Homocysteine and cardiovascular disease: cause or effect? 1, 2

Lars Brattström and David EL Wilcken

ABSTRACT Both markedly and mildly elevated circulating homocysteine concentrations are associated with increased risk of vascular occlusion. Here we review possible mechanisms that mediate these effects. Inborn errors of homocysteine metabolism result in markedly elevated plasma homocysteine (200–300 μmol/L) and thromboembolic (mainly venous) disease: treatment to lower but not to normalize these concentrations prevents vascular events. Mild homocysteine elevation (> 15 μmol/L) occurs in ≈20–30% of patients with atherosclerotic disease. Usually, this is easily normalized with oral folate and ongoing trials are assessing the effect of folate treatment on outcomes. Although there is evidence of endothelial dysfunction with both markedly and mildly elevated homocysteine concentrations, the elevated homocysteine concentration in atherosclerotic patients is also associated with most standard vascular risk factors, and importantly, with early decline in renal function, which is common in atherosclerosis. Decline in renal function alone causes elevated plasma homocysteine (and cysteine). These observations suggest that mild hyperhomocysteinemia could often be an effect rather than a cause of atherosclerotic disease. Data on the common C677T methylenetetrahydrofolate reductase polymorphism supports this, in that, although homozygosity is a frequent cause of mild hyperhomocysteinemia when plasma folate is below median population concentrations, it appears not to increase cardiovascular risk. Indeed, there is recent evidence suggesting an acute antioxidant effect of folic acid independent of its effect on homocysteine concentrations. This antioxidant mechanism may oppose an oxidant effect of homocysteine and be relevant to treatment of patients with vascular disease, especially those with chronic renal insufficiency. Such patients have moderately elevated plasma homocysteine and greatly increased cardiovascular risk that is largely unexplained. Am J Clin Nutr 2000;72:315–23.

KEY WORDS Homocysteine, cardiovascular disease, methylenetetrahydrofolate reductase, folic acid, oxidative stress, thromboembolism, renal function

INTRODUCTION Despite the impressive epidemiologic evidence that mild hyperhomocysteinemia is an independent risk factor for atherosclerotic and atherothrombotic vascular disease, we have become increasingly doubtful as to whether modest elevations of plasma homocysteine may be causally involved in the pathogenesis of atherosclerosis. As will be outlined in this review, there are now substantial indications that a modest elevation of plasma homocysteine is usually benign and is a consequence rather than a cause of atherosclerosis.

A REVIEW OF THE EVIDENCE

Homocystinuria and vascular disease

First, it must be emphasized that the vascular disease in homocystinuria due to cystathionine β-synthase (CBS) deficiency, methylenetetrahydrofolate reductase (MTHFR) deficiency, or inborn errors in cobalamin metabolism bears little resemblance to the atherosclerotic and atherothrombotic vascular disease seen in the adult general population. Atherosclerosis is characterized by a thickening of the arterial wall due to smooth muscle cell proliferation, lipid deposits, and fibrosis (1). Rupture of the lipid-containing atherosclerotic plaques results in thrombosis (atherothrombosis) and leads to myocardial infarction and stroke (1). In contrast, homocystinuria seems to be associated with a primary thrombotic disorder that affects veins more often than arteries (2). Stroke in homocystinuric patients is frequently due to intracranial venous thrombosis, which is a rare cause of stroke in the general population (3–10). Multifocal old and fresh mural thromboses in different stages of organization are found post-mortem (7, 8, 11–15). The changes in the arterial wall are patchy, lack lipid deposits in young patients and have an appearance that may represent the arterial wall repair response to repeated mural thrombosis (8, 11, 15–17). Therefore, homocystinuria seems to be associated with a factor or factors that primarily cause venous and arterial thrombosis. Whether the same factor (that in homocystinuria does not seem to cause the characteristic changes of primary atherosclerosis) would be atherogenic in much lower concentrations in the general population remains uncertain.

In 1969, McCully (18) put forward the homocysteine theory of arteriosclerosis. This was based on the findings of arterial changes in an infant with homocystinuria due to CBS deficiency similar to those in an infant with homocystinuria due to a remethylation defect, one an inborn error involving the transsul-
furation pathway (CBS deficiency) and the other an inborn error affecting the remethylation of homocysteine to methionine. The changes in the arterial wall lacked lipid deposits and were classified as arteriosclerosis (not atherosclerosis) (18). Because the 2 different disorders shared markedly elevated homocysteine concentrations as a metabolic consequence of the inborn errors but with widely different concentrations of methionine, homocysteine or a derivative of homocysteine was considered to be the common factor leading to arterial damage (18).

