Proceedings of the Nutrition Society (2015), **74**, 46–55 doi:10.1017/S0029665114001554
 First published online 5 November 2014

The Nutrition Society 23rd Annual Irish Section Postgraduate Meeting was held at University College, Dublin on 13–14 February 2014

Irish Postgraduate Winners

Emerging roles for folate and related B-vitamins in brain health across the lifecycle

C. McGarel, K. Pentieva*, J. J. Strain and H. McNulty
Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK

Nutrition plays a fundamental role in supporting the structural and functional development of the human brain from conception, throughout early infancy and extending into later life. A growing body of evidence suggests that folate and the metabolically related B-vitamins are essential for brain health across all age groups, owing to their specific roles in C_1 metabolism and particularly in the production of *S*-adenosylmethionine, a universal methyl donor essential for the production of neurotransmitters. Emerging, though not entirely consistent, evidence suggests that maternal folate status throughout pregnancy may influence neurodevelopment and behaviour of the offspring. Furthermore optimal B-vitamin status is associated with better cognitive health in ageing. Of note, a recent clinical trial provided evidence that supplementation with folic acid and related B-vitamins over a 2-year-period reduced global and regional brain atrophy, as measured by MRI scan in older adults. In terms of potential mechanisms, the effects of these B-vitamins on cognitive health may be independent or may be mediated by nutrient–nutrient and/or relevant gene–nutrient interactions. Furthermore, a new area of research suggests that the *in utero* environment influences health in later life. Folate, an important cofactor in C_1 metabolism, is indirectly involved in DNA methylation, which in turn is considered to be one of the epigenetic mechanisms that may underlie fetal programming and brain development. The present review will explore the evidence that supports a role for folate and the related B-vitamins in brain health across the lifecycle, and potential mechanisms to explain such effects.

C_1 metabolism: Maternal folate: B-vitamins: Cognitive performance: Cognitive health

Brain development is subject to complex lifelong processes of interactions between genetic and environmental factors. Although many environmental factors contribute to brain development, nutrition is of particular importance through the role that nutrients play in specific metabolic pathways and structural components[1]. It is known, for example, that a dietary deficiency during critical periods of development can result in permanent changes to the brain[2]. Of particular importance are folate and the related B-vitamins that are involved in C_1 metabolism and in the production of *S*-adenosylmethionine (SAM), a universal methyl donor required for various reactions, including the production of neurotransmitters[3]. B-vitamins are required for essential brain metabolic pathways and are fundamental in all aspects of brain development and maintenance of brain health throughout the lifecycle[4].

This review will explore the evidence linking maternal folate status with neurocognitive development in the offspring and will consider the associated explanatory mechanisms. In addition, the roles of B-vitamins and relevant genetic interactions in relation to cognitive health in older adults will also be examined.

Abbreviations: IGF2, insulin-like growth factor 2; MTHFR, methylenetetrahydrofolate reductase; RCT, randomised controlled trial; SAM, *S*-adenosylmethionine.

***Corresponding author:** Dr Kristina Pentieva, email k.pentieva@ulster.ac.uk

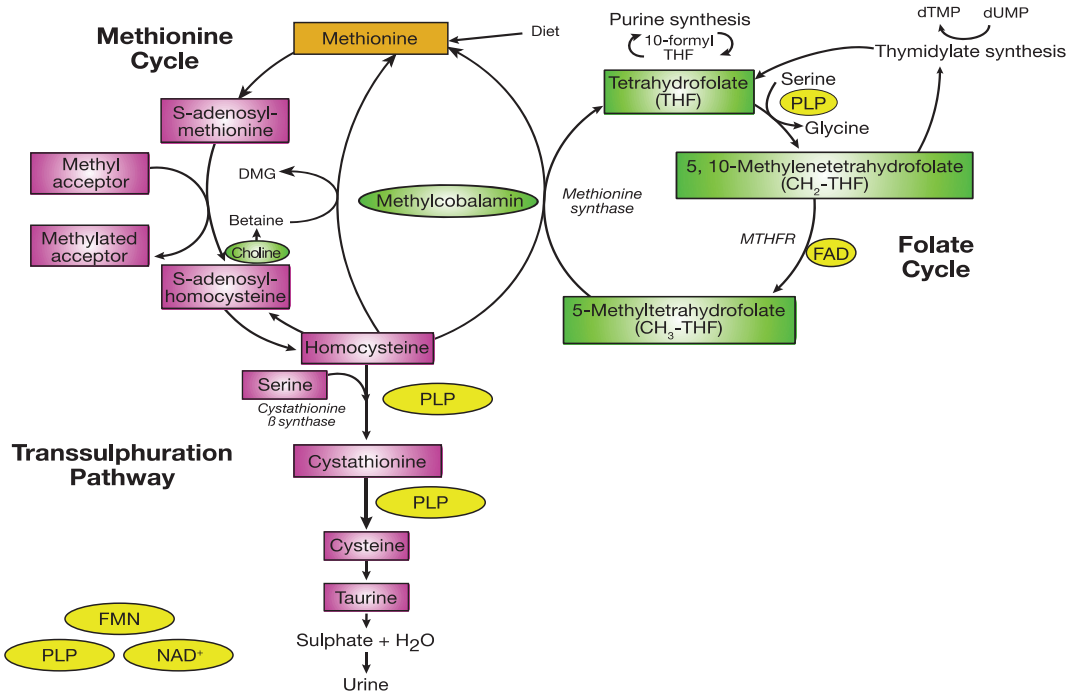

Fig. 1. (colour online) C_1 metabolism. PLP, pyridoxal 5′ phosphate; MTHFR, methylenetetrahydrofolate reductase; DMG, dimethylglycine; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate. (Adapted from Clarke *et al.*[85])

