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Homocysteine Hypothesis for Atherothrombotic Cardiovascular Disease

Not Validated

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Homocysteine has been implicated in promoting atherosclerotic and thrombotic vascular disease. During the last decade, the utility of homocysteine in predicting risk for atherothrombotic vascular disease has been evaluated in several observational studies in a large number of patients. These studies show that the overall risk for vascular disease is small, with prospective, longitudinal studies reporting a weaker association between homocysteine and atherothrombotic vascular disease compared to retrospective case-control and cross-sectional studies. Furthermore, randomized controlled trials of homocysteine-lowering therapy have failed to prove a causal relationship. On the basis of these results, there is currently insufficient evidence to recommend routine screening and treatment of elevated homocysteine concentrations with folic acid and other vitamins to prevent atherothrombotic vascular disease. This review outlines the metabolism and pathophysiology of homocysteine, highlights the results of homocysteine observational and interventional trials, and presents areas of uncertainty and potential future work. (J Am Coll Cardiol 2006;48:914–23) © 2006 by the American College of Cardiology Foundation

The homocysteine “hypothesis of arteriosclerosis” was first proposed by McCully (1) in 1969, when he observed premature atherothrombosis of the peripheral, coronary, and cerebral vasculature in children with homocystinuria, an inborn error in methionine metabolism. In 1976, Wilcken and Wilcken (2) provided the first evidence of a relationship between abnormal homocysteine metabolism and coronary artery disease (CAD) in the general population. Since these seminal observations, results from a large number of laboratory and epidemiologic investigations have implied a pathogenetic role for homocysteine in atherosclerotic cardiovascular disease (CVD) (3–8).

Ideally, the declaration of homocysteine as a causal risk factor for CVD should be predicated on fulfilling 3 requirements: 1) establishment of a strong, consistent, and independent association between elevated homocysteine levels and adverse cardiovascular outcomes, derived from robust epidemiologic assessment; 2) elucidation of a coherent mechanism by which homocysteine directly promotes atherothrombosis; and 3) demonstration of cardiovascular risk modification based on prospective, randomized intervention trials aimed at reducing homocysteine levels. In this review, we deconstruct the current evidence base for homocysteine’s role in atherosclerotic vascular disease with respect to these criteria. Our analysis shows that the current data do not support the validity of the homocysteine hypothesis.

HOMOCYSTEINE METABOLISM AND DETERMINANTS OF ELEVATED HOMOCYSTEINE

Homocysteine is a sulfur-containing amino acid produced in the metabolism of the essential amino acid methionine (3). Homocysteine is metabolized through 1 of 2 vitamin-dependent pathways—remethylation (requiring folate and vitamin B₁₂), which converts homocysteine back to methionine, and transsulfuration (requiring vitamin B₁₂), which converts homocysteine to cysteine and taurine (Fig. 1). An alternative remethylation pathway in the liver and kidney utilizes betaine instead of folate (3,4).

Total plasma (or total serum) homocysteine (tHcy) reflects the combined pool of free, bound, reduced, and oxidized forms of homocysteine in the blood (3). Normal tHcy levels range between 5 and 15 $\mu\text{mol/l}$ (12 $\mu\text{mol/l}$ being the upper reference limit for populations on a folic acid-fortified diet, as in North America) with elevations of 16 to 30 $\mu\text{mol/l}$, 31 to 100 $\mu\text{mol/l}$, and >100 $\mu\text{mol/l}$ classified as mild, moderate, and severe hyperhomocysteinemia, respectively (9). Blood levels of tHcy are optimally measured during fasting. However, measurement after methionine load may be more sensitive in identifying mild disturbances in homocysteine metabolism (10).

Several dietary and lifestyle factors, genetic defects, nutritional deficiencies, and other etiologies can cause elevations in homocysteine (3,4) (Fig. 1). A thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity (C677T mutation) is the most common form of genetic hyperhomocysteinemia (5% to 14% of the general population is homozygous for this mutation). However, an association of this mutation with increased CVD risk is manifest only in populations characterized by low baseline folate levels (8). Deficiency of folic

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Abbreviations and Acronyms

CAD	= coronary artery disease
CVD	= cardiovascular disease
HOPE-2	= Heart Outcomes Prevention Evaluation-2 trial
IHD	= ischemic heart disease
MACE	= major adverse cardiac event
MI	= myocardial infarction
MTHFR	= methylene tetrahydrofolate reductase
NORVIT	= Norwegian Vitamin Trial
tHcy	= total homocysteine
VISP	= Vitamin Intervention for Stroke Prevention study

