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C₁ metabolism and CVD outcomes in older adults

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CVD is the most common cause of death in people over 65 years. This review considers the latest evidence for a potential protective effect of C₁ donors (folate and the metabolically related B-vitamins) in CVD. Such an effect may or may not be mediated via the role of these nutrients in maintaining plasma homocysteine concentrations within a desirable range. Despite predictions from epidemiological studies that lowering plasma homocysteine would reduce cardiovascular risk, several secondary prevention trials in at-risk patients published since 2004 have failed to demonstrate a benefit of homocysteine-lowering therapy with B-vitamins on CVD events generally. All these trials were performed in CVD patients with advanced disease; thus current evidence suggests that intervention with high-dose folic acid is of no benefit in preventing another event, at least in the case of heart disease. The evidence at this time, however, is stronger for stroke, with meta-analyses of randomised trials showing that folic acid reduces the risk of stroke, particularly in people with no history of stroke. Genetic studies provide convincing evidence to support a causal relationship between sub-optimal B-vitamin status and CVD. People homozygous for the common C677T variant in the gene encoding the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR), typically have a 14–21% higher risk of CVD. Apart from folate, riboflavin is required as a co-factor for MTHFR. New evidence shows that riboflavin intervention results in marked lowering of blood pressure, specifically in patients with the MTHFR 677TT genotype. This novel gene–nutrient interaction may provide insights as to the mechanism that links C₁ metabolism with CVD outcomes.

Cardiovascular risk: Homocysteine: MTHFR C677T polymorphism: Folate and folic acid: Riboflavin

The B-vitamins folate, vitamins B₁₂ and B₆ and riboflavin are the source of coenzymes which participate in C₁ metabolism. This metabolic process involves the transfer and utilisation of C₁ units for DNA and RNA biosynthesis, methylation reactions and the metabolism of amino acids including methionine. Homocysteine is a precursor of methionine synthesis, in turn required for the generation of the universal methyl donor S-adenosyl methionine. Normal homocysteine metabolism requires an adequate supply of

up to four B-vitamins (Fig. 1). When the status of folate and related B-vitamins is sub-optimal, plasma homocysteine concentration is invariably found to be elevated, while supplementation with the relevant B-vitamins can lower homocysteine, in healthy and diseased populations, by about 25% with folic acid⁽¹⁾ and to a generally lesser extent with vitamins B₁₂⁽²⁾ and B₆⁽³⁾ and riboflavin⁽⁴⁾. Thus, the measurement of plasma homocysteine concentration provides a reliable functional marker of B-vitamin status.

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; RCT, randomised controlled trials.

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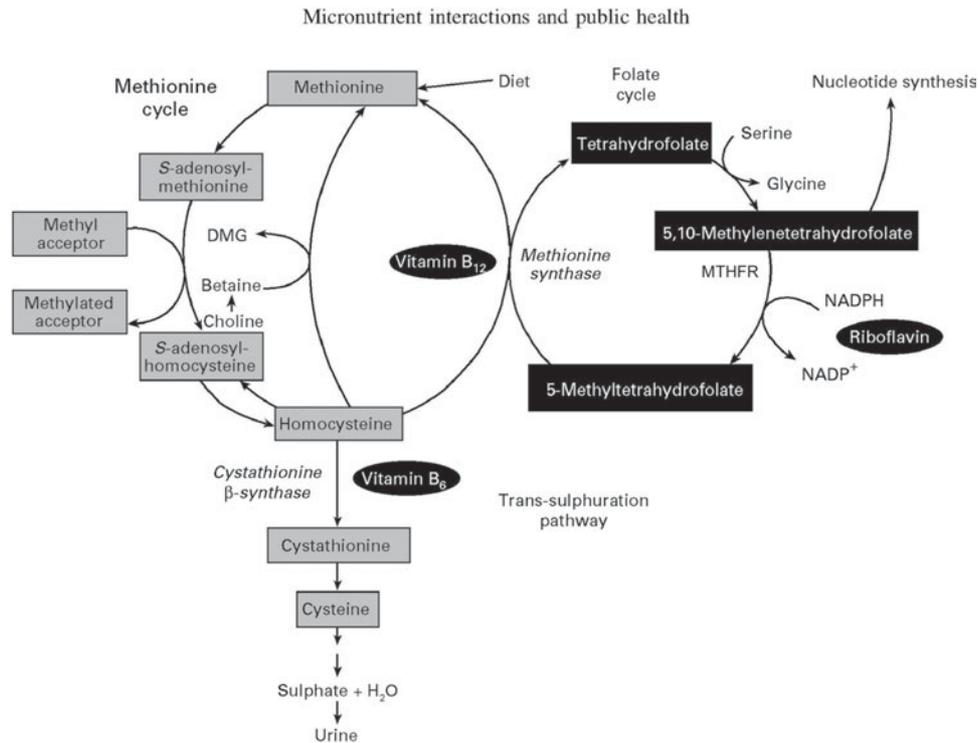


Fig. 1. Pathways for the metabolism of homocysteine. MTHFR, methylenetetrahydrofolate reductase; DMG, dimethylglycine. Reproduced from Strain *et al.*⁽⁵⁾.

