Homocysteine and cardiovascular disease: Biological mechanisms, observational epidemiology, and the need for randomized trials

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Basic research indicates that homocysteine causes endothelial dysfunction and damage, accelerates thrombin formation, inhibits native thrombolysis, promotes lipid peroxidation through free radical formation, and induces vascular smooth muscle proliferation and monocyte chemotaxis. Most, but not all, observational epidemiological studies indicate that individuals with higher homocysteine levels have increased risks of cardiovascular disease. The magnitude ranges from approximately 20% in prospective studies to approximately 80% in retrospective case-control studies. In all observational epidemiological studies, however, the amount of uncontrolled and uncontrollable confounding is as large as the postulated small to moderate effect size. Thus, the totality of evidence should include randomized trials of sufficient sample size and duration with clinical end points. Folic acid reduces levels of homocysteine, but at present, despite several plausible biological mechanisms and a large body of observational epidemiological data, it is unclear whether supplementation will reduce risks of cardiovascular disease. It is also unclear whether any benefit of folic acid is attributable to lowering homocysteine levels. The current evidence is necessary, but not sufficient to judge causality. Such judgments await the availability of data from large-scale randomized trials. The availability of such data would permit rational clinical decision-making for individual patients and policy decisions for the health of the general public. (Am Heart J 2004;148:34–40.)

As is the case with homocysteine and risk of cardiovascular disease (CVD), advances in medical knowledge proceed on several fronts, optimally simultaneously.1 In general, basic research answers the question of why and analytic epidemiology answers the question of whether there is an association between an exposure and disease. For most hypotheses, randomized trials are neither necessary nor desirable. When the effect size is small to moderate, however (20%–50%) the amount of uncontrolled and uncontrollable confounding inherent in all observational analytic study designs is about as big as the postulated effect size. In such circumstances, randomized trials represent the most reliable design strategies.

In this report, we review various plausible biological mechanisms by which elevated plasma homocysteine may increase the risk of CVD, the existing clinical and epidemiological data, and discuss the published and ongoing large-scale randomized trials with clinical CVD end points.

Background

Homocystinuria is an autosomal recessive disorder, the manifestations of which in childhood include abnormalities of the long bones, mental retardation, ocular lens dislocation, and accelerated atherosclerosis.2,3 Patients with homocystinuria have a defect of the enzyme cystathionine β synthetase, responsible for the transulfuration of homocysteine.4 As a result, patients with homocystinuria have dramatically increased plasma homocysteine levels.3 This finding has led to the hypothesis that elevated levels of plasma homocysteine may cause accelerated atherosclerosis and premature CVD, in particular coronary heart disease (CHD) and stroke.5–7 Uncontrolled clinical observations have suggested that plasma homocysteine levels increase in patients with CVD who are challenged with methionine, a precursor of homocysteine.8

Basic research

Basic research has provided several plausible mechanisms by which homocysteine enhances thrombosis, the proximate cause of CVD.
Homocysteine causes endothelial dysfunction and damage

Endothelial dysfunction in the presence of elevated homocysteine levels may be mediated by oxidative stress by impairing intracellular glutathione peroxidase-1 activity and expression. This effect reduces the ability of the endothelial cell to detoxify itself of hydroxyl radicals and permits further oxidative damage. This effect may also be seen extracellularly. Homocysteine has been shown to inhibit superoxide dismutase and may increase lipid peroxidation and oxidized low-density lipoprotein cholesterol, thereby adding to the oxidative burden of the endothelium.

Homocysteine may also enhance endothelial dysfunction by decreasing tumor necrosis factor (TNF)-α ability to induce production of endothelial intercellular adhesion molecules such as ICAM-1, VCAM-1, and E-selectin. This increases endothelial permeability to expose underlying vascular smooth muscle and tissue factor, which may lead to thrombosis.

Homocysteine may also cause endothelial damage and apoptosis. In-vitro, homocysteine has been shown to upregulate the expression of programmed cell death genes within endothelial cells. This effect may be mediated by impairing the ability of the endoplasmic reticulum to properly fold newly synthesized proteins because of an increased oxidative burden.

Endothelial growth may be impaired by elevated homocysteine levels. In-vitro studies have shown that homocysteine inhibits the production of proteins, such as cyclin A, that are responsible for progression within the cell growth cycle. This effect causes the arrest of endothelial cell growth and prevents the replacement of damaged endothelial cells.

