Homocysteine, Folate, and Cardiovascular Disease

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Abstract: Cardiovascular disease (CVD) continues to be one of the main causes of mortality in the western world, however approximately only two-thirds of all episodes can be attributed to traditional environmental and genetic risk factors. Over the past decade it has emerged that a moderate elevation in plasma concentrations of the amino acid homocysteine (tHcy) constitutes a risk factor for atherosclerotic vascular disease in the coronary, cerebral and peripheral vessels. Furthermore, this association is a graded one with no apparent threshold and is independent of, but may enhance the effect of conventional risk factors.

Plasma homocysteine is determined by both genetic and nutritional factors. The B-vitamins folate, B-12 and B-6 all play a key role in homocysteine metabolism and in fact it has been proposed that about two-thirds of all cases of hyperhomocysteinemia are due to an inadequate status of one or all of these vitamins. Of the three, folate appears to be the most important determinant and has been shown to significantly lower homocysteine concentration when administered at doses ranging from 0.2 to 10 mg/d in both healthy and hyperhomocysteinemic subjects.

There is considerable variation in the rate of CVD mortality between northern and southern European countries. A common dietary element in regions with lower CVD incidence i.e. southern European countries appears to be the higher consumption of fruit and vegetables. In the past this protective effect of fruit and vegetables has been primarily attributed to antioxidants. Fruit and vegetables are however also one of the main sources of folate in the diet, contributing to more than 30% of total dietary folate intake (even in countries where consumption of fruit and vegetables is low). Thus, in light of the evidence that folate may play a role in primary prevention of CVD via homocysteine-lowering the protective effect of fruit and vegetables may be partly explained by folate.

Key words: Homocysteine, folate, fruit and vegetables, cardiovascular disease

Introduction

Approximately only two-thirds of all episodes of vascular disease can be attributed to established risk factors including age, family history, sex and hypercholesterolaemia. The possible involvement therefore of other risk factors is currently receiving much attention. There is now considerable evidence to indicate that even moderately raised plasma levels of the sulphur amino acid homocysteine are associated with an increased risk of vascular disease in the general population.

Homocysteine: historical perspective

Homocysteine, the sulphur amino acid, was first discovered by DuVigneaud in 1932 [1] as a demethylation product of methionine. In the 1960's the condition homocysteinuria (markedly elevated of homocysteine in urine) was described in mentally retarded infants with congenital abnormalities [2, 3]. These early discoveries initiated numerous investigations into the clinical features and biochemical basis of homocysteinuria and led to reports describing the susceptibility of such patients to life-threat-

ening vascular disease [4, 5]. In 1969 McCully made the clinical observation linking elevated homocysteine concentration with vascular disease [6] following detailed autopsy evidence of extensive arterial thrombosis and atherosclerosis in two children with elevated plasma homocysteine. On the basis of his observations he subsequently proposed the homocysteine theory of atherosclerosis, however, it wasn't until 1976 that Wilcken & Wilcken published results which indicated that even a moderate elevation in homocysteine concentration might have a role to play in the pathogenesis of coronary artery disease [7].

Homocysteine: Reference range

Approximately 70–80% of homocysteine in plasma exists bound to plasma proteins, 1% circulates as free homocysteine, approximately 20–30% combines with itself to form the dimer homocysteine while the remaining (< 5%) combines with other thiols, including cysteine to form disulphide or circulates as the free thiol compound [8]. There is currently no established reference range for homocysteine however a value for total homocysteine between 5 and 15 μmol/l is generally considered normal, 15 to 30 μmol/l indicates moderate hyperhomocysteinaemia and 30–100 μmol/l intermediate hyperhomocysteinaemia [9]. More recently Selhub and colleagues have suggested that these values may be set too high. In a cross-sectional prevalence study plasma homocysteine concentration was assessed in a nationally (US) representative sample of 3563 males and 4523 females aged 12 years or over, participating in the National Health and Nutrition Examination Survey (NHANES 3) [10]. A high homocysteine concentration was defined as at least 11.4 μmol/l for male participants and 10.4 μmol/l for female participants.

The prevalence of hyperhomocysteinaemia is estimated to be 5% in the normal population and 13–47% among patients with atherosclerotic disease [11]. Homocysteine levels are higher in men than in pre-menopausal women [12, 13] and homocysteine levels are known to increase significantly with age [14].

Homocysteine Metabolism

The only exogenous source of homocysteine is methionine, an essential sulphur containing amino acid supplied through the catabolism of dietary proteins [15]. Methionine is converted to S-adenosylmethionine (SAM) in a reaction catalysed by the enzyme methionine adenosyltransferase. SAM acts as a methyl donor in the numerous transmethylation reactions, which take place in all mammalian cells including the methylation of transfer ribonucleic acid and deoxyribonucleic acid. The demethylated product of SAM, S-adenosylhomocysteine is further hydrolysed to adenosine and homocysteine.

