Hyperhomocysteinemia in health and disease: where we are now, and where do we go from here?

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Abstract

Homocysteine is a sulfur-containing amino acid, which is synthesized from the precursor methionine through a multi-step process, and then reconverted to methionine or catabolized into cysteine. The presence of vitamin B9 (folic acid), vitamin B6 (pyridoxine) and vitamin B12 (cobalamin) is essential in homocysteine metabolism, wherein deficiency of one or more of these nutrients is associated with various degree of hyperhomocysteinemia. There is little doubt that hyperhomocysteinemia is associated with several human disorders, such as cardiovascular disease, neurodegenerative disorders, pregnancy complications and fractures, so that its measurement might be useful for risk assessment. Nevertheless, several randomized homocysteine-lowering therapy trials have failed to show that supplementation with vitamins B substantially modifies (and – more importantly – improves) the end points and the related outcomes. According to the current state of scientific knowledge, it seems thus reasonable to conclude that lowering homocysteine alone is probably insufficient to mitigate the risk of thromboembolic, cardiovascular and neurodegenerative disorders inasmuch as this bizarre amino acid acts in strict synergy with other probably more powerful risk factors. Several lines of evidence suggest, however, that its measurement may be helpful for identifying subjects at greater risk of disease, who may thus benefit from a more aggressive treatment of other modifiable risk factors, as recently shown by result of the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial.

Keywords: cardiovascular disease; homocysteine; hyperhomocysteinemia; risk factor; venous thrombosis.

Homocysteine in health and disease

Homocysteine (Hcy) is a relatively simple sulfur-containing amino acid (HSCH₂CH₂CH[NH₂]CO₂H), homologue to cysteine, from which it only differs for the presence of an adjunctive methylene group. Hcy cannot be considered as a traditional nutrient, since it needs to be endogenously synthesized from the precursor methionine through a multi-step process which principally encompasses removal of the terminal C β methyl group (Figure 1). The compound can then be alternatively reconverted to methionine, or converted into cysteine through processes catalyzed by methionine synthase or betaine homocysteine methyltransferase in the former case, and by cystathionine β-synthase in the latter, respectively. Throughout the metabolic cycle of Hcy, the presence of vitamins, such as vitamin B9 (commonly known as folic acid or folate), vitamin B6 (also known as pyridoxine) and vitamin B12 (also known as cobalamin) is essential (Figure 1) (1), so that deficiency of one or more of these vitamins is associated with various degree of hyperhomocysteinemia. Other important determinants of serum Hcy concentration are genetic polymorphisms, especially those involving the enzyme methylenetetrahydrofolate reductase (MTHFR), methionine synthase, methionine synthase reductase, and cystathionine β-synthase (2), as well as ageing, renal disease and cigarette smoking (3).

The important contribution of homocysteine in human disease has been acknowledged since the early 1960s, when cases of homocystinuria were associated with vascular disorders. In particular, the analysis of archival cases of homocystinuria in 1933 and cobalamin C disease in 1968 led Kilmer S. McCully to first hypothesize that Hcy may be involved in the pathogenesis of vascular disease by a direct effect of the amino acid on arterial cells and tissues (1). This earlier conjecture has catalyzed a large number of cross-sectional and prospective studies over the ensuing decades, aimed to definitely establish whether this sulfur-containing amino acid could be considered a powerful predictor of cardiovascular disease. Before analyzing the potential association between Hcy and thrombosis, we should however bear in mind that the generic term “thrombosis” underlines rather different entities, i.e., venous (4), arterial (5) and even lymphatic thrombosis (6), which are characterized by a specific pathogenesis and only marginally share predisposing conditions and relative risk factors (7). Although a certain condition may henceforth be considered a risk factor for arterial thrombosis (e.g., periodontal disease) (8), it does not automatically translate into a risk factor for venous thrombosis and vice versa (e.g., antithrombin deficiency) (9, 10). One notable exception is probably Hcy, since several studies have now contributed to raise the issue that hyperhomocysteinemia may be associated with both venous and arterial thrombosis. It is noteworthy that Hcy has also been implicated in the pathogenesis of other non-classically “thrombotic” disorders, including neurodegenerative diseases, such as Alzheimer’s disease, vascular dementia, cognitive impairment or stroke (11, 12), pregnancy complications (13)
as well as bone loss, decreased bone strength, and increased risk of fracture (14) among others.