Furthermore, homocysteine reduces endothelium dependent vasodilatation by both elevating plasma levels of asymmetric dimethylarginine (ADMA), a potent inhibitor of nitric oxide (NO) synthase, and decreasing endothelin-1 production. These changes are likely to interfere with the ability of the endothelium to play a role in vasodilatation. In clinical studies, patients with elevated plasma homocysteine levels had blunted endothelial dependent vasodilatation.

Homocysteine accelerates thrombin formation

Homocysteine may also increase thrombosis by increasing activity of factors XII and V and increasing tissue factor expression on endothelium and vascular smooth muscle. Furthermore, homocysteine has been shown to reduce activity of Protein C and thrombomodulin while increasing platelet aggregation, all of which may lead to increased risk of CVD.

These effects of elevated plasma homocysteine on endothelial dysfunction and damage and increased intravascular exposure to tissue factor and elevated clotting factors also lead to platelet aggregation and activation. Thus, homocysteine may accelerate thrombus formation by increasing activity of the clotting cascade and enhancing platelet aggregation. Homocysteine may also initiate and propagate atherosclerosis through several additional mechanisms.

Homocysteine induces vascular smooth muscle proliferation

In vitro studies have shown that homocysteine added to human endothelial and vascular smooth muscle cell cultures induces expression of the c-myb and the c-fos genes, both of which are involved in upregulating diacylglycerolalddehyde associated protein kinase C. This is thought to cause increased DNA synthesis and increased vascular smooth muscle proliferation.

Homocysteine promotes monocyte chemotaxis through interleukin-8 and monocyte chemoattractant protein-1

Homocysteine is also involved in the migration of monocytes, the precursors to foam cells. Elevated levels of homocysteine lead to production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1. These molecules are responsible for monocyte migration. Thus, homocysteine may also act by recruiting the basic elements needed for the formation of the atherosclerotic plaque.

Homocysteine induces lipid peroxidation through free radical formation

Elevated plasma levels of homocysteine increase production of superoxide dismutase and hydroxyl radicals, which accelerates atherogenesis by oxidation of low-density lipoprotein cholesterol and lipid peroxidation.

Increased plasma homocysteine levels inhibit native thrombolysis

Homocysteine inhibits thrombolysis by decreasing the effectiveness of tissue plasminogen activator (tPA).

Observational epidemiological studies

Most, but not all, observational epidemiologic studies and their meta-analyses indicate that individuals with high levels of plasma homocysteine have an increased risk of the development of cardiovascular disease (CVD). The magnitude of this risk varies from approximately 20% in prospective cohort studies to 80% in case-control studies. In 1 such study by Nygård et al, 587 patients with angiographically proven coronary artery disease were screened for po-
Homocysteine-lowering therapy in patients with known CHD

In 1 trial, 593 patients with a history of stable atherosclerotic heart disease were randomized to receive open label folic acid or usual care. The primary end point was a composite of vascular events. These events were defined as vascular death (sudden death, fatal recurrent MI, fatal stroke, and other cardiovascular deaths), noncardiovascular death, recurrent MI, or invasive coronary procedures (percutaneous coronary intervention, coronary artery bypass grafting, cerebrovascular accident (CVA), transient ischemic attack (TIA), or any other vascular surgery such as carotid endarterectomy, abdominal aneurysmectomy, or peripheral vascular surgery including limb amputation for vascular reasons. Patients were treated and observed for an average of 24 months. The rates were 10.3% for the homocysteine-treated group and 9.6% for the usual care group (RR, 1.05; 95% CI, 0.63–1.75; \( P = .85 \)).

Homocysteine-lowering therapy as primary prevention for patients with a family history of premature CHD and elevated plasma homocysteine levels

In another randomized trial, by Vermeulen et al, 167 patients with known premature CHD (defined as age \(< 56 \) years) or obstetric complications and post-methionine loading hyperhomocysteinemia (\( > 2 \) SDs greater than the mean of apparently healthy control individuals) were asked to provide information on their living siblings. Once contacted, these 627 siblings were then invited to undergo post-methionine loading, homocysteine plasma level screening. Those with elevated post-test homocysteine levels (\( n = 104 \)) or normal post-test homocysteine levels (\( n = 54 \)) were randomized to receive homocysteine-lowering therapy or placebo. Randomized subjects were tested at baseline, at 1 year, and at 2 years for post-methionine loading homocysteine plasma levels, exercise stress testing, ankle-brachial systolic pressure index (ABI), and an ultrasound of femoral and carotid arteries.