Homocysteine as a risk factor for CVD: Lines of evidence

Homocysteine has been considered for many years to be a risk factor for CVD when its concentration in plasma is elevated but this is somewhat controversial. Apart from the responsiveness of plasma homocysteine to the lowering effects of B-vitamin intervention^(1–4), folate and related B-vitamins may have roles in CVD which are independent of their effects on homocysteine. Thus, while the literature in this area focuses predominantly on homocysteine *per se* as the cardiovascular risk factor, it is possible that elevated plasma homocysteine is merely a marker of sub-optimal B-vitamin status rather than being causatively linked with CVD. In any case, the value of plasma homocysteine as a measurement lies in the fact that its concentration has been investigated in numerous human studies of CVD and such studies may not necessarily have also included biomarker measurements of the various B-vitamins that act as C₁ donors. It is beyond the scope of this review to separate homocysteine-lowering from enhanced folate status as the relevant physiological event of potential benefit and one that responds to intervention. Instead, in describing the evidence, the appropriate phraseology is used to indicate that any potential beneficial effect of homocysteine-lowering may well relate to enhanced B-vitamin status.

Evidence from observational studies

Clinical studies of patients with CVD published over the past 20 years provide much evidence to link low folate

status and/or elevated plasma homocysteine with an increased risk of CVD. The data from numerous such studies, both retrospective and prospective, were used to generate two important meta-analyses, both published in 2002^(6,7). They predicted that lowering homocysteine by 3 $\mu\text{mol/l}$ (or by about 25% based on typical homocysteine concentrations of 12 $\mu\text{mol/l}$) would reduce the risk of heart disease by 11–16% and stroke by 19–24%^(6,7). Apart from clinical studies, population data from North America showed an improvement in stroke mortality corresponding to the timing of the introduction of mandatory food fortification with folic acid in 1996–98 and no similar improvement over the same time period in the UK where no such fortification policy was put in place⁽⁸⁾. Thus, the temporal decline in stroke-related mortality in the US and Canada which coincided with the introduction of folic acid fortification was consistent with a beneficial effect of folic acid or homocysteine-lowering on CVD⁽⁸⁾. Randomised trials, however, were required in order to prove a cause-and-effect relationship.

Evidence from randomised controlled trials

Since 2004, the results of a number of much-awaited secondary prevention trials in at-risk patients began to appear in high-profile medical journals^(9–15). All these failed to demonstrate a benefit of homocysteine-lowering therapy on CVD events generally (Table 1). Some argued that these trials lacked sufficient statistical power to detect a beneficial effect, given that the magnitude of the association between homocysteine and CVD risk on

Table 1. Randomised controlled trials (RCT) of homocysteine-lowering and CVD events: secondary prevention

Authors/Trial/Country	Population studied	Treatment (mg/d)	Main result/Conclusion
Toole <i>et al.</i> ⁽⁹⁾ /Vitamin Intervention for Stroke Prevention (VISP) trial/USA, Canada, Scotland	Patients (<i>n</i> 3680) with non-disabling cerebral infarction	2.5 FA, 0.4 B12, 25 B6 or 0.02 FA, 0.006 B12, 0.2 B6 for 2 years	RR 1.0 (95% CI 0.8, 1.3) for ischaemic stroke RR 1.0 (95% CI 0.8, 1.1) for any stroke, CHD event or death No effect on vascular outcomes
Lonn <i>et al.</i> ⁽¹⁰⁾ /Heart Outcomes Prevention, Evaluation 2 (HOPE 2) study/Canada, USA, Brazil, Western Europe, Slovakia	Patients (<i>n</i> 5522) with vascular disease or diabetes	2.5 FA, 50 B6, 1 B12 or placebo for 5 years	RR 0.95 (95% CI 0.84, 1.07) No reduction in the risk of death from cardiovascular causes
Bonaa <i>et al.</i> ⁽¹¹⁾ /Norwegian Vitamin (NORVIT) trial/Norway	Acute MI patients (<i>n</i> 3749)	0.8 FA, 0.4 B12, 40 B6 or 0.8 FA, 0.4 B12 or 40 B6 or placebo for 40 months	RR 1.22 (95% CI 1.00, 1.50) for FA, B12, B6 RR 1.08 (95% CI 0.93, 1.25) for FA, B12 RR 1.14 (95% CI 0.98, 1.32) for B6 No lowering in the risk of recurrent MI, stroke and sudden death attributed to CAD
Ebbing <i>et al.</i> ⁽¹²⁾ /Western Norway B-vitamin Intervention Trial (WENBIT)/Norway	Patients (<i>n</i> 3096) undergoing coronary angiography	0.8 FA, 0.4 B12, 40 B6 or 0.8 FA, 0.4 B12 or 40 B6 or placebo for 38 months	HR 1.09 (0.9–1.32) for FA, B12 HR 0.90 (0.74–1.09) for B6 No effect of treatment on total mortality or cardiovascular events (non-fatal acute MI, acute hospitalisation for unstable angina pectoris and non-fatal thromboembolic stroke)
Albert <i>et al.</i> ⁽¹³⁾ /Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS)/USA	Women (<i>n</i> 5442) with a history of CVD or three or more coronary risk factors	2.5 FA, 1 B12, 50 B6 or placebo for 7.3 years	RR 1.03 (95% CI 0.90, 1.19) No reduction in MI, stroke, coronary revascularisation or CVD mortality
SEARCH Collaborative Group 2010 ⁽¹⁴⁾ /Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)/UK	MI patients (<i>n</i> 12 064)	2 FA, 1 B12 or placebo for 6.7 years	RR 1.04 (95% CI 0.97, 1.12) No beneficial effects on coronary death, MI, coronary revascularisation, fatal or non-fatal stroke or non-coronary revascularisation
The VITATOPS Trial Study Group 2010 ⁽¹⁵⁾ /VITamins TO Prevent Stroke (VITATOPS) trial/20 countries on five continents	Patients (<i>n</i> 8164) with recent stroke or TIA	2 FA, 0.5 B12, 25 B6 or placebo for 3.4 years	RR 0.91 (95% CI 0.82, 1.00) No significant reduction in incidence of stroke, MI or vascular death

