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The Treatment of Hyperhomocysteinemia

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Abstract

The unique biochemical profile of homocysteine is characterized by chemical reactivity supporting a wide range of molecular effects, and a tendency to promote oxidant stress-induced cellular toxicity. Numerous epidemiological reports have established hyperhomocysteinemia as an independent risk factor for cardiovascular disease, cerebrovascular disease, dementia-type disorders, and osteoporosis-associated fractures. Although combined folic acid and B-vitamin therapy substantially reduces homocysteine levels, results from randomized placebo-controlled clinical trials testing the effect of vitamin therapy on outcome in these diseases are mixed, but have generally fallen short of expectations. These results have led some to abandon homocysteine monitoring in the management of patients with cardiovascular or cognitive disorders. These trials, however, have generally included patients with only mildly elevated homocysteine levels, and have not addressed several clinical scenarios in which homocysteine level reduction may be effective, including the primary prevention of atherothrombotic disease in individuals at low- or intermediate-risk, or those with severe hyperhomocysteinemia.

Keywords

B-vitamins; folic acid; cardiovascular risk factor; cognitive impairment; clinical trials; osteoporosis

INTRODUCTION

The demonstration of a concentration-dependent relationship between plasma homocysteine levels and cardiovascular disease risk has generated interest in characterizing to what extent lowering homocysteine levels may improve vascular disease-associated morbidity and mortality. Elevated levels of circulating homocysteine increase the risk for developing atherothrombotic coronary artery disease (CAD), peripheral vascular disease, myocardial infarction (MI), and stroke (1,2). Data in support of this conclusion, however, are derived largely from population-based observational and cross-sectional analyses. Nevertheless, these data, together with the characterization of cellular mechanisms by which homocysteine promotes oxidant stress-induced vascular dysfunction (3-5), have provided ample evidence to support clinical trials of homocysteine lowering with B-vitamins as a novel therapeutic approach to patients with vascular disease.

In addition to cardiovascular disease, hyperhomocysteinemia has been recently identified as a mediator in the development of other clinical entities for which a vasculopathic or microangiopathic substrate is implicated. For example, the adverse effects of homocysteine on

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vascular function and neuroexcitation may contribute to the development and progression of dementia-type disorders (6,7). In fact, homocysteine-associated cellular toxicity has been linked to diseases that include involvement of nearly all organ systems. Children with homocystinuria, in which homocysteine levels are severely elevated, present with a clinical syndrome of dislocated optic lens, premature osteoporosis, mental retardation, in addition to atherothrombosis of medium and small arterial vessels (8). Epidemiological data in adults parallel these observations to some extent: elevated levels of homocysteine are associated with premature diabetic retinopathy (9,10), osteoporosis-induced bone fractures (11), and dementia-type disorders (7).

The identification of homocysteine as a risk factor for CAD and stroke carries important public health implications. If effective, the simplicity, availability, and presumably favorable side effect profile of hyperhomocysteinemia treatment with combined folic acid and oral B₆- and B₁₂-vitamin supplementation (collectively referred to in this text as vitamin therapy) makes this an attractive addition to standard medical therapy for cardiovascular diseases. Indeed, folic acid fortification (140 µg/100g) of the U.S. grain supply in 1996 by the Food and Drug Administration (FDA) is estimated to have decreased the populations' plasma homocysteine levels by ~53% (12); this policy is also believed to have fulfilled its intended goal of decreasing significantly the prevalence of pregnancy-associated neural tube defects associated with low plasma folic acid levels in pregnant woman (RR=0.28, 95% CI 0.12-0.71) (13).

In the past decade, multiple randomized clinical trials have tested the efficacy of homocysteine-lowering therapy on various outcome measures including the secondary prevention of cardiovascular (1,14-18) and cerebrovascular disease (19), progression of cognitive decline (20), and prevention of osteoporosis-related bone fractures (21). Within these outcome measures, conclusions from different trials are mixed, but have, in general, reported negative results. Rather than discounting altogether the potential therapeutic benefit of homocysteine reduction therapy, however, results from these studies may be useful for the enhanced targeting of those in whom hyperhomocysteinemia is a modifiable risk factor for disease. This review provides a comprehensive assessment of current principles in homocysteine pathobiology and summarizes recently published clinical data evaluating the efficacy of treatment for hyperhomocysteinemia.

