Proceedings of the Nutrition Society (2014), **73**, 330–339 doi:10.1017/S0029665114000044
© The Authors 2014 First published online 26 February 2014

The Nutrition Society Annual Summer Meeting was held at Newcastle University on 15–18 July 2013

Conference on 'Nutrition and healthy ageing' The 2013 Summer Meeting Postgraduate Winners

B-vitamins and bone in health and disease: the current evidence

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Osteoporosis, a metabolic skeletal disease characterised by decreased bone mass and increased fracture risk, is a growing public health problem. Among the various risk factors for osteoporosis, calcium and vitamin D have well-established protective roles, but it is likely that other nutritional factors are also implicated. This review will explore the emerging evidence supporting a role for certain B-vitamins, homocysteine and the 677C→T polymorphism in the gene encoding the folate-metabolising enzyme methylenetetrahydrofolate reductase, in bone health and disease. The evidence, however, is not entirely consistent and as yet no clear mechanism has been defined to explain the potential link between B-vitamins and bone health. Coeliac disease, a common condition of malabsorption, induced by gluten ingestion in genetically susceptible individuals, is associated with an increased risk both of osteoporosis and inadequate B-vitamin status. Given the growing body of evidence linking low bone mineral density and/or increased fracture risk with low B-vitamin status and elevated homocysteine, optimal B-vitamin status may play an important protective role against osteoporosis in coeliac disease; to date, no trial has addressed this possible link.

B-vitamins: homocysteine: *MTHFR* 677C→T polymorphism: bone: coeliac disease

Osteoporosis, a metabolic skeletal disease characterised by reduced bone mass and deterioration of bone micro-architecture, predisposes affected individuals to an increased risk of fragility fracture[1]. It is a major public health problem, recently estimated to cost £5.5 billion in the UK alone[2], with one in two women and one in five men over the age of 50 years expected to experience an osteoporotic fracture[3]. Bone is a dynamic tissue in a constant state of remodelling. Bone formation generally outweighs bone resorption in the first three decades of life, and peak bone mass (i.e. the maximum bone mass achieved in adulthood) is accrued during this period. After this time, bone resorption is favoured and bone loss ensues, which in turn predisposes older individuals to weaker bones and increased risk of fracture[4].The most common osteoporotic fracture sites are at the spine, hip and wrist, with both spine and hip fractures accompanied by considerable disability and increased morbidity and mortality[5].

The measurement of bone mineral density (BMD) through dual-energy X-ray absorptiometry scanning is currently the basis of diagnosing osteoporosis. The WHO defines osteoporosis as a BMD of less than or equal to 2·5 SD below the reference mean of a young adult with peak bone mass (i.e. a *T*-score of ≤−2·5 SD), and osteopenia (the precursor stage to osteoporosis) as a BMD of less than 1 SD but greater than 2.5 SD below the reference mean (i.e. a *T*-score between −1 and −2·5 SD)[6]. A BMD greater than or equal to 1 SD below the reference mean is defined as normal (i.e. a *T*-score of ≥−1 SD) according to the WHO criteria[6]. Each SD fall in BMD (i.e. per unit *T*-score) below the reference mean corresponds to an estimated 2-fold increase in fracture risk[7,8]. While low BMD is an important predictor of osteoporosis, fracture incidence is the most important clinical end point for osteoporosis. In recent years, the FRAX® tool, an algorithm used to assess the fracture probability by integrating weighted clinical risk

Abbreviations: BMD, bone mineral density; CD, coeliac disease; MTHFR, methylenetetrahydrofolate reductase; RCT, randomised controlled trials.
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https://doi.org/10.1017/S0029665114000044 Published online by Cambridge University Press