The role of folate and related B-vitamins in C_1 metabolism

Folate, a substrate of various enzyme reactions, along with vitamins B_{12}, B_6 and riboflavin in their co-factor forms, are involved in C_1 metabolism, which comprises a network of interrelated biochemical pathways that donate and regenerate C_1 units, including methyl groups (Fig. 1). Within the folate cycle tetrahydrofolate acquires a carbon unit from serine in a vitamin B_6-dependent reaction, which subsequently forms 5, 10-methylenetetrahydrofolate. The latter is involved in the synthesis of thymidine, which in turn is incorporated into DNA or it is converted to 5-methyltetrahydrofolate which participates in a riboflavin (i.e. FAD)-dependent reaction, catalysed by the enzyme methylenetetrahydrofolate reductase (MTHFR). 5-Methyltetrahydrofolate then serves as single carbon donor, feeding into the C_1 pathway by donating its methyl group to homocysteine to form methionine, via the vitamin B_{12}-dependent enzyme methionine synthase. Methionine is a precursor for the synthesis of SAM, a methyl donor required for the methylation of DNA, proteins, chromosomes and phospholipids, production of myelin and the synthesis and activation of neurotransmitters, including catecholamine and serotonin[5]. Upon donating its methyl group, SAM is converted to *S*-adenosylhomocysteine and homocysteine, which can be further metabolised in the transsulphuration pathway to form cysteine, a B_6-dependent process, or remethylated back to methionine.

It is important to note that some genetic factors may affect the function of enzyme activity, leading to disturbances in B-vitamin absorption, transport and uptake. Of particular interest is the common 677C→T polymorphism in the gene coding for MTHFR, and this polymorphism is an important genetic determinant of plasma homocysteine. Individuals with the homozygous mutant *MTHFR* 677TT genotype have reduced MTHFR enzyme activity. Homozygosity for this polymorphism alters B-vitamin requirements and has been linked to a number of degenerative diseases, including cognitive dysfunction[6].

C_1 metabolism, B-vitamins and early brain health

The role of folate in fetal brain development

Optimal folate status is essential throughout pregnancy for placental and fetal growth and development. During pregnancy there is a decline in maternal folate concentrations to approximately 50 % of non-pregnant concentrations[7]. This is partly owing to the increased folate requirements for rapid cell proliferation and tissue growth of the uterus and placenta, growth of the fetus and for the expansion of maternal blood volume[8]. Irrefutable evidence has shown that supplementation with folic acid protects against both first occurrence[9] and recurrence[10] of neural tube defects, leading to government recommendations, which are in place worldwide, advising all women planning a pregnancy to consume 400 μg folic acid/d from preconception until the end of the first trimester of pregnancy[11,12]. This protective effect of folic acid supplementation relates to the

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

Table 1. Observational studies investigating the association between maternal folate status with cognitive performance of the offspring

Reference	Sample size	Maternal folate status	Age of child	Cognitive assessment	Main outcome
Gross *et al.*[14]	14	Diagnosed megaloblastic anaemia, third trimester	6 weeks–4 years	DDST	Maternal folate deficiency associated with abnormal neurodevelopment
Julvez *et al.*[15]	420	Self-reported, first trimester	4 years	MSCA	FA associated with improved cognition
Venna *et al.*[16]	536	Blood samples at 30th GW	9–10 years	KABC^{TM}-II	FA in late pregnancy associated with better cognitive performance
Roth *et al.*[17]	39 954	Self-reported, first trimester	3 years	Language Grammar Scale	FA associated with reduced language delay
Chatzi *et al.*[18]	553	Self-reported, first trimester	18 months	Bayley-III	FA associated with vocabulary, communication and verbal skills
Villamor *et al.*[19]	1210	Self-reported, first trimester	3 years	PPVT-III & WRAVMA	FA associated with increased cognitive performance

FA, folic acid; GW, gestational week; PPVT, Peabody Picture Vocabulary Test; WRAVMA, Wide Range Assessment of Visual Motor Abilities; DDST, Denver Development Screening Test; MSCA, McCarthy Scales of Children's Abilities; KABC^{TM} -II, Kaufman Assessment Battery, 2nd edition; Bayley-III, Bayley Scales of Infant and Toddler Development, 3rd edition.

early stages of pregnancy when the closure of the neural tube occurs (about 21–28 d post-conception); however, little is known as to whether continuing folic acid usage throughout pregnancy confers any long-term beneficial effects to the offspring.

There is a growing body of evidence from observational and experimental studies, suggesting that nutrition *in utero* may affect later cognitive development in the offspring. As the fetal brain develops rapidly, poor maternal intake of key nutrients during pregnancy can influence the development of the structure and components of the brain. Folate and the metabolically related B-vitamins are fundamental throughout brain development via their participation in transcription, nucleotide synthesis, neurotransmitter production and methylation processes, including DNA methylation[13].

Evidence linking maternal folate status with offspring cognitive performance

In recent years, the association between maternal folate and/or related B-vitamin status with later cognitive performance of the offspring has become a topic of interest. To date most of the literature in this area is derived from observational (Table 1) and animal studies. Given the recognised protection of folic acid in the prevention of neural tube defect during early fetal development, the majority of these studies reported peri-conceptional use of folic acid by women and are focused in the early stages pregnancy. Only a few studies have investigated maternal folate status in the second and third trimesters of pregnancy, i.e. after the recommended period, in relation to later cognitive performance of the child, and no available human evidence has addressed this question in a randomised trial in pregnancy.

A number of human studies have shown positive associations between self-reported folic acid supplement use during the first trimester of pregnancy and cognitive performance of the child[15,17–19]. Julvez *et al.*[15] demonstrated that folic acid use in pregnancy was associated with improved neurodevelopment, verbal performance and motor development in children aged 4 years. Similarly, a large longitudinal study (*n* 1210) examined the association between maternal intake of methyl donor nutrients, including folate and vitamin B_{12}, during the first trimester of pregnancy in relation to the cognitive performance of the offspring. The authors estimated that each 600 μg/d increment in total folate intake (from food and supplements combined) was associated with a 1·6 point higher score in cognitive performance in the child, assessed at age 3 years[19]. Likewise, another observational study indicated significantly higher verbal comprehension, vocabulary development and communication skills in Greek children born to mothers taking high dose folic acid (5 mg/d) in pregnancy, compared with those whose mothers did not take supplements[18]. Most notably, evidence from a large prospective study (*n* 38 954) in the US-linked maternal folic acid supplement use from 4 to 8 weeks after conception with a reduced risk of severe language delay evaluated by self-reported parental questionnaires assessing the grammar of the child, at age 3 years[17].

