Intake and status of folate and related B-vitamins: considerations and challenges in achieving optimal status

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Folate and the metabolically related B-vitamins, vitamin B_{12} and riboflavin, have attracted much scientific and public health interest in recent years. Apart from a well established role in preventing neural tube defects (NTDs), evidence is emerging to support other potential roles for folate and/or related B-vitamins in protecting against cardiovascular disease (especially stroke), certain cancers, cognitive impairment and osteoporosis. However, typical folate intakes are sub-optimal, in that although adequate in preventing clinical folate deficiency (i.e. megaloblastic anaemia) in most people, they are generally insufficient to achieve a folate status associated with the lowest risk of NTDs. Natural food folates have a limited ability to enhance folate status as a result of their poor stability under typical cooking conditions and incomplete bioavailability when compared with the synthetic vitamin, folic acid (as found in supplements and fortified foods). Current folate recommendations to prevent NTDs (based primarily on folic acid supplementation) have been found to be ineffective in several European countries. In contrast, in North America and Chile, the policy of mandatory folic acid-fortification has proven itself in terms of lowering the prevalence of NTD, but remains controversial because of concerns regarding potential risks of chronic exposure to high-dose folic acid. In the case of vitamin B_{12}, the achievement of an optimal status is particularly difficult for many older people because of the common problem of food-bound B_{12} malabsorption. Finally, there is evidence that riboflavin status is generally low in the UK population, and particularly so in younger women; this warrants further investigation.

Folate: Vitamin B_{12}: Vitamin B_2 (riboflavin): Homocysteine

In recent years there has been a growing interest in folate and the metabolically related B-vitamins (vitamin B_{12}, vitamin B_6 and riboflavin). Apart from a well established role in the prevention of neural tube defects (NTDs), an optimal status of folate and related B-vitamins may protect against cardiovascular disease (particularly stroke) and certain cancers, and may have other important roles in cognitive function and bone health. These effects may/may not be mediated via homocysteine, the metabolism of which requires an adequate status of all four relevant B-vitamins. However, in the case of folate, vitamin B_{12} and riboflavin, the achievement of an optimal status presents particular challenges. This review will consider these nutrients, their potential roles in disease prevention, the assessment of status and the challenges involved in achievement of an optimal nutritional status.

Folate

Folate has a major role in one-carbon metabolism, involving the transfer and utilisation of one-carbon units in DNA and RNA biosynthesis, methylation reactions and amino acid metabolism. Folate deficiency leads to megaloblastic anaemia, characterised by larger than normal red cell precursors (megaloblasts) in bone marrow, macrocytes in the peripheral blood and giantism in the morphology of proliferating cells.

Folate status is routinely assessed by measurement of folate concentrations in plasma/serum or red cells. Red cell folate is considered to be the best index of longer term status (i.e. over the previous months), while serum folate reflects more recent dietary intake. The measurement of plasma total homocysteine concentration provides a reliable functional marker of folate status, on the basis that normal homocysteine metabolism requires an adequate supply of folate. When folate status is low or deficient, elevated plasma homocysteine is invariably observed.

Typical folate intakes are found to be sub-optimal in the diets of many people when considered from the perspective of achieving an optimal folate status, i.e. a folate level associated with lowest risk of folate-related disease (e.g. NTDs), rather than merely preventing overt folate deficiency (i.e. megaloblastic anaemia). This widespread under-provision of folate is generally attributed to the poor bioavailability of natural food folates. Although there is general agreement among experts that the bioavailability of natural food folates is incomplete when compared with the synthetic vitamin folic acid (FA), reported estimates of relative bioavailability vary greatly among human studies[1].