BIOCHEMISTRY

Nomenclature

A variety of unstable homocysteine species exist in human plasma, presented here in descending relative concentration: protein (albumin)-bound, free circulating disulfide, and sulfhydryl forms (22). Current laboratory methods detect the presence of all three forms and report this as total homocysteine concentration, but reference intervals published for clinical practice may be misleading since they are generally not corrected for factors known to influence circulating homocysteine levels (e.g., ethnicity, gender, etc.). According to the American Heart Association (AHA) advisory statement, normal homocysteine concentrations range from 5-15 µmol/L, although 12 µmol/L is regarded as elevated by others (22,23). *Intermediately* elevated homocysteine levels are between 31-100 µmol/L, while *severely* elevated levels are >100 µmol/L, and are essentially pathognomonic for the presence of an inborn error of homocysteine metabolism causing homocystinuria.

Genetic Etiologies of Hyperhomocysteinemia

Homocysteine is a sulfhydryl-containing amino acid produced from the metabolism of the essential amino acid methionine. Homocysteine can undergo autooxidation, resulting in the formation of key, biologically reactive products (24) that participate in signaling pathways

associated with increased cell toxicity. It follows that homocysteine has been identified as a contributor to four fundamental mechanisms of disease: thrombosis (25) oxidant stress (24), apoptosis (26), and cellular proliferation (27).

Homocysteine metabolism occurs via three pathways; i) remethylation of homocysteine to form methionine by methionine synthase in a vitamin B₁₂ and folate-dependent reaction, ii) the transsulfuration pathway, by which, after the addition of a serine group, homocysteine is converted to cystathionine by cystathionine β -synthase (CBS), and, in certain tissues such as liver and kidney, by iii) remethylation of homocysteine to methionine via betaine:homocysteine methyltransferase (BHMT) (Figure 1) (28).

Several genetically inherited enzyme mutations responsible for elevated homocysteine levels have been described, perhaps the most common of which encodes a cytosine to thymine mutation at nucleotide 677 (C677T) of *N*⁵,*N*¹⁰-methylenetetrahydrofolate reductase (MTHFR). MTHFR facilitates conversion of homocysteine to methionine by methionine synthase; impaired MTHFR activity can increase homocysteine levels by ~25%, and may occur in up to 40% of some U.S. subpopulations (29). Despite a prevailing consensus that the homozygous T/T genotype is an independent risk factor for hyperhomocysteinemia (30,31), evidence from observational studies has been unable to link definitively this mutation to an increased risk for CAD.

There are over 100 disease-associated CBS mutations, making this the most common genetic cause of severely elevated homocysteine levels (32,33). The I278T mutation accounts for ~25% of all homocystinuric alleles, and is a common cause of homocystinuria, a genetically inherited (autosomal recessive) inborn error of metabolism (1:200,000 live U.S. births) (22). The cornerstone clinical features of patients homozygous for homocystinuria are mental retardation, ectopia lentis, and skeletal abnormalities, although premature death is typically a consequence of thromboembolic events (36).

Nutritional etiologies of hyperhomocysteinemia

Normal homocysteine metabolism is dependent upon adequate stores of three dietary vitamins: folic acid, vitamin B₁₂ (cobalamin), and vitamin B₆ (pyridoxal phosphate). Folic acid, or pteroylmonoglutamic acid, is a substrate for cellular production of tetrahydrofolate (THF), a precursor to 5-methyl-THF that is required for normal methionine synthase enzyme activity. By increasing S-adenosylmethionine (SAM) levels, folates transfer 1-carbon moieties to various organic compounds (37), thus contributing to the synthesis of key macromolecules (e.g., purines) integral to basic cellular processes, such as cell growth and proliferation (38).

Folic acid is found primarily in green leafy vegetables and in some animal products (e.g., egg yolk) (38,39). The minimum daily requirement of folic acid is 50 μ g, although the current recommended intake is 400 μ g/d for the average adult and 600 μ g/d during pregnancy (~10 cups of lettuce/day) (8). Because folic acid stores in normal individuals are only 5-20 mg, familiarity with clinical circumstances that either increase the dietary demand of folic acid or impair an individual's ability to absorb folic acid in the proximal jejunum may be useful (Table 1) (38,39).