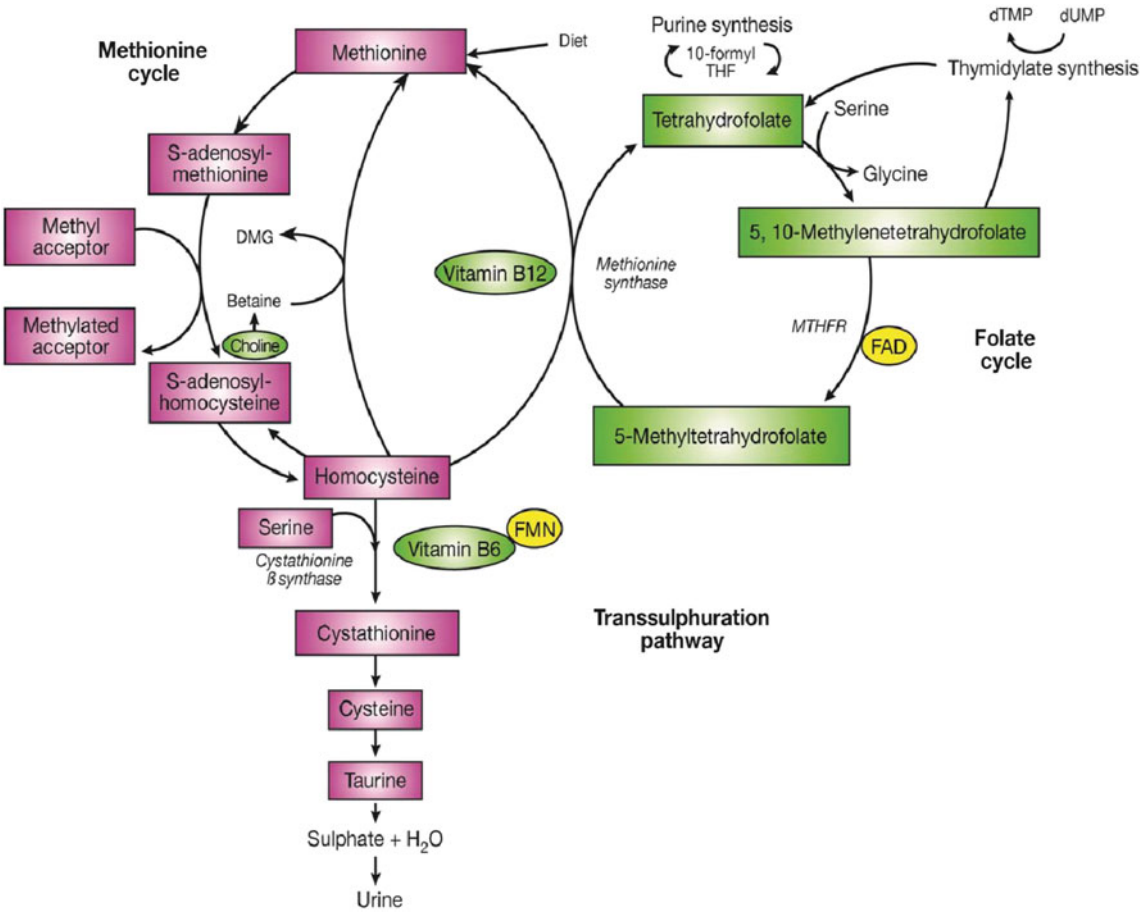

Fig. 1. (Colour online) The metabolism of homocysteine. MTHFR, methylenetetrahydrofolate reductase; DMG, dimethylglycine.

factors of disease (with or without BMD), was developed by the WHO and has been used to assess osteoporotic fracture risk[9].

The aetiology of osteoporosis is multi-factorial, with genetic heritability estimated to account for approximately 30 % of osteoporotic fracture risk[10]. Non-modifiable factors including age, parental history of hip fracture and genetically determined traits, such as Caucasian ethnicity and female sex, increase risk[2]. Similarly, various modifiable factors such as low body weight, physical inactivity, smoking, excessive alcohol intake and the long-term use of certain medications (e.g. glucocorticoids and some anti-convulsant drugs) increase the risk of osteoporosis. Considering the general trend towards ageing populations, and the corresponding increases in osteoporosis-related costs[2], investigation of modifiable risk factors, along with the development of intervention strategies to reduce the incidence of disease, is vital. In terms of nutritional intervention, combined calcium and vitamin D supplementation are proven to reduce bone loss and fracture incidence[11]. However, it is also possible that the nutritional factors not typically linked to bone health could play protective roles. Emerging evidence in generally healthy cohorts worldwide suggests a protective effect of certain B-vitamins, and a detrimental effect of homocysteine and the 677C→T polymorphism in the gene encoding the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR), on bone health and fracture risk[12,13].

