Recent data are not in conflict with homocysteine as a cardiovascular risk factor

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An elevated level of plasma total homocysteine is an independent, graded and strong risk factor for cardiovascular disease, which shows a strong interactive effect with conventional risk factors. It is a predictor of cardiovascular disease events in high-risk populations, but is weakly associated with risk in healthy individuals. A common polymorphism in the MTHFR gene is a major determinant of the total homocysteine level, but is unrelated to cardiovascular disease risk in most studies. This observation is in accordance with the view that high total homocysteine itself may not be particularly deleterious, but provokes vascular occlusion under conditions predisposing to cardiovascular disease. Curr Opin Lipidol 9:533–539. © 1998 Lippincott Williams & Wilkins

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Abbreviations

CAD	coronary artery disease
CVD	cardiovascular disease
EVD	endothelial-dependent vasodilatation
MTHFR	methylenetetrahydrofolate reductase
tHcy	total homocysteine

Introduction

An elevated homocysteine level or hyperhomocysteinemia was first reported to be associated with cardiovascular disease (CVD) in patients with homocystinuria, a group of inborn errors in metabolism leading to severely elevated homocysteine levels [1]. Based on the clinical and pathological findings in these patients, McCully in 1969 [2] proposed the homocysteine theory for atherosclerosis, which suggested that moderate elevation in homocysteine is also a CVD risk factor. In 1976, Wilcken and Wilcken [3] found that patients with coronary artery disease (CAD) had an abnormal homocysteine metabolism, and in the following 15 years, retrospective case–control studies have shown that homocysteine is associated with occlusive disease in the coronary, cerebral and peripheral vessels and with venous thrombosis [4-5]. In 1995, Boushey *et al.* [6] published a meta-analysis based on results from 27 studies which convincingly showed that total homocysteine (tHcy) is an independent, graded risk factor for CVD. Since then, there has been an increasing number of studies investigating tHcy as a risk factor [7**]. The majority support the conclusions of Boushey *et al.* but also emphasize thrombogenicity and interactive effects as consequential traits of elevated tHcy.

Total homocysteine as an interactive factor provoking the acute event

It has frequently been suggested that moderate hyperhomocysteinemia promotes atherosclerosis and acts independently of conventional risk factors [6]. Two papers challenge this view [8**,9**].

The European COMAC project on homocysteine and vascular disease [8**] is a multi-centre case–control study comprising 750 patients with confirmed CVD and 800 control individuals. This study showed that an increased tHcy level confers an independent and graded risk of CVD of similar strength to that of smoking or hyperlipidemia. A novel finding was that hyperhomocysteinemia interacts with several conventional risk factors, in particular smoking and hypertension, thereby markedly enhancing their effect [8**].

The second article describes a Norwegian prospective study on tHcy as a predictor of mortality in 587 CAD patients who underwent coronary angiography in 1991–1992 [9**]. The tHcy level measured at the time of the angiography was significantly associated with

a previous history of myocardial infarction but, surprisingly, it was only weakly associated with the extent of coronary stenosis. After 5 years, 11% had died, but the mortality ratio showed a strong and dose-dependent relation with tHcy. In patients with a tHcy <9 µmol/l, only 4% had died compared with nearly 27% in those with a tHcy ≥20 µmol/l. Furthermore, high tHcy was a stronger predictor of mortality than conventional risk factors, including high cholesterol, elevated lipoprotein (a), and hypertension, and actually equaled low ventricular ejection fraction and reduced renal function.

The COMAC study emphasized that an elevated tHcy level should not be evaluated independently of other risk factors, while the Norwegian mortality study suggested that a high tHcy level causes an acute event rather than atherosclerosis, but both studies indicate that an elevated tHcy level is particularly important in patients with high CVD risk.

The C677T MTHFR polymorphism, cardiovascular disease and longevity

Homozygosity for the C677T transition in the methylenetetrahydrofolate reductase (MTHFR) gene (TT genotype) causes thermolability of the enzyme, reduces enzyme activity and thereby inhibits the formation of 5-methyltetrahydrofolate which serves as a methyl donor during remethylation of homocysteine to methionine. This explains why the TT individuals have elevated tHcy under conditions of impaired folate status [10–12,13••].