Cobalamin is an organometallic compound and a key cofactor required for normal methionine synthase activity. Cobalamin cannot be synthesized *de novo* in humans, and adequate stores are maintained by nutritional sources (39). In contrast to folic acid, cobalamin is found exclusively in animal meats or dairy foods derived from animals, placing vegans at risk for cobalamin deficiency (8). In addition, absorption of cobalamin in the distal jejunum requires adequate levels of intrinsic factor that is released in parallel with hydrochloric acid in the stomach. Therefore, in patients at risk for achlorhydria, low intrinsic factor levels, pancreatic

exocrine insufficiency, and inflammatory bowel disease history, or who have a history of gastric surgery may be at increased risk for cobalamin deficiency (40). Perhaps, in part, for these reasons the elderly are particularly prone to cobalamin deficiency; by some estimates, 15% of individuals over age 65 years are cobalamin-deficient (41). In concert with this observation, homocysteine levels appear to increase with age by $\sim 1 \mu\text{mol/L/decade}$ (8).

A hypcobalamin-induced methionine synthase enzymopathy has two effects on homocysteine metabolism. First, homocysteine levels are directly increased by impaired methionine synthase activity. Second, decreased methionine synthase activity also impairs 5-methyl-THF metabolism, which, in turn, increases tetrahydrofolate levels. Under this circumstance, however, tetrahydrofolate is unable to serve efficiently as a precursor to 5-methyl-THF, and so despite adequate nutritional supplies of folic acid, the expected biologic effect of folic acid on homocysteine level reduction is not achieved (*i.e.*, folic acid 'trap') (38,39,42). For this reason, homocysteine lowering therapy often includes folic acid *and* cobalamin supplementation; this combination ensures that methionine synthase cofactor stores are adequate, thereby promoting normal tetrahydrofolate metabolism and remethylation of homocysteine to methionine.

Pyridoxine phosphate is a cofactor necessary for normal CBS enzyme activity. Pyridoxine phosphate is stored hepatically and is found in all food groups, making nutritional deficiency uncommon. However, in patients with a combination of liver disease and poor nutritional status, such as for alcoholics (1.5 g alcohol/kg/d, >5 years) (43), the risk for pyridoxine deficiency is increased (44). Clinical manifestations of pyridoxine phosphate deficiency are typically unrelated to hyperhomocysteinemia, but observational studies have linked low pyridoxine phosphate levels with an increased risk for first-time and recurrent venous thromboembolism (45). Data from an uncontrolled intervention trial suggested that high dose pyridoxine supplementation decreases the incidence of thromboembolism in patients with homocystinuria (RR=0.09, 95% CI 0.02-0.38) (46); however, the benefit of routine surveillance or treatment of low pyridoxine phosphate levels in the general population remains unknown.

PRIMARY DISEASE-ASSOCIATED ETIOLOGIES OF HYPERHOMOCYSTEINEMIA

End stage renal disease

Hyperhomocysteinemia is strongly associated with end-stage renal disease (ESRD) (47). In one case-controlled study, 85% of hemodialysis patients demonstrated homocysteine levels above the 95th percentile for normal controls (48); and hyperhomocysteinemia also significantly increased the risk for vascular comorbidities, (OR=2.9; 95% CI, 1.4 to 5.8), supporting similar conclusions made by others (49,50). The complete mechanism of ESRD-induced hyperhomocysteinemia is unresolved, although high levels of protein-bound homocysteine may impair the glomerular filtration rate (GFR) (51), possibly inducing a circular phenomenon whereby homocysteine-induced GFR reduction further increases circulating homocysteine levels.

Several randomized, placebo-controlled clinical trials (52,53) have tested the effect of homocysteine lowering therapy on reducing the vascular event rate in ESRD patients. Most of these studies, however, included <500 patients and, therefore, were not sufficiently powered to detect a significant benefit in outcome. A multi-centered Veterans Administration collaboration study, however, prospectively examined the effect of vitamin therapy (40 mg of folic acid, 100 mg of Vitamin B₆, and 2 mg of Vitamin B₁₂) or placebo on mortality and vascular comorbidity in over 2,000 ESRD patients (creatinine clearance <30 ml/min) with homocysteine

levels $>15 \mu\text{mol/L}$ (mean $=24 \mu\text{mol/L}$) (47). Despite a decrease in the circulating homocysteine concentration by 25%, vitamin therapy was not associated with a reduction in mortality, MI, stroke, amputations plus mortality, or time to dialysis.