With such uncertainty regarding folate bioavailability from food, setting dietary folate recommendations is problematic for policy makers. The approach adopted in the United States[2] is based on an adjustment for the differences in

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bioavailability between natural food folates and the synthetic vitamin, with the introduction of 'Dietary Folate Equivalents' (DFE). The DFE is defined as the quantity of natural food folate plus 1·7 times the quantity of FA in the diet, a definition based on the assumption that the bioavailability of FA added to food is greater than that of natural food folate by a factor of 1·7. This estimation, however, is highly dependent on one metabolic study in non-pregnant women which estimated the bioavailability of food folates to be no more than 50 % relative to that of FA[3], plus other evidence showing that FA added to food had about 85 % the bioavailability of free FA[4]. Subsequent bioavailability studies have addressed the robustness of the US DFE value, with the findings of some[5] being generally supportive of the estimates used, whereas others have concluded that food folate bioavailability is far greater than that assumed in generating the US DFE[6]. Emerging dietary folate recommendations in other countries may adopt a similar approach to that used in the United States, and the folate bioavailability literature will need to be carefully considered. Apart from their poor bioavailability, natural food folates (particularly green vegetables) can be unstable under typical cooking conditions, and this can substantially reduce the vitamin content before it is even ingested[1]. This is an important (but often overlooked) additional factor limiting the ability of natural food folates to enhance folate status. In contrast to food folates, FA (as found in supplements and fortified foods) provides a very stable and highly bioavailable form of the vitamin.

Vitamin B_{12}

Vitamin B_{12} is required as a cofactor for two mammalian enzymes: methionine synthetase and methylmalonyl CoA mutase. In vitamin B_{12} deficiency, reduced function of the latter enzyme causes levels of the substrate methylmalonylCoA to build up and form the by-product methylmalonic acid (MMA) which is then excreted into the circulation; plasma MMA is thus a functional marker that is specific to vitamin B_{12} and will not be affected by intake/status of other B-vitamins. In the other reaction, vitamin B_{12} (as methylcobalamin) acts as a cofactor for methionine synthetase which catalyzes the remethylation of homocysteine to methionine. However this metabolic step is not only dependent on vitamin B_{12} but also on folate. Measurement of plasma homocysteine can therefore provide a functional biomarker of vitamin B_{12} status, but it is not specific for vitamin B_{12} and will be principally affected by changes in folate status (and also by vitamin B_6).

Measurement of serum/plasma total vitamin B_{12} concentrations is the standard screening test for vitamin B_{12} deficiency, but it may not be the most sensitive marker of B_{12} status[7]. It is proposed that the most relevant measure of vitamin B_{12} status is holotranscobalamin (holoTC) as this represents the fraction of circulating B_{12} that is attached to transcobalamin, the protein that delivers vitamin B_{12} to cells. In recent years, a reliable method to measure holoTC using a radio immuno-assay (RIA) was developed and validated[8]. Numerous studies have since been published, strongly suggesting that holoTC may be a more reliable marker of B_{12} status than the typically used marker of status, serum total B_{12} concentration. The method has been

the basis of an EU project framework 5 QLRT-2001-01775 "Demonstration of the clinical utility of holoTC as an early marker of vitamin B_{12} deficiency" aimed at determining the clinical utility of holoTC as a marker of vitamin B_{12} deficiency[9]. Some preliminary data from this project have been published, confirming that holoTC may be a better marker of B_{12} status than serum total B_{12} concentration[10].

There is a high prevalence of low/deficient vitamin B_{12} status among healthy elderly people which becomes more common with increasing age. One recent population-based study estimated that as many as 10 % and 20 % of British people aged 65–74 and >75 y, respectively, were at high risk of vitamin B_{12} deficiency[11]. In other studies the prevalence of mild vitamin B_{12} deficiency is reported to be as high as 45 %, depending on the diagnostic criteria used[12]. However, the low status of B_{12} found in older people is not explained by inadequate dietary intakes of vitamin B_{12}. In fact, among older adults living in the UK, the average daily intake of vitamin B_{12} was found to be 5·2 μg/d, an intake which is at least as good as that found in younger adults[13] and markedly higher than the reference nutrient intake (RNI) value of 1·5 μg/d. Thus, the average diet of older people provides vitamin B_{12} levels which appear to be well in excess of needs.