During the growth spurt in pregnancy (about 24–42 gestational weeks), the developing brain, owing to the sequence of developmental stages, including neuronal proliferation and myelination, is particularly vulnerable to adequate nutrition[20]. Throughout the late fetal and early postnatal periods areas such as the hippocampus, auditory and visual cortices and the striatum undergo rapid growth by morphogenesis and synaptogenesis which makes them functionally active[21]. All nutrients are important for brain development, but it is proposed that certain nutrients, including folate, have greater effects during the late fetal development. Only a few studies, however, have investigated the impact of maternal folate status in the later stages of pregnancy on the child's neurodevelopment. Nearly 40 years ago, Gross *et al.*[14] reported that children born to mothers with diagnosed megaloblastic anaemia during the third trimester of pregnancy had abnormal neurodevelopment and lower intellectual abilities compared with infants born to mothers with optimal folate status. Several decades later a study investigating the impact of maternal blood folate, B_{12} and homocysteine concentrations, at 30 gestational

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

weeks, in relation to cognitive performance of 9–10-year-old children (*n* 536), showed that higher maternal folate status during late pregnancy predicted better cognitive performance in children[16]. No association was observed for maternal B_{12} or homocysteine status on the overall cognitive ability of the children[16].

To date no randomised controlled trial (RCT) has investigated the effect of maternal folate status during the later stages of pregnancy and subsequent cognitive performance of the offspring. A pilot study (*n* 39), conducted by our group, examined the effect of folic acid supplementation in the second and third trimester of pregnancy and subsequent cognitive performance of the child. The novel results showed that children (aged 3 years) born to mothers supplemented with folic acid, scored significantly higher in the cognitive domain of the Bayley's development assessment[22]. These highly promising results from this RCT now need to be confirmed on a larger scale.

Mechanistic studies in animal models also provide evidence that prenatal folate deficiency may be causally related to adverse structural changes in the brain[23,24]. Craciunescu *et al.*[24] reported that the offspring of rats fed a folic acid deficient diet during days 11 and 17 of gestation (i.e. corresponding to mid- and late stages of human pregnancy), had a reduction in progenitor cells in the fetal neocortex (an area responsible for complex behaviours, including cognition) suggesting that the developing fetal brain is vulnerable to maternal folate deficiency, which may adversely affect cognitive performance in the later life[24]. Similarly, another study showed that gestational B-vitamin deficiency resulted in an accumulation of homocysteine with 'concomitant apoptosis' in selective brain areas, the cerebellum, striatum and hippocampus, which altered motor function and learning and memory abilities in rats[25].

Evidence regarding the impact of maternal folate status on the offspring neurodevelopment is further strengthened by genetic studies. The common 677C→T polymorphism in the gene coding for the folate metabolising enzyme MTHFR is an important genetic determinant of plasma homocysteine and individuals homozygous for the polymorphism (*MTHFR* 677TT genotype), are prone to low folate status and elevated plasma homocysteine concentrations. Recent studies from Mexico, where the frequency of the *MTHFR* 677TT genotype is reported to be the highest in the world[26], showed that the maternal *MTHFR* 677TT genotype is a predictor of poor child neurodevelopment[27] especially in combination with low maternal folate intake (<400 μg/d) during the first trimester of pregnancy[28]. However, research in this area is limited and further studies are required to investigate whether children born to mothers genetically susceptible to impaired folate status are more at risk of impaired neurodevelopment.

Not all studies support the association between maternal folate status and the neurodevelopment of the child. A Hungarian RCT investigating pregnant women consuming a multi-vitamin containing folic acid (0·8 mg/d) before conception until the second month of pregnancy did not find any evidence of improved 'mental development' of children aged 6 years[29]. The present study, however, was primarily designed to investigate the effect of folic acid on neural tube defects during the very early stages of pregnancy. Likewise a longitudinal study found no evidence of an association between maternal blood folate status (low ≤11 nmol *v.* normal >11 nmol/l) in the later stages of pregnancy (19, 26 and 37 gestational weeks) and cognitive performance of children aged 5 years[30]. Possible reasons to explain these inconsistencies may include the influence of socio-economic status, a well-known confounder for cognitive development and which could potentially confound any effect of folic acid[30]. Furthermore, the studies by Dobo & Czeiel[29] and Tamura *et al.*[30] used a multi-vitamin approach, rather than folic acid alone; an approach, which again could impact the findings.

Overall, the observational and animal evidence appears to be supportive for a role of maternal folate status in later cognitive performance of the child. Aside from cognitive health, there are also studies linking low maternal folate status with higher offspring behavioural[31], inattention and hyperactivity problems[32] and emotional problems[33], which warrant further investigation. It is important to note however that much of the observational evidence is based on self-reported folic acid usage, usually during the early fetal development stages[15,17–19], with only a few studies exploring the effect of supplementation during later stages of pregnancy[14,16] and none doing so using a RCT. Considering that there is rapid structural and synaptic development in key areas of the brain, including the hippocampus, during the growth spurt in pregnancy, further studies are required to more fully investigate the potential effect of folate throughout pregnancy and to determine if the effect is specific to certain stages of pregnancy or perhaps the effect is mediated throughout all trimesters of pregnancy.

Other evidence supporting folate status in relation to brain health in the young

The effect of dietary and blood folate status on cognitive performance has also been investigated in young children and adolescents. A recent study by Strand *et al.*[34] reported that low plasma folate and vitamin B_{12} concentrations were associated with poorer cognitive performance, measured in children aged 12–18 months. Furthermore, an investigation of Swedish adolescents (age 15 years) showed that higher dietary folate intakes was positively associated with academic achievements[35]. Nguyen *et al.*[36] also reported that higher serum folate measured in children aged 6–16 years was associated with higher performance in reading and also in block design in participants from the National Health and Nutrition Examination Survey III cohort in the USA.