The potential clinical benefits of homocysteine reduction may be offset by the protracted time course and multifactorial pathophysiology of atherosclerotic disease in ESRD. Quantifying the attributable risk of homocysteine in the progression of ESRD-associated vascular disease may not be possible by virtue of epidemiological studies alone, and the results of this study suggest that homocysteine lowering is unable to overcome other mediators of vascular disease in these patients (i.e., dyslipidemia, diabetes mellitus-induced microangiopathy, hyperaldosteronism). Sufficiently powered studies are needed to test the effect of homocysteine lowering therapy for prevention of vascular events in patients with mildly or moderately reduced creatinine clearance.

Other medical etiologies of hyperhomocysteinemia

Hypothyroidism and estrogen deficiency states are associated with hyperhomocysteinemia (8). Estrogen replacement therapy decreases elevated homocysteine levels in post-menopausal women (54), although it remains unresolved as to whether a cardioprotective benefit results from the homocysteine lowering effects of this treatment. Additional diseases associated with hyperhomocysteinemia include acute lymphoblastic leukemia and psoriasis, while the use of tobacco and medications, such as phenytoin, sulfasalazine, and methotrexate, have also been shown to increase homocysteine levels either by directly depleting folate stores, or impairing synthesis of enzyme cofactors required for normal homocysteine metabolism (33).

HOMOCYSTEINE AND CLINICAL DISEASE EXPRESSION

Vascular Disease

Numerous epidemiological reports have established an increased risk for CAD, MI, stroke, venous thromboembolism, and peripheral vascular disease in patients with elevated levels of homocysteine. An early meta-analysis that included 27 retrospective and prospective studies showed an incremental increase in risk of CAD per $5 \mu\text{mol/L}$ increase in tHcy concentration (men, OR=1.6, CI 95% 1.4-1.7; women OR=1.8, CI 95% 1.3-1.9) (1). From this result, the authors extrapolated that 10% of the populations' CAD risk was attributable to hyperhomocysteinemia, and that up to 50,000 deaths from CAD could be prevented annually by homocysteine level reduction. These conclusions were supported by findings from the Homocysteine Studies' Collaboration meta-analysis (2) that showed a risk reduction for ischemic heart disease by 11% and for stroke by 19% per $3 \mu\text{mol/L}$ reduction in homocysteine concentration.

By and large, despite successful reductions in plasma homocysteine levels, vitamin therapy has not improved vascular disease outcome in randomized placebo-controlled clinical trials for patients with mildly elevated homocysteine levels. For example, The Vitamin Intervention For Stroke Prevention (VISP) (19), Heart Outcomes Prevention Evaluation (HOPE)-2 (55), and Norwegian Vitamin Trial (NORVIT) (56) tested the effect of vitamin therapy on the risk of recurrent MI, stroke, and CHD-associated sudden death. In these studies, the composite mean baseline homocysteine level was $12.7 \pm 0.85 \mu\text{mol/L}$, and vitamin therapy decreased homocysteine levels by an average of 21%; however, this was not accompanied by a reduction in primary endpoint outcome measures.

A meta-analysis by Bazzano and colleagues (57) examined 12 randomized, controlled clinical trials (not including HOPE-2 or NORVIT) that tested the effect of folic acid supplementation ($\pm$ vitamins B₆ and B₁₂) for the secondary prevention of cardiovascular and cerebrovascular events (mean treatment time was 26 months, folic acid dose range was 0.5-15 mg/d). In total,

the relative risk for cardiovascular disease was 0.95 (95% CI, 0.88-1.03); for coronary heart disease, 1.04 (95% CI, 0.92-1.17); and for stroke, 0.86 (95% CI, 0.71-1.04) (57). Moreover, a relative risk reduction for CHD of ≤ 0.75 was found in only one of these 12 studies, reported by Schnyder and colleagues (58), who evaluated the effect of vitamin therapy (1 mg/d of folic acid, 400 μ g/d of vitamin B₁₂, 10 mg/d of vitamin B₆) after percutaneous transluminal coronary angioplasty (PTCA) in patients with a mean homocysteine level of 11.3 μ mol/L. A 7.4% decrease in the risk for death, nonfatal myocardial infarction, and need for repeat revascularization was observed (RR=0.68; 95% CI, 0.48-0.96), although others have reported negative results or a greater loss of lumen at follow-up angioplasty in similar patients (59). The differential effect of folic acid therapy in patients who undergo PTCA and receive a coronary stent vs. balloon angioplasty remains a topic of controversy; some have argued that the antithrombotic effect of folic acid may favor those receiving coronary stents, but the routine use of folic acid supplementation in post-PTCA patients is not currently recommended regardless of the type of intervention.