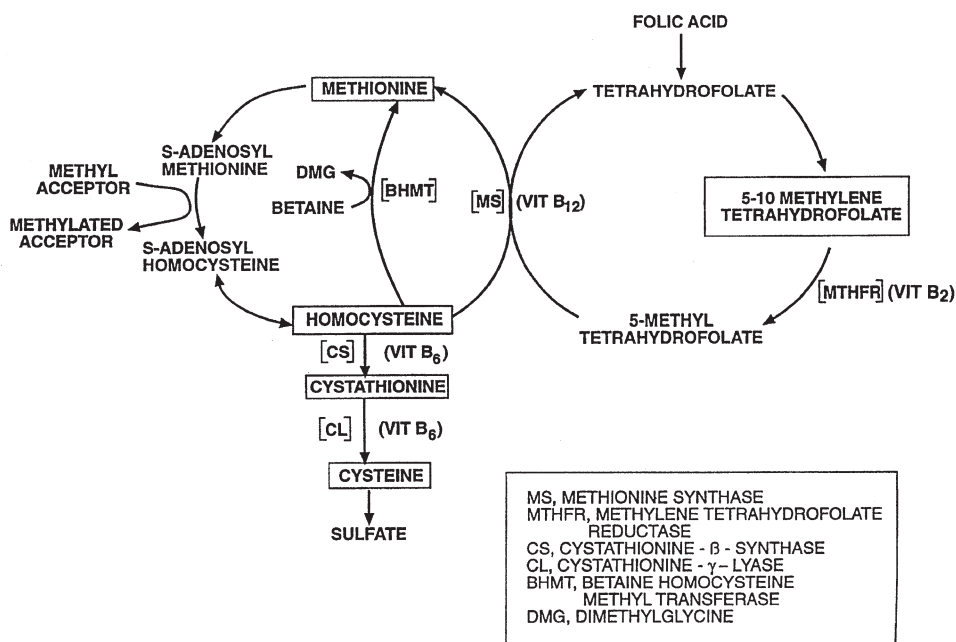
acid, vitamin B₆, and vitamin B₁₂ accounts for the majority (two-thirds) of cases of elevated homocysteine in the general population (11).

The therapeutic options for lowering elevated homocysteine are summarized in Figure 1. Folate supplementation (0.5 to 5 mg/day) significantly reduces tHcy concentration

by 25% in patients with mild to moderate hyperhomocysteinemia (12). Supplementation with vitamin B₁₂ produces a small additional effect (7%), whereas vitamin B₆ treatment alone only reduces post-methionine load concentrations (12). Betaine (trimethylglycine) reduces fasting homocysteine by 12% to 20% without altering folate levels (13). Choline, a precursor to betaine, decreases fasting and post-methionine load homocysteine levels. Both betaine and choline can have an adverse impact on lipid profile.

EPIDEMIOLOGICAL EVIDENCE LINKING HOMOCYSTEINE AND ATHEROTHROMBOTIC VASCULAR DISEASE

Observational studies. Case-control and prospective studies demonstrate a graded and independent association between tHcy and cardiovascular risk. The results of these investigations have been compiled into 2 large, independent meta-analyses (7,8).



Causes of Hyperhomocysteinemia

- Genetic enzyme polymorphisms MTHFR, methionine synthase, cystathionine B synthase
- Dietary deficiency
Folic acid, vitamin B12, vitamin B6, methionine
- Lifestyle factors
Chronic alcohol intake, smoking, high coffee intake
- Renal failure
- End-stage diabetes
- Systemic lupus erythematosus
- Hyperproliferative disorders
- Medications (methotrexate, sulfonamides, antacids)

Therapeutic Options to Lower Homocysteine

- Folic acid, 500 to 5000 mcg
- Vitamin B6, 10 to 500 mg
- Vitamin B12, 1000 to 3000 mcg
- Trimethylglycine (TMG), 500 to 9000 mg
- Choline, 250 to 3000 mg
- Inositol, 250 to 1000 mg
- Zinc, 30 to 90 mg
- S-adenosyl-methionine, 200 to 800 mg

Figure 1. Outline of methionine/homocysteine metabolism, causes of hyperhomocysteinemia, and therapeutic options for lowering homocysteine. Vitamin coenzymes and substrates: THF, tetrahydrofolate; B₂, riboflavin; B₆, vitamin B₆ as its biologically active form, i.e., pyridoxal 5'-phosphate; and B₁₂, methyl cobalamin. Intermediate metabolite: DMG, dimethylglycine. Adapted, with permission, from Malinow et al. (48).

The Homocysteine Studies Collaboration (8) pooled evidence from 12 prospective and 18 retrospective studies from 1966 to 1999. A total of 5,073 CAD events and 1,113 stroke events were observed among 16,786 healthy individuals. The results showed that a 25% increase in the serum homocysteine concentration (an increase of approximately 3 $\mu\text{mol/l}$) is associated with a 49% higher risk of ischemic heart disease (IHD) in the retrospective studies. In contrast, a weaker association was observed in the prospective studies (20% risk increase) (Table 1). With respect to stroke, the strength of association was reversed—a nonsignificant 16% higher risk in retrospective studies compared with a significant 30% higher risk in prospective studies (Table 1). After adjustment for confounding by known cardiovascular risk factors and regression dilution bias, the strength of association was attenuated—from a 49% to a 12% increase in the risk of IHD and from a 30% to a 23% increase in the risk of stroke (Table 1).

An independent meta-analysis of 72 retrospective studies by Wald et al. (7), in which the prevalence of a mutation in the MTHFR gene was determined in 16,849 people, showed increased odds of IHD and stroke for homozygotes for the mutation (TT) compared with wild-type homozygotes (Table 1). A 5- $\mu\text{mol/l}$ increase in tHcy level was associated with an increased risk of IHD and stroke (Table 1). Similar to the findings observed in the previous meta-analysis, the increase in adjusted risk for IHD was lower in the prospective (19%) compared with the retrospective studies (43%) (Table 1). On the basis of this meta-analysis, a decrease in serum homocysteine of 3 $\mu\text{mol/l}$ (achievable by daily intake of about 0.8 mg folic acid) would reduce the risk of IHD by 16% and stroke by 24% (8).

