Homocysteine and Cardiovascular Risk: Considering the Evidence in the Context of Study Design, Folate Fortification, and Statistical Power

The hypothesis that moderately increased plasma total homocysteine (tHcy) concentrations are causally related to cardiovascular disease (CVD) originated from observations of vascular disease in patients with homocystinuria *(1)*. tHcy concentrations are ~10-fold higher in patients with untreated homocystinuria than in the general population, and these patients often suffer from CVD in early life. Homocystinuria may arise from one of several rare defects in genes involved in methionine metabolism, resulting in high tHcy concentrations, with cystathionine β-synthase (*CBS* gene) deficiency being the most common. In responsive cases of homocystinuria, dietary supplementation with B-vitamins and betaine is remarkably effective at lowering plasma tHcy concentrations and decreasing the risk of CVD *(2)*. In addition to suggesting that extremely high tHcy concentrations may be causally related to CVD in affected individuals with homocystinuria, McCully also suggested that moderately increased tHcy concentrations may be related to CVD risk in the general population *(1)*.

A single discrete mechanism of vascular injury has not been identified, but high homocysteine may have adverse effects on platelet function and clotting factors and may increase vascular smooth muscle cell proliferation. Furthermore, increased homocysteine concentrations provoke endothelial dysfunction, possibly mediated by oxidative stress or interference with nitric oxide function *(3, 4)*.

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 *(5)*. Although results of prospective cohort studies (in which blood for tHcy determination was collected before the onset of disease) have been weaker and more inconsistent than those of retrospective studies (in which the blood was collected after the onset of disease), prospective studies are more reliable because they are not vulnerable to bias due to the effect of disease on tHcy ("reverse causality") and because they control for confounding from established risk factors. A metaanalysis of prospective studies reported that after adjustment for established risk factors for CVD, a 25% lower plasma tHcy concentration was associated with an 11% lower risk of CHD and a 19% lower risk of stroke *(5)*.

The strength of the association of tHcy with CHD and stroke observed in prospective studies is consistent with that observed in the so-called Mendelian randomization studies *(6–11)*, which investigated the CVD risk associated with the methylenetetrahydrofolate reductase (*MTHFR*) 677 C->T polymorphism. The design of the Mendelian randomization studies exploits the fact that homozygosity for the variant allele (TT genotype), which occurs at a rate of ~10% in most ethnic groups, leads to 25% higher tHcy concentrations than occur in individuals with the common genotype (CC). This approach should not be affected by reverse causality bias or confounding due to other factors affecting tHcy concentrations. However, as additional evidence on *MTHFR* and CVD risk has accrued in more recent years, results of individual studies have shown greater heterogeneity *(6–11)*. A metaanalysis of 80 studies involving 26 000 CHD cases *(8)* 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 *(8)*. Stronger associations and less heterogeneity were observed in the studies of *MTHFR* and risk of stroke. A metaanalysis of 111 studies involving 6324 stroke cases reported that persons with the TT genotype had a 26% higher risk of stroke than did those with the CC genotype *(9)*. Another recent metaanalysis on *MTHFR* and stroke found an additive influence of T-allele dose on risk. In examining the consistency of the results across ethnic groups as a proxy of folate status, the same metaanalysis found the strongest associations in European and Asian studies, whereas a similar trend among North American studies did not reach statistical significance *(10)*. 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 *(11)*, but it may also be related to effect modification by other B-vitamins in addition to folate *(12)*.

Dietary supplementation with B-vitamins that lower plasma tHcy concentrations *(13)* is expected to lower risk of CVD, and in the late 1990s, several large-scale trials of B-vitamin supplementation in people with prior stroke or CHD or renal disease were designed to test the homocysteine hypothesis of CVD *(14–16)*. One of the earliest trials addressing this question involved 3318 individuals in Northern China *(17)*, a population with a low folate status. In this study, multivitamin supplementation containing B-vitamins was associated with a 47% decrease in stroke risk *(17)*. Among the ongoing large-scale randomized trials (involving more than 1000 participants) that were initiated in Western countries, the results of 4 trials [Vitamin Intervention for Stroke Prevention (VISP) *(18)*, the Norwegian Vitamin (NORVIT) trial *(19)*, Heart Outcomes Prevention Evaluation-2 (HOPE-2) trial *(20)* and the Second Cambridge Heart Antioxidant Study (CHAOS-2)] have been published. Two of these trials (NORVIT and CHAOS-2) were conducted in populations without folic acid fortification and investigated the effects of folic acid and vitamin B_{12} alone or in combination with vitamin B_6. Metaanalysis of all completed trials involving 16 958 CVD patients reported relative risks associated

