1.1.1 ***Folate***

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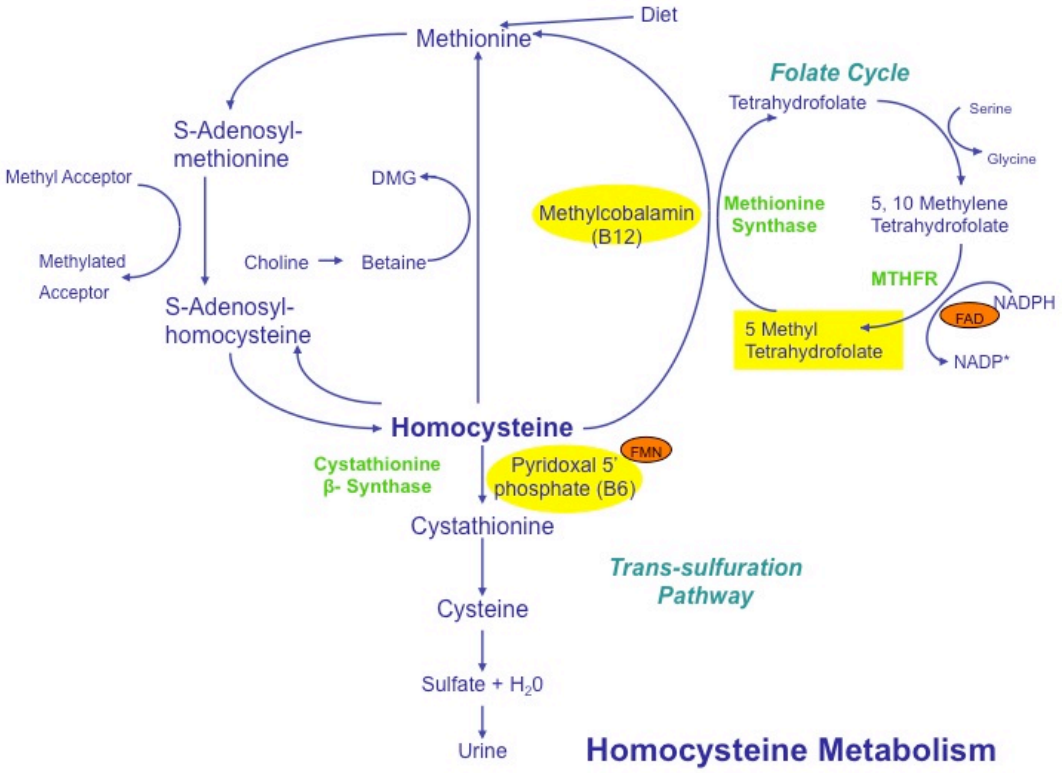
1.1.1.1 Summary

In recent years there has been much interest in the potential protective effect of the B-vitamin folate in cardiovascular disease. Such an effect may or may not be mediated via the role of folate (and the metabolically related vitamins B12, B6 and B2) in maintaining healthy plasma homocysteine concentrations. A number of recent secondary prevention trials in at-risk patients have, however, failed to show a benefit of homocysteine-lowering therapy on CVD events generally. Given that all of these trials were performed in patients with well-established pathology, current evidence appears to suggest that the administration of high dose folic acid (with or without B-related vitamins) to CVD patients is of no benefit in preventing another event, at least in the case of heart disease. The evidence supporting a beneficial role for those B-vitamins at this time is however somewhat stronger for stroke, with one meta-analysis of randomised trials showing that homocysteine-lowering with folic acid reduced the risk of stroke by 18% and by an overall 25% in patients with no history of stroke (but with pre-existing cardiovascular or renal diseases). The decline in stroke-related mortality in the North American population that relates to the timing of introduction of mandatory folic acid fortification, adds further support to the potential benefit of enhancing folate status and/or lowering homocysteine in the prevention of stroke. Evidence supporting a causal relationship between elevated homocysteine (or sub-optimal folate) and CVD also comes from genetic studies. The most important genetic determinant of homocysteine in the general population is the common 677C→T variant in the gene encoding for the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR). People homozygous for this polymorphism (TT genotype)—about 10% of populations worldwide—typically have higher plasma homocysteine levels and a 14 to 21% higher risk of CVD. Apart from folate, riboflavin (vitamin B2) is required as a co-factor for MTHFR, and has a major modulating effect on the expected (high homocysteine) phenotype in people with the TT genotype. New evidence shows that riboflavin intervention results in marked lowering of blood pressure specifically in patients with the TT genotype, an effect that may be independent of the homocysteine-lowering effect of riboflavin also seen only in individuals with the TT genotype. Of note, the fact that these effects of riboflavin are achievable with intervention at dietary levels, suggests that there may be important implications for dietary riboflavin requirements specifically in individuals with this common genetic variant in folate metabolism. The latest evidence linking folate and related B-vitamins to CVD, the relevant gene-nutrient interactions and the practical considerations in relation to achieving optimal B-vitamin status aimed at preventing CVD will be considered.