The high prevalence of vitamin B_{12} deficiency among older people, despite adequate intakes, relates to changes in the gut with age that may prevent its release from natural food sources. Absorption of vitamin B_{12} from food requires the action of the stomach, pancreas, and small intestine and is therefore dependent upon normal functioning throughout the gastrointestinal tract. Less than 2 % of cases of B_{12} deficiency can be explained by pernicious anaemia, a condition characterised by loss of secretion of gastric intrinsic factor (GIF) leading to very marked B_{12} depletion as the vitamin must bind to GIF in order to be absorbed in the terminal ileum[14]. To effectively treat pernicious anaemia, therefore, patients must be given regular B_{12} injections which are continued for life. However, a much more prevalent but largely unrecognised cause of mild B_{12} deficiency is considered to be food-bound malabsorption. This is mainly caused by atrophic gastritis, an age-related condition resulting in decreased gastric acid production (hypochlorhydria). Because gastric acid is required for the release of vitamin B_{12} from proteins in food, vitamin B_{12} absorption from foods is diminished in older people with hypochlorhydria[14]. In theory, older adults with mild (pre-clinical) B_{12} deficiency due to food-bound malabsorption should be able to absorb free (crystalline) vitamin B_{12} because it is not bound to protein and because intrinsic factor is still secreted. In fact, on the basis of this assumption, the Institute of Medicine in the US[2] recommends that people aged 50 years and over consume most of their vitamin B_{12} from crystalline B_{12} found in fortified foods and supplements. No such recommendation exists in the UK and no study to date has directly addressed this issue in order to provide a firm basis for introducing one.

The issue of low/deficient B_{12} status among older people is also relevant to emerging policy on mandatory fortification of food with FA. A number of European countries have opted not to proceed with mandatory fortification or have delayed a decision on it because of potential health risks to the elderly, primarily the concern that high dose FA might mask the anaemia of vitamin B_{12} deficiency in older people, while allowing

the associated irreversible neurological symptoms to progress[15]. Although some reports concluded that mandatory FA fortification has not had any adverse effect with respect to the diagnosis of vitamin B_{12} deficiency since its introduction over 10 years ago in the US[16,17], one recent study from the post-fortification era showed that among older American adults with a low vitamin B_{12} status, a higher serum folate concentration was associated with an increased risk of cognitive impairment[18]. Regardless of whether mandatory fortification with FA goes ahead in European countries, however, preventing the high prevalence of low vitamin B_{12} status due to food-bound malabsorption among older adults is an important public health issue that needs to be addressed.

Riboflavin

Riboflavin is the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both of which are essential for several reduction-oxidation enzymes. Classical riboflavin deficiency (presenting as angular stomatitis, cheilosis and glossitis) rarely occurs in isolation and usually occurs in association with other vitamin deficiencies[19]. Riboflavin status is determined by erythrocyte glutathione reductase activation coefficient (EGRac). This is a functional assay which measures the activity of glutathione reductase before and after *in vitro* reactivation with its prosthetic group FAD[20]. EGRac is calculated as a ratio of FAD-stimulated to unstimulated enzyme activity, with values of 1·3 or more generally indicative of suboptimal riboflavin status. Advantages of EGRac include stability and high sensitivity to small degrees of cofactor desaturation. The UK COMA Panel on Dietary Reference Values[21] decided to base RNI values for riboflavin (1·3 mg/d for men and 1·1 mg/d for women) on those intakes at which all but two corresponding EGRac results were less than 1·3 in British adult survey data available at that time[22]. Similarly, in the US, the relevant Committee[2] used evidence from intake studies showing normal EGRac values when setting its reference values.