The two most important pathways of homocysteine metabolism are the transsulfuration and remethylation pathways [15]. In the transsulfuration pathway homocysteine is catabolised to cysteine in two reactions, both of which require vitamin B-6 in the active form, pyridoxal phosphate (PLP). Alternatively homocysteine is remethylated to methionine in a reaction catalysed by either methionine synthase or betaine homocysteinemethyltransferase. The former enzyme which is widely distributed requires the active forms of both folate and vitamin B-12 as methyl donor and co-factor respectively. Not surprisingly therefore, conditions that limit the availability of these B-vitamins may lead to hyperhomocysteinaemia.

Causes of Hyperhomogysteinemia

Genetic

The most common genetic cause of moderately raised homocysteine concentration is a point mutation (C to T substitution at nucleotide 677) in the coding region of the gene for methylene-tetra-hydro-folate-reductase (MTHFR), a key enzyme in folate metabolism [16]. This results in a thermolabile variant of the enzyme with impaired activity. The frequency of this homozygous thermolabile genotype in different populations ranges from 5 to 16% and in association with sub-optimal folate status will result in elevated homocysteine concentration [17].

Nutritional

Two thirds of all cases of moderate hyperhomocysteinaemia are thought to be due to inadequate blood levels of one or more of the vitamins, folate B12 or B6, which is not surprising given their important role in homocysteine metabolism. An inverse association exists between homocysteine and the three B-vitamins, and this has consistently been shown to be strongest for folate. One of the earliest reports of the association between sub-optimal folate status and elevated homocysteine was by Kang and colleagues in 1987 who reported significantly higher homocysteine concentration in subjects with sub-normal folate status compared to controls [18]. Ubbink and colleagues examined the prevalence of suboptimal vitamin B12, B6 and folate status in men aged 40–60 years with elevated

plasma homocysteine and found it to be 56.8%, 25% and 29.1% respectively [19]. Furthermore a negative correlation was found with plasma folate. In the same year Selhub and colleagues investigated the contribution of B-vitamin intake and status to elevated homocysteine concentration in an elderly population (n = 1160) in the Framingham study [20]. Mean plasma homocysteine in the two lowest deciles of plasma folate was significantly greater than the mean for subjects in the highest decile.

Homocysteine and vascular disease: epidemiological evidence

Since the pioneering work of Wilcken & Wilcken [7] numerous studies have investigated the relationship between plasma homocysteine. These will be discussed under the headings of prospective studies and cross-sectional/case-control studies.

Prospective studies

Prospective studies provide the strongest epidemiological evidence for an association between homocysteine and vascular disease. Results to date however are not consistent. One of the early prospective studies to report a positive association was the Physicians Health Study where a relative risk of 3.1 was estimated for subjects compared to controls for fatal and non-fatal Myocardial Infaction (MI) and CHD [21]. It should however be noted that the association was confined to those with homocysteine concentrations in the top 5% of those studied. The Tromso study demonstrated a clear positive association between homocysteine concentration and coronary heart disease (CHD) in 123 subjects versus 492 matched controls [22]. Furthermore there was no threshold above which homocysteine was associated with CHD. A positive graded association has also been demonstrated for plasma homocysteine and risk of Ischaemic Heart Disease [23] and stroke [24].

A positive association has not been shown, however, in all studies. A follow-up study carried out on the Physicians failed to demonstrate a significant association between homocysteine and risk of MI/CHD after a more prolonged follow-up and in addition, no association was observed between plasma homocysteine and stroke or angina in this cohort [25]. The US physicians do however represent a relatively well-nourished sample and thus this may help explain the lack of association in this group. Other prospective studies which showed no association included the MRFIT study [26] and the ARIC study [27].

Cross-sectional and case-control studies

Numerous case-control and cross sectional studies have been carried out which examined the relationship between homocysteine and vascular disease. In 1995 Boushey and colleagues carried out a meta-analysis of 27 studies which included 19 case-control and 5 cross-sectional studies [28]. They concluded that elevated homocysteine was associated with an increased risk of coronary artery, cerebral vascular and peripheral vascular disease. The association was found to be a graded one, independent of other risk factors and the authors estimated that a 5 μmol/l increase in homocysteine would result in a 33% increase in vascular disease risk. These findings were confirmed in the European Concerted action project [29]; an elevated plasma homocysteine was demonstrated as being a risk factor for CVD with an estimated odd's ration of 2.2. Cross-sectional and case-control studies have also demonstrated a significant association between homocysteine levels and the extent of vascular disease in the coronary [30], peripheral [31] and carotid [32] arteries.