In homocystinuria, there is evidence that the very high homocysteine concentrations are thrombogenic. However, in both CBS deficiency and inborn errors of homocysteine remethylation, the precursor of homocysteine, S-adenosylhomocysteine (SAH), will most likely also accumulate. SAH is the demethylated product of numerous S-adenosylmethionine (SAM)-dependent transmethylation reactions and a potent feedback inhibitor of the same reactions (19). It is possible that SAH accumulation leads to hypomethylation of some essential components (19), the tissue-specific sensitivity of which may explain both similarities (thrombosis) and dissimilarities (ectopia lentis, skeletal deformities, and osteoporosis) between the 2 forms of homocystinuria (ie, that due to CBS deficiency or that due remethylation defects). In homocystinuria, therapy that lowers plasma homocysteine concentration also reduces SAH and restores impaired transmethylation reactions. In patients with CBS deficiency, this has been shown to effectively reduce the risk of thrombotic events, although plasma homocysteine concentrations frequently remain well above normal values (20–22). This suggests that the threshold of homocysteine concentration for thrombogenesis is clearly higher than the modestly elevated concentrations of homocysteine found in patients with cardiovascular disease.

**Mild hyperhomocysteinemia and vascular disease**

The important core question is: does a modest elevation of plasma homocysteine concentration (eg, from 15 to 20 μmol/L) contribute to the pathogenesis of atherosclerotic vascular disease, or is it merely a marker for increased risk? Several studies established that the association between plasma homocysteine concentration and the risk of cardiovascular disease or severity of atherosclerosis is graded throughout the normal range from low to mildly elevated concentrations (23, 24). If this graded relation reflects a pathogenic role of homocysteine in the development of cardiovascular disease, one could assume that any cause of longstanding, mild hyperhomocysteinemia would also be associated with increased cardiovascular risk. Well-known, common causes of hyperhomocysteinemia are low serum or red cell folate concentrations (25, 26), vitamin B-12 deficiency (26, 27), decline in renal function (28, 29), and the TT genotype for the common C677T/MTHFR polymorphism in conjunction with low folate status (30). Moreover, heterozygosity for CBS deficiency is associated with normal or mild elevation of basal homocysteine concentration and frequently with an abnormal response to methionine loading with increased postload hyperhomocysteinemia (31–33).

The clinical symptoms of untreated vitamin B-12 and folate deficiency are well-known. However, whereas hyperhomocysteinemia in these conditions may be moderate (> 30 μmol/L) or even severe (> 100 μmol/L) (26), vascular disease is not a known complication of folate or vitamin B-12 deficiency. Folate status (serum or red cell folate or folate intake) is considered to be one of the most important determinants of plasma homocysteine concentration, and folic acid supplementation decreases plasma homocysteine concentration in almost all subjects (34–37). Much of the relation between folate status and plasma homocysteine concentration seems to be dependent on the genotype of the C677T/MTHFR polymorphism (30). There are several ongoing, randomized, blinded intervention studies with folic acid or placebo treatment of patients with vascular disease to determine whether homocysteine-lowering therapy reduces cardiovascular risk and improves outcome (38).

There were a few longitudinal studies in which homocysteine was not measured that assessed cardiovascular outcome related to folate status, with varying results (39–44). In addition, there were many studies in which both plasma homocysteine and folate status were assessed. Only one of these studies showed that a low folate status together with elevated homocysteine may constitute a risk factor for cardiovascular disease (45). Paradoxically, this small retrospective study and another prospective study showed increased incidence or risk of mortality and coronary heart disease in subjects with elevated serum vitamin B-12 concentrations (43, 45). A few other studies indicated that a poor folate status itself may be associated with increased risk but that this risk is only marginally (46–48) or only partially (49, 50) mediated by homocysteine. Although the bulk of studies confirmed the strong negative relation between folate status and plasma homocysteine concentration on one hand and the relation between increased homocysteine concentrations and cardiovascular disease on the other, the results of these studies do not suggest that the former relation is coupled to the latter. In other words, elevation of plasma homocysteine concentrations due to poor folate status seems to be benign with regard to risk or severity of cardiovascular disease (51–68). It follows from this that if ongoing intervention studies were to show that folic acid therapy reduces cardiovascular risk, an additional question would be whether the risk reduction was due to lowering of homocysteine or to some other effect of the vitamin. Recently, a prospective study in women showed that an elevated plasma homocysteine concentration predicts myocardial infarction and stroke. Self-reported multivitamin supplement use at baseline was associated with markedly lower concentrations of homocysteine but not with a lower risk of myocardial infarction or stroke during follow-up compared with nonusers (69).