1.1.1.2 Introduction

This section considers the latest evidence in relation to the potential roles played by folate and related B-vitamins in preventing cardiovascular disease and relevant gene-nutrient interactions that appear to be implicated. These effects may be mediated via homocysteine, the metabolism of which requires an adequate status of up to four B-vitamins (Figure 1). Plasma homocysteine is very responsive to intervention with the relevant B-vitamins, in particular folate[1] but also (albeit to a lesser extent) vitamins B12,[2] B6[3] and B2 (riboflavin).[4] Although the literature in this area tends to focus on plasma homocysteine as the cardiovascular risk factor, it is also possible that folate and related B-vitamins have roles in CVD that are independent of their effects on homocysteine and that elevated plasma homocysteine may merely be a marker of sub-optimal B-vitamin status.

Figure 1 Homocysteine metabolism showing role of folate and related B-vitamins



1.1.1.3 Suboptimal folate status

Folate is required for one-carbon metabolism, involving the transfer of one-carbon units for DNA and RNA biosynthesis, methylation reactions and amino acid metabolism. Classically folate deficiency results in megaloblastic anaemia. Although this is relatively rare in the absence of an underlying clinical cause, many otherwise healthy populations in Europe and elsewhere have inadequate folate intakes when considered from the perspective of achieving an optimal folate status (a status associated with lowest risk of folate-related disease), rather than merely preventing overt folate deficiency (megaloblastic anaemia).[5] On the basis that normal homocysteine metabolism requires an adequate supply of folate, the measurement of plasma homocysteine concentration provides a reliable functional marker of folate status. When folate status is sub-optimal, plasma homocysteine concentration is invariably found to be elevated and

supplementation with folic acid can lower homocysteine levels in healthy and diseased populations by about 25%.[1]