Mechanisms

Although the exact mechanism by which elevated homocysteine causes vascular disease is unknown studies indicate it is both atherogenic and thrombogenic [33]. Homocysteine appears to play a role in atherogenesis by facilitating oxidative arterial injury, damaging the vascular matrix, and promoting the proliferation of smooth muscle [34]. Homocysteine may promote thromboembolic disease by causing oxidative injury to the endothelium, altering anticoagulant pathway and endothelial anticoagulant function.

Treatment of Hyperhomocysteinaemia

Of the three vitamins folate, B12 and B6, folic acid has been shown consistently to reduce homocysteine concentration, even in subjects who are not folate deficient [35, 36]. A recent meta-analysis of 12 randomised controlled trials, including 1114 individuals demonstrated that folic acid in the range 0.5 to 5 mg/d lowers plasma homocysteine by approximately 25% with 0.5 mg/d shown to be as effective as 5.0 mg/d [37]. Furthermore it was shown that the greatest lowering was observed in those with the highest baseline homocysteine concentration. These doses, however, could only ever be achieved by supple-

mentation, therefore, the homocysteine lowering effect of lower doses is of interest i.e. doses that could be achieved through dietary modification or folic acid fortification. Ward and colleagues investigated the response of plasma homocysteine to folic acid administered daily at increasing doses of 100 μg (6 weeks) to 200 μg (6 weeks) to 400 μg (14 weeks) in healthy male subjects (n = 30) [38]. Results indicated that, while homocysteine concentration decreased significantly in response to 100 μg/d folic acid, increasing the dose to 200 μg/d resulted in further lowering. No additional lowering was however achieved in response to 400 μg/d folic acid and this was despite the fact that intervention at the highest dose was maintained for a period of 14 weeks. When the results of this study were expressed in tertiles of baseline homocysteine, the lowering was shown to occur only in the top two tertiles; homocysteine and interestingly folate (serum or red-cell) showed no response to intervention with any of the doses in the bottom tertile. It was concluded therefore that of the three doses, folic acid administered at 200 μg/d was the optimal homocysteine-lowering dose. Interestingly, 200 μg/d folic acid is also the dose predicted by Daly and colleagues to be optimal in reducing neural tube defect risk in women of child-bearing age, based on results of an intervention study which, examined the response of folate (serum and red-cell) to folic acid administered at doses ranging from 100 to 400 μg/d [39].

Folate: Current intakes

In a recent review it was estimated that current dietary folate intakes for European males and females range from 180 to 300 μg/d with mean reported intakes of 291 μg/d for men and 247 μg/d for women [40]. While clinical folate deficiency is relatively uncommon in such populations, folate status appears to be sub-optimal with respect to disease prevention. This is supported by the fact that significant reductions in plasma homocysteine, in association with increased red-cell folate concentration can be achieved in healthy subjects in response to physiological doses of folic acid. Furthermore this has been demonstrated in subjects with mean folate intakes estimated to be 281 ± 60 μg/d (i.e. meeting current dietary recommendations for folate) [38]. In the Nurses Health study, which examined intakes of folate in relation to non-fatal MI and fatal CHD, each 100 μg increase in folate in the population was associated with a 5.8% lower risk of CHD [41]. Furthermore, the lowest risk of CHD was among women who had a total folate intake (from food and supplements) greater than 400 μg/d. A total folate intake of 400 μg/d has also been reported by Selhub and colleagues to be associated with the lowest concentration of homocysteine in 1160 participants of the Framingham study [20].

The main dietary sources of folate are liver, yeast, green leafy vegetables and certain fruits. These foods may not however necessarily be the predominant contributors of dietary folate e.g. the major contributors of folate in the UK and Ireland are vegetables, bread and potatoes [42]. Throughout Europe highest folate intakes have been reported in France, Spain and Portugal while lowest intakes have been reported in the UK, Sweden and Ireland. Intakes less than 200 μg/d have been reported for Irish men aged 40 years or over [40]. It has been suggested that these differences in folate intake may partly reflect differences in dietary habits among European countries and that, compared to Northern European diets, Mediterranean diets contain greater portions of fruit and vegetables. It is well known that Coronary Heart Disease (CHD) mortality rates are also lower in Mediterranean countries. Traditionally this has been associated with a low saturated fat, high antioxidant and high dietary fibre intake, characteristic of Mediterranean diets. More recently however, it has been hypothesised that the lower incidence of CHD may be partly attributed to an increased folate intake from fruit and vegetables [43].

In conclusion, elevated plasma homocysteine is an established, independent risk factor for vascular disease. Treatment with physiological folic acid alone or in combination with vitamin B-6 and B-12 results in significant homocysteine-lowering. The results of a number of randomised clinical trials are currently awaited which will evaluate the effect of decreasing homocysteine concentration on major cardiovascular events [11]. Meanwhile it would appear worthwhile to recommend an increased consumption of folate from natural sources.

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