Where do we stand, now?

The evidence of a link between hyperhomocysteinemia and different human disorders produced an unprecedented scale of clinical studies that were aimed to explore the epidemiological association of serum Hcy and venous thrombosis, cardiovascular disease, neurodegenerative disorders and pregnancy complications.

Venous thrombosis and cardiovascular disease

The most recent meta-analysis linking hyperhomocysteinemia with venous thrombosis was published by Den Heijer et al., who identified case-control or nested case-control studies by searches of electronic literature for relevant reports published before July 2003 (15). The following meta-analysis of 24 retrospective (n=3289 cases) and three prospective studies (n=476 cases) showed that a 5 \( \mu \)mol/L higher measured Hcy level was associated with a 27% [95% confidence interval (95% CI) 1%–59%] higher risk of venous thrombosis in prospective studies, and a 60% (95% CI 10%–134%) higher risk in retrospective studies. The relationship between hyperhomocysteinemia and coronary heart disease has been eventually meta-analyzed by Humphrey, who performed a MEDLINE search 1966 through March 2006 for prospective cohort studies that measured Hcy and incidence of disease in the general adult population (16). Twenty-six articles of good or fair quality were included in the final model, which showed 20%–50% increased risk of coronary heart disease for each 5 \( \mu \)mol/L higher measured Hcy level. The resulted combined risk ratio (RR) for coronary events was 1.18 (95% CI 1.10–1.26) for each increase of 5 \( \mu \)mol/L in Hcy level, independently of traditional cardiovascular risk factors. As regards the relationship between hyperhomocysteinemia and peripheral arterial disease, Khandanpour et al. searched MEDLINE, EMBASE and Cochrane databases from 1950 to December 2007, selecting observational studies and trials that evaluated Hcy levels in patients with peripheral arterial disease compared with unaffected controls (17). The meta-analysis of 14 relevant studies showed that Hcy was significantly elevated (pooled mean difference +4.31 \( \mu \)mol/L; 95% CI 1.71–6.31 \( \mu \)mol/L) in patients as compared with controls. The relationship between Hcy levels and stroke was explored by the Homocysteine Studies Collaboration in 2002 (18). The MEDLINE database was searched for articles published from January 1966 to January 1999. Overall, data from prospective or retrospective studies involving a total of 1113 stroke events were included in a meta-analysis of individual participant data. After adjustment for cardiovascular risk factors and regression dilution bias in prospective studies, 25% lower usual Hcy level (i.e., ~3 \( \mu \)mol/L) was associated with a 19% [odds ratio (OR), 0.81; 95% CI 0.69–0.95] lower risk.
Neurodegenerative diseases

Van Dam et al. performed a search through large literature and trial databases on the relationship between hyperhomocysteinemia and Alzheimer’s disease (20). Nine qualitatively good case-control studies (631 patients, 703 controls) were identified and meta-analyzed, producing a pooled standardized mean difference of Hcy levels of 1.04 μmol/L (0.44–1.63 μmol/L) in favor of patients with Alzheimer’s disease. The analysis of prospective cohort studies including 2569 subjects revealed a pooled RR for Alzheimer’s disease in hyperhomocysteinemia of 2.5 (95% CI 1.38–4.56). Muntjewerff et al. performed a meta-analysis of eight retrospective studies (812 cases and 2113 control subjects) to assess the potential relationship between Hcy and schizophrenia, and found that 5 μmol/L higher measured Hcy level was associated a 70% (95% CI 27–129) higher risk of disease (21). Almeida et al. performed a systematic review and meta-analysis of PubMed articles published between 1966 and November 2007 that investigated the association between hyperhomocysteinemia and depression or depressive symptoms (22). Nine cross-sectional studies were identified, showing that older adults with high Hcy had an OR of 1.70 (95% CI 1.38–2.08) for depression. More recently, Ho et al. carried out random-effects meta-analyses on studies investigating the relationship between Hcy and the risk of developing dementia/cognitive decline (23). Seventeen pertinent studies (13 cross-sectional and 4 prospective) including 6122 participants were finally included in the meta-analysis. Overall, Hcy was found to be significantly higher in both patients with Alzheimer’s disease (pooled standardized mean difference 0.59 μmol/L; 95% CI 0.38–0.80 μmol/L) and vascular dementia (pooled standardized mean difference: 1.30 μmol/L; 95% CI 0.75–1.84 μmol/L) than in controls. A trend towards a higher risk of developing dementia in patients with hyperhomocysteinemia was also found in the analysis of the four prospective studies (OR 1.34; 95% CI 0.94–1.91).