Since the TT genotype is common (with a prevalence of approximately 10% in Caucasians) and causes elevated tHcy [13••], it is logical to investigate its relation to CVD. Recent studies in Japanese populations, comprising approximately 1000 patients and control individuals, indicate that the TT genotype is a significant risk factor for CVD [14–17], whereas data from Caucasian populations suggest that the relation to CVD is weak or absent [13••]. A meta-analysis confined to studies with CAD patients (2478 cases and 2481 control individuals) showed that the TT genotype was a weak but significant risk factor (odds ratio = 1.22, P <0.05) [18•]. However, in another meta-analysis, which also included patients with stroke and peripheral artery disease (5869 patients and 6644 control individuals), the relative risk was only 1.12, and not significant [13••].

If the TT genotype predisposes to major causes of mortality such as CVD, the TT prevalence would be expected to be low in the very old. One study on approximately 1000 Japanese individuals demonstrated that the TT prevalence decreased significantly with age [19•]; however, a meta-analysis comprising approximately 1388 elderly and 1415 younger individuals showed nearly the same prevalence of the TT genotype in young versus very

old individuals (13.3% versus 11.3%, P > 0.05), suggesting that the C677T MTHFR polymorphism does not markedly affect longevity [20•].

In view of the many negative studies on MTHFR and CVD, two pioneers in the homocysteine field [3,21], David Wilcken and Lars Brattström, recently questioned the status of hyperhomocysteinemia as a CVD risk factor [22,23••].

Is high total homocysteine caused by the C677T MTHFR transition harmless?

The mean tHcy level is approximately 25% higher in the TT than in the CC genotype [13••]. This difference corresponds to an estimated increase in vascular risk of approximately 25% [6,24,25•]. However, the TT genotype confers no or only marginal CVD risk in most ethnic groups [13••]. This finding, together with high and the large interethnic variability in the TT genotype frequency [26] suggests that this enzyme variant represents a nutritional genetic adaptation which may have a contingent health promoting effect. For example, the TT genotype is associated with a lower von Willebrand factor, which may protect against CVD [27], and is also associated with decreased risk of colorectal cancer [28,29]. On the other hand, it may promote CVD risk by interacting with certain genetic traits [30], as demonstrated for the factor V Leiden mutation in some [31] but not all [32] populations.

Conceivably, hyperhomocysteinemia caused by the TT genotype may have a different association with CVD than elevated tHcy which is a result of other causes. The COMAC study demonstrated that hyperhomocysteinemia in TT individuals is associated with less CVD risk than a similar tHcy elevation in CC/CT individuals [Meleady *et al.* (1998), unpublished observations]. This unexpected observation was explained when the prevalence of conventional risk factors was compared in TT and CC/CT individuals with tHcy ≥ 15 µmol/l. The hyperhomocysteinemic TT individuals had a relatively low CVD risk profile, and the high tHcy level was attributed to MTHFR genotype in combination with low vitamin status. In contrast, the hyperhomocysteinemic CT/CC individuals had a high prevalence of conventional risk factors. The data may indicate that hyperhomocysteinemia associated with TT genotype is less harmful than hyperhomocysteinemia which is accompanied by a high risk factor level. This corroborates the powerful interactive effects of high tHcy described in the COMAC report [8••].

Prospective studies

Approximately 15 prospective studies based on community or occupational cohorts, including a total of 3500 cases and 5000 control individuals, have been published to date [7••,25•,33•–35•,36]. Approximately 80% of these studies

show a positive trend, and about 60% have reported significant positive results. The tHcy level was lower in cases compared with control individuals in only one study [33•].

Notably, the prospective studies based on community/occupational cohorts demonstrate weaker associations between tHcy and CVD than the retrospective case–control studies. For example, in the prospective British United Provident Association study [25] on CAD-related mortality, a 5 μmol/l increase in tHcy was associated with a 41% increase in risk. The corresponding increase in risk based on results from eight retrospective studies on myocardial infarction was 85% [25•]. There is also a trend demonstrating that tHcy is less predictive of the first event than the second or later events [34•], and that the predictive value of tHcy decreases with time from blood sampling to the event [33•,34•,37].

In contrast to the generally weak results obtained from community based cohorts, results from the prospective studies based on patients with CAD [9••], systemic lupus erythematosus [38] or renal failure [39–41] demonstrate that high tHcy is a very strong predictor of mortality or CVD-related complications. On the other hand, the tHcy level does not predict, or only weakly predicts, progression of atherosclerosis or restenosis after PTCA [42,43].