**The C677T/MTHFR polymorphism, homocysteine, and vascular disease**

A common polymorphism (C677T) in MTHFR leads to thermolability of the enzyme and increased sensitivity to low folate concentrations (30). About 12% of the white population is homozygous (TT genotype) (30). Several studies established that TT homozygotes on average have ~3.5 μmol/L or 35% higher plasma homocysteine concentrations than do wild-type homozygotes (CC genotype) (70). Because high plasma homocysteine concentrations are confined to individuals with serum or plasma folate concentrations in the lower range, these TT homozygotes have even higher homocysteine elevations than those given above (70). The TT genotype is present in ~30% of individuals in the population who have plasma homocysteine concentrations > 18 μmol/L; of those with homocysteine values > 40 μmol/L, ~70% are TT homozygotes (30). Considering that the TT genotype is present from birth and that dietary habits in adults tend to be stable over many years, TT homozygotes with low folate
intakes would indeed be expected to be exposed to markedly elevated plasma homocysteine concentrations for decades. Therefore, during the past few years, >40 investigations, including several thousands of cardiovascular patients and control subjects, studied whether the TT genotype is associated with increased risk or severity of arterial disease (30, 44, 62–64, 68, 70–86). Our conclusions from these studies are that the hyperhomocysteinemia associated with the TT genotype, even in the presence of low folate status (44), seems to be benign and not related to the risk or severity of cardiovascular disease.

Early in the homocysteine-cardiovascular disease era, the hypothesis studied was whether heterozygotes for CBS deficiency have increased risk of cardiovascular disease. As indicated above, obligate heterozygote parents of patients with homocystinuria have normal or only slightly increased fasting plasma homocysteine concentrations but commonly respond to oral loads of methionine with abnormally high plasma homocysteine concentrations (31–33, 51). Several studies have shown that patients with premature cardiovascular disease frequently respond abnormally to methionine loads and that postload hyperhomocysteinemia is a risk factor for cardiovascular disease (32, 33, 51, 87). However, an international survey did not show increased cardiovascular risk in obligate heterozygotes, although the study had relatively low power (2). Later, when DNA-based methods became available, it could not be confirmed that cardiovascular disease patients with abnormal responses to methionine were predominantly heterozygotes for common mutations leading to CBS deficiency (88, 89) or that such CBS mutations were more frequent in patients than in control subjects (90, 91). These findings suggest that abnormally high plasma homocysteine concentrations after methionine loading may not be related to an important degree to cardiovascular disease because heterozygotes for CBS deficiency who have such responses to methionine appear not to have elevated cardiovascular disease risk.

What, then, is the explanation for the frequent finding of mild hyperhomocysteinemia in association with atherothrombotic cardiovascular disease? The Hordaland Study, the largest population-based study of the relation between plasma homocysteine and risk factors for cardiovascular disease, provided important data relevant to this question (92). The study showed that elevated plasma homocysteine was strongly and positively related to major components known to be associated with atherosclerosis and cardiovascular risk, ie, age, male sex, smoking, blood pressure, elevated total cholesterol, and lack of exercise. Many other studies in patients with cardiovascular disease and healthy control subjects showed similar associations. Importantly, plasma homocysteine seems to be related to blood pressure (59, 89, 93–97), which is a well-known major risk factor not only for atherothrombotic cardiovascular disease but also for nephrosclerosis and decline in renal function (98–101). Also, atherosclerosis is considered to contribute to the nephrosclerotic process (100–103).

Renal function and mild hyperhomocysteinemia

Serum creatinine, a marker with low sensitivity for early decline in renal function, is a strong determinant of plasma homocysteine concentration (29, 53, 104–107). Almost every study has shown a highly significant positive correlation between the concentrations of creatinine and homocysteine. In renal disease, both the creatinine and homocysteine concentrations increase, and in end-stage renal disease, plasma homocysteine concentrations may become 3–5 times higher than normal (28, 29, 108). From studies in both humans and animals, it was estimated that the kidneys account for ≈70% of plasma clearance of homocysteine (109, 110). The importance of renal function for plasma homocysteine concentration has been shown in subjects with normal serum creatinine concentrations (105–107, 111). Mean plasma homocysteine concentrations were 4 μmol/L, or 45% higher in subjects with normal serum creatinine who had glomerular filtration rates (GFRs) in the lower range, assessed directly or with cystatin C, than in subjects with normal serum creatinine and GFRs in the upper range (105, 107). Moreover, in multivariate analysis, GFR predicts plasma homocysteine concentration much more accurately than does serum creatinine (105–107, 111). This is consistent with serum creatinine being an insensitive indicator of early renal decline. As a consequence, controlling for serum creatinine to adjust statistically for the effect of mildly impaired renal function on circulating homocysteine does not provide an accurate assessment of the effect of mildly impaired renal function on plasma homocysteine concentrations.