Role of B-vitamins in homocysteine metabolism

The major determinant of plasma homocysteine concentration is the biomarker status of the metabolically related B-vitamins, primarily folate, and to lesser extent vitamins B_{12} and B_6 (Fig. 1). Homocysteine is formed by the demethylation of dietary methionine. Homocysteine may then be metabolised to form cystathionine as the part of the trans-sulphuration pathway, requiring a vitamin B_6 co-factor, or regenerate methionine, and in turn *S*-adenosyl methionine (a universal methyl donor). The latter reaction is catalysed by methionine synthase and requires 5-methyltetrahydrofolate, the principal circulating form of folate and vitamin B_{12} in its co-factor form, methylcobalamin. The formation of 5-methyltetrahydrofolate is catalysed by the enzyme MTHFR, which has the riboflavin-derived FAD as a prosthetic group. Supplementation with folic acid can lower homocysteine concentrations by an estimated 25 %, with vitamins B_{12} and B_6 having additional homocysteine lowering effects (i.e. by a further 7 % each) in

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Table 1. Observational evidence to support a role for homocysteine (Hcy) and B-vitamin status in fracture risk

Author, Country	Sample size, age, gender	Design	Main findings; Risk of fracture (95 % CI)
Van Wijngaarden *et al.*[12], International	*n* 11 511, NA, male/female	Meta-analysis; eight studies	RR 1.04 (1.02, 1.07) per 1 μmol/l increase in Hcy
Dai *et al.*[29], Singapore	*n* 63 257, 45–74 years, male/female	Cohort study	HR 0.78 (0.66, 0.93) in women in the highest quartile intake of pyridoxine compared with the lowest
Yang *et al.*[30], International	*n* 14 863, *NA*, male/female	Meta-analysis; nine studies	RR 1.59 (1.30, 1.96) and 1.67 (1.17, 2.38) for all fractures and hip fractures, respectively, in highest Hcy quartile compared with lowest
Enneman *et al.*[31], The Netherlands	*n* 503, ≥55 years, female	Cohort study	Incidence of fracture was associated with increasing Hcy quartile, $P=0.047$
LeBoff *et al.*[32], USA	*n* 800, mean 70 years, female	Case-control study	OR 1.38 (1.14, 1.66) at hip, per SD increase in serum Hcy
Gjesdal *et al.*[33], Norway	*n* 4766, 65–67 years, male/female	Prospective study	HR 2.42 (1.43, 4.09) in women and 1.37 (0.63, 2.98) in men with Hcy ≥15 μmol/l compared with ≤9 μmol/l. HR 2.40 (1.50, 3.84) in women with low compared with high folate status
Yazdanpanah *et al.*[34], The Netherlands	*n* 5304, ≥55 years, male/female	Cohort study	HR 0.58 (0.38, 0.90) in women in the highest quartile intake of pyridoxine compared with the lowest
Dhonukshe-Rutten *et al.*[35], The Netherlands	*n* 1252, ≥65 years, male/female	Prospective study	RR 3.80 (1.20, 11.60) in men and 2.80 (1.30, 5.70) in women with high Hcy and low B_{12} compared with normal concentrations.
Van Meurs *et al.*[36], The Netherlands	*n* 2406, ≥55 years, male/female	Cohort study	RR 1.40 (1.20, 1.60) per SD increase in log Hcy concentrations. RR 2.00 (1.40, 2.70) for fracture in highest Hcy quartile compared with the lower quartiles
McLean *et al.*[37], USA	*n* 1999, 55–91 years, male/female	Cohort study	HR 3.84 (1.38, 10.70) in men and 1.92 (1.18, 3.10) in women, for highest Hcy quartile compared with the lowest quartile

NA, not applicable; RR, relative risk; HR, hazard ratio.

folate replete, and folate and riboflavin replete individuals, respectively[14,15].