C_1 metabolism, B-vitamins and brain health in ageing

Cognitive dysfunction and dementia

Cognitive dysfunction is a common problem among the ageing population and ranges in severity from mild

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

cognitive impairment to more progressive types of dementia; the latter referring to a state in which the disease is sufficient to impair normal way of living[37,38]. Although some degree of cognitive decline is considered a normal and an unpreventable aspect of ageing, the development of dementia and Alzheimer's disease is attributable to diseases of the brain, resulting in changes to the brain structure sufficient to interfere with normal life activities. Globally an estimated 35·6 million people suffer from dementia, affecting 7 % of individuals aged over 65 years and 30 % of those over 80 years[39]. Given the increase in life expectancy, these figures are expected to double worldwide by 2025[39] and represents a major public health challenge for future generations.

As brain changes start to progress long before the diagnosis of dementia is overt, it is important to find early biomarkers that would enable timely interventions to delay the onset or slow the progression of the disease[40]. Well-established non-modifiable risk factors include increasing age, family history and genetic factors. However, evidence has now amassed from long-term epidemiological studies linking potentially modifiable lifestyle factors, including smoking status, physical inactivity and nutritional factors with cognitive dysfunction. Emerging evidence suggests that suboptimal status of folate and the metabolically related B-vitamins and/or elevated homocysteine concentrations, owing to their essential roles in C_1 metabolism may be linked with cognitive dysfunction and dementia.

Evidence linking B-vitamins with brain health in ageing

The majority of epidemiological studies generally support an association between suboptimal status of folate, the metabolically related B-vitamins or elevated concentrations of the metabolite homocysteine with cognitive dysfunction in older adults. Indeed, a review by Smith [41] some years ago reported that 90 out of 100 published cross-sectional and prospective studies showed a link between elevated homocysteine and/or low B-vitamins concentrations with cognitive dysfunction. Most of these studies have focused on elevated plasma homocysteine concentrations[42–46], and/or a combination of suboptimal status of folate and vitamin B_{12}[47–54] and to a much lesser extent on vitamin B_6[55,56]. To the authors' knowledge, no published study thus far has focused on riboflavin alone as a potential contributor to cognitive health. Notably, there are a number of limitations associated with these studies; typically, only one cognitive assessment tool was used, rather than a battery of tests providing information for multiple cognitive domains; depression and anxiety (known confounders) are not measured and there is a lack of data on vitamin supplement usage, all of which can limit the reliability of reported results.

It is also important to take into consideration recent concerns regarding mandatory folic acid fortification and the potential 'masking' effect of vitamin B_{12} deficiency among older adults. In B_{12} deficiency, the activity of methionine synthase is reduced; therefore 5, methyltetrahydrofolate cannot be converted to tetrahydrofolate (the active form of folate) and becomes trapped in an unusable form. Evidence from the National Health and Nutrition Examination Survey reported that although higher folate was generally associated with better cognitive health, a combination of high plasma folate (>59 nmol/l) with low plasma B_{12} (<148 pmol) and elevated methylmalonic acid (a B_{12}-specific functional biomarker), was actually associated with poorer cognitive performance compared with individuals with normal concentrations of these biomarkers[57]. Furthermore, Moore *et al.*[58] showed participants with high red cell folate and low serum B_{12} were three times more likely to have impaired cognitive performance. In contrast, however, analysis from the Hordaland Health study failed to confirm this association in their analysis[59]. The disagreement between studies is possibly linked to the relatively small sample of participants with the combination of high folate and low B_{12} status available for analysis in these studies. In addition, high and low status of folate and B_{12} is defined differently among various published studies.

Following the positive associations from epidemiological evidence a number of RCT have investigated the potential benefits of B-vitamin supplementation on cognitive health (Table 2). Many of these trials however were of insufficient power and duration to detect an effect or included participants with existing optimal B-vitamin status or with advanced dementia, where a beneficial effect is not likely[60,62,67–70].

Notably, two similarly designed homocysteine-lowering trials have yielded conflicting results. The Folic Acid and Carotid Intimamedia Thickness (FACIT) trial found that supplementation with 800 μg folic acid/d over 3 years in healthy adults' (*n* 818) improved global cognitive function, in particular memory, information processing speed and sensorimotor speed[61]. In contrast, a 2-year trial also conducted in healthy individuals (*n* 276) supplemented with a combination of folate (1000 μg/d), B_{12} (500 μg/d) and B_6 (10 mg/d) or placebo, reported no significant effect on cognitive performance, across the eight cognitive assessments used[60]. Although these studies were of similar design, sufficiently powered, had comparable baseline cognitive scores and used similar exclusion criteria, it is important to note the difference in the baseline folate status of participants between these two trials. The baseline folate (12 nmol/l) was lower in the FACIT trial compared with the McMahon study (baseline folate 22·6 nmol/l), which may suggest that any benefits of folic acid on cognitive health in ageing may arise from correcting suboptimal folate status, whereas supplementing with additional folic acid to those with optimal status may not provide any further benefit to cognitive function. More recently, a significant improvement in overall cognitive performance was found following a 2-year intervention with B-vitamins in participants with elevated psychological distress[64]. The majority of the reported intervention trials used questionnaire-based assessments of cognitive performance; very few clinical trials have investigated the effect of B-vitamins on cognitive dysfunction using direct methods, such as brain imaging techniques, which may provide a more robust measure of long-term brain changes, including the impact of nutritional interventions.