The role of homocysteine lowering therapy in the primary prevention of cardiovascular disease generally remains untested. Data from one meta-analysis that examined the effect vitamin therapy in the primary prevention of stroke, however, are encouraging of an effect (60). In this analysis, 8 randomized clinical trials that included stroke as an endpoint were analyzed. Data on the effect of treatment in 7 trials that upon entry into the study did not identify subjects with pre-existing stroke were compared with data from one trial that recorded subjects' stroke history. Despite the uneven nature of this comparison, folic acid supplementation decreased the relative risk for stroke by 18% (RR=0.82, 95% CI, 0.68-1.00), and a greater benefit was observed with treatment duration of >36 months. Sufficiently powered studies measuring the effect of homocysteine-lowering therapy in the primary prevention of CHD, MI, and stroke are ongoing.

There are several possible reasons to explain inconsistencies between conclusions drawn from epidemiological studies and intervention trials. First, randomized clinical trials have, by and large, included only patients with mildly elevated levels of homocysteine (≤ 15 μ mol/L). Atherothrombotic vascular disease is a consequence of multiple dysregulated cell signaling pathways that produce changes in vascular architecture and function over a long period of time. Accurately characterizing the attributable risk of mildly elevated homocysteine levels in atherogenesis may not be adequately defined by observational studies alone. The odds of successfully targeting patients for vitamin therapy could be improved by the determination of a clinically salient homocysteine concentration 'threshold,' above which the attributable risk of homocysteine to the progression of vascular disease is substantial and modifiable.

Second, the diverse biological effects of folate (and other B-vitamins) may offset the intended benefit of its use. For example, folic acid increases levels of *S*-adenosylmethionine that serves as a methyl donor group for protein arginine N-methyltransferases, which yields the precursor for asymmetrical dimethylarginine (ADMA) (Figure 2). ADMA is associated with hypercholesterolemia and decreased bioavailable nitric oxide levels, thereby promoting proatherogenic changes in vascular endothelial cells (61). This may be one mechanism by which folic acid treatment adversely influences vascular reactivity, and may, in part, explain the failure of folic acid to improve vascular event rates in patients with pre-existing cardiovascular disease. In addition to this effect, folate and vitamin B₁₂ promotes DNA synthesis, supporting cell proliferation, which may lead to neointimal proliferation in individuals with established atherosclerosis.

Third, measurement of highly reactive species (i.e., non-protein bound) may be superior to total homocysteine measurement for the clinical assessment of cardiovascular risk (22). Oxidation of homocysteine to form derivative homocysteine species may result in the

formation of homocysteine-mixed disulfides with other thiols (e.g., homocysteine-glutathione) in a process that increases reactive oxygen species (ROS) generation, and thereby disrupting the cell redox state, antioxidant enzyme function (e.g., glutathione peroxidase-1) (3), and vascular cell phenotype (Figures 3a, 3b). Specifically, for example, thiolactone formation and protein homocysteinylation are processes directly associated with endothelial toxicity in humans (62).

Homocysteine, the central nervous system, and cognitive disorders

Alzheimer's disease (AD) is the leading cause of dementia (incidence $\approx$ 400,000 new cases/year) (63), and evidence from retrospective, population-based studies has suggested an association between cerebrovascular disease and AD. For example, data from the Rotterdam study showed that large vessel atherosclerotic disease (e.g., carotids) significantly increased the risk for developing AD (OR=3.0; 95% CI, 1.5-6.0) (64). The presence of basal ganglia, thalamus, or deep white matter infarcts at autopsy are also highly correlated with a history of AD (OR=20.7; 95% CI 1.5-288.0) (65). The identification of homocysteine as a risk factor for cerebrovascular disease, therefore, raised speculation that elevated levels of homocysteine could also be an independent risk factor for AD and non-AD dementias.

