Evidence Based Medicine

Insufficient Evidence for the Efficacy of Homocysteine-Lowering Treatment on Cardiovascular Disease: The Homocysteine Hypothesis Revisited

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Abstract

A large body of observational studies has suggested that a moderate increase of plasma total homocysteine (tHcy) concentration is a modest risk factor for CHD and a somewhat stronger risk factor for stroke, at least in nonfortified populations. Folic acid and vitamin B6 and B12 supplementation can lower tHcy levels. However, recent clinical trials in populations at high risk for CVD have failed to show treatment benefits with homocysteine-lowering dietary interventions. There is continued controversy with regard to whether high homocysteine level is a cause of CVD and whether homocysteine-lowering therapy can protect against the development of CVD. *[N A J Med Sci. 2008; 1(1):30-33.]*

Key Words:

Cardiovascular Disease, Homocysteine

History

Based on the earlier observations of vascular disease in patients with homocystinuria, McCully (1969) first proposed a hypothesis that increased tHcy concentrations might be causally related to CVD risk in the general population.[1] Homocystinuria is a rare autosomal recessive disorder, which is usually caused by homozygous deficiency of cystathionine β-synthase, an enzyme required in methionine metabolism for the conversion of homocysteine to cystathionine. Patients with untreated homocystinuria have at least 10-fold higher tHcy concentrations than healthy subjects, and they are prone to develop premature atherosclerosis and thromboembolism in early life. Accumulating data from epidemiological studies suggested that elevated tHcy levels are very common in the general population and individuals with even moderate elevated levels of homocysteine (>16 umol/L) have small to moderate increased risks of CVD.

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Unknown Pathophysiologic Mechanisms

Although the exact mechanism of vascular injury has not been identified, high homocysteine may promote atherosclerosis by impairing coronary microvascular dilator function, by vascular smooth muscle cell proliferation, platelet activation, thrombogenesis, and collagen synthesis.

Furthermore, increased homocysteine concentrations provoke endothelial dysfunction, possibly mediated by oxidative stress or interference with nitric oxide function. In vitro and in vivo studies suggest that tHcy is a potent inducer of inflammation and is involved with inflammatory functions of endothelial cells at the level of gene expression.

Epidemiological Observational Studies Support a Modest Association of tHcy and CVD

Over the last 3 decades, many observational epidemiological studies have reported associations between increased tHcy concentrations and risk of coronary heart disease (CHD) and stroke. In 1995, a meta-analysis involving a total of 2297 CHD cases indicated that a 5 μmol/L higher homocysteine level was associated with about a 70% increase in the risk of CHD.[2] The included studies were mainly retrospective in design (in which blood for tHcy determination was collected after the onset of disease) and limited by reverse causality (the atherosclerotic disease process might itself have increased tHcy levels). It is difficult to establish whether increased tHcy concentrations precede clinical CVD. It is also unable to fully control for the effects of confounding factors. In 1998, an updated meta-analysis reported weaker associations in prospective studies (in which the blood was collected after the onset of disease) than in retrospective studies; a 5 μmol/L higher tHcy levels were associated with only a 30% increased risk of CHD within the prospective studies.[3] In 2002, the Homocysteine Studies Collaboration by performing a meta-analysis based on individual data from observational studies reported that, after adjustment for known cardiovascular risk factors (age, sex, blood pressure, cigarette smoking, and total cholesterol) and for the effects of measurement error, each 25% lowering of tHcy (about 3 μmol/L) was associated with only an 11% (odds ratio [OR], 0.89; 95% confidence interval [CI], 0.83-0.96) lower risk of CHD and a 19% (OR, 0.81; 95% CI, 0.69-0.95) lower risk of stroke.[4] The reduction in the risk of stroke was greater than that for CHD. Although case-control studies have tended to report strong associations between tHcy and CVD, many prospective studies have shown weaker or no associations.

The prospective study design allowed us to minimize biases due to recall or selection that may limit case-control studies. Prospective studies are also more reliable because they are not vulnerable to bias due to the effect of disease on tHcy ("reverse causality"). Because tHcy levels are closely correlated with established risk factors for vascular disease, including socioeconomic status, it is very important to adjust for cardiovascular risk factors and socioeconomic indicators in observational studies including meta-analyses. Overall, the association of tHcy with CVD tended to be markedly attenuated towards the null after controlling for confounding from established risk factors.