MI, myocardial infarction; CAD, coronary artery disease; TIA, transient ischaemic attack; RR, relative risk; HR, hazard ratio; FA, folic acid; B12, vitamin B₁₂; B6, vitamin B₆.

Table 2. Randomised controlled trials (RCT) of homocysteine-lowering and risk of stroke: a meta-analysis

	Wang <i>et al.</i> ⁽¹⁸⁾		Lee <i>et al.</i> ⁽¹⁹⁾	
	(8 RCT; <i>n</i> 16 841)		(13 RCT; <i>n</i> 39 005)	
	Relative risk (95% CI)	<i>P</i> value	Relative risk (95% CI)	<i>P</i> value
Overall	0.82 (0.68, 1.00)	0.045	0.93 (0.85, 1.03)	0.16
Duration of intervention				
<3 years	1.00 (0.83, 1.21)	0.95	1.03 (0.84, 1.27)	NS
≥ 3 years	0.71 (0.57, 0.87)	0.001	0.87 (0.78, 0.98)	0.02
Homocysteine lowering				
<20%	0.89 (0.55, 1.42)	0.62	1.02 (0.86, 1.22)	NS
≥ 20%	0.77 (0.63, 0.94)	0.012	0.87 (0.77, 0.98)	0.02
History of stroke*				
Yes	1.04 (0.84, 1.29)	0.71	1.04 (0.84, 1.29)	NS
No	0.75 (0.62, 0.90)	0.002	0.89 (0.79, 0.99)	0.03
Gender effect				
Predominantly women	N/a	N/a	1.11 (0.83, 1.49)	NS
Predominantly men	N/a	N/a	0.84 (0.74, 0.94)	0.003

N/a, not applicable.

*Referred to as secondary and non-secondary stroke prevention in Lee *et al.* meta-analysis⁽¹⁹⁾.

re-evaluation of the epidemiological evidence was later found to be more modest than originally estimated at the time the trials were being designed⁽¹⁶⁾. The relationship for homocysteine and stroke, however, was always estimated to be stronger than that for heart disease^(6,7). In fact one of the aforementioned trials, the HOPE 2 trial⁽¹⁰⁾, had shown a clear benefit of B-vitamin intervention in reducing the risk of stroke, a result that for some reason was largely overlooked in the original report. Instead, the positive results of the trial in relation to stroke risk were separately reported 3 years after the original trial findings⁽¹⁷⁾, but these later results were not as widely recognised as the original report of the HOPE 2 trial. Of greater note, a meta-analysis of randomised controlled trials (RCT) showed that homocysteine-lowering by folic acid reduced the risk of stroke in general by 18%, but with significantly greater reductions observed (by 23–29%) in those trials of longer treatment duration or with greater homocysteine-lowering or in patients with no history of stroke (Table 2)⁽¹⁸⁾. Likewise, a more recent meta-analysis also showed that the beneficial effect of folic acid on stroke risk was to be found in trials longer than 3 years in duration, with a homocysteine-lowering effect of 20% or greater and in non-secondary prevention of stroke (Table 2)⁽¹⁹⁾. In addition the latter meta-analysis suggested for the first time that folic acid intervention might be more beneficial in men than in women; however, this finding requires further verification as none of the trials evaluated had exclusively investigated men or women. The evidence from these meta-analyses of RCT, together with the aforementioned North American population data suggesting a beneficial effect of increased folate status (through mandatory fortification) in preventing stroke, means that the case for enhanced folate and/or homocysteine-lowering in preventing CVD is stronger for stroke than for heart disease, and perhaps strongest for the primary prevention of stroke.