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

Table 2. Summary of randomised trials assessing the effect of B-vitamin treatment on cognitive function in older adults

Reference	Sample size (*n*)	Age (years)	Population	Duration	Treatment	Main outcome
Questionnaire-based assessment						
McMahon *et al.*[60]	276	≥65	Healthy individuals hcy ≥13 μmol/l	2 years	1000 μg FA, 500 μg B_{12}, 10 mg B_6	No significant effects on cognition
Durga *et al.*[61]	818	50–70	Healthy individuals hcy ≥13 μmol/l	3 years	800 μg FA	Improved domains of cognitive function
Aisen *et al.*[62]	304	≥50	Mild to moderate AD	18 months	5 mg FA, 1 mg B_{12}, 25 mg B_6	No significant effects on cognition
deJager *et al.*[63]	233	≥70	MCI	2 years	800 μg FA, 500 μg B_{12}, 20 mg B_6	B-vitamin treatment slowed cognitive decline
Walker *et al.*[64]	900	60–74	Elevated psychological distress	2 years	400 μg FA, 100 μg B_{12}	B-vitamin treatment improved cognitive function
Brain-imaging assessment						
Smith *et al.*[65]	168	≥70	MCI	2 years	800 μg FA, 500 μg B_{12}, 20 mg B_6	B-vitamin treatment slowed the rate of brain atrophy
Douaud *et al.*[66]	156	≥70	MCI	2 years	800 μg FA, 500 μg B_{12}, 20 mg B_6	B-vitamin treatment reduced grey matter atrophy is regions associated with AD

FA, folic acid; hcy, homocysteine; AD, Alzheimer's disease; MCI, mild cognitive impairment.

The strongest evidence to date originates from the VITACOG trial in Oxford, in which patients with mild cognitive impairment (without dementia) were supplemented with folic acid (800 μg/d), B_{12} (500 μg/d) and B_6 (20 mg/d) or placebo over a 2-year period. The results reported that supplementation with B-vitamins slowed the rate of cognitive decline, assessed by questionnaire-based cognitive tests[63]. Of greater importance is the fact that the same study also found that B-vitamin treatment reduced brain atrophy, as measured by MRI scans, by approximately 30 %[65]. The effect was greatest in participants with the highest baseline homocysteine concentrations (>13 μmol/l), among whom an overall reduction of brain atrophy rate of 53 % was reported, while no effect was found in those in the bottom quartile (≤9·5 μmol/l)[65]. Moreover, when a subsample of VITACOG cohort were further analysed, the investigators reported that B-vitamin treatment reduced the cerebral atrophy, by as much as 7-fold, specifically in grey matter areas of the brain vulnerable to Alzheimer's disease, including bilateral hippocampus and cerebellum[66]. Finally, when participants were analysed by quartiles for brain shrinkage, it was reported that participants with the highest rate of brain shrinkage displayed the most cognitive decline[71]. Importantly, folate, vitamin B_{12} and vitamin B_6 are all required to lower concentrations of homocysteine and perhaps it is the combination of B-vitamins, and not a monotherapy B-vitamin approach, which is required to optimise C_1 metabolism. This research has paved the way for future RCT in this area; more research is however warranted in both healthy and cognitively impaired groups to investigate the proposed effect further.

In summary, results from large observational studies conducted in healthy and cognitively impaired cohorts provide strong evidence of a possible relationship between suboptimal B-vitamin status, elevated homocysteine (independently or as a marker of B-vitamin status) and cognitive dysfunction. At present the most promising evidence supporting a cause and effect relationship comes from the VITACOG trial[63,65,66,71]. These findings now require replication in other population groups to confirm the role played by B-vitamins in cognitive health. If cognitive dysfunction can in fact be slowed or prevented by B-vitamins, in healthy older people, then this could offer a cost-effective preventative public health strategy in ageing populations.

Potential mechanisms linking B-vitamins with brain health

Potential mechanism explaining the role of B-vitamins in early brain development

B-vitamins appear to have direct roles on brain development and maintenance, through their involvement in C_1 metabolism. A new emerging area of research concerns the role of epigenetics, which is broadly defined as the 'heritable changes in gene function that cannot be explained by changes in the DNA sequence'[72]. Epigenetic alternations *in utero* may have the potential to programme diseases in adulthood. Studies have recently begun to investigate whether epigenetic modification, through nutritional interventions, can influence an individual's health in later life. DNA methylation is the most widely studied epigenetic mechanism and occurs through C_1 metabolism, which is dependent on folate and several cofactors, including the related B-vitamins.

A number of animal studies have addressed the issue of maternal folate status and subsequent epigenetic effect on the offspring. Waterland & Jirlte[73] demonstrated that in pregnant agouti mice a high methyl diet, resulted in offspring with mottled brown coats, which were less

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

obesogenic and less prone to diseases. Interestingly, methyl donor supplementation of agouti pregnant dams appeared to not only affect the fetus, but also the subsequent generation, suggesting that the maternal diet may influence several generations[74]. Recently, Cho *et al.*[75] also provided evidence of the epigenetic effects of folate supplementation in pregnancy and showed that a high folate diet throughout pregnancy and weaning resulted in dams less exposed to an obseogenic phenotype. Collectively, these studies show that restriction in folate can influence DNA methylation in the offspring and in turn influence gene expression and disease related phenotypes.

The results from human studies, although limited, are also generally supportive of the effect of folate on epigenetic processes. Evidence from the Dutch Hunger Winter study has shown that malnutrition (in this case because of extreme famine) during pregnancy may induce permanent epigenetic changes in *IGF2*[76,77]. Recent studies suggest that *IGF2* epigenetic changes *in utero* are associated with long-term metabolic health risk in human subjects[77]. The results from Steegers-Theunissen *et al.*[78] showed that reported maternal folic acid supplement usage was associated with an increased methylation (4·5 %) of *IGF2* differentially methylated region of the offspring, 17 months after delivery. Moreover, maternal SAM concentrations were related to the offspring *IGF2* differentially methylated region's methylation levels, indicating that the maternal environment had a greater influence on the methylation of *IGF2* in the offspring[78]. In further support, results from an observational study (*n* 913) investigating the effect of folic acid use after the recommended 12 gestational weeks, reported that supplement use was associated with significant, albeit small, elevation in *IGF2* methylation and reduced methylation in paternally expressed 3 (*PEG3*) and LINE-1[79]. No effect was observed before 12 weeks gestation; perhaps suggesting an epigenetic effect of folate occurred during the later stages of pregnancy. Considering that *IGF2* is involved in placental and fetal development and *PEG3* is a gene highly expressed in the brain and also involved in the development of the fetal hypothalamus[80,81], alterations in the methylation of these genes may subsequently exert influence on their expression, which in turn might have an impact on the offspring's brain development. However, further studies are required to expand on the current knowledge of nutrition and disease prevention through epigenetic mechanisms.