Restenosis studies. Despite the potential involvement of hyperhomocysteinemia in the restenotic process suggested by experimental studies (14,15), clinical studies have failed

to demonstrate this relationship after stent implantation. In a recently published report of pooled analysis of 1,429 patients, 383 (26.8%) had hyperhomocysteinemia ($>15 \mu\text{mol/l}$) that was not associated with higher rates of in-stent restenosis (29.0% vs. 29.5%, $p = 0.47$) (16) (Table 1).

Dietary studies. Finally, large observational studies correlating diet with long-term risk of vascular events among more than 50,000 healthy individuals suggest that a decreased dietary intake of folate is associated with an increased risk of ischemic stroke and cardiovascular events, independent of major lifestyle and other dietary factors (17).

In summary, the totality of evidence from epidemiologic observations suggests a graded and independent relationship between homocysteine and atherothrombotic vascular risk. Stronger associations are found in studies that used less robust methods (case-control), and weaker associations were reported by more robust prospective cohort studies. It is quite plausible that the relationship between hyperhomocysteinemia and CVD is indirect, and is confounded by other factors (e.g., deficiencies of folate, vitamin B₁₂, or vitamin B₆ and renal insufficiency) that influence both homocysteine levels and cardiovascular risk. Results from long-term follow-up of the Kuopio Ischemic Heart Disease Risk Factor Study (18) demonstrate that low folate concentrations were associated with increased risk of CAD events independent of homocysteine concentrations. Therefore, homocysteine may be overrated as a causal risk factor for atherothrombotic vascular disease. Findings that tHcy levels typically rise after an acute vascular event in response to tissue damage or repair and remain elevated for months following the acute event (19,20) suggest that elevated homocysteine may be a “consequence” rather than a “cause” of vascular disease (20).

Table 1. Summary of Meta-Analyses Studying Relationship of Serum Homocysteine and Ischemic Heart Disease, Stroke, and In-Stent Restenosis

Source	Study Types (No. of Studies)	Sample Size	Variable	Rate Ratio (95% CI)
Homocysteine Studies Collaboration, 2002 (6)	Retrospective (18)	7,761	+3 $\mu\text{mol/l}$ tHcy*	1.49 (1.41–1.61): IHD 1.16 (0.99–1.37): stroke
	Prospective (12)	9,025	+3 $\mu\text{mol/l}$ tHcy*	1.20 (1.12–1.30): IHD 1.30 (1.11–1.52): stroke 1.12 (1.04–1.20): IHD† 1.23 (1.05–1.45): stroke‡
Wald et al. 2002 (7)	Retrospective (72)	16,849	MTHFR (TT vs. wild-type)	1.21 (1.06–1.39): IHD 1.31 (0.80–2.15): stroke
	Prospective (20)	3,820	+5 $\mu\text{mol/l}$ tHcy†	1.43 (1.11–1.84): IHD 1.65 (0.66–4.13): stroke 1.19 (1.12–1.25): IHD† 1.32 (1.18–1.49): stroke‡
Homocysteine and In-Stent Restenosis Pooled Analysis, 2005 (16)	Retrospective (4)	383	$>15\mu\text{mol/l}$ tHcy	1.10 (0.85–1.43): in-stent restenosis
		1,046	$\leq 15\mu\text{mol/l}$ tHcy	

*Denotes a 3- $\mu\text{mol/l}$ increase in serum homocysteine concentration between study groups (the original data have been modified to show higher risk with higher homocysteine).
†Signifies a 5- $\mu\text{mol/l}$ increase in serum homocysteine concentration between study groups. ‡Adjusted for traditional cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes, etc., and regression dilution bias.

CI = confidence interval; IHD = ischemic heart disease; MTHFR = methylene tetrahydrofolate reductase; tHcy = total homocysteine levels.

PATHOPHYSIOLOGIC MECHANISMS LINKING HOMOCYSTEINE AND ATHEROTHROMBOTIC VASCULAR DISEASE

The mechanisms by which elevated homocysteine impairs vascular function are not completely understood. Laboratory investigations have revealed several potential mechanisms (Table 2), including impairment of endothelial function (21,22), oxidation of low-density lipids (23), increased monocyte adhesion to the vessel wall (4), increased lipid uptake and retention (4), activation of the inflammatory pathway (4,24), stimulatory effects on smooth-muscle proliferation (4), and thrombotic tendency mediated by activation of coagulation factors (21) and platelet dysfunction (25). The atherogenic and thrombogenic potentials of homocysteine have been implicated in promoting endothelial dysfunction induced by acute hyperhomocysteinemia after methionine loading in human subjects (26), facilitating the progression of atherosclerotic plaque in apolipoprotein E-deficient mice (24), promotion of prothrombotic state (4), and exacerbation of intimal hyperplasia and restenosis after balloon injury of arteries (14,15).

These findings provide a coherent and biologically plausible basis for a direct role for homocysteine in promoting atherothrombosis.

