Plasma total homocysteine: instigator or indicator of cardiovascular disease?

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Personal View

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Abstract

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in developed countries. However, traditional risk factors cannot fully account for this. In the last 20 years, there has been an explosion of interest in plasma total homocysteine (tHcy) as a potential modifiable risk factor for CVD. Recent meta-analyses of epidemiological studies support the concept that increased tHcy concentrations are associated with CVD. This has led to the 'homocysteine hypothesis', which states that lowering plasma tHcy using folic acid and other B-vitamins will reduce the risk of CVD. In experimental studies, homocysteine has been shown to cause oxidative stress, endothelial cell dysfunction and promote thrombogenesis. However, data from recent large randomized controlled trials have shown that there is no clinical benefit to lowering plasma tHcy concentrations with folic acid and other B-vitamins. This lack of effect of tHcy lowering strongly suggests that homocysteine is not an *instigator* but merely an *indicator* of CVD.

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Introduction

Homocysteine is a sulphur-containing amino acid produced during the metabolism of the dietary amino acid methionine and once formed can be metabolized via two pathways: (1) remethylation to methionine, in a series of reactions that are dependent on folate and the vitamins B_{12} and B_2 as co-factors, and (2) trans-sulphuration to cysteine via the enzyme cystathionine β-synthase, which requires vitamin B_6 as a cofactor. In 1969, a report linking marked elevations of the amino acid homocysteine in urine (homocystinuria) to thromboembolism and arteriosclerosis in children with inborn errors of homocysteine metabolism led to the concept of homocysteine as an instigator of vascular and thrombotic disease.[1] In homocystinuria, it is clear that the grossly elevated plasma total homocysteine (tHcy) concentrations (>200 μmol/L) play a direct role in the development of vascular and thrombotic disease. In the general population, although tHcy concentrations are much lower (<15 μmol/L), epidemiological studies demonstrated that a mild to moderate elevation in plasma tHcy (hyperhomocysteinaemia) was associated with an increased risk of cardiovascular disease (CVD).[2,3] This evidence led to the homocysteine hypothesis of CVD, which assumed an elevated plasma tHcy played a causal role in its development and by implication, a reduction in tHcy would result in a reduced risk of CVD.

Evidence linking homocysteine and cardiovascular disease

Early retrospective and cross-sectional studies have shown a strong relationship between plasma tHcy concentrations and cardiovascular events, which contrasts with the more recent long-term prospective studies.[2] The difference in the strength of these associations between retrospective studies, cross-sectional studies and prospective studies may be explained by the fact that data and blood samples are collected before the event in prospective studies and therefore exclude the influence of the disease process, lifestyle and dietary habits. Consistent associations between plasma tHcy and the risk of CVD are found only in those prospective studies where subjects already have a high risk of CVD, are older in age and are followed up for a short period.[4] However, it has been argued that the reduction in risk estimate in long-term prospective studies may be due to treatment, lifestyle and dietary changes during follow-up. These factors may modify tHcy concentrations during the course of the study and may underestimate/attenuate any true association between plasma tHcy concentrations and CVD risk.[5] Therefore, results from some of the long-term prospective studies appear to indicate that elevated concentrations of plasma tHcy may only be a short-term risk factor in subjects with a high risk of CVD or an indicator of the degree of the underlying vascular disease. The question of

whether homocysteine *per se* is responsible for these associations with CVD remains unanswered.

Effect of homocysteine lowering on cardiovascular disease outcome

Many studies have confirmed the effects of folic acid and other B-vitamins on tHcy lowering. In a large meta-analysis consisting of 25 randomized controlled trials involving data from 2596 individuals, it was concluded that daily doses of 0.8 mg folic acid were typically required to achieve the maximal reduction in plasma tHcy concentrations, while daily doses of 0.2 and 0.4 mg were associated with 60% and 90%, respectively, of this maximal effect.[6] During the last five years, several large prospective intervention trials have been initiated to investigate the effect of plasma tHcy lowering using folic acid and other B-group vitamins on cardiovascular events. Several of these have recently been reported. Three prospective intervention trials: the Norwegian Vitamin trial[7] (3749 patients), the Heart Outcomes Prevention Evaluation 2 trial[8] (5522 patients) and the Vitamin Intervention for Stroke Prevention trial[9] (3680 patients) all showed a significant decrease in plasma tHcy but no significant reduction in the composite primary endpoint of myocardial infarction, stroke or sudden death from CVD.

A recent meta-analysis of completed trials involving 16,958 CVD patients reported relative risks (95% confidence intervals) of outcomes for patients treated with folic acid compared with controls of 0.95 (0.88–1.03) for CVDs, 1.04 (0.92–1.17) for coronary heart disease (CHD) and 0.86 (0.71–1.04) for stroke, demonstrating that lowering plasma tHcy did not decrease the risk of CHD or stroke.[10] However, some have argued that a meta-analysis involving 50,000 subjects would be needed to have sufficient statistical power to confirm or refute the hypothesis.[11]