More recently, however, the Scientific Advisory Committee on Nutrition has expressed particular concern about the high proportion of the UK adult population with apparently poor riboflavin status as determined by EGRac values. In the National Diet & Nutrition Survey (NDNS), a major proportion of adults were found to have abnormal (>1·3) EGRac: 82 % of men aged 19 to 24 years decreasing to 54 % in those

aged 50 to 64 years; 77 % of women in the youngest age group decreasing to 50 % in the oldest age group[23]. Some would argue that these abnormal EGRac values are simply a characteristic of the sensitivity of the assay procedure used, as in general, they did not correspond with dietary data, which indicated that only 3 % of men and 8 % of women had a riboflavin intake below the lower reference nutrient intake (LRNI) value of 0·8 mg/d[13]. However, the NDNS shows some evidence of low intakes in young adults, particularly young women, with some 15 % of women aged 19 to 24 years having a mean intake of riboflavin below the LRNI[13]. Consistent with NDNS findings, a recent study in Northern Ireland (Table 1) showed generally poor riboflavin status, and again particularly so among younger women.

Whether there is a general problem of poor riboflavin status in the UK population, as indicated by the large proportion with high EGRac values, is unclear and requires further investigation. Although EGRac is well established as a valid (often considered gold-standard) marker of riboflavin status, its measurement requires very specific treatment at the time of sampling and is therefore limited to those studies which have set out to measure EGRac. It does not enable riboflavin status to be determined retrospectively in large clinical studies which may have available a stored plasma sample, for example, on which B_2 vitamers can be directly measured[24].

B-vitamin status and disease prevention

Neural tube defects (NTDs)

NTDs are major malformations in which there is a failure of the neural tube to close properly. Importantly, the neural tube normally closes in the first few weeks of pregnancy and therefore the NTD malformations may have occurred before a woman even knows that she is pregnant. Folate has a well established protective role against both first occurrence and recurrence of NTDs, resulting in clear recommendations in the UK and elsewhere which have been in place for over 15 years. For the prevention of first occurrence of NTD (i.e. primary prevention), official bodies worldwide recommend women to take an additional 400 µg/d folate/FA from before conception up to the 12th gestational week. However, the achievement of this recommendation is proving to be very problematic in practice. Compliance with FA supplements is poor[25] and consequently they are generally ineffective. Recent evidence shows that current recommendations have not had any measurable impact

Table 1. Homocysteine and related B-vitamin status in the UK: comparison of data from a convenience sample in Northern Ireland (NI)* and the National Diet and Nutrition Survey (NDNS) of British adults aged 19–64 years†

	NI sample: males 19–64 y ($n = 215$)	NDNS males 19–64 y	NI sample: females 19–64 y ($n = 220$)	NDNS females 19–64 y
tHcy (µmol/l)	10·5 ± 4·6	11·7 ± 4·6	9·6 ± 2·7	10·1 ± 4·8
SF (nmol/l)	25·0 ± 14·8	20·8 ± 8·6	24·1 ± 15·2	22·1 ± 9·5
RCF (nmol/l)	905 ± 360	694 ± 288	867 ± 335	685 ± 293
B_{12} (pmol/l)	270 ± 97	298 ± 123	278 ± 108	288 ± 185
EGRac	1·35 ± 0·15	1·38 ± 0·17	1·39 ± 0·18	1·40 ± 0·19

Values are presented as mean ± SD.

Abbreviations: tHcy, plasma total homoysteine; SF, serum folate; RCF, red cell folate; B_{12}, serum vitamin B_{12}; and EGRac, erythrocyte glutathione reductase activation coefficient, a functional indicator of riboflavin status. A higher EGRac ratio indicates lower riboflavin status (values ≥1·3 considered to indicate sub-optimal status).

* Adapted from Hoey *et al.* [54].

† Ruston *et al.* [23].

on the rates of NTDs in some 13 European countries (including the UK) over a 10-year period, before and after the recommendations were introduced[26]. In contrast, the population-based policy of mandatory FA fortification of cereal grains implemented in North America and some non-European countries has substantially increased folate status[27] and reduced the occurrence of NTDs[28,29]. Despite this evidence, many governments elsewhere have decided against implementing similar population-based policies because of safety concerns regarding chronic exposure to FA.