Potential mechanism explaining the role of B-vitamins with brain health in ageing

Mechanisms linking B-vitamins and cognitive health in ageing are speculative and several hypotheses have been suggested. Folate and vitamin B_{12} are required for the production of SAM, which in turn is required for various methylation reactions and adequate production of neurotransmitters. Deficiencies in these vitamins may lead to disturbances in important methylation reactions, which may affect pathways associated with cognitive health. It is also proposed that deficiencies in folate, vitamins B_{12} and B_6 can disrupt the remethylation of homocysteine to methionine, resulting in hyperhomocysteinemia. Studies have linked elevated plasma homocysteine concentrations with atrophy of the hippocampus; an area of the brain required for memory consolidation[82]. It is therefore suggested that high homocysteine concentrations may have direct neurotoxic effects, resulting in apoptosis and possibly impairing pathways associated with cognition[64,83]. In addition, optimal B_6 status plays an important role in brain health through its essential role in transamination and decarboxylation reactions required for the metabolism of several neurotransmitters, including serotonin, γ-aminobutyric acid, dopamine, noradrenaline and histamine[84].

Conclusion

Given the importance of C_1 metabolism in a wide array of processes, including the production of neurotransmitters and DNA methylation, it is not surprising that disturbances in this cycle may have profound effects on both the developing and ageing brain. Mechanistically it is clear that folate and the related B-vitamins are critical for brain function throughout the lifecycle. Some important questions, however, still need to be considered. The potential role between maternal folate status during pregnancy and later neurodevelopment of the offspring needs to be explored through well-designed RCT. A clearer understanding of the role of folate in early brain health will help to inform future policies in relation to folic acid recommendations in pregnancy. Compelling evidence from epidemiological studies and RCT show that there is a strong association between suboptimal B-vitamin status and/or elevated homocysteine with an increased risk of cognitive dysfunction in older adults. Further research from well-conducted RCT which includes brain-imaging techniques is warranted to shed light on some fundamental questions. In conclusion, current evidence suggests that folate and the metabolically related B-vitamins may be important contributors to brain health across the lifecycle. Improving knowledge of potential epigenetic mechanisms during pregnancy and postnatal life will help provide the important mechanistic links between B-vitamins and brain health.

Financial Support

This work was supported by the funding from the Northern Ireland Department for Employment and Learning which funded the PhD studentship for C. M. G. The Northern Ireland Department for Employment and Learning had no role in the design, analysis or writing of this article.

Conflict of Interest

None.

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

Authorship

C. M. G. drafted the manuscript. K. P., H. McN. and J. J. S. critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

References

1. Georgieff MK (2007) Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr* **85**, 614S–620S.
2. Anjos T, Altmäe S, Emmett P *et al.* (2013) Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. *Eur J Nutr* **52**, 1825–1842.
3. Dominguez-Salas P, Cox SE, Prentice AM *et al.* (2012) Maternal nutritional status, C_1 metabolism and offspring DNA methylation: a review of current evidence in human subjects. *Proc Nutr Soc* **71**, 154–165.
4. van de Rest O, van Hooijdonk LWA, Doets E *et al.* (2012) B Vitamins and *n*-3 fatty acids for brain development and function: review of human studies. *Ann Nutr Metab* **60**, 272–292.
5. Schaevitz L, Berger-Sweeney J & Ricceri L (2014) One-carbon metabolism in neurodevelopmental disorders: using broad-based nutraceutics to treat cognitive deficits in complex spectrum disorders. *Neurosci. Biobehav Rev* (Epublication ahead of print version)
6. Ford AH, Flicker L, Hankey GJ *et al.* (2012) Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism and cognitive impairment: the health in men study. *Mol Psychiatry* **17**, 559–566.
7. McNulty B, Pentieva K, Marshall B *et al.* (2011) Women's compliance with current folic acid recommendations and achievement of optimal status for preventing neural tube defects. *Hum Reprod* **26**, 1530–1536.
8. McNulty H, McPartlin JM, Weir DG *et al.* (1993) Folate catabolism is increased during pregnancy in rats. *J Nutr* **123**, 1089–1093.
9. Czeizel AE & Dudás I (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* **327**, 1832–1835.
10. MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131–137.
11. Centers for Disease Control Prevention (1992) Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *Morb Mortal Wkly Rep* **41**, 1–8.
12. Department of Health (1992) Folic acid and the prevention of neural tube defects. *Report from an expert advisory group* No.1. London: Department of Health.
13. Reynolds E (2006) Vitamin B_{12}, folic acid, and the nervous system. *Lancet Neurol* **5**, 949–960.
14. Gross RL, Newberne PM & Reid JVO (1974) Adverse effects on infant development associated with maternal folic acid deficiency. *Nutr Rep Intern* **10**, 241–248.
15. Julvez J, Fortuny J, Mendez M *et al.* (2009) Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. *Paediatr Perinat Epidemiol* **23**, 199–206.
16. Veena SR, Krishnaveni GV, Srinivasan K *et al.* (2010) Higher maternal plasma folate but not Vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9- to 10-year-old children in South India. *J Nutr* **140**, 1014–1022.
17. Roth C, Magnus P, Schjølberg S *et al.* (2011) Folic acid supplements in pregnancy and severe language delay in children. *J Am Med Assoc* **306**, 1566–1573.
18. Chatzi L, Papadopoulou E, Koutra K *et al.* (2012) Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother-child cohort 'Rhea' study in Crete, Greece. *Public Health Nutr* **15**, 1728–1736.
19. Villamor E, Rifas-Shiman SL, Gillman MW *et al.* (2012) Maternal intake of methyl-donor nutrients and child cognition at 3 years of age. *Paediatr Perinat Epidemiol* **26**, 328–335.
20. Isaacs EB (2013) Neuroimaging, a new tool for investigating the effects of early diet on cognitive and brain development. *Front Hum Neurosci* **7**, 445.
21. Thompson RA & Nelson CA (2001) Developmental science and the media. Early brain development. *Am Psychol* **56**, 5–15.
22. Pentieva K, McGarel C, McNulty B *et al.* (2012) Effect of folic acid supplementation during pregnancy on growth and cognitive development of the offspring: a pilot follow-up investigation of children of FASSTT study participants. *Proc Nutr Soc* **71**, E139.
23. Whitley JR, O'Dell BL & Hogan AG (1951) Effect of diet in maze learning in second generation rats; folic acid deficiency. *J Nutr* **45**, 153–160.
24. Craciunescu CN, Brown EC, Mar MH *et al.* (2004) Folic acid deficiency during late gestation decreases progenitor cell proliferation and increases apoptosis in fetal mouse brain. *J Nutr* **134**, 162–166.
25. Blaise SA, Nédélec E, Schroeder H *et al.* (2007) Gestational Vitamin B deficiency leads to homocysteine-associated brain apoptosis and alters neurobehavioral development in rats. *Am J Pathol* **170**, 667–679.
26. 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 worldwide. *J Med Genet* **40**, 619–625.
27. Pilsner JR, Hu H, Wright RO *et al.* (2010) Maternal *MTHFR* genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. *Am J Clin Nutr* **92**, 226–234.
28. del Río Garcia C, Torres- Sanchez L, Chen J *et al.* (2009) Maternal *MTHFR 677C>T* genotype and dietary intake of folate and vitamin B(12): their impact on child neurodevelopment. *Nutr Neurosci* **12**, 13–20.
29. Dobó M & Czeizel AE (1998) Long-term somatic and mental development of children after periconceptional multivitamin supplementation. *Eur J Pediatr* **157**, 719–723.
30. Tamura T, Goldenberg RL, Chapman VR *et al.* (2005) Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age. *Pediatrics* **116**, 703–708.
31. Roza SJ, van Batenburg-Eddes T, Steegers EAP *et al.* (2010) Maternal folic acid supplement use in early pregnancy and child behavioural problems: the Generation R Study. *Br J Nutr* **103**, 445–452.
32. Schlotz W, Jones A, Phillips DIW *et al.* (2010) Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. *J Child Psychol Psychiatry* **51**, 594–602.
33. Steenweg-de Graaff J, Roza SJ, Steegers EAP *et al.* (2012) Maternal folate status in early pregnancy and child