Evidence from genetic studies

Other evidence for a causal relationship between low folate status or elevated homocysteine and CVD comes from genetic studies. The most important genetic determinant of homocysteine in the general population is the common C677T variant in the gene encoding the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR) required for the formation of 5-methyltetrahydrofolate which, in turn is required in the remethylation of homocysteine to methionine. People who are homozygous for this polymorphism (TT genotype), have reduced MTHFR activity resulting in impaired folate metabolism and higher homocysteine concentrations *in vivo*⁽²⁰⁾; a phenotype that is found to be particularly marked in people who also have low status of folate⁽²¹⁾. The MTHFR 677 TT genotype has a reported frequency of 10% worldwide, ranging from 4 to 26% in Europe, 20% in Northern China to as high as 32% in Mexico⁽²²⁾. Evidence from various meta-analyses of genetic studies including data for homocysteine concentrations and CVD events shows that individuals with the MTHFR 677 TT genotype have a significantly higher risk of CVD (by 14–21%) compared with those without this polymorphism^(7,23,24). The trend towards increasing risk of disease shown among individuals with wild-type (CC), heterozygous (CT) and homozygous mutant (TT) genotypes is consistent with the increasing gradient in homocysteine concentrations typically found among the three genotypes^(7,23), as would be expected if there is a causal relationship between elevated homocysteine and risk of disease. Analysis of the OR among different countries, however, shows a large geographical variation in the extent of association between this polymorphism and CVD risk. Meta-analyses, which have examined this variation in relation to heart disease, show that the excess risk is not significant in North American

populations, while the TT genotype carries a significantly increased (but variable) risk of CHD elsewhere in the world^(23,24). The largest and most recent investigation in this area, a collaboration of genetic studies consisting of data on almost 60 000 individuals including 20 885 stroke events, reported that the association between the *MTHFR* 677TT genotype and stroke was evident only in regions of low folate, whereas in regions with folic acid fortification policies the effect of *MTHFR* genotype was null⁽²⁵⁾. Thus, the general conclusion emerging from genetic studies to date is one of effect modification by population dietary folate on the association between this polymorphism and CVD risk.

The mechanism whereby this genetic variant is linked with an increased risk of CVD is not generally dealt with in these reports but is generally assumed to relate to the phenotype of elevated plasma homocysteine in some way^(23–25). Riboflavin, the co-factor for *MTHFR*, has been largely overlooked as a potential modulator of the phenotype, or indeed the cardiovascular risk, associated with the *MTHFR* C677T polymorphism. This omission is despite evidence that intervention with riboflavin lowers plasma homocysteine specifically in individuals with the *MTHFR* 677TT genotype, and unlike folate and vitamin B₁₂, riboflavin does not have a homocysteine-lowering effect in the other *MTHFR* genotype groups⁽⁴⁾. This finding suggests that riboflavin intervention can in some way stabilise *MTHFR* activity in individuals with the *MTHFR* 677TT genotype and restore enzyme activity to levels found in those without this genotype.

C₁ metabolism and hypertension, a major CVD risk factor

Hypertension (i.e. a blood pressure of 140/90 mmHg or greater) is estimated to carry an almost threefold greater risk of developing CVD⁽²⁶⁾, while treating hypertension substantially reduces cardiovascular events, and stroke in particular⁽²⁷⁾. As reviewed by us recently, plasma homocysteine concentrations are found to be associated with blood pressure in most (though not all) large cohort studies⁽²⁸⁾. Most published studies in this area, however, have not considered the role of the *MTHFR* C677T polymorphism, but emerging evidence shows an important association between this common genetic factor and hypertension⁽²⁸⁾. Moreover, new evidence has identified that riboflavin can play an important role in determining blood pressure specifically in CVD patients with the *MTHFR* 677TT genotype⁽²⁹⁾. Intervention with riboflavin (at dietary levels, 1.6 mg/d) was found to result in marked lowering of blood pressure (from 144/87 to 131/80 mmHg) in CVD patients with the TT genotype and no response in the other *MTHFR* genotype groups⁽²⁹⁾. These findings could have important implications for the prevention and management of hypertension among the 10% of individuals worldwide⁽²²⁾ who carry this genotype.