Pregnancy complications

Nelen et al. identified 10 case-control studies published between January 1992 and November 1999, which assessed Hcy levels in women with two or more pregnancy losses before 16-week menstrual age (24). The resulting pooled ORs of recurrent early pregnancy loss for hyperhomocysteinemia, fasting and after methionine loading, were 2.7 (95% CI 1.4–5.2) and 4.2 (95% CI 2.0–8.8), respectively. Hogeveen et al. performed a systematic review and meta-analysis on the association between maternal Hcy and birth weight, retrieving data from English, German, and French publications with the use of the PubMed database (January 1966–July 2010) (25). The literature search produced 19 reliable studies, consisting of 21,326 individuals, and the consequent pooled analysis resulted in a crude OR of 1.25 (95% CI 1.09–1.44) for hyperhomocysteinemia. After expression of the estimate as a continuous measure, a 31 g decrease in birth weight was associated with 1 SD increase in maternal Hcy.

Where do we go from here?

Although the outcomes of most several meta-analyses seem to concur in attributing a clear role to Hcy in human disease, especially in cardiovascular or neurodegenerative disorders and pregnancy complications (Table 1), yet vast challenges remain to bridge the gap between epidemiological observational and interventional studies. Basically, it must be established as to whether Hcy can be effectively considered a “cause” of human pathology, an indirect “marker”, or both. The answer to this question is nothing but ancillary, since the lack of a causal relationship between hyperhomocysteinemia and disease would still fulfill the basic requirements for a justifying a population screening, but not for establishment of a widespread and even aggressive lowering therapy.

To establish whether Hcy-lowering intervention in patients with and without pre-existing cardiovascular disease is effective for preventing cardiovascular events and death, is safe for preventing cardiovascular events and differ in efficacy or safety, Martí-Carvajal performed a comprehensive search of randomized controlled trials with a follow-up period of 1 year or longer, including adults at risk of or with established cardiovascular disease, who were administered vitamin B6, B9 or B12 (alone or in combination) (26). Eight randomized clinical trials, including 24,210 participants with a low risk of bias, were finally analyzed, yielding a RR of 1.03 (95% CI 0.94–1.13) for non-fatal or fatal myocardial infarction, 0.89 (95% CI 0.73–1.08) for stroke, and 1.00 (95% CI 0.92–1.09) for death by any cause, thus concluding that there is no sufficient evidence to support the use of Hcy-lowering therapy for preventing cardiovascular events. Clarke et al. recently performed a meta-analysis of published results of eight Hcy-lowering trials for preventing vascular disease, including 37,485 patients and providing comparisons of the effects of B vitamins on 5074 coronary heart disease events, 1483 stroke events, 2692 incident cancer events, and 5128 overall deaths (27). Interestingly, allocation to B vitamins had no beneficial effects on any cardiovascular events, with hazard ratios (HR) of 1.01 (95% CI 0.96–1.07) for coronary heart disease, 0.96 (95% CI 0.87–1.07) for stroke, 1.08 (95% CI 0.99–1.17) for cancer, and 1.02 (95% CI 0.97–1.07) for death from any cause. Hence it seems reasonable to conclude that although identification of hyperhomocysteinemia might be somehow useful, lowering its concentration may
not substantially modify (and – more importantly – improve) cardiovascular outcomes.