Table 2. Pathophysiologic Mechanisms Relating Homocysteine to Atherothrombosis

Atherogenesis

- Induces vascular inflammation via expression of TNF- α and iNOS
- Increases oxidative stress
- Induces DNA hypomethylation and gene expression for cell growth and differentiation
- Promotes the oxidation of low-density lipoprotein
- Enhances uptake of modified lipoproteins by macrophages
- Induces endothelial dysfunction via increased oxidant stress, increased ADMA, increased inflammation, and decreased bioavailability of NO
- Promotes lipid accumulation via induction of HMG-CoA reductase
- Stimulates vascular smooth muscle cell (VSMC) DNA synthesis and proliferation
- Directly toxic to endothelial cells

Thrombogenesis

- Induces tissue factor activity
- Promotes leukocyte-endothelial interactions via MCP-1 and IL-8 expression
- Enhances endothelial-cell-associated factor V activity
- Impairs inactivation of factor Va by activated protein C
- Inhibits the binding of antithrombin III to the endothelium
- Reduces endothelial binding sites for tissue plasminogen activator
- Enhances binding of lipoprotein (a) to fibrin
- Decreases cell surface thrombomodulin and protein-C activation
- Increases platelet aggregation

ADMA = asymmetric dimethylarginine; HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA; IL = interleukin; iNOS = inducible nitric acid synthase; MCP = monocyte chemoattractant protein; NO = nitric oxide; VSMC = vascular smooth muscle cell.

IMPACT OF LOWERING ELEVATED HOMOCYSTEINE ON ATHEROTHROMBOTIC VASCULAR DISEASE

Several randomized controlled trials have investigated the effect of folic acid and/or vitamins B₆ and B₁₂ supplementation on multiple surrogate markers of CVD, restenosis, and serious clinical outcome events, such as stroke, myocardial infarction (MI), or death.

Impact on surrogate outcomes. Several lines of evidence demonstrate that lowering of homocysteine may favorably alter surrogate cardiovascular outcomes. First, supplementation with folate and vitamin B₁₂ has been shown to prevent post-prandial endothelial dysfunction in subjects with normal homocysteine levels and to improve endothelial function in patients with hyperhomocysteinemia, CAD, or risk factors for CAD (27). It is not clear whether this effect is directly related to lowering of homocysteine or mediated via an indirect homocysteine-independent mechanism (5,28–30). For example, folate stimulates tetrahydrobiopterin regeneration (28) and counteracts homocysteine inhibition of endothelial nitric oxide synthase (29), thereby improving nitric oxide bioavailability. In addition, vitamin B₆, via its effects on the glutathione antioxidant system (30), could attenuate the oxidant stress associated with hyperhomocysteinemia, thereby leading to improved endothelial function. Second, vitamin treatment decreases the incidence of positive stress electrocardiograms in healthy siblings of patients with atherothrombotic disease without improving peripheral arterial abnormalities (Table 3) (31). Third, patients treated with a combination of folate, vitamin B₁₂, and vitamin B₆ show a significant regression in carotid plaque area, even those with homocysteine concentrations <14 $\mu\text{mol/l}$ (32). In renal transplant recipients, treatment with B-vitamins decreased carotid intima-media thickness by 32% compared to a 23% increase in those treated with placebo (Table 3) (33).

In contrast to the positive studies, a recent study failed to demonstrate any beneficial effect of homocysteine lowering on inflammatory markers such as C-reactive protein, soluble intercellular adhesion molecule-1, oxidized low-density lipoprotein, and autoantibodies against oxidized low-density lipoprotein that have been implicated in atherothrombosis (34). The null findings were observed despite a 4-fold increase in serum folate and a 25% reduction in homocysteine levels.

Impact on clinical outcomes. The evidence that lowering of homocysteine by vitamin supplementation reduces cardiovascular clinical outcomes in patients with elevated homocysteine comes primarily from secondary prevention randomized trials—2 evaluating the impact on restenosis and 3 evaluating the impact on hard clinical outcomes (Table 3).

Homocysteine-lowering and restenosis. A prospective, double-blinded, randomized clinical trial in 205 patients with baseline normal-to-mild hyperhomocysteinemia (11 $\mu\text{mol/l}$) provided the first evidence of reduction of angiographic restenosis 6 months after coronary angioplasty and/or bare-metal

Table 3. Summary of Randomized Controlled Trials Investigating the Impact of Homocysteine-Lowering Therapy on Cardiovascular Outcomes