Seshardi and colleagues (7) published the first sufficiently powered prospective epidemiological study to examine the relationship between homocysteine and AD. Using data from the Framingham Heart Study, 1,092 non-demented individuals (mean age=76) were followed $\geq$ 8 years. The risk for developing AD was doubled in patients with homocysteine levels >14 μ mol/L. Overall, the relative risk of developing AD by homocysteine levels was 1.8 for per 1 SD above baseline homocysteine levels (95% CI, 1.3-2.5), and a 5 μ mol/L increase in homocysteine concentration elevated the risk of developing AD by 40%. Similar trends have been described in different populations; Ravaglia and colleagues (66) showed that in a dementia-free Italian cohort, homocysteine levels >15 μ mol/L were prospectively associated with a significantly increased risk of developing AD (RR=2.11; 95% CI, 1.19-3.76). Mildly elevated homocysteine levels may also increase the risk of developing non-AD dementia (67), vascular dementia, Parkinson's disease-associated dementia, and multiple sclerosis-associated cognitive decline (68).

Aberrant homocysteine-GABA A/B nerve receptor interactions in the central nervous system cause increased microvascular permeability and expose circulating homocysteine to neuronal tissue (69). Homocysteine reduces transition metal ions (e.g., Cu^{2+}) in a reaction that promotes ROS generation; pathological concentrations of H_2O_2 induce synaptic failure, and may represent one mechanism by which homocysteine induces cellular changes typical of AD pathogenesis. At circulating concentrations between 10-100 μ mol/L, homocysteine also influences nerve cell function directly by overstimulation of the *N*-methyl-D-aspartate (NMDA) receptor (70,71). This increases Ca^{+2} influx and promotes ROS formation, thereby impairing vascular endothelial cell and vascular smooth muscle cell function of cerebral blood vessels.

Despite epidemiological and mechanistic evidence in support of a relationship between hyperhomocysteinemia and other diseases of cognitive impairment, the translation of homocysteine level reduction into clinical benefit remains elusive. Two early trials examined the short-term effect ($\leq$ 6 months treatment duration) of homocysteine reduction on cognitive performance in patients at high risk for dementia, but found no improvement in cognitive performance on standardized testing (72,73). Others have reported negative results for vitamin therapy in the prevention of dementia progression in those with pre-existing AD (74).

The use of vitamin therapy for the primary prevention of cognitive decline has produced conflicting results. In the Folic Acid and Carotid Intima-Media Thickness (FACIT) study, a double blinded, randomized placebo-controlled trial from the Netherlands suggested that in

healthy individuals between 50-70 years old with homocysteine concentrations of 11–14 $\mu\text{mol/L}$, daily supplementation with 800 μg of folic acid improved memory, information processing speed, and sensorimotor speed as compared to placebo (75); however, a similarly designed trial by McMahon and colleagues (20) drew different conclusions. In this study, 276 healthy subjects >65 years old with plasma homocysteine levels >13 $\mu\text{mol/L}$ received placebo or daily vitamin therapy with folate (1000 μg), vitamin B₁₂ (500 μg) and B₆ (10 mg). No improvement in cognitive function was detected over a 2 year period, despite a 26% (4.4 $\mu\text{mol/L}$) decrease in homocysteine levels after vitamin therapy.

There are several possible reasons to explain the failure of homocysteine level reduction to improve cognitive function or decrease progression of AD. First, decreasing circulating homocysteine levels addresses only one of several pro-inflammatory mechanisms identified to promote oxidant stress and decrease the threshold for neurotoxicity. Second, randomized clinical trials in this arena have included patients with only mildly elevated homocysteine levels. The role of homocysteine reduction in patients with more robustly elevated homocysteine levels for both primary prevention and therapeutic treatment of dementia has not yet been conducted. Finally, elevated homocysteine levels may be a marker of other processes more closely associated with the neurotoxicity of dementia. For example, the transsulfuration pathway facilitates metabolism of homocysteine to the antioxidant glutathione. An abnormally reciprocal relationship between homocysteine and glutathione has been observed in neurodegenerative diseases, raising the possibility that increased homocysteine levels in dementia reflect impaired transsulfuration, and are in actuality a surrogate for decreased cellular concentrations of neuroprotective antioxidants (76).