The 677C→T polymorphism in *MTHFR* is widely reported as the most common genetic determinant of mildly elevated plasma homocysteine concentrations in healthy populations[16]. Individuals with the homozygous mutant TT genotype experience a decrease in MTHFR enzyme activity, believed to be through the loss of FAD[17], leading to hyperhomocysteinemia (>15 μmol/l)[18,19] and low erythrocyte folate concentrations[19,20]. Riboflavin supplementation has however been shown to significantly lower homocysteine concentrations specifically in individuals with the *MTHFR* 677TT genotype[21]. The frequency of the *MTHFR* 677TT genotype varies greatly worldwide, ranging from 4 to 18 % in the US populations, 10 % in many European populations and up to 32 % in Mexico[22,23].

Hyperhomocysteinemia mainly reflects low or deficient status of the relevant B-vitamins, or the *MTHFR* genotype, but it can also reflect a decline in renal function with age[24].

B-vitamins, homocysteine and bone health

Historical evidence

Homocystinuria, a rare autosomal recessive disease, is a condition in which the metabolism of homocysteine is severely impaired leading to excessive accumulation of homocysteine in the blood (>100 μmol/l) and urine[25]. The most common cause of homocystinuria is cystathionine-*β*-synthase deficiency. Among the major clinical features of the disease (including mental retardation and severe occlusive vascular disease) premature osteoporosis is well described, and homocystinuric patients are reported to have a 50 % increased risk of developing osteoporosis by the age of 16[25]. This premature onset of osteoporosis lends support to the hypothesis that mild elevations of homocysteine (and/or impaired status of related B-vitamins), may play a role in the aetiology of osteoporosis in the general population[26].

Early studies in patients with pernicious anaemia provided the first evidence to support a specific role for vitamin B_{12} deficiency in osteoporosis. One small study in post-menopausal women with pernicious anaemia (*n* 21) reported lower BMD in patients compared with controls[27]. Further evidence from a slightly larger cohort of post-menopausal women (*n* 131) demonstrated an almost 3-fold increase in the risk of osteoporotic fracture among pernicious anaemia patients compared with the general community[28].

Evidence from observational studies

In the past decade, numerous large observational studies have provided evidence to support a role for homocysteine and/or low B-vitamin status in fracture risk (Table 1)[12,29–37]. In 2004, two landmark studies performed in the Netherlands and the USA produced consistent findings in terms of the magnitude of increased risk of fracture associated with mild elevations in homocysteine[36,37]. In subsequent years, further studies from

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Table 2. Observational evidence to support a role for homocysteine (Hcy) and B-vitamin status in low bone mineral density (BMD) and bone loss

Author, Country	Sample size, age, gender	Design	Main findings
Kim *et al.*[38], Korea	*n* 460, ≥30 years, male/female	Longitudinal study	Significantly greater bone loss in highest Hcy quartile compared with lowest in pre-menopausal women and men
Zhu *et al.*[39], Australia	*n* 1213, 70–85 years, female	Longitudinal study	Significantly greater bone loss in highest Hcy tertile compared with the middle and lowest
Gjesdal *et al.*[40], Norway	*n* 5329, 47–50, 71–75 years, male/female	Cohort study	OR 2.19 (95 % CI 1.48, 3.25) and 1.55 (95 % CI 1.07, 2.23) for low BMD among subjects in highest quartile compared with lowest quartile of Hcy and lowest quartile compared with highest quartile of folate, respectively, in women only
McLean *et al.*[41], USA	*n* 714, mean 75 years, male/female	Longitudinal study	Participants with deficient plasma vitamin B_6 (<20 nmol/l) had greater mean bone loss compared with those with normal status
Morris *et al.*[42], USA	*n* 1550, >55 years, male/female	Cohort study	Serum vitamin B_{12} related to BMD in a dose response fashion to 220 pmol/l. Hcy level (≥20 μmol/l) compared with low level (<10 μmol/l) associated with lower BMD
Tucker *et al.*[43], USA	*n* 2576, mean 58 years, male/female	Cohort study	Low vitamin B_{12} (<148 pmol/l) associated with lower average BMD than those with vitamin B_{12} above this cut-off
Cagnacci *et al.*[44], Italy	*n* 161, mean 53 years, female	Cross-sectional study	Folate but not Hcy/B_{12}, was associated with BMD