The effect of Hcy lowering by supplementation of B vitamins on the risk reduction of venous thromboembolism has been investigated by den Heijer in a randomized, placebo-controlled, double-blind trial (28). Overall, the rate of recurrent venous thrombosis did not significantly differ between patients treated with vitamins and those with placebo (i.e., 12.2% vs. 14.3%). The relative HR associated with vitamin treatment was 0.84 (95% CI 0.56–1.26), thus showing that Hcy lowering therapy seems also ineffective to prevent venous thrombosis. Analogous results were obtained by Mei et al, who recently published the results of a meta-analysis of randomized controlled trials on the effect of Hcy lowering therapy and risk of cerebrovascular events (29). After comprehensive search of MEDLINE and OVID databases from January 1966 to December 2008, 17 trials involving 39,107 patients with cardiovascular disease were included, but results of the following meta-analysis failed to show any significant difference between the intervention group and the control population, the RR for patients treated with folic acid or B vitamins supplementation compared with controls being 1.01 (95% CI 0.97–1.05) for overall cardiovascular events, 1.01 (95% CI 0.94–1.07) for coronary heart disease, and 0.94 (95% CI 0.85–1.04) for stroke. As regards neurodegenerative disorders, Hcy is indeed a surrogate marker for B vitamin deficiency and a neurotoxic agent, so that the notion of improving clinical outcomes by lowering Hcy with B supplementation would be unquestionably appealing (11). Likewise thrombotic disorders, the outcomes of two recent randomized placebo controlled trials are however disappointing. Sun et al. performed a randomized, double-blind, placebo-controlled study in Taiwanese patients with mild to moderate Alzheimer’s dementia, and found that although a 26-week multivitamin supplement containing vitamins B6, B12 and folic acid was effective to decrease Hcy concentrations, no significant differences in cognition or performance of activities of daily living function were found between multivitamin and placebo (30). In the latter study, Kwok et al. found the concentration of Hcy was reduced to nearly 9 μmol/L in a group of mild to moderate Alzheimer’s disease patients supplemented with methylcobalamin and folic acid for 24 months as compared with a placebo group, but no significant difference in any of the neuropsychological scores could be recorded between supplement group and placebo (31). Since it is now well established that periconceptional folate supplementation is effective to reduce the risk of neural tube defects, the question as to whether Hcy-lowering therapy would be also effective for reducing the risk of other pregnancy complications is probably pleonastic and anachronistic (32). Regardless of speculative considerations, the conclusions of several observational studies on vitamin B supplementation and adverse pregnancy outcomes including miscarriage, gestational diabetes, premature rupture of the membranes, placental abruption, have been questioned for a variety of methodological drawbacks, which preclude drawing definitive conclusions (33).

Therefore, we are now facing a tangible paradox, whereby epidemiological evidence strongly supports the association between Hcy and human disease, reliable biological mechanisms have been provided to justify the pathogenetic role of this sulfur-containing compound (e.g., direct injury to vascular structure and function, or promoting atherosclerosis by enhancing the atherogenicity of cholesterol-rich lipoproteins, especially lipoprotein[a]) (34), but yet most intervention trials have failed to show any significant clinical benefit from homocysteine-lowering therapy. It is thereby reasonable to reiterate the question as to whether Hcy has definitively shrunk (35)? In this issue of Clinical Chemistry and Laboratory Medicine, we publish an article by Herrmann and collaborators, which prospectively investigated the relationship between Hcy and deep venous thrombosis or pulmonary embolism in 9522 participants of the 5-year Fenoﬁbrate Intervention and Event Lowering in Diabetes (FIELD) trial (36). Fenoﬁbrate is a lipid regulating agent of the fibrate class, which is administered to lower triglycerides