Genetic Evidence Support the Role of the *MTHFR* 677 C->T Polymorphism as a Major Determinant of tHcy Concentrations and a Possible Genetic Factor for Stroke

Methylenetetrahydrofolate reductase (MTHFR) is the enzyme that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a substrate for homocysteine remethylation to methionine. Genetic studies have pointed to the importance of the *MTHFR* 677 C->T polymorphism as a major determinant of tHcy concentrations in nonfortified populations. Individuals with a C to T substitution at base 677 of the gene that encodes MTHFR have reduced enzyme activity, and tHcy levels are typically 20% higher in those with the TT genotype compared with the CC genotype in nonfortified populations. The T allele has been associated with higher CVD risk in some studies but not others. Meta-analyses of case-control studies indicate that a 3 µmol/L difference in tHcy levels among individuals with the TT and CC genotypes is associated with about a 10% to 15% difference in CHD risk and a 20% to 25% difference in stroke risk.[5] Moreover, results of individual studies have shown greater heterogeneity. The meta-analysis also reported strong associations of *MTHFR* with risk of CHD in Asian and Middle Eastern populations, whereas the associations in Europe, Australia, and North America attenuated toward the null.[5] Stronger associations and less heterogeneity were observed in the studies of *MTHFR* and risk of stroke. However, several well-designed prospective studies provide convincing evidence that *MTHFR* 677C->T polymorphisms are not important risk factors for CVD in healthy individuals, although the *MTHFR* 677 C->T polymorphism was associated with tHcy concentrations. This geographic variation in the association of *MTHFR* 677C->T polymorphism with CHD and stroke risk may reflect the difference in tHcy concentrations between the *MTHFR* genotypes and decreased effects in more folate-replete populations. It has been suggested that the MTHFR T allele carriers may be at increased CVD risk in the setting of low dietary intake of folate or B-vitamins. Mandatory folate fortification in the North America may have reduced the frequency of low folate and lowered plasma tHcy concentrations in some study populations and may explain some null findings.

Available Evidence from Randomized Clinical Trials Does Not Support the Efficacy of Lowering Homocysteine Treatments among Individuals at High Risk of CVD

The homocysteine hypothesis has attracted considerable interest because plasma homocysteine can be reduced by folic acid and vitamin B_{12}, raising the prospect that dietary supplementation with B vitamins could reduce the risk of CHD and stroke. In the general population, the most important modifiable determinants of tHcy are dietary folate and B vitamins. Randomized controlled trials of tHcy-lowering vitamins for cardiovascular endpoints may clarify whether tHcy is causative in the pathogenesis of atherosclerosis or is simply related to other confounding cardiovascular risk factors. Since the mid-1990s, a number of large-scale trials (involving more than 1000 participants) of B vitamin supplementation have been designed and conducted to test this homocysteine hypothesis. Most trials were done among people with prior stroke or CHD or renal disease as a secondary prevention strategy. All 5 large trials that have recruited more than 1000 participants have reported their results to date (Second Cambridge Heart Antioxidant Study [CHAOS-2],[8] Vitamin Intervention for Stroke Prevention [VISP],[9] Norwegian Vitamin Trial [NORVIT],[10] Heart Outcomes Prevention Evaluation-2 [HOPE-2][11]), and the Homocysteinemia in Kidney and End Stage Renal Disease (HOST),[12] have so far failed to demonstrate significant reductions in their primary outcomes (although the HOPE-2 study did report a significant reduction in stroke, which was a secondary outcome). A meta-analysis of randomized controlled trials, which included the 4 previously reported large trials and 8 smaller trials, reported that folic acid supplementation did not reduce risk of CVD or all-cause mortality in individuals with pre-existing vascular or kidney disease.[13] The summary relative risk (RR) for CHD was 1.04 (95% CI, 0.92-1.17) and for stroke was 0.86 (95% CI, 0.71-1.04). If these results are updated with the results of the HOST trial, then the overall OR per each 3 µmol/L reduction in tHcy levels for a mean duration of treatment of 3.1 years is 1.00 (95% CI, 0.92-1.09) for CHD events (in 12 trials) and 0.88 (95% CI, 0.78-1.00) for stroke (in 9 trials).[14] A recent meta-analysis in which the efficacy of folic acid supplementation in stroke was specifically assessed has shown that lowering tHcy with folic acid may actually reduce risk of stroke by 18% (0-32%, p=0.045), and that the beneficial effect was greater (23%-29%) in those trials with a longer treatment duration (>36 months), larger homocysteine-lowering effects (>20%), no or partial grain fortification, and no history of stroke.[15] Although this meta-analysis only included 8 trials, their conclusions are strengthened by their sensitivity analyses showing that no individual study findings influenced the overall results.

Conclusions and Future Implications

Observational studies including genetic association studies support an independent association between tHcy and CVD, but this association is generally weaker in the more robust prospective studies and is weaker for CHD than for stroke. Homocysteine is not likely to be as important in determining

CHD risk as traditional CVD risk factors such as low-density lipoprotein, high-density lipoprotein, triglycerides, smoking, diabetes mellitus, and blood pressure. Most of randomized clinical trials assessing the efficacy of folate in combination with vitamin B6 and/or B12 supplementation on the risk of CVD have yielded negative results, although there was a suggestion that homocysteine-lowering treatments could have a greater protective effect in stroke risk. The current evidence does not support the routine use of homocysteine-lowering vitamin supplements for the prevention of CVD events among individuals at high risk for vascular disease. Several ongoing large randomized trials will provide further clarification on this issue.