It could be that the results of several important studies on homocysteine and cardiovascular disease or death would have been different if it had been possible to adjust for GFR instead of serum creatinine. In one prospective study that showed that plasma homocysteine strongly predicted cardiovascular mortality in patients with type 2 diabetes, homocysteine concentrations were related to GFR, but after adjustment for GFR and other covariates, the predictive value of homocysteine was completely eliminated (112).

We have put forward the view that impaired renal function due to hypertension and atherosclerosis is an important cause of the elevated plasma homocysteine found in vascular disease patients (30, 53). The reasons are as follows. Atherogenesis and elevation of blood pressure commonly develop silently over many years before the emergence of clinically evident vascular events. These processes also lead to nephrosclerosis and a degree of deterioration of renal function (98–103), and this is highly relevant to the plasma clearance of homocysteine (109). For these reasons, the presence of vascular disease itself may contribute to an elevation in circulating homocysteine by leading to a decline in renal function (53). This means that because of reduced renal function, patients with either occult or clinically evident cardiovascular disease and normal serum creatinine concentrations may have elevated circulating homocysteine concentrations. This could also explain the graded relation between plasma homocysteine and severity of atherosclerosis.

A recently published study by McQuillan et al (64) emphasized much of the points above. In 1111 subjects, carotid intima-media thick (IMT), an index of generalized atherosclerosis, was determined and found to be strongly and positively related to plasma homocysteine concentration. Among several factors, IMT was also related to advanced age, elevated blood pressure, and plasma creatinine. All of these are well-known to be associated with some decline in renal function. A strong relation between increased IMT and low GFR has been shown in diabetic patients (113). In contrast, in the study by McQuillan et al, although serum folate, folate intake, and the TT genotype of the C677T/MTHFR polymorphism were strong determinants of plasma homocysteine concentration, none of these factors alone or in combination was related to IMT (64).

Thus, we have reason to believe that much of the epidemiologic association found in prospective and retrospective studies and in cross-sectional studies between modestly elevated plasma homocysteine concentrations and cardiovascular risk or severity
of atherosclerosis is explained by the renal mechanism outlined above (Figure 1). This constitutes the basis for our reverse causality hypothesis, which implies that mild hyperhomocysteinemia in association with atherosclerotic cardiovascular disease is a consequence and not a cause of the disease. Our hypothesis is also supported by the lack of consistent evidence that mild hyperhomocysteinemia due to reduced folate or vitamin B-12 status, heterozygosity for CBS deficiency, or homozygosity for the C677T/MTHFR gene polymorphism is associated with increased cardiovascular risk.

Mild hyperhomocysteinemia and venous thromboembolism

There is, however, considerable epidemiologic evidence that mild hyperhomocysteinemia is a risk factor for venous thromboembolic disease, as reviewed recently (114) and supported by other recent studies (115, 116). These are interesting findings because homocystinuria (severe hyperhomocysteinemia) seems to be associated primarily with thrombotic disease, which predominantly affects veins. Although on one hand the mild hyperhomocysteinemia associated with the usual form of vascular disease may be a reflection of an associated modest renal impairment, this mechanism does not explain a possible relation between mild hyperhomocysteinemia and venous thromboembolism because the latter is not usually associated with reduced renal function. Thus, it is possible that mild hyperhomocysteinemia itself may enhance the risk of both venous and arterial primary thrombogenesis.

To explore this possibility, we performed a meta-analysis of 15 published reports (68, 115, 117–130) on the common C677T/MTHFR polymorphism and venous thrombosis (Figure 2). The analysis included a total of 2683 patients with venous thromboembolism and 3306 control subjects. A sixteenth study was not included because of uncertainty regarding the ethnic origin of the patients and control subjects (125). Although the TT genotype is a major cause of mild hyperhomocysteinemia (70, 118), the prevalence of the TT genotype was the same in patients with venous thromboembolism (14.9%) as in control subjects (14.3%). The odds ratio (OR) after adjustment for heterogeneity between studies (30) was 1.09 (95% CI: 0.907, 1.316). Therefore, these findings do not support the hypothesis that mild hyperhomocysteinemia is causally related to primary venous or arterial thrombosis, assuming that the polymorphism is not in some way protective against it.