Homocysteine and hip fractures

Hip fractures are a major cause of morbidity and mortality in elderly patients, and account for a substantial portion of annual health care-related costs in the U.S. (77). Spondylo-epimetaphyseal dysplasia, characterized by accelerated skeletal growth, osteopenia, and elongation of the appendicular skeleton (78), is a clinical feature of homocystinuria. Homocysteine-induced collagen-crosslinking dysregulation and Type I collagen fibril enlargement have been suggested as possible mechanisms to explain the pathogenesis of bone disease in patients with homocystinuria (79). Several prospective, observational studies have established that hyperhomocysteinemia is an independent risk factor for osteoporotic fractures in elderly men and women. For example, data from the Rotterdam study demonstrated an association between circulating homocysteine levels and the risk of incident osteoporotic fracture in 2,406 subjects > 55 years of age (RR=1.4 per 1 SD increase in the natural-log-transformed homocysteine level, 95 % CI, 1.2 to 1.6) (80). Furthermore, data from the Framingham Heart Study cohort suggest that, compared to individuals with normal or low-normal homocysteine levels, moderate elevations in homocysteine (>20 $\mu\text{mol/L}$ for men and >18 $\mu\text{mol/L}$ for woman) confer a sizeable risk increase for bone fracture (4.1-fold men, 1.9 fold for women) (81).

The effect of vitamin therapy on decreasing hip fracture rates in patients with moderately elevated homocysteine concentrations was tested in a randomized controlled trial of 628 post-stroke, osteoporotic patients in Japan (mean=19.9 $\mu\text{mol/L}$) (21). Post-stroke patients were selected because hemiplegia significantly increases the incidence of bone fractures in the affected limb. Therapy with 5 mg of folate and 1,500 μg of methylcobalamin resulted in an absolute risk reduction for fracture of 7.1% (95% CI, 3.6%-10.8%), with a number needed to treat to prevent one fracture was 14 (95% CI, 9-28). The 4.5% fracture rate observed in the placebo group, however, is several fold higher than the predicted fracture rate in an age- and sex- cohort of normal controls, which has led some to argue against the application of these findings to routine clinical practice. The benefit of vitamin therapy for fracture risk has not

been reproduced in mildly hyperhomocysteinemic individuals ($<15 \mu\text{mol/L}$) without a stroke history (82). In addition, vitamin therapy does not appear to influence measures of bone turnover in healthy, elderly individuals, although its effect in hyperhomocysteinemics is unknown (83). Thus, at this time, vitamin therapy may be useful for the prevention of fractures in certain high risk patients, but there are insufficient data to support its use in primary prevention of fractures in elderly individuals with mildly elevated homocysteine levels.

CONCLUSIONS

Data from randomized clinical trials have been unable to translate convincingly homocysteine lowering into improved clinical outcome for the secondary prevention of cardiovascular disease or in the prevention or treatment of dementia-type disorders. Vitamin therapy may decrease the risk of osteoporosis-induced bone fracture in selected, high risk patients. Thus, although the efficacy of combined folic acid, B₆-, and B₁₂-vitamin supplementation in decreasing circulating homocysteine levels is not in dispute, identification of patients who are most likely to benefit from this treatment is a subject of continued investigation. Most randomized intervention trials published to date have studied groups with only mildly elevated homocysteine levels, thus targeting clinical entities for which the attributable risk of homocysteine is unknown or is likely small compared to other factors. Importantly, the role of vitamin therapy has not been tested in key patient groups for which it may prove useful, such as in the primary prevention of coronary artery disease, or in the prevention of other, homocysteine-associated disease in patients with moderately or severely elevated homocysteine levels.

SUMMARY POINTS

1. Epidemiologic reports have established that elevated levels of homocysteine are an independent risk factor for atherothrombotic cardiovascular disease, stroke, cognitive impairment, and osteoporosis-induced bone fracture.
2. Oxidation of homocysteine promotes reactive oxygen species formation that may impair vascular function via decreased bioavailable nitric oxide levels, depletion of nitric oxide synthase cofactors, or impairment of normal antioxidant enzyme function.
3. Homocysteine overexcitation of *N*-methyl-D-aspartate receptors in the central nervous system may promote reactive oxygen species formation to induce synaptic failure in brain tissue.
4. Randomized clinical trials have shown that oral supplementation with the combination of folic acid, B₆-, and B₁₂-vitamins substantially lowers circulating homocysteine levels, but does not appear to improve outcome in the secondary prevention of cardiovascular disease or dementia.
5. Combination vitamin therapy may have a role in preventing osteoporosis-induced bone fractures in selected, high risk patients.
6. The diverse biologic effects of folic acid include indirectly increasing asymmetrical dimethylarginine (ADMA) levels, a molecule associated with impaired vascular function.

FUTURE ISSUES

1. Can a clinically salient proatherogenic homocysteine concentration threshold be identified for improved targeting of individuals who may benefit from combination B-vitamin therapy?