Presumably the effect of riboflavin in individuals with the *MTHFR* 677TT genotype is to stabilise the variant enzyme that is known from molecular studies to become inactive as a result of having an increased propensity to

dissociate from its FAD co-factor⁽³⁰⁾. The mechanism, however, which links riboflavin with blood pressure in this genotype group is as yet unknown. The potent vasodilator NO may possibly be implicated. Vascular tissue concentrations of 5-methyltetrahydrofolate (rarely measured in human studies) were recently found to be lower in patients with the *MTHFR* 677TT genotype and were associated with NO regulation and endothelial function in these patients⁽³¹⁾. By stabilising the variant *MTHFR* enzyme, it is possible that riboflavin supplementation could restore 5-methyltetrahydrofolate concentrations in vascular tissues, improve NO bioavailability and in turn lower blood pressure specifically in patients with the TT genotype. Clearly, further investigation to determine the precise mechanism is required, but the elevated homocysteine phenotype typically associated with this polymorphism is unlikely to be directly implicated. Despite the significant associations that are found between plasma homocysteine and blood pressure in several observational studies, intervention studies to lower homocysteine have shown little or no corresponding blood pressure response⁽²⁸⁾, suggesting that there is no causative link between elevated homocysteine concentrations *per se* and hypertension.

Whatever the mechanism, this novel genotype-specific effect of riboflavin on blood pressure may, if confirmed, also help to explain the aforementioned inconsistencies in the evidence as to the role of the *MTHFR* C677T polymorphism in CVD generally^(23–25). The policy of population-wide riboflavin fortification of food, which has existed for over 50 years in North America, would ensure higher and generally less variable intakes of riboflavin compared with elsewhere. The higher riboflavin status could in turn be predicted to neutralise any effect of the variant *MTHFR* enzyme. The reported differences among countries as to whether this polymorphism represents an increased risk of CVD may thus relate, not only to differences in the prevailing folate status as commonly suggested^(23–25) but also to the modulating effect of riboflavin. Based on the genotype-specific effect of riboflavin on blood pressure recently shown⁽²⁹⁾, it could be predicted that individuals with the TT genotype who also have low riboflavin status would have an excess risk of CVD, whereas TT homozygotes with optimal riboflavin status may not show the expected risk. No study, however, has addressed this possibility.

Potential for prevention of stroke through optimisation of B-vitamin status

Folate

Taken together, the evidence showing a decline in stroke-related mortality in North America corresponding to the introduction of mandatory folic acid-fortification⁽⁸⁾, along with the evidence from RCT showing beneficial effects of folic acid in people with no previous history of stroke^(18,19), suggest that folic acid may have an important role to play in the primary prevention of stroke. Thus, although aimed at preventing neural-tube defects, folic acid fortification on a population-wide basis may also prevent stroke. Randomised trials that have failed to show

beneficial effects of folic acid on stroke have in general been conducted in countries with a high prevailing folate status⁽²⁵⁾. Thus, it is unlikely that further increases in folate intake will be effective in a population whose folate status is generally optimal. However, while overt folate deficiency (i.e. megaloblastic anaemia) is relatively rare in the absence of an underlying clinical cause, many otherwise healthy populations have inadequate folate intakes when considered from the perspective of achieving optimal status rather than merely preventing deficiency of folate⁽³²⁾. The poor stability and poor bioavailability of folate from natural food sources compared with folic acid (the synthetic vitamin form found in fortified foods and supplements) means that achieving optimal folate status in a population in the absence of a folic acid fortification policy can be a major challenge⁽³²⁾. Thus, in populations with limited or no access to fortified foods where consumers are dependent on natural food folate sources to meet their dietary intakes, folate status could be predicted to be low⁽³³⁾. Intervention could be anticipated to have the greatest benefit in reducing the risk of stroke in these regions, and it is suggested that any future RCT of homocysteine-lowering intervention for stroke prevention should focus on such populations with low prevailing folate intake and status⁽²⁵⁾.

Balanced against any potential benefits of folic acid in preventing stroke, however, are concerns regarding potential adverse effects of long-term exposure to high folic acid levels. Thus, mandatory folic acid fortification (aimed at preventing neural-tube defects) remains very controversial, despite the relative ineffectiveness of alternative strategies to increase folate status through health promotion campaigns⁽³⁴⁾. The major concern traditionally focused on the potential risk that high folic acid intake might mask the anaemia of vitamin B₁₂ deficiency⁽³⁵⁾, but more recently the concern emerged that it could be associated with an increased risk of cognitive dysfunction in older people with low-vitamin B₁₂ status⁽³⁶⁾. Furthermore, despite considerable evidence that higher folate within the dietary range plays a protective role against cancer at various sites^(37–39), other evidence suggests that exposure to high-dose folic acid (>1 mg/d) may promote colorectal tumorigenesis in patients with pre-existing lesions⁽⁴⁰⁾ or even increase cancer risk generally^(41,42). Thus, some remain concerned that excessively high folic acid intakes could have a cancer-promoting effect in segments of aging populations⁽⁴³⁾, and policy-makers worldwide (including the UK government) have delayed decisions to implement population-based folic acid fortification policies similar to those introduced in 1998 in North America.