the USA, the Netherlands and Norway similarly demonstrated increases in fracture risk associated with elevated homocysteine concentrations[31–33,35]. Confirming these findings, a recent meta-analysis involving nine prospective studies and 14 863 participants reported a relative risk of 1.59 (95 % CI 1.30, 1.96) and 1.67 (95 % CI 1.17, 2.38) for all fractures and hip fractures, respectively, in those within the highest homocysteine quartile compared with the lowest[30]. Most recently, a dose–response meta-analysis including eight observational studies and 11 511 participants estimated a 4 % increase in fracture risk for every 1 μmol/l increase in homocysteine concentration[12]. Given the close metabolic relationship of the B-vitamins with homocysteine, studies have also demonstrated inverse associations between the status of the B-vitamins, folate, vitamins B_{12} and B_6 and fracture risk in large cohorts of men and women[29,33–35].

Observational studies have also reported the significant associations of homocysteine concentrations and/or B-vitamin status with BMD (or loss of BMD over time; Table 2)[38–44]. Evidence from large USA and Norwegian cohorts demonstrated a significant inverse association of homocysteine concentrations with BMD[40,42]. Additionally, a recent 3-year longitudinal study in a Korean population reported an accelerated rate of bone loss in a dose-dependent manner with increasing homocysteine concentration, an effect that was shown in men and pre-menopausal (but not post-menopausal) women[38]. In contrast to the latter study that showed no homocysteine relationship in post-menopausal women, Zhu *et al.* in 2009 reported that high homocysteine concentrations were associated with greater bone loss after 4 years in 1213 post-menopausal Australian women[39]. Perhaps these apparently conflicting findings might be explained by the larger sample size (*n* 1213) and older age of the Australian women (mean age 75 years) compared with the much smaller sample (*n* 138) of younger (mean age 56 years) post-menopausal Korean women, as well as a longer duration of follow-up in the Australian study[38,39]. In terms of the B-vitamins and BMD, analysis of the Hordaland Homocysteine Study found low plasma folate to be associated with low BMD among Norwegian women[40]. Furthermore, the analysis of data from the Framingham Offspring Osteoporosis Study (*n* 2576), a large USA cohort, demonstrated a relationship between low vitamin B_{12} status and low BMD[43]. Findings from the USA National Health and Nutrition Examination Survey (*n* 1550), also demonstrated that poorer vitamin B_{12} (but not folate) status was associated with lower BMD[42]. Additionally, a study of 714 older American men and women showed that the participants with deficient plasma vitamin B_6 concentrations (<20 nmol/l) had greater mean annual bone loss than those with normal B_6 concentrations[41].