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

emotional and behavioral problems: the Generation R Study. *Am J Clin Nutr* **95**, 1413–1421.
34. Strand TA, Taneja S, Ueland PM *et al.* (2013) Cobalamin and folate status predicts mental development scores in North Indian children 12–18 mo of age. *Am J Clin Nutr* **97**, 310–317.
35. Nilsson TK, Yngve A, Bottiger AK *et al.* (2011) High folate intake is related to better academic achievement in Swedish adolescents. *Pediatrics* **128**, E358–E365.
36. Nguyen CT, Gracely EJ & Lee BK (2013) Serum folate but not Vitamin B-12 concentrations are positively associated with cognitive test scores in children aged 6–16 years. *J Nutr* **143**, 500–504.
37. Graham JE, Rockwood K, Beattie BL *et al.* (1997) Prevalence and severity of cognitive impairment with and without dementia in an elderly population. *Lancet* **349**, 1793–1796.
38. 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.
39. Alzheimer's Society UK (2013) Statistics; available at https://www.alzheimers.org.uk/statistics
40. Kivipelto M, Ngandu T, Laatikainen *et al.* (2006) Risk score for the predicition of dementia risk in 20 years among middle-aged people: a longitudinal, population-based study. *Lancet Neurol* **5**, 735–741.
41. Smith AD (2008) The worldwide challenge of the dementias: a role for B vitamins and homocysteine? *Food Nutr Bull* **29**, 2 Suppl, S143–S172.
42. Ravaglia G, Forti P, Maioli F *et al.* (2003) Homocysteine and cognitive function in healthy elderly community dwellers in Italy. *Am J Clin Nutr* **77**, 668–673.
43. Ford AH, Flicker L, Singh U *et al.* (2013) Homocysteine, depression and cognitive function in older adults. *J Affect Disord* **151**, 646–651.
44. Allam M, Fahmy E, Elatti SA *et al.* (2013) Associations between total plasma homocysteine level and cognitive functions in elderly Egyptian subjects. *J Neurol Sci* **322**, 86–91.
45. Seshadri S, Beiser A, Selhub J *et al.* (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* **346**, 476–483.
46. Miller JW, Green R, Ramos MI *et al.* (2003) Homocysteine and cognitive function in the Sacramento Area Latino Study on Ageing. *Am J Clin Nutr* **78**, 441–447.
47. Ramos MI, Allen LH, Mungas DM *et al.* (2005) Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. *Am J Clin Nutr* **82**, 1346–1352.
48. Campbell AK, Jagust WJ, Mungas DM *et al.* (2005) Low erythrocyte folate, but not plasma B-12 or homocysteine, is associated with dementia in elderly Latinos. *J Nutr Health Aging* **9**, 39–43.
49. de Lau LML, Refsum H, Smith AD *et al.* (2007) Plasma folate concentration and cognitive performance: Rotterdam Scan Study. *Am J Clin Nutr* **86**, 728–734.
50. Duthie SJ, Whalley LJ, Collins AR *et al.* (2002) Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr* **75**, 908–913.
51. Kado DM, Karlamangla AS, Huang MH *et al.* (2005) Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of successful aging. *Am J Med* **118**, 161–167.
52. Nurk E, Refsum H, Tell Gs *et al.* (2005) Plasma total homocysteine and memory in the elderly: the Hordaland Homocysteine Study. *Ann Neurol* **58**, 847–857.
53. Haan MN, Miller JW, Aiello AE *et al.* (2007) Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. *Am J Clin Nutr* **85**, 511–517.
54. Hooshmand B, Solomon A, Kåreholt I *et al.* (2012) Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study. *J Intern Med* **271**, 204–212.
55. Riggs KM, Spiro A III, Tucker K *et al.* (1996) Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* **63**, 306–314.
56. Moorthy D, Peter I, Scott TM *et al.* (2012) Status of Vitamins B-12 and B-6 but not of folate, homocysteine, and the methylenetetrahydrofolate reductase C677T polymorphism are associated with impaired cognition and depression in adults. *J Nutr* **142**, 1554–1560.
57. Morris MS, Jacques PF, Rosenberg IH *et al.* (2007) Folate and vitamin B-12 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.
58. Moore EM, Ames D, Mander AG *et al.* (2014) Among vitamin B_{12} deficient older people, high folate levels are associated with worse cognitive function: combined data from three cohorts. *J Alzheimers Dis* **39**, 661–668.
59. Doets EL, Ueland PM, Tell GS *et al.* (2014) Interactions between plasma concentrations of folate and markers of vitamin B_{12} status with cognitive performance in elderly people not exposed to folic acid fortification: the Hordaland Health Study. *Br J Nutr* **111**, 1085–1095.
60. McMahon JA, Green TJ, Skeaff CM *et al.* (2006) A controlled trial of homocysteine lowering and cognitive performance. *N Engl J Med* **354**, 2764–2772.
61. Durga J, van Boxtel MPJ, Schouten EG *et al.* (2007) Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet* **369**, 208–216.
62. Aisen PS, Schneider LS, Sano M *et al.* (2008) High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. *J Am Med Assoc* **300**, 1774–1783.
63. de Jager CA, Oulhaj A, Jacoby R *et al.* (2012) Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial. *Int J Geriatr Psychiatry* **27**, 592–600.
64. Walker JG, Batterham PJ, Mackinnon AJ *et al.* (2012) Oral folic acid and vitamin B-12 supplementation to prevent cognitive decline in community-dwelling older adults with depressive symptoms-the Beyond Ageing Project: a randomized controlled trial. *Am J Clin Nutr* **95**, 194–203.
65. Smith AD, Smith SM, de Jager CA *et al.* (2010) Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. *PLoS ONE* **5**, e12244.
66. Douaud G, Refsum H, de Jager CA *et al.* (2013) Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. *Proc Natl Acad Sci USA* **110**, 9523–9528.
67. Lewerin C, Matousek M, Steen G *et al.* (2005) Significant correlations of plasma homocysteine and serum methylmalonic acid with movement and cognitive performance in elderly subjects but no improvement from short-term vitamin therapy: a placebo-controlled randomized study. *Am J Clin Nutr* **81**, 1155–1162.
68. Pathansali R, Mangoni AA, Creagh-Brown B *et al.* (2006) Effects of folic acid supplementation on psychomotor performance and hemorheology in healthy elderly subjects. *Arch Gerontol Geriatr* **43**, 127–137.