Fasting and post-methionine total concentrations decreased by 49.7% and 46.2%, respectively (\( P < .001 \)), versus baseline in the treatment group and by 38.3% (95% CI, 27.0–49.6; \( P < .001 \)) and 30.6% (95% CI, 20.7–40.5; \( P < .001 \)), respectively, in the placebo group. Those patients in the treatment group also had a decreased risk of developing an abnormal stress test result at follow-up (odds ratio [OR], 0.40; 95% CI, 0.17–0.93; \( P = .035 \)). However, vitamin treatment, when compared with placebo, was not associated with improved ABI (OR, 0.87; 95% CI, 0.56–1.33) or with progression of femoral atherosclerosis (OR, 1.02; 95% CI, 0.26–4.05) or carotid atherosclerosis (OR, 0.86; 95% CI, 0.47–1.59).

Randomized trials

Percutaneous coronary intervention and homocysteine lowering therapy

Recently, there have been several, small, randomized trials of homocysteine-lowering therapy in patients who underwent percutaneous coronary intervention (PCI). In The Swiss Heart Study, 55 553 patients who underwent a successful PCI were randomized to receive vitamin B complex therapy or placebo. These patients were observed for a mean of 11 months, and a composite end point of major adverse events (death, nonfatal MI, and need for repeat revascularization) was evaluated at 6 months and 1 year. At the end of follow-up, 14% of patients treated with B complex vitamins underwent repeat revascularization versus 19.6% in the placebo group (relative risk [RR], 0.52; 95% CI, 0.32–0.86; \( P = .01 \)). However, there was no difference seen in the 2 groups in the rate of death from cardiac causes or nonfatal myocardial infarction.

In another randomized trial by Schnyder et al, 56 205 patients who had undergone successful PCI of native coronary stenosis \( \geq 50 \% \) were randomized to receive homocysteine-lowering therapy with folate (1mg), vitamin B12 (400 \( \mu \)g), and pyridoxine (10 mg) or placebo for 6 months. Follow-up angiography was performed at 6 months, or earlier when symptoms recurred. Quantitative angiographic evaluation was performed in 2 orthogonal views after intracoronary nitroglycerin injection. The primary end point was the presence or absence of restenosis \( \geq 50 \% \). In the group assigned to B complex vitamin treatment, 19.6% reached the primary end point, as compared with 37.6% in the control group (RR, 0.52; 95% CI, 0.32–0.86; \( P = .01 \)). However, there was no difference seen in the 2 groups in the rate of death from cardiac causes or nonfatal myocardial infarction.

Potential cardiovascular risk factors, including plasma homocysteine, and observed for a median of 4.6 years. After adjustments for potential confounders, patients with higher plasma homocysteine levels had higher rates of mortality. In subgroup analyses, in patients with plasma homocysteine levels <9 \( \mu \)mol/L, the mortality rate was 3.8%. For the subgroups of patients with plasma homocysteine levels of 9 to 14.9 \( \mu \)mol/L and \( > 15 \mu \)mol/L, the overall mortality rates were 8.6% and 24.7%, respectively (\( P \) for trend \(< .001 \)).
Homocysteine-lowering therapy on progression of carotid atherosclerosis

In a trial by Hackam et al,\textsuperscript{39} 101 patients with known vascular disease were randomized to receive 2.5 mg of folate, 25 mg of vitamin B6, and 250 μg of vitamin B12 daily or placebo and underwent ultrasound scanning. The mean duration of treatment before initiation of vitamin therapy was 2.6 ± 1.4 years (range, 0.9–6 years); the post-treatment period was 1.8 ± 0.7 years (0.8–3.3 years). In nonrandomized subgroup analyses of patients with plasma homocysteine levels >14 μmol/L, the rate of disease progression was 0.21 ± 0.41 cm²/year and −0.049 ± 0.24 cm²/year, before and after treatment, respectively (P = .0001). In the subgroup of patients with a <14 μmol/L plasma homocysteine level, the rate of disease progression was 0.13 ± 0.24 cm²/year and −0.024 ± 0.29 cm²/year, before and after treatment, respectively (P = .022).

Arterial/endothelial function and homocysteine-lowering therapy

In a trial by Woo et al,\textsuperscript{60} patients with a fasting total homocysteine level >75 percentile (mean, 9.8 ± 2.8 μmol/L; n = 17) were randomized in crossover trial to receive 10 mg of folic acid or placebo daily for 8 weeks, each separated by a 4-week washout period. Patients underwent high-resolution ultrasound scanning for flow-mediated endothelium-dependent dilatation of the brachial artery, before and after folic or placebo supplementation. When compared with placebo, folic acid supplementation improved endothelium-dependent dilatation (8.2% ± 1.6% vs 6% ± 1.3%; mean absolute difference, 2.2% ± 1.3%, P = .001).