Some studies, however, have not reported associations between B-vitamins status (and/or homocysteine) and BMD[45], suggesting that the increased osteoporotic fracture risk may be independent of an effect on BMD. Indeed, Van Meurs *et al.*[36] found a significant increase in fracture risk associated with the highest (compared with the lowest) quartile of homocysteine concentrations, but no significant differences in BMD values across homocysteine quartiles. Conversely, some studies have found an association of B-vitamin biomarkers with BMD but not with fracture risk. For example, the analysis of an Australian population by Zhu *et al.* demonstrated a significant association of homocysteine with BMD but not with fracture risk; although the latter study, however, may have lacked sufficient statistical power to observe an effect on fracture[39]. Additionally, some studies have found an effect on bone (through fracture risk and/or BMD) for folate but not for vitamin B_{12}[33,44] or an effect for vitamin B_{12} but not for folate[42]. Perhaps any beneficial effect of the B-vitamins may be the most evident in those with compromised status, and therefore the B-vitamin (i.e. folate or vitamin B_{12}) that emerges as a risk factor may be more reflective

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Table 3. The relationship between the 677C→T polymorphism in *MTHFR* and bone health outcomes bone mineral density (BMD) and fracture risk

Author, Country	Sample size, age, gender	Design	Main findings; risk of fracture (95 % CI)
Zhu *et al.*[39], Australia	*n* 1213, 70–85 years, female	Cohort study	No relationship between the polymorphism and BMD or fracture, even after stratification by dietary folate, riboflavin and erythrocyte folate
Yazdanpanah *et al.*[47], The Netherlands	*n* 5035, ≥55 years, male/female	Cohort study	In the lowest quartile of riboflavin intake, RR 1.80 (1.20, 2.90) and 2.60 (1.30, 5.10) for osteoporotic and fragility fracture, respectively, in women with TT compared with CC genotype
Hong *et al.*[48], China	*n* 1899, mean 54 years, female	Cohort study	RR 2.50 (1.20, 4.90) in the CT/TT genotype compared with the CC genotype. No relationship between the polymorphism and BMD
Abrahamsen *et al.*[49], Denmark	*n* 1700, mean 50 years, female	Cohort study	BMD associated with the TT genotype in the lowest quartile of riboflavin intake. Similar threshold effects were shown for folate, vitamins B_{12} and B_6
MacDonald *et al.*[50], UK	*n* 1241, 45–54 years, female	Cohort study	A significant interaction among quartile of energy-adjusted riboflavin intake, the TT genotype, and BMD ($P=0.01$ for baseline femoral neck, $P=0.02$ at follow-up)
McLean *et al.*[51], USA	*n* 1632, mean 59 years, male/female	Cohort study	Individuals with the TT genotype and low folate status had lower BMD than those with the TT genotype and higher folate status
Abrahamsen *et al.*[52], Denmark	*n* 1748, mean 51 years, female	Prospective study	The TT genotype was associated with significantly lower BMD and a 2-fold increase in fracture risk
Miyao *et al.*[53], Japan	*n* 307, 46–91 years, female	Cohort study	BMD significantly lower in individuals with the TT genotype compared with the CC genotype ($P<0.05$)

MTHFR, methylenetetrahydrofolate reductase; RR, relative risk.
The TT and CT genotypes refer to individuals homozygous and heterozygous for the 677C→T polymorphism in *MTHFR* respectively, whereas the CC genotype refers to homozygosity for the wild type.

of the presence of sub-optimal vitamin status in the population being studied. For instance, folate status may vary greatly between populations dependent on whether the folic acid fortification policy in place is mandatory (e.g. USA), voluntary (e.g. most European countries) or not permitted on any basis (e.g. Scandinavia). Apart from differences in the vitamin status, disparities in findings between studies may reflect fundamental differences in the populations under investigation including, although not limited to, differences in ethnicity, sex, age and menopausal status. Furthermore, methodological constraints pertaining to sample size, inclusion and exclusion criteria, methods of measuring B-vitamin status (such as the use of dietary intake data as opposed to more robust blood biomarker measures) and choice of outcome measures, may also contribute to inconsistent findings among studies[46].

Genetic evidence

Epidemiological studies investigating the relationship between the *MTHFR* 677C→T polymorphism and bone health add further evidence to support the view that impaired folate metabolism (and the related phenotypes of elevated homocysteine and lower folate concentrations[19,20]) may be implicated in adverse outcomes (Table 3)[39,47–53].