1.1.1.4 Latest evidence linking low folate/elevated homocysteine with CVD

1.1.1.4.1 Evidence from population studies and randomised trials

There is much evidence over the past 15 years to link low folate status and/or elevated plasma homocysteine with an increased risk of CVD. In 2002, two major meta-analyses of numerous retrospective and prospective studies predicted that lowering homocysteine by 3μmol/l (or by about 25% based on typical homocysteine concentrations in Western populations of 12μmol/l) would reduce the risk of heart disease by 11-16% and stroke by 19-24%.[6,7] However, since 2004, a number of secondary prevention trials in at-risk patients published in high profile journals failed to demonstrate a benefit of homocysteine-lowering therapy on CVD events generally.[8,9,10,11,12] The most recent of these, the SEARCH trial, again found no significant effects of combined folic acid and vitamin B12 on major coronary events (RR 1.05 95%, CI 0.97-1.13) despite the large sample size (n=12064).[13] Thus the balance of evidence suggests that the administration of high dose B-vitamins to CVD patients with established disease is of little/no benefit in preventing another event, particularly in the case of heart disease. One of the aforementioned negative trials, however, did show a clear benefit of B-vitamin intervention in reducing the risk of stroke but for some reason the authors largely overlooked this result in their original report.[9] Instead, the beneficial effect of B-vitamins in relation to stroke risk were separately reported three years after the original report.[14] Likewise, a recent meta-analysis of 13 randomised trials by Lee and colleagues, predominantly concerning secondary prevention found a mild beneficial effect of folic acid in reducing the overall risk of stroke but this did not reach statistical significance when all 13 trials were considered together (RR 0.93, 95% CI 0.85-1.03; P=0.16).[15] However, another meta-analysis of randomised trials considered stroke events separately in patients with a history of stroke and in patients without a history of stroke but with pre-existing cardiovascular or renal diseases. This meta-analysis showed that homocysteine-lowering by folic acid reduced the risk of stroke by an overall 18%, but to a much greater extent (by 25%) in patients with no history of stroke (RR 0.75, 95% 0.62-0.90; *P*=0.002).[16] Consistent with the idea that folic acid may have a beneficial effect in preventing stroke (though by no means conclusive evidence) are population data from North America (1990-2002), showing an improvement in stroke mortality that relates to the timing of introduction of mandatory folic acid fortification, but no corresponding improvement over the same time period in the UK where no such fortification policy was put in place.[17] Thus current evidence to support the case for folic acid/homocysteine-lowering in CVD is stronger for stroke than for heart disease.

1.1.1.4.2 Evidence from genetic studies

Other evidence for a causal relationship between low folate status/elevated homocysteine and CVD comes from genetic studies. The most important genetic

determinant of homocysteine in the general population is the common 677C→T variant in the gene encoding the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR) required for the formation of 5-methyltetrahydrofolate, which in turn is required to convert homocysteine to methionine. People who are homozygous for this polymorphism (TT genotype), the reported frequency of which varies from 3% to 32% in populations worldwide,[18] have reduced MTHFR activity (resulting in impaired folate metabolism) and higher homocysteine concentrations *in vivo*;[19] an effect which is found to be particularly marked in people with low folate intake.[20] Importantly, evidence from various meta-analyses involving over 25,000 cases shows that individuals with the MTHFR 677 TT genotype have a significantly higher risk of CVD (by 14 to 21%) compared to those without this genetic factor.[7,21,22] There does, however, appear to be a large geographical variation in the extent of excess cardiovascular disease risk associated with this polymorphism. Studies that have examined this variation show that the excess risk of coronary heart disease is not significant in North American populations, while the polymorphism carries a significantly increased (but variable) risk of coronary heart disease elsewhere in the world including in European populations.[22,21]

Although largely overlooked, riboflavin (vitamin B2) is required as a co-factor for the MTHFR enzyme and therefore is needed to generate 5-methyltetrahydrofolate. Evidence shows that enhancing riboflavin status (through intervention with low-dose riboflavin) results in a marked lowering in homocysteine specifically in people with the MTHFR 677 TT genotype.[4] Presumably this effect of riboflavin works by neutralising the variant form of the enzyme which is reported to become inactive as a result of having an increased propensity to dissociate from its FAD (riboflavin) cofactor.[23] Thus ensuring optimal riboflavin intake and status may be important in modulating the risk of CVD specifically in people who have the TT genotype, i.e. 10% of Europeans generally, and much higher in certain sub-populations such as southern Italy where the reported frequency is as high as 26%.[24]

1.1.1.4.3 Folate related B-vitamins and blood pressure

Hypertension (i.e a blood pressure of 140/90mmHg or greater) is estimated to carry an almost three-fold greater risk of developing CVD,[25] while treating hypertension significantly reduces cardiovascular events, and stroke in particular.[26] Despite the significant associations that are observed between homocysteine and blood pressure in observational studies, intervention studies to lower homocysteine have shown little or no corresponding blood pressure response, suggesting that there is no causative relationship between elevated homocysteine concentrations *per se* and high blood pressure.[27] However, most published studies in this area have not considered the role of the MTHFR 677C→T polymorphism, but evidence is now emerging showing an important association between this common genetic factor and hypertension.[27] Moreover, evidence just published shows for the first time that riboflavin is an important determinant of blood pressure specifically in individuals with the TT genotype.[28] Of greater note, riboflavin intervention (at dietary levels, 1.6 mg/d) was shown to result in marked

lowering of blood pressure in CVD patients with the TT genotype (from 144/87 to 131/80mmHg), with no response observed in the other MTHFR genotype groups.[28] Thus riboflavin is effective in reducing blood pressure specifically in patients with the MTHFR 677 TT genotype. There may be important implications of these findings for the prevention and treatment of hypertension among the 4-26% of Europeans[24] who carry this genotype.

