

Homocysteine and Cardiovascular Risk: The Perils of Reductionism in a Complex System

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Patients with genetic defects causing severe hyperhomocysteinemia have a high rate of mortality from cardiovascular diseases at an early age, irrespective of the specific genetic lesion. Long-term therapy with B vitamins or betaine in patients with homocystinuria due to cystathionine β -synthase deficiency decreases homocysteine and reduces the rate of vascular events by 90%. Kilmer McCully studied the vascular pathology in young patients who died with homocystinuria and in 1969 proposed that a moderate increase in circulating homocysteine could be involved in the pathogenesis of vascular occlusive disease in the general population, i.e., the so-called homocysteine theory of atherosclerosis. This theory was investigated in the early 1990s in many clinical and epidemiologic studies. A metaanalysis of studies of homocysteine concentration and vascular risk concluded that an increase of 0.68 mg/L (5 μ mol/L) in plasma homocysteine had an impact on cardiovascular risk that was equivalent to a 19-mg/dL (0.5-mmol/L) increase in total cholesterol. In general, the risk estimates from the prospective studies are weaker than those obtained from case-control and cross-sectional studies (1, 2), yet they clearly support plasma homocysteine as an independent cardiovascular risk factor.

The possible role of homocysteine in the development of vascular disease inspired research on the biochemical and molecular effects on the vasculature of homocysteine and the determinants of circulating homocysteine concentrations. Experimental studies revealed numerous homocysteine effects relevant to the pathogenesis of vascular disease, including induction of endothelial dysfunction, inhibition of biological methylation through accumulation of S-adenosylhomocysteine, homocysteinylolation and posttranslational modification of proteins, increased

oxidant stress, and decreased availability of nitric oxide (1). In addition, plasma homocysteine positively correlates with a variety of biochemical parameters, clinical traits, and lifestyle factors associated with increased cardiovascular risk, including dyslipidemia, increased blood pressure, impaired renal function, a sedentary lifestyle, and an unhealthy diet. These associations point to possible mechanisms or potential confounders in studies of homocysteine and cardiovascular disease (3).

Among nutrients, B vitamins, particularly folate, effectively reduce plasma homocysteine (3). The secondary prevention of cardiovascular disease by homocysteine-decreasing B vitamins was investigated in numerous randomized controlled trials, beginning in the late 1990s. Folic acid, a synthetic folate form that does not exist in nonsupplemented individuals, was the cornerstone of these intervention studies. A recent metaanalysis involving 37 485 individuals reported a mean 25% reduction in circulating homocysteine after treatment with folic acid; however, despite this reduction, there was no effect on cardiovascular events after a median follow-up of 5 years (4). These disappointing results weaken the argument that folic acid supplementation has potential for preventing cardiovascular disease. Importantly, however, inference beyond that of a lack of short-term benefit from folic acid supplementation in patients with established cardiovascular disease simply cannot be made or justified on the basis of the available data. A protective effect from long-term treatment in healthy individuals (primary prevention) is not precluded by these studies. More importantly, folic acid supplementation is a complex intervention with the potential to affect numerous folate-dependent processes beyond that of facilitating remethylation of homocysteine to methionine. Some of these processes may promote vascular disease, such as enhanced cell proliferation and growth within the atherosclerotic plaque and altered S-adenosylmethionine-dependent methylation reactions (1). Furthermore, a recent study demonstrated that folic acid caused the expected decrease in plasma homocysteine but had no effect on intracellular homocysteine concentrations, results that led the authors to suggest that folic acid disrupts the regulation of methylenetetrahydrofolate reductase by S-adenosylmethionine (5). Notably, an observation

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from the WENBIT⁴ (Western Norway B Vitamin Intervention Trial) and NORVIT (Norwegian Vitamin Trial) intervention trials appears to have escaped attention: Although baseline plasma homocysteine concentrations did not predict future cardiovascular events among the participants allocated to treatment with folic acid plus vitamin B₁₂, plasma homocysteine concentrations measured after 1–2 months of treatment were predictive (3). These observations highlight the fact that intracellular homocysteine resides at a metabolic branch point connecting various critical pathways. One fraction of the total plasma homocysteine is linked to homocysteine remethylation to methionine, which requires 5-methyltetrahydrofolate as a methyl donor (1), and this folic acid-responsive fraction does not predict cardiovascular disease progression.

