVD patients not supplemented with B-vitamins. Secondary tHcy-lowering B-vitamin intervention trials have so far demonstrated no significant reduction CVD events [40]. Our study demonstrated that treatment with folic acid/vitamin B12 or vitamin B6 had no detectable effects on levels of neopterin, sCD40L, IL-6 or CRP. Failure to reverse inflammatory processes associated with atherosclerosis may partly explain the negative results of B-vitamin intervention in patients with established CVD treated with conventional therapy.

Conflict of interest statement

P. M. Ueland reports having received consulting fees from Nycomed and is a member of the steering board of both the nonprofit 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 3 March 2005; P. M. 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.

Acknowledgements

We thank Elin Strand, Janne Dyngeland, Gry Kvalheim and their colleagues for organising patient follow up and blood processing and Jannicke Igland for statistical advice.

This study is part of the Biomed project 'Homocysteine Determination in Laboratory Diagnostics' (BMH4-98-3549). Alpharma A/S, Copenhagen, Denmark has provided the study medication free of charge. The project has additionally been financed with the aid of extra funds from Alpharma A/S, Copenhagen, Denmark, The Norwegian Foundation for Health and Rehabilitation, The Norwegian Heart and Lung Association (LHL), Advanced Research Program, Norway, The Norwegian Research Council, The Regional Committee of Health, Region West, Norway and the Foundation to promote research into functional B12-deficiency, Norway.

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