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press

69. Eussen SJ, de Groot LC, Joosten LW *et al.* (2006) Effect of oral vitamin B_{12} with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial. *Am J Clin Nutr* **84**, 361–370.
70. Kwok T, Lee J, Law CB *et al.* (2011) A randomized placebo controlled trial of homocysteine lowering to reduce cognitive decline in older demented people. *Clin Nutr* **30**, 297–302.
71. de Jager CA (2014) Critical levels of brain atrophy associated with homocysteine and cognitive decline. *Neurobiol Aging* **35**, Suppl. 2, S35–S39.
72. Russo VEA, Martienssen RA & Riggs AD (1996) *Epigenetic Mechanisms of Gene Regulation*. Cold Springs Harbor, New York: Cold Springs Harbor Laboratory Press.
73. Waterland RA & Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* **23**, 5293–5300.
74. Cropley JE, Suter CM, Beckman KB *et al.* (2006) Germ-line epigenetic modifications of the murine A^{vy} allele by nutritional supplementation. *Proc Natl Acad Sci USA* **103**, 17308–17312.
75. Cho CE, Sánchez-Hernández D, Reza-López SA *et al.* (2013) High folate gestational and post-weaning diets alter hypothalamic feeding pathways by DNA methylation in Wistar rat offspring. *Epigenetics* **8**, 710–719.
76. Roseboom TJ, Painter RC, van Abeelen AFM *et al.* (2011) Hungry in the womb: what are the consequences? Lessons from the Dutch famine. *Maturitas* **70**, 141–145.
77. Heijmans BT, Tobi EW, Stein AD *et al.* (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* **105**, 17046–17049.
78. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D *et al.* (2009) Periconceptional maternal folic acid use of 400 µg per day is related to increased methylation of the *IGF2* gene in the very young child. *PLoS ONE* **4**, 1–5.
79. Haggarty P, Hoad G, Campbell DM *et al.* (2013) Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. *Am J Clin Nutr* **97**, 94–99.
80. Ivanoca E & Kelsey G (2011) Imprinted genes and hypothalamic function. *J Mol Endocrinol* **47**, R67–R74.
81. Keverne B (2009) Monoallelic gene expression and mammalian evolution. *BioEssays* **31**, 1318–1326.
82. den Heijer T, Vermeer SE, Clarke R *et al.* (2003) Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain* **126**, 170–175.
83. Clarke R, Smith AD, Jobst KA *et al.* (1998) Folate, vitamin B_{12}, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* **55**, 1449–1455.
84. di Salvo ML, Contestabile R & Safo MK (2011) Vitamin B_6 salvage enzymes: mechanism, structure and regulation. *Biochim Biophys Acta* **1814**, 1597–1608.
85. Clarke M, Ward M, Strain JJ *et al.* (2014) B-vitamins and bone health and disease: the current evidence. *Proc Nutr Soc* **73**, 330–339.

https://doi.org/10.1017/S0029665114001554 Published online by Cambridge University Press