2. Are derivative species of homocysteine oxidation, such as thiolactone, superior to total homocysteine as a measure of cardiovascular risk?
3. Does homocysteine level lowering have a role in decreasing vascular event rates or delaying hemodialysis in patients with mild to intermediately decreased creatinine clearance?

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Figure 1. The homocysteine metabolic cycle

The sulfhydryl-containing amino acid homocysteine is an intermediate of normal methionine metabolism. Mutations in the 5,10 methyl-tetrahydrofolate reductase and cystathionine β-synthase genes impair homocysteine conversion to methionine and cystathionine, respectively. Alternatively, elevated levels of homocysteine may result from nutritional deficiencies of folic acid, vitamin B₆, and vitamin B₁₂ that are key enzyme cofactors required for normal homocysteine metabolism. Folic acid replenishes the tetrahydrofolate pool and is required for normal methionine synthase activity; however, folic acid also increases S-adenosylmethionine. This methyl donor is a precursor for asymmetrical dimethylarginine (ADMA), an inhibitor of endothelial nitric oxide synthase, which leads to uncoupling of the enzyme, decreased bioavailable nitric oxide, and vascular endothelial dysfunction. Finally, the antioxidant glutathione is an end product of cysteine metabolism; aberrant transsulfuration pathway activity has been implicated in decreased cellular antioxidant protection, which may represent one way by which elevated homocysteine levels serve as a surrogate for downstream processes that more specifically characterize the oxidative pathophysiology of various diseases linked to hyperhomocysteinemia. Adapted from Maron and Loscalzo (22).

Figure 2. Folate and asymmetrical dimethylarginine

Folic acid indirectly increases cellular stores of *S*-adenosylmethionine, which, in turn, facilitate the synthesis of asymmetrical dimethylarginine (ADMA). ADMA uncouples eNOS, resulting in increased oxidant stress, decreased bioavailable NO•, and impaired vascular function.

Dimethylarginine dimethylaminohydrolase degrades ADMA, although activity of this enzyme is decreased by various forms of oxidant stress, including directly by homocysteine. THF, tetrahydrofolate; SAM, *S*-adenosylmethionine; *S*-AdoHcy, *S*-adenosyl-homocysteine; DDAH, Dimethylarginine dimethylaminohydrolase; eNOS, endothelial nitric oxide synthase. *Adapted from Palm F, et al. (85).*

Figure 3. Homocysteine autoxidation

(A) Under oxidizing conditions, such as after exposure to plasma, homocysteine autoxidizes to form homocysteine or homocysteine-mixed disulfides (upper pathway). Homocysteine may also cyclize under acidic conditions to form homocysteine thiolactone (lower pathway). Hydroxyl radicals (OH^- , $\text{OH}^{\cdot}$) and superoxide anion ($\text{O}_2^{\cdot-}$) are byproducts of these reactions; the latter can be converted to H_2O_2 in the presence of superoxide dismutases or spontaneously undergoes dismutation to H_2O_2 . Homocysteine also promotes oxidant stress by directly impairing glutathione peroxidase expression (Gpx-1), an antioxidant enzyme that reduces H_2O_2 to water. (B) Homocysteine-induced reactive oxygen species formation decreases levels of bioavailable $\text{NO}^{\cdot}$ either by reducing the availability of key NOS cofactors, such as tetrahydrobiopterin (BH_4), or by inducing conversion of $\text{NO}^{\cdot}$ to peroxynitrite (ONOO^-). SOD, superoxide dismutase; eNOS, endothelial nitric oxide synthase.

Table 1

The common anatomic and metabolic causes of folate deficiency. *Adapted from Hoffbrand (84).*

Malabsorption	Increased utilization	Increased loss
Tropical sprue	Pregnancy	Congestive heart failure (↑ urinary losses)
Gluten-induced enteropathy	Lactation	Active liver disease
Intestinal megaloblastosis	Prematurity	Hemodialysis, peritoneal dialysis
Extensive jejunal resection, gastrectomy	Alcoholism	Alcoholism
Inflammatory bowel disease (e.g., Crohn's Disease)	Chronic hematologic diseases of increased cell turnover: hemolytic anemia, sickle cell anemia, myelofibrosis	Chronic inflammatory conditions that increase folate demands, but decrease appetite: TB, Crohn's disease, psoriasis,
Diabetic enteropathy	Chronic oncologic diseases of increased cell turnover: carcinoma, lymphoma, leukemia, myeloma	