Study	Sample Size	Study Duration	Treatment Groups	Outcomes Rate Ratio (95% CI)
Surrogate outcomes				
Vermeulen et al., (31) 2000 (volunteers)	78	24 months	5 mg FA, 250 mg B ₆	0.4 (0.2–0.9): + stress ECG
	80		vs. placebo	0.9 (0.6–1.3): ankle-brachial index 1.0 (0.3–4.1): PAD 0.9 (0.5–1.6): carotid stenosis
Marcucci et al., (38) 2003 (post-renal transplant)	25	6 months	5 mg FA, 50 mg B ₆ , 4 mg B ₁₂	32 ± 13% decrease in CIMT
	28		vs. placebo	23 ± 21% increase in CIMT
Clinical outcomes				
Schnyder et al., (35) 2001 (post-PTCA/stenting)	272	6 months	1 mg FA, 10 mg B ₆ , 0.4 mg B ₁₂	0.46 (0.28–0.73): restenosis
	281		vs. placebo	0.52 (0.28–0.98): MACE 0.48 (0.25–0.94): TLR
The Swiss Heart Study, Schnyder et al., (36) 2002 (post-PTCA/stenting)	272	12 months	1 mg FA, 10 mg B ₆ , 0.4 mg B ₁₂	0.68 (0.48–0.96): MACE
	281		vs. placebo (× 6 months)	0.62 (0.40–0.97): TLR 0.60 (0.24–1.51): nonfatal MI 0.52 (0.13–2.04): cardiac death 0.54 (0.16–1.70): mortality
Lange et al., (37) 2004 (post-stenting)	316	6 months	1.2 mg FA, 48 mg B ₆ , 60 μg B ₁₂ *	1.30 (1.0–1.69): restenosis
	320		vs. placebo	1.53 (1.03–2.28): MACE
VISP Trial, (40) 2004 (post-stroke)	1,827	24 months	2.5 mg FA, 25 mg B ₆ , 0.4 mg B ₁₂	1.0 (0.8–1.3): recurrent stroke
	1,853		vs. 0.02 mg FA, 0.2 mg B ₆ , 6 μg B ₁₂	0.9 (0.7–1.2): MACE 0.9 (0.7–1.1): mortality
NORVIT trial, (42) 2005 (post-MI)	937	40 months	0.8 mg FA, 40 mg B ₆ , 0.4 mg B ₁₂ (group A)	1.08 (0.93–1.25) MI, stroke, SCD†
	935		0.8 mg FA, 0.4 mg B ₁₂ (group B)	1.02 (0.83–1.26): death†
	934		40 mg B ₆ (group C)	1.06 (0.91–1.24): MI†
	943		Placebo (group D)	1.02 (0.68–1.51): stroke†
				1.22 (0.88–1.70): cancer† 1.22 (1.00–1.50): MI, stroke, SCD‡ 1.21 (0.91–1.61): death‡ 1.23 (0.99–1.52): MI‡ 0.83 (0.47–1.47): stroke‡ 1.02 (0.65–1.58): cancer‡
HOPE trial, (43) 2006 (vascular disease, diabetes)	2,758	60 months	2.5 mg FA, 50 mg B ₆ , 1.0 mg B ₁₂	0.95 (0.84–1.07): CV death, MI, stroke
	2,764		vs. placebo	0.96 (0.81–1.13): CV death 0.98 (0.85–1.14): MI 0.75 (0.59–0.97): stroke

*Patients received 1 initial intravenous bolus of 1 mg FA, 5 mg vitamin B₆, and 1 mg of vitamin B₁₂ given on day 1. The oral dosages listed were continued thereafter. †The results are shown as comparisons between groups A and B vs. groups C and D. ‡The results are shown as comparisons between group A vs group D. The primary end point for each trial is shown in **bold**.

CIMT = carotid intima-media thickness; CV = cardiovascular; ECG = electrocardiogram; FA = folic acid; HOPE = Heart Outcomes Prevention Evaluation trial; MACE = major adverse cardiac event; MI = myocardial infarction; NORVIT = Norwegian Vitamin Trial; PAD = peripheral arterial disease; PTCA = percutaneous transluminal coronary angioplasty; SCD = sudden cardiac death; TLR = target lesion revascularization; VISP = Vitamin Intervention for Stroke Prevention study.

stenting associated with vitamin supplementation (35). The restenosis benefit was primarily observed in patients undergoing angioplasty (10.3% vs. 41.9%, $p < 0.001$), but not bare-metal stenting (20.6% vs. 29.9%, $p = 0.32$).

An extension of this study (Swiss Heart Study) aimed to evaluate the impact of homocysteine-lowering therapy on a major adverse cardiac event (MACE) in 553 patients treated with angioplasty, with or without stenting (Table 2) (36). Although therapy did show significant risk reduction in MACE compared to placebo, this was primarily driven by a reduction in target lesion revascularization, with no significant impact on nonfatal MI or death.

The second study was also a placebo-controlled trial, in which Lange et al. (37) examined the effect of vitamin therapy at higher doses on 636 patients after successful coronary stenting with bare-metal stents. The multi-vitamin therapy successfully reduced serum homocysteine levels, but was associated with a paradoxical increase in restenosis (target vessel revascularization) and MACE at 6 months, particularly in patients with homocysteine levels in the normal range ($<15 \mu\text{mol/l}$) (36.2% vs. 25.3%, $p = 0.02$), whereas slight benefits were observed in patients with elevated homocysteine (27.2% vs. 31.7%, $p = \text{NS}$). Clinical end points such as death and nonfatal MI were not affected

significantly. The divergent findings of this study compared with previous observations may likely be related to the use of: 1) a higher treatment dose of folate and vitamins, especially vitamin B₆ (including intravenous loading); 2) 100% use of bare-metal stents (50% in past studies); 3) longer lesion length; 4) lower-risk patient population (more smokers, diabetic patients, and patients with prior MIs in former studies); 5) higher serum homocysteine levels; and 6) limited angiographic follow-up (76% compared with 100%). Although the increased risk of bare-metal stent-associated restenosis with high-dose homocysteine-lowering therapy is of some concern, it is unclear what impact this will have in clinical practice given the current widespread adoption of drug-eluting stents over bare-metal stents in the U.S. (>85% to 90% of all coronary interventions).

Homocysteine lowering and hard vascular event outcomes. Two smaller open-label studies had previously failed to show any beneficial effects of administration of folic acid added to statin therapy on cardiovascular events in a population of post-acute MI patients (FOLARDA [Folic Acid on Risk Diminishment After Acute Myocardial Infarction] trial, n = 283) (38) or stable CAD (the Goes study, n = 583) (39) despite significant lowering in homocysteine levels.