The precise mechanism linking the MTHFR 677C→T polymorphism, riboflavin and blood pressure is as yet unclear, although it is possible that nitric oxide (NO), a potent vasodilator, may be implicated. Vascular concentrations of 5-methlytetrahydrofolate were recently shown be important in regulating NO and endothelial function in patients with the MTHFR 677 TT genotype.[29] By stabilising the variant MTHFR enzyme, riboflavin supplementation may restore 5-methyltetrahydrofolate levels in vascular cells, improve NO bioavailability and in turn lower blood pressure specifically in patients with the MTHFR 677 TT genotype.

This novel genotype-specific effect of riboflavin on blood pressure may also partly explain the inconsistencies in the evidence as to the role of this common polymorphism in cardiovascular disease generally.[21,22] The policy of population-wide riboflavin fortification of food that has existed for over 50 years in North America would ensure higher and less variable intakes of riboflavin (compared with European populations), which could in turn be predicted to neutralise any phenotypic effect of the variant MTHFR enzyme. Thus differences in prevailing nutrient intakes in different regions could result in this polymorphism carrying an increased risk of CVD in Europe, but not in North America.[21,22]

1.1.1.5 Public health considerations in relation to folate and related B-vitamins

In practice achieving an optimal status of the B-vitamins folate, vitamin B12 and riboflavin can present particular challenges.

1.1.1.5.1 Achieving optimal folate status

Achieving optimal folate status in a population is a major challenge because of the poor stability and poor bioavailability of folate from natural food sources (e.g. green leafy vegetables, green beans, liver) compared with folic acid (the synthetic form of the vitamin found in fortified foods and supplements).[3] Thus there is a particular challenge for certain European populations with limited or no access to fortified foods where consumers are dependent on natural food folate sources as a means to optimise status. The decline in stroke-related mortality in the US and Canada relating to the introduction of mandatory fortification of food with folic acid[17] suggests that, although primarily aimed at preventing neural tube defects, folic acid fortification may also play a role in

the primary prevention of stroke. However, there are also safety concerns regarding long-term exposure to high dose folic acid. Traditionally these involved the potential risk that high folic acid intake might mask the anaemia of vitamin B12 deficiency while allowing the associated irreversible neurological symptoms to progress,[30] but more recently the new concern was raised that it may be associated with an increased risk of cognitive impairment in older people with low vitamin B12 status.[31] Furthermore, despite considerable evidence that folate within the dietary range plays a protective role against various cancers,[5] recent evidence has raised the concern that high dose folic acid (1 mg/d) may promote colorectal tumorigenesis in patients with pre-existing lesions[32] or significantly increase the risk of prostate cancer in men.[33] Because of these concerns, many governments worldwide have delayed decisions to implement population-based folic acid fortification policies as in the United States and Canada.

Thus mandatory (population-wide) folic acid fortification remains very controversial. However alternative strategies to increase folate status through health promotion and educational campaigns have generally been found to be ineffective, even in the case of preventing neural tube defects where the evidence for a beneficial effect is beyond any doubt.[3] Optimising folate should be a priority for public health, but will only be achieved with levels of intake of the vitamin greater than those currently provided by a typical diet as eaten in most European countries.