Mild hyperhomocysteinemia and endothelial function

Recent data on interrelations between endothelium-dependant vasodilatation mediated by nitric oxide release and plasma homocysteine raise important issues. After the initial study by Celermajer et al (131) showing that endothelium-dependant vasodilatation is reduced in homocystinuric patients but not in their obligate heterozygote parents, several groups established that an ~3-fold increase in circulating homocysteine after a standard methionine load transiently reduces endothelium-dependant vasodilatation. In a careful study, Bellamy et al (132) also showed that a reduction of plasma total homocysteine from 14.9 ± 7.4 to 8.7 ± 2.5 μmol/L after 6 wk of oral folate supplementation (5 mg/d) enhanced endothelium-dependant brachial artery dilatation in 18 healthy adults. These effects may be mediated by a reduction in homocysteine-induced oxidative stress.

The recent findings of Kanani et al (133) support this possibility. They showed that oral ascorbic acid, a potent antioxidant, prevented endothelial dysfunction associated with a 2–3-fold increase in homocysteine after a standard methionine load (133). Chambers et al (134) reported similar findings. And in the coronary circulation, Schachinger et al (135) documented an inverse relation between a range of plasma homocysteine concentrations...
and endothelium-dependent coronary blood flow (135). There is also evidence that elevated homocysteine may be associated with enhanced oxidative stress. The findings of Bellamy et al (132) could be explained by folate itself having an antioxidant effect that opposes homocysteine-induced oxidative changes, as suggested by Verhaar and Rabelink (136), and in this way influences endothelial nitric oxide–mediated vasodilatation. The results of a study by Usui et al (137) provide support for this concept. They showed that a single, large (10 mg) oral folic acid dose reversed impaired flow-mediated dilatation after a standard methionine load without affecting the associated acute increase in homocysteine concentration (137).

The long-term implications of these observations for the pathogenesis of vascular disease are of course unknown. However, the persisting endothelial dysfunction associated with this order of homocysteine elevation seen in selected patient groups—particularly in patients with advanced renal failure and a small proportion of vascular patients—could enhance the atherogenic effects of other standard risk factors. This may be particularly relevant in chronic renal failure. Such patients are at greatly increased cardiovascular risk that is largely unexplained and, as mentioned, commonly have circulating homocysteine concentrations 3-fold higher than normal. Homocysteine-associated endothelial dysfunction could also increase vascular risk when occurring together with common prothrombotic factors. Factor V Leiden is an example of such a factor. It is common, with a prevalence of 4% in the Australian white population (138), and confers an increased risk of venous (but not arterial) thrombosis, a risk that may well be amplified when linked with endothelial dysfunction.

CONCLUSIONS

It has been established that lowering the markedly elevated circulating homocysteine concentrations found in patients with the inborn error of homocystinuria due to CBS deficiency, even to suboptimal concentrations, greatly reduces cardiovascular risk (20, 139). This finding defines a key role for grossly elevated homocysteine concentrations in the pathogenesis of vascular disease. The relevance to vascular risk of mild hyperhomocysteinemia is, however, still undetermined. The results of the many ongoing homocysteine-lowering trials with folic acid in vascular patients may certainly clarify whether folate therapy is relevant to cardiovascular risk in the general population and will provide much important information (38). However, if the trials show a positive effect of supplementation they will not of course separate the effects of oral folate supplementation from those of lowering homocysteine concentration. This might be done by comparing folic acid and betaine therapy in such patients because both lower circulating homocysteine but by different mechanisms. This would be an extremely interesting study but one that is unlikely to be done.

We acknowledge Lars Brudin, head of the Department of Clinical Physiology, County Hospital, Kalmar, Sweden, for his invaluable help with the meta-analysis.

REFERENCES


FIGURE 2. Meta-analysis of 15 studies on the risk of venous thromboembolism in association with the commonly hyperhomocysteinemic TT genotype of the C677T methylenetetrahydrofolate reductase polymorphism compared with the normally normohomocysteinemic CT and CC genotypes. Odds ratio: 1.093 (95% CI: 0.907, 1.316). The analysis was adjusted for heterogeneity between studies (30).