Convincingly evidence from a recent meta-analysis of twenty studies (3525 cases and 17909 controls) found that the 677C→T polymorphism was associated with BMD at all measured sites, and with a 23 % increased risk for all fractures in individuals with the *MTHFR* 677TT genotype compared with those with the CT or CC genotypes[13]. In 2000, the first report linking the 677C→T polymorphism in *MTHFR* with low BMD was published. This study of 307 healthy post-menopausal Japanese women demonstrated that those with the TT genotype (18·6 % of participants) had lower BMD than those with the CC genotype[53]. Confirming these findings in a European population, the Danish Osteoporosis Prevention Study demonstrated that among 1748 post-menopausal Danish women, BMD at the spine and hip were significantly lower, and fracture incidence more than two times greater, in individuals with the TT genotype (affecting 8·7 % of participants) compared with the CT or CC genotypes[52]. One subsequent study reported that individuals with the TT genotype and low folate status (<9 nmol/l), had a lower BMD compared with those with the CC or CT genotypes and low folate status, suggesting an important gene–nutrient interaction[51]. Furthermore, Gjesdal *et al.* in 2007 reported an almost 3-fold increased risk of fracture among Norwegian men and women with the TT genotype and low folate status compared with those with the TT genotype and high folate status[33].

Considering that riboflavin plays an integral role in homocysteine metabolism specifically in those with the *MTHFR* 677TT genotype[21], riboflavin intakes have also been investigated in relation to this polymorphism and bone health. In a longitudinal study, involving a subset of 1241 Scottish women from the Aberdeen Osteoporosis Screening Study, low riboflavin intake in combination with the TT genotype had an adverse effect on femoral neck BMD, at baseline and after a mean follow-up of 6·6 years[50]. In support of these findings, further analysis of the Danish Osteoporosis Prevention Study cohort concluded that the BMD in individuals with the TT genotype was only significantly reduced

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with low dietary intakes of several B-vitamins including folate, B_{12}, B_6 and riboflavin[49]. In terms of fracture risk, evidence from the Rotterdam Study (*n* 5035) demonstrated that women with the TT genotype in the lowest quartile of riboflavin intakes had 1·8 times greater risk of osteoporotic fracture, and 2·6 times greater risk of fragility fracture (defined as a fracture at the hip, pelvis or proximal humorus), than those in the same quartile of riboflavin intakes with the CC genotype[47]. Such findings provide evidence to support a detrimental effect of the polymorphism combined with low B-vitamin intakes on robust bone health outcomes (i.e. BMD and fracture risk), and indicate that B-vitamins may have the potential to modulate any negative effect of this polymorphism on bone health.

To date the majority of bone studies that have considered gene–nutrient interactions have stratified B-vitamin status using dietary intake data, which are prone to a number of sources of error[39,48]. For instance, Hong *et al.* in 2007 reported an increased fracture risk associated with the *MTHFR* 677TT genotype, but no effect of the polymorphism on BMD, even after stratification using food groups representative of B-vitamin intakes[48]. Additionally Zhu *et al.* in 2009 failed to find an interactive effect of the polymorphism combined with low B-vitamin intakes on BMD or indeed fracture risk[39]. Perhaps these null findings might be explained by the inadequacy of B-vitamin intakes to accurately represent B-vitamin status. Other factors, such as differences in ethnicity (considerable variation in the frequency of this polymorphism between populations) and variation in the age of the populations investigated, may also contribute to inconsistencies among studies. Unfortunately, however, a recent meta-analysis showing an association between the *MTHFR* 677TT genotype and BMD and fracture risk, did not address any potential interactive effects of the polymorphism with low B-vitamin status and/or intakes, as most of the studies included did not provide the relevant data to allow this sub-analysis to be performed[13].