Overall, the prospective studies indicate that an elevated tHcy level is a strong risk factor for CVD events in high risk populations, but a much weaker predictor of CVD events in presumably healthy individuals.

Total homocysteine as a cause or as an indicator of vascular disease?
Total homocysteine and the cardiovascular disease risk profile
An elevated tHcy level fulfills most of the defined criteria of causality, such as consistency, strength, temporality, and biological plausibility [44]. However, an elevated tHcy level is influenced by and shows a remarkable relation to several CVD risk factors [45]. Women have lower tHcy levels than men, and tHcy increases with age and after menopause [7••,45]. Physical activity and a diet rich in fruit and vegetables are associated with a low plasma tHcy, whereas smoking and caffeinated coffee increase the tHcy level [46,47••]. Plasma tHcy correlates with cholesterol and blood pressure [7••,45]. High tHcy levels are invariably present in individuals with impaired renal function [48], but have also been associated with reduced left ventricular function [9••,49,50], and hypothyroidism [51], i.e. conditions associated with increased CVD risk (Table 1).

Recent reports suggest that tHcy is not related to CVD in populations with a favourable risk profile. A study on healthy and active 80-year-old individuals demonstrated

Table 1. **Total homocysteine as a risk factor for cardiovascular disease**

Prevalent in the general population
Acquired and genetic determinants
Associated with both venous and arterial disease
Predicts thrombosis and acute CVD events
Strong and independent
Dose-dependent, no threshold
Interactive effects with other CVD risk factors
Relatively harmless in the absence of CVD or predisposing factors
Mechanism(s) still unknown, but plausible theories exist

CVD, cardiovascular disease.

that they had overall favourable levels of the conventional CVD risk factors but their tHcy was high [52]. Similarly, 95 CAD patients with a low risk profile were found to have tHcy levels equal to those of matched control individuals [53]. These data, together with reports of MTHFR genotypes in CVD patients, some negative prospective studies and the relations between tHcy and other risk factors, have raised the question of whether an elevated tHcy is just an epiphenomenon reflecting the overall CVD risk [23••].

Homocystinuria
The case of homocystinuria brings strong support to the homocysteine theory. These inborn errors of metabolism are caused by cystathionine β-synthase deficiency, severe MTHFR deficiency or defects in intracellular cobalamin metabolism. Irrespective of the site of defect, these patients have a high incidence of vascular occlusive disease. Moreover, intervention with tHcy lowering vitamins markedly reduces the risk of a fatal thromboembolic event [1].

The question has been raised as to whether high tHcy alone is sufficient for development of CVD. In Israeli homocystinurics, Mandel et al. [54] reported that thromboembolic events occurred only in patients with the factor V Leiden mutation. The possibility of an interactive phenomenon is supported by the finding of increased risk of venous thrombosis in hyperhomocysteinemic individuals with the factor V Leiden mutation [31,55,56], but such an interaction has been questioned [57,58]. A recent study [59] found the Leiden mutation in only one out of six homocystinuria individuals with thrombotic events, but a remarkably high prevalence (50%) of the TT MTHFR genotype. This suggests that a combination of genetic defects renders these individuals susceptible to vascular events.

In 1997, Wilcken and Wilcken published their results on the follow-up of 40 homocystinuria patients [60••]. Overall, there was an estimated 90% reduction in CVD events in the 32 individuals receiving effective homocysteine lowering therapy. Even among the 15 pyridoxine non-responsive individuals with a mean tHcy of approximately

100 μmol/l, no events were observed. Except for high tHcy, the CVD risk profile is presumably low in these young individuals. The data indicate that elevated tHcy causes disease, but in the absence of other risk factors, even a very high tHcy level seems relatively harmless, emphasizing the multifactorial genesis of CVD.

Mechanisms of action

To date, there is no unifying hypothesis for the vascular effects of homocysteine, but proposed mechanisms include promotion of platelet activation, hypercoagulability, increased smooth muscle cell proliferation, cytotoxicity, stimulation of LDL oxidation and endothelial dysfunction [61,62,63**]. A recent study indicated that homocysteine-mediated responses on vascular smooth muscle cells are coupled to diacylglycerol-dependent protein kinase C activation via the N-methyl-D-aspartate receptor [64*]. Literature on mechanisms of action has been critically reviewed by Lentz [63**].