Cardiovascular disease

There is considerable interest in plasma homocysteine as a cardiovascular disease (CVD) risk factor, with meta-analyses of prospective studies predicting that lowering concentrations by 3 (mol/l (i.e. a 25 % reduction in current levels) would reduce the risk of heart disease by up to 16 % and stroke by up to 24 %[30,31]. Good evidence for a causal relationship between elevated homocysteine and CVD comes from genetic studies. The most important genetic determinant of homocysteine in the general population is the common $677C \rightarrow T$ variant in the gene coding for the folate-metabolising enzyme MTHFR which results in impaired folate metabolism and higher homocysteine concentrations *in vivo*[32]. Importantly, people with the homozygous mutant (TT) genotype (typically 10 % of Western populations)[33] are found to have a significantly higher CVD risk (by 14 to 21 %) compared to those without this genetic factor[34,35].

A recent population based study showed that the decline in stroke-related mortality in recent years in the US and Canada related to the introduction of mandatory FA fortification[36]. Although the secondary prevention trials published to date have failed to confirm a benefit of homocysteine-lowering therapy on CVD events generally, they are now widely recognised as being substantially underpowered to detect a significant effect for heart disease risk, and therefore cannot be interpreted as evidence that no relationship exists. In support of this, evidence just published from a meta-analysis of clinical trials showed that homocysteine-lowering (by FA) reduced the risk of stroke by 18 % overall and by 25 % in those trials in people without a history of stroke[37]. Thus, although primarily aimed at reducing NTD, FA fortification may have an important role in the primary prevention of CVD, in particular stroke, via homocysteine-lowering.

Cancer

Folate is thought to play an important role in cancer prevention, with epidemiological evidence consistently showing inverse relationships between higher folate intakes and a reduced risk of cancer, most notably colorectal, but also pancreatic and breast cancer[38,39]. These observational studies from humans are substantiated by animal and experimental studies, with the latter focusing on the mechanisms explaining the possible cancer-preventative properties of folate. These have been attributed to its function in the de novo synthesis of thymidylate and purines, nucleotides required for DNA replication and repair. Folate is also required for the production of S-adenosylmethionine a universal donor of methyl groups for numerous methylation reactions including

the methylation of DNA which is central to gene silencing. In addition, genetic studies show that polymorphisms in folate metabolism are important in influencing folate status, related one-carbon metabolism and cancer risk[38].

Of note, the issue of folate and cancer has received much recent public health and scientific attention as a result of the publication of a randomised controlled trial which provided preliminary evidence, though as yet unconfirmed, suggesting that exposure to high dose FA (1 mg/d for 5 years) could promote colorectal tumorigenesis specifically in patients with pre-existing neoplasms[40]. This in turn supports the view that FA might have a dual role in carcinogenesis: it might be protective before any precancerous lesions have developed and in populations with low folate status, whereas at high doses it might stimulate the further development of existing lesions in populations already exposed to high FA intakes (through fortification and supplementation). Thus the role of folate in carcinogenesis may be more complicated than the observational studies suggest; the timing, and particularly the dose of FA, appear to be highly relevant.

Cognitive impairment

Impaired cognitive function is a common problem of ageing, ranging in severity from mild memory loss to dementia, the latter characterised by a decline in memory and thinking to a level that impairs activities of daily living. Recent interest has focussed on the potential role of homocysteine related B-vitamins in maintaining cognitive function in ageing. Observational studies in both healthy and cognitively impaired older adults show that lower status of B-vitamins and/or higher homocysteine is associated with poorer cognitive function. However, it is difficult to interpret much of the evidence from observational studies, since poor diet may be both a cause and a consequence of poor cognitive function. Evidence from randomised trials of homocysteine-lowering with B-vitamins is lacking and inconclusive, with many of the studies in this area being of insufficient duration or sample size. Two well designed, randomised placebo-controlled trials of relatively long duration in older adults were recently published, but their findings are conflicting. One trial reported no benefit of B-vitamins (FA, vitamin B_{12} and vitamin B_6 for two years) on cognitive performance[41], whereas the other concluded that supplementation with FA for three years significantly improved cognitive function[42]. Further evidence is required to confirm whether optimal B-vitamin status has a role in preventing cognitive impairment or dementia.