5-Methyltetrahydrofolate, the prevailing folate species in plasma, is formed via the action of methylenetetrahydrofolate reductase (MTHFR), which is encoded by the *MTHFR*⁵ [methylenetetrahydrofolate reductase (NAD(P)H)] gene. Homozygosity of the variant T allele of the common *MTHFR* 677 C→T polymorphism (TT genotype) is associated with low enzyme activity, which explains the lower circulating concentrations of folate (mainly 5-methyltetrahydrofolate) and the higher plasma homocysteine concentrations (10%–20% higher) in individuals with the TT genotype compared with those with the CC genotype. This metabolic phenotype has been exploited in so-called Mendelian randomization studies, which often have demonstrated an increased cardiovascular risk in those with the TT genotype compared with the CC genotype (2). The metaanalysis of Clarke and colleagues (4) of the risk of coronary heart disease included 86 published studies of 28 617 cases and 41 857 controls, as well as 19 unpublished data sets of 48 175 cases and 67 961 controls. The unpublished data sets included 67 to hundreds of thousands of polymorphisms from studies on coronary heart disease risk in which the *MTHFR* polymorphism was not of primary interest. Analyses of the published studies showed a weak association between *MTHFR* genotype and risk, whereas the analyses of the unpublished studies showed a null effect (4).

This important work of Clarke and colleagues (4) strongly suggests that the *MTHFR* 677 C→T polymorphism and the associated increase in plasma homocysteine is not related to the risk of coronary heart disease. The large study populations and the statistical power of

the analysis make a type II error unlikely. Thus, the prior published finding of an increased risk related to the number of T alleles likely reflects publication bias (4). Importantly, however, the authors have inappropriately interpreted the lack of an effect of the *MTHFR* polymorphism on cardiovascular risk as a lack of direct adverse effects of homocysteine on the cardiovascular system. This erroneous inference regarding mechanism may partly be based on the misconception that MTHFR is a homocysteine-metabolizing enzyme. Methylenetetrahydrofolate reductase serves as a metabolic switch through which the flow of 1-carbon units from methylenetetrahydrofolate is directed either to the methionine/S-adenosylmethionine methylation cycle or to the synthesis of thymidylate. The low enzyme activity associated with the TT genotype may therefore favor DNA synthesis and cell proliferation at the expense of methylation of a variety of compounds (pleiotropy), which may in turn affect the progression of vascular disease (2). Furthermore, to date no data exist on the intracellular concentrations of homocysteine with respect to *MTHFR* genotype.

What Are the Take-Home Points?

1. The striking reduction in cardiovascular morbidity and mortality from homocysteine lowering in patients with inborn errors of homocysteine metabolism points to a role for homocysteine in the development of vascular occlusive disease, a role that is well supported by results from experimental studies.
2. Plasma homocysteine is a predictor of cardiovascular disease in the general population and shows remarkable associations with a variety of risk factors, including lifestyle, nutrition, and disorders that affect cardiovascular health.
3. The negative results in secondary-intervention studies have demonstrated no benefit from supplementation with B vitamins in patients with established cardiovascular disease. Therefore, B vitamin supplementation for cardiovascular patients without overt vitamin deficiency cannot be recommended.
4. No inferences on mechanism regarding possible adverse effects from increased (intracellular) homocysteine can be made from the results of the published intervention trials or the Mendelian randomization studies. Thus, whether increased homocysteine is an epiphenomenon or is directly responsible for adverse cardiovascular effects remains an open question.

The initial clinical and experimental results within the homocysteine field created excitement and a search for a simple solution. The lack of a protective effect from B vitamin supplementation is disappointing, yet the error of this reductionist approach to vascular dis-

⁴ Nonstandard abbreviations: WENBIT, Western Norway B Vitamin Intervention Trial; NORVIT, The Norwegian Vitamin Trial; MTHFR, methylenetetrahydrofolate reductase.

⁵ Human genes: *MTHFR*, methylenetetrahydrofolate reductase (NAD(P)H).

ease should not be compounded by the erroneous reductionist conclusion that homocysteine does not directly promote atherothrombosis. Nature, diseases, and therapeutic strategies (6) all operate within complex (patho)biological networks that must be examined holistically if one hopes to gain an optimal understanding of effective treatments. It is also important to have a balanced view of the existing literature.

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