Most mechanistic studies are carried out *in vitro*. The relevance of some of these studies to the development of vascular occlusion *in vivo* should be questioned because high homocysteine concentrations (in the millimolar range) are often used, similar effects can be obtained by other thiols [63**] and the in-vitro model systems usually do not reflect the disulfide exchange and redox reactions that take place *in vivo* [65]. Furthermore, the in-vitro data frequently do not explain the vascular pathology associated with hyperhomocysteinemia, in particular because homocystinuria and moderate hyperhomocysteinemia are associated with both venous and arterial occlusive disease [7**] whereas specific platelet or clotting abnormalities cause vascular lesions preferentially at either the venous or the arterial side [66]. Finally, plaque formation and atheroma are attributed to hypercholesterolemia and increased LDL oxidation, but are rarely observed in patients with homocystinuria [1,62].

A mechanism involving endothelial dysfunction seems attractive because it is relevant for both arterial and venous occlusion, and has been found in hyperhomocysteinemic patients *in vivo* [62,63**]. In 1976, Harker *et al.* [67] reported that endothelial desquamation occurs in baboons infused with Hcy. Endothelial lesions or increased circulating endothelial cells have also been observed in animal models [68,69] and in healthy individuals receiving a standard methionine load [70*]. Notably, impaired endothelial-dependent vasodilatation (EVD) has recently been demonstrated *in vivo* in patients with homocystinuria [71], transiently following methionine loading [72**,73] and in moderate hyperhomocysteinemia [74,75]. Folate supplementation improves EVD both in hyperhomocysteinemic [76] and hypercholesterolemic individuals [77], but not in hyperhomocysteinemic monkeys [78,79]. No relationship between tHcy and EVD was observed in renal failure patients [80] or in healthy individuals with chronic high methionine intake [81]. Conceivably, various experimental techniques may give different results, adaptive processes may exist, and the disease may modify the EVD response.

Elevated tHcy may affect EVD by forming an S-nitrosohomocysteine adduct, which itself is a potent vasodilator. Nitric oxide may also be regarded as a homocysteine scavenger, and when depleted, free homocysteine can exert its toxic effect on the vascular endothelium as well as the smooth muscle cells [63**]. In addition, the prooxidant effects of high homocysteine levels may inhibit formation or enhance degradation of nitric oxide [61]. This idea is now supported by two studies that have shown that the effect of homocysteine on EVD can be prevented by vitamin C [82*].

Perspectives

Findings during the past year have both challenged and refined the concept of tHcy as a risk factor. Clinical trials with homocysteine reducing therapy are now required. The beneficial effects of such therapy are indicated by the observations that a high intake of folate or vitamin B6 is associated with lower CVD risk [83,84], and may delay the progression of atherosclerosis [85,86].

Different strategies for intervention have been proposed, but all studies currently in progress include folic acid. A daily supplement of 0.5 mg folic acid per day is expected to reduce plasma tHcy by approximately 25%, and the reduction is largest in individuals with high tHcy levels [87*]. A lower folic acid dose may be equally effective [88]. Overall, vitamin B12 will probably only lead to a small additional tHcy reduction [87*], but should be included because it may protect against undiscovered vitamin B12 deficiency. Vitamin B6 does not affect fasting tHcy levels [7**], but may lower post-load tHcy levels and have a cardioprotective effect unrelated to tHcy [35*,89].

Conclusion

The case of homocystinuria strongly suggests that severely elevated tHcy levels are a causal risk factor for thromboembolic events, which can be prevented by homocysteine reducing therapy.

Recent studies suggest that moderate hyperhomocysteinemia enhances the effect of other risk factors and that it is more strongly associated with thrombosis or acute complications of CVD than with the slowly developing atherosclerotic process.

In the absence of other risk factors, moderate hyperhomocysteinemia seems to cause little harm, but it may promote CVD and the effects of coexisting risk factors.

The mechanism by which elevated homocysteine exerts its vascular effect is still unclear, but increasing evidence suggests that it interferes with normal endothelial function and impairs the nitric oxide-dependent vasodilatory response.

The results from intervention trials with homocysteine reducing therapy will be crucial for our view on moderate hyperhomocysteinemia as a risk factor for CVD in the general population.

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