Osteoporosis

In recent years evidence is emerging from large cohort studies in the USA, the Netherlands and Norway linking elevated homocysteine levels (and/or lower status of related B-vitamins) to lower bone mineral density and a higher rate of osteoporotic fracture. More convincing evidence is provided by a randomised trial showing that combined treatment with FA and vitamin B_{12} was effective in reducing the risk of hip fracture following stroke[43], offering considerable support for the hypothesis that folate and/or vitamin B_{12} may delay the progression of osteoporosis, or that high homocysteine levels may promote it. If the link between elevated homocysteine and osteoporosis is

confirmed as being a causative one, there is considerable scope for prevention of bone disease by lowering homocysteine through enhanced B-vitamin status.

Homocysteine as a functional biomarker of B-vitamin status

Apart from its potential role as a risk factor in CVD and other diseases, plasma homocysteine is very responsive to intervention with the B-vitamins required for its metabolism: folate[44], and to a lesser extent, vitamin B_{12}[45], vitamin B_6[46] and riboflavin[47]. A recent meta-analysis of intervention studies examining the effect of B-vitamins on homocysteine-lowering, showed that vitamin B_{12} produces an additional one-third (7 %) lowering of homocysteine, over and above that achieved with FA alone (typically 20–25 % lowering)[48]. Thus vitamin B_{12} is generally considered to be a far less effective determinant of homocysteine concentrations compared with folate. However, evidence from a study in healthy subjects, supplemented with low dose FA[49], and from studies in the era of mandatory FA fortification of cereal grains in the US[50], shows that vitamin B_{12} becomes the main nutritional determinant of homocysteine once folate status is optimised.

The typical phenotype associated with homozygosity (TT genotype) for the MTHFR 677C → T polymorphism is elevated homocysteine levels[32]. Individuals with the TT genotype are considered to have increased dietary folate requirements on the basis that they have lower red cell folate levels compared to those without this genetic variant[51], and the expected increase in homocysteine is found to be most marked among those with lower folate status[52]. Apart from folate, however, riboflavin (FAD) is required as a cofactor for the MTHFR enzyme. The reduced activity of the variant form of MTHFR has been shown in vitro to result from the inappropriate loss of its FAD cofactor[53]. Recent results, showing a genotype-specific response of homocysteine to riboflavin supplementation, now confirm that riboflavin is an independent modifier of homocysteine in people with the TT genotype. Significant lowering of homocysteine in response to riboflavin supplementation was observed in healthy individuals with the TT genotype, with levels decreasing by as much as 22 % overall, and markedly so (by 40 %) in those with lower riboflavin status at baseline[47]. No homocysteine response to intervention was observed in non-homozygous individuals (CC or CT genotypes), despite a significant improvement in riboflavin status and the pre-selection of subjects with sub-optimal riboflavin status at baseline. The responsiveness of homocysteine to riboflavin is therefore specific to individuals with the MTHFR 677 TT genotype and represents a new gene-nutrient interaction.

Thus homocysteine can be considered a reliable functional marker of the status of folate in particular, of vitamin B_{12} in those optimised in folate, and of riboflavin specifically in those with the MTHFR 677 TT genotype.

Public health challenges in achieving optimal B-vitamin status

Optimal folate status has an established role in preventing NTDs and possible preventative roles in stroke and other diseases. Thus, the achievement of optimal folate status

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. Strategies to increase folate status are generally ineffective if based on health promotion and educational campaigns, and are controversial if based on mandatory fortification of foods with FA. There is a particular challenge for some European and other populations without access to fortified foods (not even on a voluntary basis in some cases) who are therefore dependent on natural food folate sources. In the case of vitamin B_{12}, 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 B_{12} malabsorption. Finally, the evidence that riboflavin status is generally low in the UK population, and particularly so in younger women, warrants further investigation.

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