In another trial by Title et al,\textsuperscript{61} patients with known coronary artery disease and a screening homocysteine level ≥9 μmol/L were randomized to 1 of 3 groups: placebo, folic acid, or folic acid plus antioxidant vitamins C and E (n = 75). Patients underwent serial brachial artery ultrasound scanning to measure endothelium-dependent flow mediated dilatation and nitroglycerin-dependent dilatation before and after treatment. Plasma folate, homocysteine, and endothelium-dependent flow mediated dilatation were unchanged in the placebo group. When compared with the placebo group, folate administration increased plasma folate levels by 475% (P < .001), reduced plasma homocysteine levels by 11% (P = .23), and improved endothelium-dependent flow mediated dilatation from 3.2% ± 3.6% to 5.2% ± 3.9% (P = .04).

When compared with placebo, folic acid plus antioxidant vitamins increased plasma folate by 438% (P = .001), reduced plasma homocysteine by 9% (P = .56), and improved endothelium-dependent flow mediated dilatation from 2.6% ± 2.4% to 4.0% ± 3.7% (P = .45).

Nitroglycerin-dependent dilatation did not change significantly in any of the groups.

In another trial by Doshi et al,\textsuperscript{62} patients with known coronary artery disease (n = 52) had been randomized in a double-blinded, crossover design. Patients were randomized to receive 5 mg of folate or placebo daily for 6 weeks. The patients then had a washout period of 4 months and were crossed over to the alternate trial arm. Patients were evaluated at baseline and at weeks 6, 22, and 28. At each visit, blood was drawn to measure levels of plasma homocysteine and lipid parameters, glucose and creatinine. Furthermore, patients underwent high-resolution brachial artery ultrasound scanning to test for endothelial-dependent flow mediated vasodilatation. In the folic acid treated group, plasma homocysteine significantly decreased (9.3 ± 2.4 vs 10.8 ± 2.4 μmol/L, P < .001), and as expected, plasma folate levels increased (310 ± 234 vs 9.1 ± 3.4 μg/L, P < .001). Endothelium-dependent flow-mediated dilatation significantly improved in the folate-treated group when compared with the placebo group (110 ± 43 vs 47 ± 35 μm respectively, P < .001).

Ongoing randomized trials

Within the next several years, the results of numerous large-scale, randomized trials such as KS-2 WEN-BIT, The Bergen Vitamin Study, CHAOS-2, PACIFIC, NORVIT, SEARCH, VITALTOPS, HOPE-2, WACS, and FAVORITE, will add important relevant information to the totality of evidence on whether supplementation will reduce risks of CVD among individuals with elevated homocysteine levels. The Vitamin Intervention for Stroke Prevention (VISP) trial\textsuperscript{63} showed no association in patients with a history of nondisabling cerebral infarction and elevated plasma homocysteine levels. It is unclear whether this and other trials conducted against the background of folic acid-fortified cereal grain flour will have adequate statistical power to detect the most plausible small to moderate effect sizes.\textsuperscript{64}

Conclusions

The principal underlying cause of CVD is atherosclerosis, and its principal proximate cause is thrombosis. Basic research has demonstrated that homocysteine enhances both atherosclerosis and thrombosis. Observational epidemiological studies, both case-control and cohort, demonstrate that patients with elevated homocysteine levels have small to moderate increased risks of CVD. Although randomized trials to date are of small sample size and appear promising, their results do not appear to be consistent. Thus, it remains unclear whether lowering plasma homocysteine levels...
will decrease risks of CVD, including myocardial infarction, stroke, or peripheral vascular disease.

The postulated small to moderate benefits of homocysteine-lowering would be clinically meaningful and have a major public health impact for a disease as common and as serious as CVD. For the observational analytic epidemiological studies, the amount of uncontrolled and uncontrollable confounding inherent in all such designs is about as large as the postulated effects. Thus, randomized trials of sufficient size and duration are necessary to detect reliably the most plausible small to moderate effects of homocysteine-lowering on risks of CVD. Failure to await randomized evidence for small to moderate effects can be grossly misleading, as was the case with female hormones and vitamin E.

Thus, basic research provides plausible mechanisms, and observational epidemiological studies are supportive of the hypothesis that increased plasma homocysteine levels increase the risk of CVD. However, at present, because of the lack of availability of sufficient data from large-scale randomized trials, it remains unclear whether lowering homocysteine levels with folic acid will reduce the risk of CVD.

All these issues require further testing in ongoing, large-scale randomized trials with clinical CVD endpoints to complete the totality of evidence. The availability of such data would permit rational clinical decision making for individual patients and policy decisions for the health of the general public.

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References


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