Three recent large, multicenter, double-blind, randomized studies have evaluated the impact of homocysteine-lowering therapy for secondary prevention of stroke and MI in high-risk individuals.

The VISP (Vitamin Intervention for Stroke Prevention) study was the first large-scale randomized interventional trial to report hard end points (40). The trial investigated the lowering of homocysteine with high-dose compared with low-dose vitamin B formulation in patients with ischemic stroke. Compared to the low-dose group, treatment with the high-dose formulation had no effect on recurrent stroke, coronary events, or deaths (Table 3). The results of the VISP trial were contrary to expectations and indicate that B-vitamins and homocysteine may not be important for development of strokes. However, there are several limitations of the VISP trial that might preclude drawing any definitive conclusions. First, a less-than-expected between-group difference of 2 $\mu\text{mol/l}$ in homocysteine concentrations, and the enrollment of patients with only mild increases in baseline homocysteine concentrations (reflecting the high prevalence of vitamin supplement use and widespread folic acid fortification in North America since the initiation of the VISP trial) may have masked the effect of treatment on stroke risk. Second, treatment of both groups with low-dose vitamin formulation in the 1-month run-in phase (the dose of vitamin B₁₂ at 2.5 \times the recommended daily allowance) may have been sufficient to normalize vitamin B₁₂ status in most patients in the low-dose group, potentially resulting in dilution of the treatment effects. Third, the rates of recurrent strokes were lower than anticipated in both study groups, and the follow-up period was short (2 years). Fourth, the trial was not placebo-

controlled, but rather a head-to-head comparison of daily high-dose against low-dose vitamin formulations. All of these factors limited the statistical power of the VISP trial to reliably identify or exclude a modest, but clinically important, therapeutic effect of vitamins.

In a post-hoc subgroup analysis confined to treatment responders (n = 2,155), the VISP investigators recently reported a 21% reduction in the risk of combined end point of ischemic stroke, coronary disease, or death in the high-dose compared with the low-dose group (unadjusted p = 0.049; adjusted for age, gender, blood pressure, smoking, and B₁₂ level, p = 0.056) (41). Analysis of vitamin B₁₂ blood levels revealed that patients with a baseline B₁₂ level \geq the median level randomized to high-dose vitamin had the best overall outcome, and those with B₁₂ < median level assigned to a low-dose vitamin formulation had the worst (p = 0.02 for combined stroke, death, and coronary events; p = 0.03 for stroke and coronary events). These hypothesis-generating data suggest that in the era of folate fortification, higher doses of B₁₂ and other treatments may be needed for some patients to reduce clinical outcomes.

The NORVIT (Norwegian Vitamin Trial) was designed as a randomized, controlled, double-blind, multicenter, secondary prevention trial, testing the hypotheses that long-term (median follow-up of 40 months) treatment with B-vitamins would lower the incidence of MI, stroke, and sudden cardiac death in patients with acute MI (42). The trial recruited 3,749 patients from Norway (where folate fortification of food is not mandatory) who were randomized into 4 groups, as shown in Table 3. The NORVIT patients were medically optimally treated (about 90% on aspirin and beta-blockers, 80% on statins) and had a >90% compliance.

Plasma homocysteine levels decreased by about 27% in patients taking folic acid (whether or not they were also taking vitamin B₆) compared with vitamin B₆- and placebo-treated patients. Plasma folate levels rose 6- to 7-fold with folic acid treatment. The primary end point occurred in approximately 18% of each of the placebo, folic acid + vitamin B₁₂, and vitamin B₆ groups. In the group that received combination therapy (folic acid + vitamin B₁₂ + vitamin B₆), however, there was a nominally significant relative increase in the primary end point by 22% (p = 0.05) and nonfatal MI by 30% (p = 0.05), and a nonsignificant 17% decrease (p = 0.52) in stroke versus placebo. The cumulative hazard ratio for the combination therapy group, as compared with the other 3 groups, was 1.20 (95% confidence interval 1.02 to 1.41). Event rates, including individual components of the primary end point, tended to be higher in the folic acid + vitamin B₁₂ group versus vitamin B₆ and the placebo group (Table 3). Cancer rates were nonsignificantly higher in both of the folic acid groups. Subgroup analyses showed no suggestion of benefit in any subgroup.

The HOPE-2 (Heart Outcomes Prevention Evaluation-2) trial was a randomized, double-blind trial including 5,522

patients 55 years of age or older with a history of vascular disease or diabetes (43). About 70% of the study population was recruited from the U.S. and Canada, where folate fortification of food is mandatory. Patients were randomized to receive a combined pill containing 2.5 mg of folic acid, 50 mg of vitamin B₆, and 1 mg of vitamin B₁₂ or placebo daily for an average of 5 years. The primary outcome was a composite of death from cardiovascular causes, MI, and stroke. Mean homocysteine levels decreased by 2.4 $\mu\text{mol/l}$ (0.3 mg/l) among those receiving the active treatment, while a slight increase of 0.8 $\mu\text{mol/l}$ was seen in the placebo group. Despite this effective homocysteine lowering, however, no significant effect was seen on the primary outcome or the individual components except for a 25% reduction in stroke. An increase in the number of patients hospitalized for unstable angina was observed with active treatment. There was no difference in outcomes in any subgroups, including those from countries with or without folate supplementation or those with higher or lower baseline homocysteine levels.