Should emerging evidence prove the role of folic acid in the primary prevention of stroke, then the public health question of setting the appropriate folic acid dose to achieve this beneficial effect in a population via food fortification will need to be answered. A lower target dose for beneficial effects will lower the risk of adverse effects (whatever the estimated size of such risk) in any emerging fortification policy. One recent trial from our Centre addressed the issue of determining the lowest dose of folic acid required to achieve a maximal lowering in plasma homocysteine in heart disease patients and age- and

sex-matched controls⁽⁴⁴⁾. The results showed that a dose of folic acid as low as 0.2 mg/d can, if given chronically, maximally lower homocysteine in patients and controls; higher doses (0.4 and 0.8 mg/d) resulted in no additional lowering. By sampling participants at both 6 and 12 weeks, in addition to the full intervention period of 26 weeks, the trial also indicated that the majority of previous studies used too short an intervention period to observe the full extent of homocysteine-lowering in response to low folic acid doses and thus concluded that the much higher folic acid dose of 0.8 mg/d was required⁽¹⁾. Thus, the potency of folic acid at low doses given chronically (as in food fortification) was previously underestimated, and these findings indicate that long-term exposure to high-dose folic acid through food fortification is unnecessary⁽⁴⁴⁾.

Vitamin B₁₂

Vitamin B₁₂, like folate, is required for the remethylation of homocysteine to methionine (Fig. 1). Thus, achieving optimal folate status alone may not lower homocysteine to desirable concentrations (<10 μmol/l)⁽⁴⁵⁾, and optimising vitamin B₁₂ may have benefits over and above the effect of folate alone. A further 7% reduction in homocysteine (additional to that with folic acid alone) can be achieved with vitamin B₁₂⁽⁴⁶⁾. 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^(6,7). Although vitamin B₁₂ intakes are generally found to be more than adequate in most healthy populations (often greatly exceeding recommended values), the achievement of an optimal status may be greatly hindered for many older people because of the common problem of age-related food-bound vitamin B₁₂ malabsorption, estimated to affect up to 40% of otherwise healthy older people^(47,48). This problem arises mainly from atrophic gastritis, a chronic inflammatory condition, resulting in decreased gastric acid production (hypochlorhydria), which diminishes vitamin B₁₂ absorption because of the essential role of gastric acid in the release of protein-bound vitamin B₁₂ from food⁽⁴⁸⁾. Unlike the treatment required for the classical (but rare) vitamin B₁₂ deficiency sign of pernicious anaemia requiring vitamin B₁₂ injections for life, older adults with low vitamin B₁₂ status owing 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 USA recommends that people aged 50 years and over consume most of their vitamin B₁₂ from crystalline vitamin B₁₂ found in fortified foods and supplements⁽⁴⁹⁾. No such recommendation exists in the UK or any European country.

Riboflavin

Riboflavin is required as a co-factor for the folate-metabolising enzyme MTHFR and therefore is needed to generate 5-methyltetrahydrofolate which, in turn, acts in the remethylation of homocysteine to methionine (Fig. 1), but the potential role of riboflavin as a C₁ donor is often overlooked. The emerging evidence reviewed here that the

higher blood pressure found in patients with a common genetic variant in *MTHFR* is highly responsive to low-dose riboflavin⁽²⁹⁾, raises the possibility that improving riboflavin status will have an important role in preventing hypertension and therefore in modulating the risk of stroke specifically in the 10% of people worldwide (and much higher in certain populations) with the relevant genotype⁽²²⁾.

The Scientific Advisory Committee on Nutrition, however, has expressed concern about the high proportion of the British adult population with apparently poor riboflavin status as determined from national survey data using the gold standard biomarker of status, erythrocyte glutathione activation coefficient⁽⁵⁰⁾. In general, dietary intakes (provided in the diet predominantly through consuming milk and dairy foods) were found to compare favourably with recommended values; however, high proportions of young women were found to have low intakes⁽⁵⁰⁾. Thus, it is unclear at this time as to whether or not the large proportion of the UK population with abnormal erythrocyte glutathione activation coefficient values indicates a generalised problem of poor riboflavin status; this requires further investigation.

Conclusions

Most of the RCT designed to show a causative relationship between elevated plasma homocysteine and CVD provide little evidence that giving folic acid or other B-vitamins to CVD patients prevents another event. These interventions were all secondary prevention trials conducted in patients with advanced disease and, given the doses employed, had little to do with nutrition. The same cannot be said for primary prevention. Emerging nutrition policy should consider nutrient requirements aimed at primary prevention and it remains probable that there is a role for folic acid and the metabolically related B-vitamins in the primary prevention of stroke.

Although elevated plasma homocysteine *per se* is unlikely to be causatively implicated in CVD, it is valuable as a sensitive functional marker of sub-optimal B-vitamin status. Correcting low folate and related B-vitamin status (rather than lowering homocysteine) is probably the relevant beneficial physiological event linked with CVD and homocysteine-lowering may well be a bystander effect. In any event, one needs to know the effective dose of folic acid necessary to correct compromised folate status, as evidenced by a return of the biomarker homocysteine to normal plasma concentrations. In practice, however, achieving an optimal status of the B-vitamins, folate, vitamin B₁₂ and riboflavin, on a population basis is challenging.