1.1.1.5.2 Achieving optimal vitamin B12 status

Dietary B12 is provided by animal foods (e.g. meat, fish, eggs, milk, cheese) and B12 intakes are generally found to be good in most healthy populations (often greatly exceeding recommended values). However, the achievement of an optimal status may present a particular difficulty for many older people because of the common problem of age-related food-bound B12 malabsorption estimated to effect up to 30% of otherwise healthy older people.[3,34] This arises mainly from atrophic gastritis, a chronic inflammatory condition resulting in decreased gastric acid production (hypochlorhydria), which diminishes B12 absorption because of the essential role of gastric acid in the release of protein-bound B12 from food. Unlike the treatment required for the classical (but rare) B12 deficiency symptom of pernicious anaemia involving B12 injections for life, older adults with low B12 status due to food-bound malabsorption should be able to absorb free (crystalline) vitamin B12 because it is not bound to protein. In fact, on the basis of this assumption, the Institute of Medicine in the United States recommends that people aged 50 years and over consume most of their vitamin B12 from crystalline B12 found in fortified foods and supplements. No such recommendation exists at this time in any European country.

Vitamin B12 is metabolically closely related to folate and, like folate, is also required for the remethylation of homocysteine to methionine. Thus achieving optimal folate status

only may not lower homocysteine to desirable levels (<10μmol/l),[35] and optimising vitamin B12 may have benefits over and above the effect of folate. A further 7% reduction in homocysteine can be achieved with vitamin B12 additional to that with folic acid alone.[1] Any small additional decrease in homocysteine could be predicted to confer a further benefit in terms of cardiovascular risk given that the relationship with homocysteine is a graded one. Of note, low biomarker status and functional deficiency of vitamin B12 (including B12-related elevated homocysteine) was found to be particularly prevalent in Asian Indians (even among non-vegetarians).[36] Thus Asian immigrants may be a vulnerable sub-group within Europe in relation to vitamin B12 status.

1.1.1.5.3 Achieving optimal riboflavin status

Riboflavin is provided in the diet predominantly through consuming milk and dairy foods. In the UK, the Scientific Advisory Committee on Nutrition has expressed particular concern about the high proportion of the British adult population (and particularly younger women) with apparently poor riboflavin status as determined from national survey data using the gold standard biomarker of status, erythrocyte glutathione activation coefficient (EGRac).[37] In general, dietary intakes of participants were found to compare favourably with recommended values with the exception of young women where high proportions were found to have low intakes.[37] Whether there is a general problem of poor riboflavin status in the UK and other European populations, as indicated by the large proportion with abnormal EGRac values, is unclear at this time, and requires further investigation. However, the new findings discussed above, that the higher blood pressure in patients with a common genetic variant in folate metabolism is responsive to low-dose riboflavin,[28] raises the possibility that improving riboflavin status may have an important role in preventing hypertension among these genetically at-risk people.

1.1.1.6 Conclusions

There is little evidence to support a beneficial role for supplemental folic acid in preventing further disease in patients with existing CVD. There remains interest in the question of whether optimal folate status and/or maintenance of lower homocysteine will have a role in the primary prevention of stroke, but further research is needed to establish this. Preliminary evidence indicates that optimising riboflavin intake may have a role in preventing hypertension specifically in people with a common genetic variant in folate metabolism, but this needs to be confirmed in much larger trials.

An adequate B-vitamin status can be achieved with a heart healthy diet that includes whole grain foods, leafy green vegetables, lean meat and low fat dairy produce. In practice, however, achieving an optimal status of the B-vitamins folate, vitamin B12 and riboflavin, can be problematic for some groups. In the case of folate, poor stability and poor bioavailability of the natural vitamin from food sources means the typical diet of most Europeans is sub-optimal in folate. In the case of vitamin B12, although dietary intakes are generally more than adequate, the achievement of an optimal status presents

a particular difficulty for many older people because of the common problem of age-related food-bound B12 malabsorption. People of Asian origin, particularly those on a vegetarian diet, may have low vitamin B12 intakes and may also be at risk of low riboflavin status because of the avoidance of milk. In order to offer maximal protection against elevated homocysteine in all individuals, including those genetically predisposed to impaired folate metabolism, an optimal status of all four relevant B-vitamins should be ensured but this remains challenging in practice.

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