In future clinical trials, several important issues need to be specifically clarified from a research and population-health perspective before definitive conclusions can be made about the role of lowering homocysteine in CVD prevention. First, future clinical trials would require larger study populations, longer periods of intervention, or greater reduction in tHcy concentrations to detect any beneficial effect. Second, there may be differential effects of homocysteine-lowering therapy on different CVD endpoints. Whether homocysteine lowering might have greater effect on stroke should be specifically addressed. Third, there seems to be an attenuation of the benefit owing to folic acid fortification of grain products in North America, where most of the trials have been conducted to date. Future trials should be done in regions without folate fortification. Fourth, optimal dosage and safety of long-term folic acid supplementation along or in combination with other B vitamins should be carefully considered in future trials. Finally, there may be subgroups of individuals with B vitamin deficiencies or common genetic variants in the pathway of homocysteine metabolisms that could particularly benefit from homocysteine lowering therapy. B vitamin status may need to be assessed in study population. A meta-analysis of all available individual data from completed and ongoing trials should have sufficient statistical power to conduct secondary analyses to confirm or refute the hypothesis.

References

1. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 56(1):111-128.
2. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. 1995;274(13):1049-1057.
3. Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. J Cardiovasc Risk. 1998;5(4):229-232.
4. The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA. 2002;288(16):2015-2022.
5. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002;288(16):2023-2031.
6. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005;331(7524):1053.
7. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. Lancet. 2005;365(9455):224-232.
8. Baker F, Picton D, Blackwood S. Blinded comparison of folic acid and placebo in patients with ischemic heart disease: an outcome trial. Circulation. 2002;106 (1):741S.
9. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA. 2004;291(5):565-575.
10. Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med. 2006;354:1578-1588.
11. Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med. 2006;354(15):1567-1577.
12. Jamison RL, Hartigan P, Kaufman JS, et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. JAMA. 2007;298(10):1163-1170.
13. Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. JAMA. 2006;296(22):2720-2726.
14. Baigent C, Clarke R. B vitamins for the prevention of vascular disease: insufficient evidence to justify treatment. JAMA. 2007;298(10):1212-1214.
15. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. Lancet. 2007; 369(9576):1876-1882.

Table 1. Observational Evidence from Major Studies Investigating this hypothesis.

Study Group	Journal, year	Subject No.	Main Results
Boushey et al. [2]	JAMA, 1995	27 studies (3 prospective studies)	5 umol/L tHcy increment→60% CHD risk (40%-70%) for men and 80% (30%-90%) for women
Danesh et al.[3]	J Cardiovasc Risk, 1998	unclear	5 umol/L tHcy increment→30%↑ CHD
The Homocysteine Studies Collaboration [4]	JAMA, 2002	30 studies (12 large prospective studies)	A 25% lower tHcy levels (3umol/L) → 11% (4-17%) lower risk of CHD and 19% lower risk for stroke
The MTHFR Studies Collaboration Group[5]	JAMA, 2002	11162 cases and 12758 controls	MTHFR 677 TT genotype versus CC genotype: 16% (5%-28%) higher risk of CHD Significant heterogeneity: 1.14 (1.01-1.28) in European populations and 0.87 (0.73-1.05) in North American populations
Lewis et al. [6]	BMJ, 2005	26000 cases and 31183 controls	MTHFR 677 TT genotype versus CC genotype: 14% (5%-24%) higher risk of CHD
Casas et al.[7]	Lancet, 2005	6324 cases and 7604 controls	MTHFR 677 TT genotype versus CC genotype: 26% (14%-40%) higher risk of stroke

Table 2. Summary of 5 large randomized trials published to date regarding the effect of homocysteine lowering interventions by folate and/or B vitamin supplementations on CVD endpoints.

Study name	Journal, year	Participants	Differences in lowering tHcy	CVD endpoints	
				Coronary events or death	Stroke
CHAOS-2	*Circulation*, 2002 [8]	1882 patients	↓tHcy levels13%	No effect	No effect
VISP	*JAMA*, 2004 [9]	3680 patients with recent ischemic stroke	↓2umol/L	No effect	No effect
NORVIT	*N Engl J Med*, 2006 [10]	3749 survivors of recent acute MI	tHcy levels↓27%	No effect	No effect
HOPE-2	*N Engl J Med*, 2006 [11]	5522 patients with a history of vascular disease or diabetes		No effect	↓25%
A meta-analysis	*JAMA*, 2006 [13]	12 trials of 16958 participants (up to July 2006)	tHcy levels↓8.9%-52%	1.04 (0.92-1.17)	0.86 (0.71-1.04)
A meta-analysis	*Lancet*, 2007[15]	8 trials of 16841 participants (up to July 2006)	tHcy levels↓11%-39%	Not available	↓18% (0.82, 0.68-1.00) ↓tHcy levels>20%→0.77 (0.63-0.94)
HOST	*JAMA*, 2007[12]	2056 patients with renal disease	tHcy levels↓26%	0.86 (0.67-1.08)	0.90 (0.58-1.40)
An updated meta-analysis	*JAMA*, 2007[14]	9 trials of 18897 participants (+HOST trial)	tHcy levels↓11%-39%	Per 3 umol/L↓ tHcy →1.00 (0.92-1.09)	0.88 (0.78-1.00)