### Table 1  Available evidences on the role of homocysteine in human disorders according to data of the most recent meta-analysis.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Risk</th>
<th>References</th>
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<tbody>
<tr>
<td>Venous thrombosis</td>
<td>27% (95% CI 1%–59%) for each 5 μmol/L of Hcy (prospective studies).</td>
<td>(15)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>Risk ratio 1.18 (95% CI 1.10–1.26) for each 5 μmol/L of Hcy.</td>
<td>(16)</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>Pooled mean difference +4.31 μmol/L (95% CI 1.71–6.31 μmol/L) in patients as compared with controls.</td>
<td>(17)</td>
</tr>
<tr>
<td>Stroke</td>
<td>OR 0.81 (95% CI 0.69–0.95) for –3 μmol/L lower Hcy level.</td>
<td>(18)</td>
</tr>
<tr>
<td>Retinal vascular occlusive disease</td>
<td>Standard difference +0.867 μmol/L (95% CI 0.735–0.999 μmol/L) in patients as compared with controls.</td>
<td>(19)</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>RR 2.5 (95% CI 1.38–4.56) for hyperhomocysteinemia.</td>
<td>(20)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>70% (95% CI 27–129) for each 5 μmol/L of Hcy.</td>
<td>(21)</td>
</tr>
<tr>
<td>Depression</td>
<td>OR 1.70 (95% CI 1.38–2.08) for hyperhomocysteinemia.</td>
<td>(22)</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>Pooled mean difference +0.59 μmol/L (95% CI 0.38–0.80 μmol/L) for hyperhomocysteinemia.</td>
<td>(23)</td>
</tr>
<tr>
<td>Recurrent early pregnancy loss</td>
<td>OR 2.7 (95% CI 1.4–5.2) for hyperhomocysteinemia.</td>
<td>(24)</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>OR 1.25 (95% CI 1.09–1.44) for hyperhomocysteinemia.</td>
<td>(25)</td>
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</table>

Hcy, homocysteine; OR, odds ratio; RR, relative risk; 95% CI, 95% confidence interval.
and cholesterol to a lesser extent. The preliminary results of the FIELD study have already highlighted a slightly higher incidence of pancreatitis, deep venous thrombosis, and pulmonary embolism associated with this drug (37), which has paved the way to further scrutiny. In this subsequent analysis of FIELD trial, the concentration of Hcy significantly increased by 6.5 μmol/L during fenofibrate therapy, a change that was fully reversible in the placebo group but persisted in the treatment group until reversing at the end of therapy (36). Throughout follow-up, the rate of venous thromboembolism was nearly double in fenofibrate than in placebo group (2.17% vs. 1.43%; p=0.006). After adjustment for potential confounders, baseline Hcy and the fenofibrate therapy persisted as significant independent predictors of thrombosis risk, and each 5 μmol/L higher baseline Hcy was associated with 19% higher risk of VTE in multivariate analysis. The interaction between fenofibrate and hyperhomocysteinemia was greater in patients with the highest baseline Hcy levels, thus providing reliable clue on the existence of a biological interaction between Hcy and fenofibrate.

Conclusions

According to the current state of scientific knowledge, it seems thus reasonable to conclude that lowering Hcy alone is probably insufficient to mitigate the risk of thromboembolic, cardiovascular and neurodegenerative disorders inasmuch as this bizarre amino acid acts in strict synergy with other probably more powerful risk factors. Several lines of evidence suggest, however, that its measurement may be helpful for identifying subjects at greater risk of disease, who may thus benefit from a more aggressive treatment of other modifiable risk factors. This is indeed in agreement with the outcome of the FIELD trial, in which Hcy was found to be a significant predictor of thrombotic events in the entire cohort (with a relative risk comparable to that previously reported by den Heijer et al., i.e., 19% vs. 27%), but not in the placebo group (36). It is also noteworthy that a positive association has been observed between dyslipidemia, especially hypercholesterolemia, and venous thromboembolism. Therapy with hypolipidemic agents has henceforth been provocatively suggested for preventing venous thrombosis on the background that statins and other cholesterol-lowering drugs may exert a pleiotropic, protective effect against hypercholesterolemia, endothelium dysfunction, platelet hyperreactivity and excessive thrombin generation (38, 39). According to the valuable data gathered from the FIELD trial, the potential benefits of fenofibrate in lowering triglycerides and cholesterol should however be carefully weighted against its potential metabolic interaction with Hcy.

Conflict of interest statement

**Authors’ conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Honoraryarium:** None declared.

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