We performed a Bayesian analysis in which prior information derived from the NORVIT study and the empirical evidence from the HOPE-2 trial were utilized to generate a posterior probability using the method described previously (44). The results are shown in Figure 2 and indicate that the probability of harm exceeds the probability of benefit for the primary composite end point and each of the individual components except for stroke, where the probability of any benefit exceeds 99% and the probability of >10% risk reduction is >93%. Thus, the Bayesian analysis helps clarify the issue raised by the HOPE-2 trial investigators that “the stroke benefit may represent either an overestimate of the real effect or a spurious result due to the play of chance” (43), and suggests a high likelihood of stroke benefit with homocysteine-lowering therapy compared to placebo. These findings are consistent with the epidemiologic observations of a stronger association of elevated homocysteine with stroke compared to IHD (6,7). However, they are in contrast to the findings of a null effect on stroke observed in the VISP trial (40). One possible explanation for the

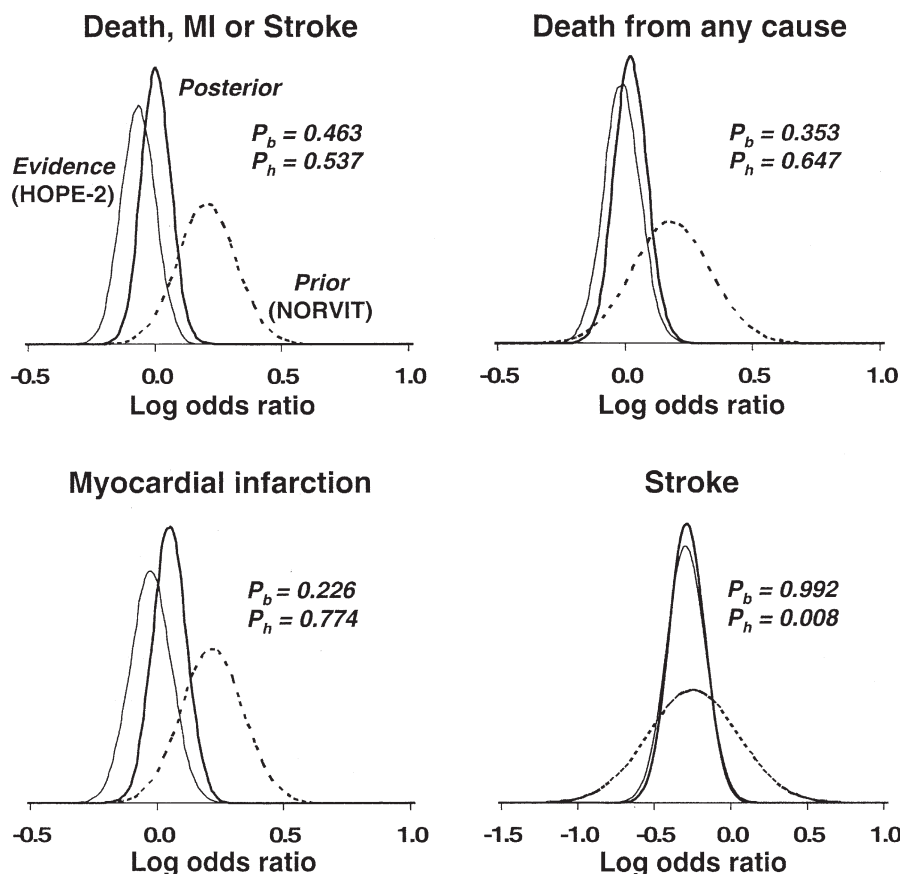


Figure 2. Bayesian analysis of the HOPE-2 trial. Triplots showing posterior (thick line) distributions derived from integrating evidence or likelihood (thin line) from the HOPE-2 trial and informative priors (dashed line) based on information derived from the NORVIT study (only data from comparison of combination therapy group vs. placebo are utilized to match the HOPE-2 trial treatment groups; see Table 3 for details) according to Bayes' theorem (44). Posterior probabilities are estimated using priors for the primary composite end point of death from cardiovascular cause, myocardial infarction (MI), and stroke (log odds ratio $\mu = 0.206$, standard deviation $\sigma = 0.115$), death from any cause ($\mu = 0.183$, $\sigma = 0.153$), myocardial infarction ($\mu = 0.219$, $\sigma = 0.122$), and stroke ($\mu = -0.246$, $\sigma = 0.293$). Probability of any effect size is calculated by computing area under the curve. The probabilities of benefit (P_b , log odds ratio <0) or harm (P_h , log odds ratio >0) are shown on the right of each plot. Superiority or inferiority is inferred at a posterior probability of benefit or harm >0.950 .

discordant results may be the lack of a placebo comparison and a lower-than-expected between-group differential in homocysteine level in the VISP trial, which might have reduced the likelihood of treatment differences. The results from ongoing trials should further clarify this issue.