The difference in results of various meta-analyses in relation to the *MTHFR* C677T polymorphism and stroke risk is generally explained in the literature by the modifying effect of prevailing folate status. Thus, high folate is seen to overcome the detrimental effect of this polymorphism but the potential role of riboflavin in correcting the associated impaired folate metabolism is generally ignored. The emerging link between *MTHFR* genotype and

hypertension, however, may provide the basis of a mechanism to explain how the common *MTHFR* C677T polymorphism could result in increased CVD risk. The elevated blood pressure associated with this polymorphism is correctable by increasing riboflavin status; thus, stroke risk could be predicted to also be responsive to riboflavin intervention targeted at this genotype group but this contention has not been addressed directly in any RCT to date.

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References

1. Homocysteine Lowering Trialists' Collaboration (2005) Dose-dependent effects of folic acid on blood concentrations of homocysteine: A meta-analysis of the randomized trials. *Am J Clin Nutr* **82**, 806–812.
2. Eussen SJ, de Groot LC, Clarke R *et al.* (2005) Oral cyanocobalamin supplementation in older people with vitamin B₁₂ deficiency: A dose-finding trial. *Arch Intern Med* **165**, 1167–1172.
3. McKinley MC, McNulty H, McPartlin J *et al.* (2001) Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr* **73**, 759–764.
4. McNulty H, Doweck le RC, Strain JJ *et al.* (2006) Riboflavin lowers homocysteine in individuals homozygous for the *MTHFR* 677C->T polymorphism. *Circulation* **113**, 74–80.
5. Strain JJ, Doweck L, Ward M *et al.* (2004) B-vitamins, homocysteine metabolism and CVD. *Proc Nutr Soc* **63**, 597–603.
6. The Homocysteine Studies Collaboration (2002) Homocysteine and risk of ischemic heart disease and stroke. *JAMA* **288**, 2015–2022.
7. Wald DS, Law M & Morris JK (2002) Homocysteine and cardiovascular disease: Evidence on causality from a meta-analysis. *BMJ* **325**, 1202–1208.
8. Yang Q, Botto LD, Erickson JD *et al.* (2006) Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation* **113**, 1335–1343.
9. Toole JF, Malinow MR, Chambless LE *et al.* (2004) Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The Vitamin Intervention for Stroke Prevention (VISIP) randomized controlled trial. *JAMA* **291**, 565–575.
10. Lonn E, Yusuf S, Arnold MJ *et al.* (2006) Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* **354**, 1567–1577.