with folic acid supplementation compared with controls of 1.04 (95% CI, 0.92–1.17) for CHD and 0.86 (95% CI, 0.71–1.04) for stroke *(16)*. Thus, the latter metaanalysis demonstrated that lowering tHcy did not decrease the risk of CHD or stroke, but the CIs are still consistent with a 10% lower risk of CHD and 20% lower risk of stroke predicted by the prospective cohort studies *(14–16)*. Several ongoing trials with large sample sizes may provide a definitive answer. A metaanalysis of all available data from ongoing and completed trials involving almost 50 000 participants should have sufficient statistical power to confirm or refute the hypothesis *(15)*.

In addition to being criticized for lack of power, interpretation of the VISP trial has been considered questionable because the null results may have been confounded by vitamin B_{12} deficiency *(20)*. A post hoc analysis of a subgroup of 2155 patients in the VISP, excluding study participants likely to have vitamin B_{12} malabsorption (vitamin B_{12} <25th percentile) and those receiving vitamin B_{12} supplements (vitamin B_{12} above 95th percentile) revealed that patients receiving B-vitamins had a significant decrease of stroke and CHD *(21)*. In the HOPE-2 trial, patients given B-vitamins not only had a significantly decreased risk of stroke but also showed a modest decrease in composite primary outcome, which became apparent after 3 years of intervention *(20)*.

A population-based cohort study with a quasi-experimental design compared the decrease in stroke mortality in the United States and Canada, where folic acid fortification was fully implemented in 1998, with the decrease in England and Wales, where folic acid fortification is not used. The decrease of stroke mortality accelerated after 1998 in the fortified population but not in the nonfortified population *(22)*. The authors attributed the decrease in stroke mortality to folic acid fortification, which also is believed to explain the postfortification decrease in neural tube defects known to be prevented by folic acid.

In the current issue of *Clinical Chemistry*, 2 separate studies provide important new evidence about the public health relevance of *MTHFR* and increased tHcy *(23, 24)*. Bathum et al. analyzed tHcy and *MTHFR* polymorphisms in 1206 Danish twin pairs *(23)*. The Danish study reported that the *MTHFR* 677 C->T polymorphism could explain half the variance in tHcy concentrations in people younger than 40 years, one quarter of the variance in tHcy concentrations at older ages, and almost all the heritability of plasma tHcy, confirming the importance of *MTHFR* as major determinant of tHcy concentrations in nonfortified populations *(23)*. Zee et al. *(24)* reported associations of CVD risk with tHcy, *MTHFR* 677C->T genotype, and dietary intake of B-vitamins in a well-designed, large, prospective study of 25 000 healthy women enrolled in the Women's Health Study that was carried out in North America between 1992 and 2005. In a 10-year follow-up, there were 812 incident CVD events. Although tHcy concentrations were associated with a 2-fold higher risk of CVD when comparing top with bottom quintiles, this association was almost completely attenuated after adjustment for established CVD risk factors, including socioeconomic status. Moreover, there was no association between the *MTHFR* polymorphism and risk of CVD in this population. Interestingly, the prevalence of hypertension was proportional to the number of T-alleles. Nevertheless, the power of this study, which is proportional to the tHcy difference between TT and CC genotypes and to the number of CVD cases rather than the total number of individuals studied, is substantially less than the power of the metaanalyses of *MTHFR* and CHD or stroke that had previously reported no associations in North American populations. The Women's Health Study provides convincing evidence that increased tHcy or *MTHFR* 677C->T polymorphisms are not important risk factors for CVD in healthy middle-aged women mainly recruited from a population after the introduction of mandatory folic acid fortification.