The results of the NORVIT and HOPE-2 trials do not support the homocysteine hypothesis and suggest that reducing plasma homocysteine is not associated with benefit and may even tend to cause harm by increasing the risk of CVD and the risk of cancer in a manner reminiscent of the vitamins A and E intervention trials (45). A likely explanation for the paradoxical findings in the NORVIT study may be related to the high doses of vitamin B₆ (40 mg), which may exert potentially detrimental effects, especially in the proinflammatory milieu encountered after acute MI. Compared to the group given no vitamin B₆, patients treated with vitamin B₆ experienced an overall 14% increase ($p = 0.09$) (and a statistically significant 28% increase in current smokers) in the primary end point, a 17% increase in MI ($p = 0.05$), and a 19% increase in death from any cause ($p = 0.11$) (42). Additional evidence in support of this finding is provided by previous reports linking high doses of vitamin B₆ (48 mg) with an increased risk of in-stent restenosis (37), compared to a reduction in post-angioplasty restenosis observed with lower doses of vitamin B₆ (10 mg) (35) and increased inflammatory responses and inhibition of angiogenesis observed in laboratory investigations (46,47). Further studies are needed to determine whether folic acid and vitamin B₆ accelerate proliferation of vascular cells and promote growth of cancer cells, particularly at high doses. Other potential explanations include alteration of methylation in vascular cells leading to a proatherogenic phenotype, methylation of L-arginine to asymmetric dimethylarginine that may neutralize the atheroprotective effects of nitric oxide, or simply a chance finding.

In summary, there are currently not enough reliable data from randomized controlled trials that show lowering plasma homocysteine concentration prevents “hard” vascular events. The available evidence is either inadequate or conflicting. Several large-scale studies totaling nearly 50,000 subjects are currently under way in the U.S., Canada, and Europe—WENBIT (the Western Norway B-vitamin Intervention Trial), SEARCH (Study of Effectiveness of Additional Reduction in Cholesterol and Homocysteine) trial, PACIFIC (Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease) trial, VITATOPS (Vitamins to Prevent Stroke) trial, and others. One will have to await the results from these trial data before finally confirming or refuting the homocysteine hypothesis in atherothrombotic vascular disease.

One important point to note is that even if the intervention trials do end up showing an overall positive effect on clinical outcomes, it is difficult to separate the effects of oral folate supplementation from those of lowering homocysteine levels. This can be accomplished via a comparison of folic acid treatment with betaine therapy because, although

both lower circulating homocysteine (by different mechanisms), the latter does not influence folate levels (9). We are not aware of any such comparison currently under evaluation in randomized trials.

CONCLUSIONS

Severe elevation of homocysteine concentration in patients with homocystinuria leads to a high incidence of premature atherothrombotic events. In vitro and in vivo studies demonstrate a plethora of biologically plausible mechanisms that implicate homocysteine in promoting atherosclerotic and thrombotic vascular disease. Numerous observational studies have also reported on the association between mild to moderately elevated homocysteine levels and vascular risk in both the general population and in those with pre-existing vascular disease. The overall risk for vascular disease is small, with prospective studies reporting weaker association compared to retrospective studies. It is unclear whether a causal relationship exists between homocysteine and cardiovascular risk, or if homocysteine is related to other confounding cardiovascular risk factors or is a marker of existing disease burden. Routine screening for elevated homocysteine is not yet recommended (Table 4) (9,48–50). However, screening may be advisable for individuals who manifest atherothrombotic disease that is out of proportion to their traditional risk factors or who have a family history of premature atherosclerotic disease. This can be done via measurement of fasting homocysteine concentrations or by evaluation of post-methionine load levels. Vitamin supplementation with folate, B₆, and B₁₂ significantly lowers homocysteine con-

Table 4. Summary of Recommendations for Screening and Treatment of Elevated Homocysteine*

Screening
<ul style="list-style-type: none"> • Routine screening not yet recommended • Screening may be advisable in individuals who <ul style="list-style-type: none"> - Manifest disease out of proportion to the traditional risk factors - Have a family history of premature atherosclerotic disease
Measurement
<ul style="list-style-type: none"> • Plasma (or serum) total homocysteine, fasting or post-methionine load (more sensitive) • Blood samples must be processed quickly (<1 h at room temperature, <8 h on ice) • Enzymatic or immunologic assays more practical than chromatographic assays • Standardization required to minimize variation among laboratories • Normal range—5 to 15 $\mu\text{mol/l}$ (12 $\mu\text{mol/l}$ as upper limit with folate fortification)
Treatment
<ul style="list-style-type: none"> • Routine treatment with vitamin supplements not yet recommended • Diet rich in folate and B vitamins encouraged • Treatment with folate (0.5 to 5 mg) and vitamin B₁₂ (0.5 to 1 mg) may be beneficial in high-risk patients • Therapeutic target in high-risk individuals <10 $\mu\text{mol/l}$ (U.S.), <13–15 $\mu\text{mol/l}$ (EU) • Vitamin B₁₂ status should be determined before starting therapy

*The recommendations are based on a European Expert Panel (9), The American Heart Association Science Advisory Statement (48), the U.S. Preventive Services Task Force (49), and the American College of Cardiology Foundation Complementary Medicine Expert Consensus Document (50).

centration and has also been shown to alter surrogate cardiovascular end points. Currently, there is no evidence that vitamin B supplementation reduces cardiovascular risk, and there may even be a suggestion of potential harm with treatment with high-dose vitamin B. These findings have brought renewed scrutiny to homocysteine's role in atherothrombotic vascular disease. Whether homocysteine is causative in the pathogenesis of atherothrombotic vascular disease will have to await the completion of a number of large, randomized controlled trials currently studying the effect of homocysteine-lowering vitamins on cardiovascular end points. Until then, the status of homocysteine as a risk factor for vascular disease remains unvalidated.

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Homocysteine Hypothesis for Atherothrombotic Cardiovascular Disease: Not Validated

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