The observation that elevated concentrations of plasma tHcy are associated with an increased risk of CVD and venous thrombosis has stimulated research designed to investigate putative pathological mechanisms of hyperhomocysteinaemia. Assessment of endothelial dysfunction has generated particular interest because of its role as a key initiating event in the development of CVD. In humans, endothelial function can be assessed non-invasively using ultrasound technology.[12] High-dose folic acid (5–10 mg daily) reverses endothelial dysfunction in patients with CVD.[12] It has been assumed that the reduction in plasma tHcy concentrations was the mechanism underlying this observed improvement. However, the reduction in plasma tHcy has been shown, in several studies, not to correlate with the improvement in endothelial function.[13] A recent meta-analysis has demonstrated that folic acid improves vascular endothelial dysfunction in a dose-dependent manner, with greater improvement being observed when higher doses of folic acid are used.[14] Furthermore, in patients with homocystinuria due to a deficiency in the enzyme cystathionine β-synthase, folic acid and vitamin B_6 lowered the grossly elevated tHcy concentrations ($>200\ \mu$mol/L) observed in these patients and significantly reduced cardiovascular events.[15] This benefit was observed despite residual plasma tHcy concentrations ($>50\ \mu$mol/L) being several times the upper limit of 'normal' ($\sim<15\ \mu$mol/L), implying beneficial actions of B-group vitamins independent of homocysteine lowering.

It should be highlighted that at the time these large intervention trials were conceived, it was not known that high-dose folic acid may have a direct beneficial effect on the vasculature independent of homocysteine lowering. These large-scale intervention trials were therefore designed to test the 'homocysteine lowering hypothesis' of vascular disease, and this is reflected in the dose of folic acid used. Most of these trials used moderate doses of folic acid in the range of 0.2–2.5 mg/day. Extrapolation of the endothelial function studies would therefore predict that 'lower' dose intervention studies would not improve vascular outcome despite lowering tHcy, whereas higher dose studies may improve outcome as a result of a direct effect of folic acid. Further studies of longer duration and the inclusion of larger sample sizes, with the appropriate dose of folic acid and with all vascular events being recorded, will be needed to clarify these results.

Homocysteine as an indicator of inflammation

The observation that an elevated plasma tHcy concentration is associated with CVD may alternatively be explained if it were related to another yet undefined confounding causal factor. Recent evidence has shown that elevated plasma tHcy concentrations are associated with increased concentrations of neopterin[16] and C-reactive protein,[17] both of which are associated with CVD,[18,19] indicating that plasma tHcy may be linked to inflammation and immune system activation. Indeed, elevated plasma tHcy concentrations are associated with numerous inflammatory diseases and immune-cell activation states such as psoriasis,[20] systemic lupus erythematosus,[21] rheumatoid arthritis,[22] malignancies[23] and dementia.[24] This fact is highlighted further in that the use of glucocorticoids in patients with rheumatoid arthritis resulted in a significant reduction in plasma tHcy and C-reactive protein concentrations.[25] Furthermore, there is evidence to suggest that homocysteine is released from damaged vascular tissue following myocardial infarction[26] and stroke.[27] The processes involved in the repair of damaged vascular tissue results in an increased requirement for methylation of DNA, RNA and proteins, reactions that lead to the generation of homocysteine as the end product. Optimal concentrations of folates are essential in that they serve as donors of 1-carbon units in the biosynthesis of the purine ring of DNA and in the production of methyl groups. In addition, there is evidence to suggest that oxidative stress resulting from immune activation may lead to the oxidation of folates, resulting in folate deficiency despite a normal dietary intake.[28] Thus, hyperhomocysteinaemia may be a consequence of inflammation, oxidative degradation of folates and as an end product of tissue repair. Plasma tHcy may therefore be an indicator of inflammatory disease processes including atherosclerosis.

Indications for the measurement of plasma total homocysteine in the clinical laboratory

In view of the recent evidence in the literature, routine testing of patients with CVD in the general population is currently *not* recommended. However, in young patients (<30 years) with a history of thromboembolic complications, plasma tHcy *must* be measured to exclude homocystinuria, as treatment can prevent further serious and life-threatening thromboembolic and vascular complications. Measurement of plasma free homocysteine and urine homocysteine to exclude homocystinuria is not recommended, as these methods of analysis show poor sensitivities for the diagnosis of mild forms of homocystinuria, resulting in missed diagnoses.[29]

At present, many clinical laboratories measure plasma tHcy in response to requests from cardiovascular risk factor and thrombophilia clinics. However, the significance of these mildly elevated concentrations (approximately $< 50\ \mu$mol/L) and their interpretation are not fully understood or appreciated. Plasma tHcy concentrations are influenced by many factors, and it should be recognized that mild elevations of plasma tHcy are frequently observed in the general population. These factors include genetic polymorphisms, nutritional intake of B-vitamins, physiological and lifestyle factors, age and gender, various diseases and drug therapy.[30] It appears that low plasma folate and/or vitamin B_{12} status or renal impairment are the main predictors of plasma tHcy concentrations. Therefore, interpretation of elevated plasma tHcy concentrations should be performed in conjunction with folate, B_{12} and renal function, drug and clinical history.

Conclusions

The view that a mildly raised plasma tHcy (15–50 μmol/L) is an instigator in the development of CVD in the general population is an attractive hypothesis if only because folic acid offers an easy, inexpensive and generally safe means of lowering it. Following results from the recent large-scale tHcy lowering trials, it would appear that a mild increase in plasma tHcy is not an *instigator* but merely an *indicator* of the CVD process. However, it is important not to discount treatment with folic acid, as it is possible that at high doses folic acid may have a beneficial effect on cardiovascular outcome via mechanisms independent of homocysteine lowering.[31]

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