In conclusion, investigations of B-vitamin–responsive homocystinuria indicate that severe hyperhomocysteinemia is causally related to CVD, and plausible mechanisms have been reported that could explain the influence of hyperhomocysteinemia on the development of vascular lesions. Prospective cohort and Mendelian randomization studies suggest that a moderate increase of plasma tHcy concentration is a modest risk factor for CHD and a somewhat stronger risk factor for stroke, at least in nonfortified populations. Completed trials investigating secondary intervention with tHcy-lowering B-vitamins have demonstrated no CVD risk decrease in patients with established vascular disease who were undergoing intensive conventional treatment. However, it is important to defer judgment on the clinical and public health relevance of the "homocysteine hypothesis" until the results of the additional ongoing trials of B-vitamins are available *(15)*.

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References

1. McCully KS. Hyperhomocysteinemia and arteriosclerosis: historical perspectives. Clin Chem Lab Med 2005;43:980–6.
2. Yap S, Naughten E. Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. J Inherit Metab Dis 1998;21:738–47.
3. Jacobsen DW, Catanescu O, Dibello PM, Barbato JC. Molecular targeting by homocysteine: a mechanism for vascular pathogenesis. Clin Chem Lab Med 2005;43:1076–83.

4. Splaver A, Lamas GA, Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. Am Heart J 2004;148:34–40.
5. Clarke R, Collins R, Lewington S, Donald A, Alfthan G, Tuomilehto J. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002;288:2015–22.
6. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325:1202.
7. 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;288:2023–31.
8. 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;331:1053.
9. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. Lancet 2005;365:224–32.
10. Cronin S, Furie KL, Kelly PJ. Dose-related association of MTHFR 677T allele with risk of ischemic stroke: evidence from a cumulative meta-analysis. Stroke 2005;36:1581–7.
11. Wald DS, Wald NJ, Morris JK, Law M. Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. BMJ 2006;333:1114–7.
12. Hustad S, Midttun Ø, Schneede J, Vollset SE, Grotmol T, Ueland PM. The methylenetetrahydrofolate reductase 677C>T polymorphism as a modulator of a B-vitamin network with major effects on homocysteine metabolism. Am J Hum Genet, in press.
13. Clarke R, Frost C, Sherliker P, Lewington S, Collins R, on behalf of the Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82:806–12.
14. Clarke R, Lewington S, Sherliker P, Armitage J. Effects of B-vitamins on plasma homocysteine concentrations and on risk of cardiovascular disease and dementia. Curr Opin Clin Nutr Metab Care 2007;10:32–9.
15. Clarke R, Armitage J, Lewington S, Sherliker P, Collins R, on behalf of the B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials. Am Heart J 2006;151:282–7.
16. 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;296:2720–6.
17. Mark SD, Wang W, Fraumeni JF Jr, Li JY, Taylor PR, Wang GQ, et al. Lowered risks of hypertension and cerebrovascular disease after vitamin/mineral supplementation: the Linxian Nutrition Intervention Trial. Am J Epidemiol 1996;143:658–64.
18. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, 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;291:565–75.
19. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;354:1578–88.
20. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med 2006;354:1567–77.
21. Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin Intervention For Stroke Prevention trial: an efficacy analysis. Stroke 2005;36:2404–9.
22. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation 2006;113:1335–43.
23. Bathum L, Petersen I, Christiansen L, Konieczna A, Sørensen TIA, Ohm Kyvik K. Genetic and environmental influences on plasma homocysteine: results from a Danish twin study. Clin Chem 2007;53:971–9.
24. Zee RYL, Mora S, Cheng S, Erlich HA, Lindpaintner K, Rifai N, et al. Homocysteine, 5,10-methylenetetrahydrofolate reductase 677C>T polymorphism, nutrient intake and incident cardiovascular disease in 24,968 initially healthy women. Clin Chem 2007;53:852–8.

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