11. Bønaa KH, Njølstad I, Ueland PM *et al.* (2006) Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* **354**, 1578–1588.
12. Ebbing M, Bleie Ø, Ueland PM *et al.* (2008) Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: A randomized controlled trial. *JAMA* **300**, 795–804.
13. Albert CM, Cook NR, Gaziano JM *et al.* (2008) Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease. A randomized trial. *JAMA* **299**, 2027–2036.
14. Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group (2010) Effects of homocysteine-lowering with folic acid plus vitamin B₁₂ vs placebo on mortality and major morbidity in myocardial infarction survivors. *JAMA* **303**, 2486–2494.
15. VITATOPS Trial Study Group (2010) B vitamins in patients with recent transient ischaemic attack or stroke in the VITamins TO Prevent Stroke (VITATOPS) trial: A randomised, double-blind, parallel, placebo-controlled trial. *Lancet Neurol* **9**, 855–865.
16. B-Vitamin Treatment Trialists' Collaboration (2006) Homocysteine-lowering trials for prevention of cardiovascular events: A review of the design and power of the large randomized trials. *Am Heart J* **151**, 282–287.
17. Saposnik G, Ray JG, Sheridan P *et al.* (2009) Homocysteine-lowering therapy and stroke risk, severity, and disability: Additional findings from the HOPE 2 trial. *Stroke* **40**, 1365–1372.
18. Wang X, Qin X, Demirtas H *et al.* (2007) Efficacy of folic acid supplementation in stroke prevention: A meta-analysis. *Lancet* **369**, 1876–1882.
19. Lee M, Hong KS, Chang SC *et al.* (2010) Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention: A meta-analysis. *Stroke* **41**, 1205–1212.
20. Frosst P, Blom HJ, Milos R *et al.* (1995) A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* **10**, 111–113.
21. Jacques PF, Bostom AG, Williams RR *et al.* (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* **93**, 7–9.
22. Wilcken B, Bamforth F, Li Z *et al.* (2003) Geographical and ethnic variation of the 677C→T allele of 5, 10 methylenetetrahydrofolate reductase (MTHFR): Findings from over 7000 newborns from 16 areas world wide. *J Med Genet* **40**, 619–625.
23. Klerk M, Verhoef P, Clarke R *et al.* (2002) MTHFR C677T polymorphism and risk of coronary heart disease: A meta-analysis. *JAMA* **288**, 2023–2031.
24. Lewis SJ, Ebrahim S & Davey-Smith G (2005) Meta-analysis of the MTHFR 677C→T polymorphism and coronary heart disease: Does totality of evidence support causal role for homocysteine and preventative potential of folate. *Br Med J* **331**, 1053–1059.
25. Holmes MV, Newcombe P, Hubacek JA *et al.* (2011) Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: A meta-analysis of genetic studies and randomised trials. *Lancet* **378**, 584–594.
26. Wang W, Lee ET, Fabsitz RR *et al.* (2006) A longitudinal study of hypertension risk factors and their relation to cardiovascular disease: The Strong Heart Study. *Hypertension* **47**, 403–409.
27. Staessen JA, Wang JG & Thijs L (2001) Cardiovascular protection and BP reduction: A meta-analysis. *Lancet* **358**, 1305–1315.
28. Wilson CP, McNulty H, Scott JM *et al.* (2010) The MTHFR C677T polymorphism, B-vitamins and blood pressure. *Proc Nutr Soc* **69**, 156–165.
29. Horigan G, McNulty H, Ward M *et al.* (2010) Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the C677T polymorphism in MTHFR. *J Hypertens* **28**, 478–486.
30. Yamada K, Chen Z, Rozen R *et al.* (2001) Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc Natl Acad Sci USA* **98**, 14853–14858.
31. Antoniadou C, Shirodaria C, Leeson P *et al.* (2009) MTHFR 677 C>T Polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. *Circulation* **119**, 2507–2515.
32. McNulty H & Scott JM (2008) Intake and status of folate and related B-vitamins: Considerations and challenges in achieving optimal status. *Br J Nutr* **99**, S48–S54.
33. Hoey L, McNulty H, Askin N *et al.* (2007) Impact of a voluntary food fortification policy on folate, related B-vitamin status and homocysteine in healthy adults. *Am J Clin Nutr* **86**, 1405–1413.
34. McNulty B, Pentieva K, Marshall B *et al.* (2011) Women's compliance with current folic acid recommendations and achievement of optimal vitamin status for preventing neural tube defects. *Hum Reprod* **26**, 1530–1536.
35. Reynolds E (2006) Vitamin B₁₂, folic acid, and the nervous system. *Lancet Neurol* **5**, 949–960.
36. Morris MS, Jacques PF, Rosenberg IH *et al.* (2007) Folate and vitamin B₁₂ status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* **85**, 193–200.
37. Kim Y-I (1999) Folate and carcinogenesis: Evidence, mechanisms, and implications. *J Nutr Biochem* **10**, 66–88.
38. Ericson U, Sonestedt E, Gullberg B *et al.* (2007) High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmo Diet and Cancer cohort. *Am J Clin Nutr* **86**, 434–443.
39. Gibson TM, Weinstein SJ, Pfeiffer RM *et al.* (2011) Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr* **94**, 1053–1062.
40. Cole BF, Baron JA, Sandler RS *et al.* (2007) Folic acid for the prevention of colorectal adenomas: A randomized clinical trial. *JAMA* **297**, 2351–2359.
41. Figueiredo JC, Grau MV, Haile RW *et al.* (2009) Folic acid and risk of prostate cancer: Results from a randomized clinical trial. *J Natl Cancer Inst* **101**, 432–435.
42. Ebbing M, Bønaa KH, Nygård O *et al.* (2009) Cancer incidence and mortality after treatment with folic acid and vitamin B₁₂. *JAMA* **302**, 2119–2126.
43. Mason JB (2011) Folate consumption and cancer risk: A confirmation and some reassurance, but we're not out of the woods quite yet. *Am J Clin Nutr* **94**, 965–966.
44. Tighe P, Ward M, McNulty H *et al.* (2011) A dose-finding trial of the effect of long-term folic acid intervention: Implications for food fortification policy. *Am J Clin Nutr* **93**, 11–18.
45. Malinow MR, Bostom AG & Krauss RM (1999) Homocyst(e)ine, diet, and cardiovascular diseases: A statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* **99**, 178–182.
46. Hoey L, Strain JJ & McNulty H (2009) Studies of biomarker responses to intervention with vitamin B-12: A systematic review of randomized controlled trials. *Am J Clin Nutr* **89**, Suppl., 1981S–1996S.

47. Clarke R, Refsum H, Birks J *et al.* (2003) Screening for vitamin B-12 and folate deficiency in older persons. *Am J Clin Nutr* **77**, 1241–1247.
48. Carmel R (1997) Cobalamin, the stomach, and aging. *Am J Clin Nutr* **66**, 750–759.
49. Institute of Medicine, Panel on Folate, Other B Vitamins, and Choline (1998) *Dietary Reference Intake; Thiamine, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press.
50. Ruston D, Hoare J, Henderson L *et al.* (2004) *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years. Vol. 4: Nutritional Status (Anthropometry and Blood Analytes), Blood Pressure and Physical Activity*. London: The Stationery Office.