Randomised controlled trials

Evidence in the form of randomised controlled trials (RCT) is limited. One notable RCT, however, found a 75 % reduction in the risk of hip fractures among 628 post-stroke Japanese patients in response to combined folic acid and vitamin B_{12} supplementation for 2 years, despite no statistical differences in the number of falls experienced in both the treatment and placebo groups[54]. Conversely one RCT investigating osteoporotic fracture in stroke patients treated with B-vitamins for more than 2 years, and performed as part of the VITATOPS (VITAmins to Prevent Stroke) study, reported no benefit of B-vitamin treatment on osteoporotic fracture[55]. As acknowledged by the authors however, this trial was insufficiently powered to detect any potential beneficial effect due to the small number of fracture events[55]. Another RCT, using participants with pre-existing CVD from the Heart Outcomes Prevention Evaluation 2 trial, failed to find a beneficial effect of B-vitamin supplementation on fracture risk[56]. It is important to note however, that the folate levels were approximately five times higher in the Heart Outcomes Prevention Evaluation 2 trial participants than in the Japanese stroke patients and so perhaps additional folate was unlikely to confer any further benefit to these individuals[56]. It is possible that certain B-vitamins, particularly folate in countries with no folic acid fortification policy, can exert protective effects on bone health that are related to, or independent of, their homocysteine lowering effects.

Potential mechanisms linking B-vitamin biomarkers with bone

There is currently no clear mechanism to explain the potential role of the B-vitamins and/or homocysteine in bone health, however various hypotheses have been investigated.

Evidence to support an effect of homocysteine on bone

Evidence from a rat model suggests that hyperhomocysteinemia induces accumulation of collagen-bound homocysteine in bone resulting in decreased bone strength[57]. In support of this relationship, an early hypothesis by McKusick suggested homocysteine had a detrimental effect on bone strength by way of disrupting collagen cross-linking (integral to bone strength)[58]. Consistent with McKusick's theory, an *in vitro* study by Kang & Trelstad in 1973, found that homocysteine interfered with collagen cross-links from purified rat skin collagen[59] and this is supported by a recent cell study demonstrating a mechanistic effect of homocysteine on lysyl oxidase (an enzyme required for collagen cross-linking)[60]. Additionally, a small study in homocystinuric patients (*n* 10) and controls provided evidence that premature osteoporosis among patients could be explained by disruption of collagen cross-linking as opposed to a reduction in collagen syntheses[58]. A case–control study by Saito *et al.* in 2006 comparing bone biopsies from twenty-five hip fracture cases and twenty-five post-mortem age- and sex-matched controls found significantly higher homocysteine concentrations, lower vitamin B_6 concentrations and lower collagen cross-links in the hip fracture cases compared with controls[61]. However, while convincing, evidence to support a role for elevated homocysteine in the reduction of collagen cross-links as a causal mechanism in fracture risk remains inconclusive.

In vitro studies have also investigated a potential effect of homocysteine on bone turnover. Evidence exists to support a stimulatory effect of mildly elevated homocysteine concentrations (as low as 10 μmol/l) on osteoclastic activity, thereby increasing bone resorption[62,63] and an inhibitory effect of homocysteine on bone formation[64]. Consistent with such *in vitro* evidence, plasma homocysteine was shown to correlate positively with markers of bone resorption, but not bone formation, in peri- and post-menopausal women[65]. Further

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observational evidence to support a relationship between homocysteine and markers of bone resorption has been described[66].

More recently, evidence from a rat model suggests homocysteine induces a reduced bone blood flow and therefore ability to repair micro-damage in bone[67]. Additionally, evidence to support a relationship between elevated homocysteine and impaired physical function suggests that homocysteine could act independently of bone, by increasing fracture risk through poor physical ability[68,69].

Evidence to support an effect of folate on bone

While folate has been linked to BMD and a reduced fracture risk[54,70], there is limited evidence to support a direct mechanistic effect of folate on bone, although one study reported a reduced trabecular thickness of bone in individuals with low folate status *v.* those with higher status[71]. Folate could have an indirect role on bone remodelling cells via its metabolic link (as a methyl source) to the methylation of DNA, proteins and other molecules and there is evidence, albeit somewhat inconsistent, to support a reduced methylation capacity as a pathomechanism responsible for poor bone health[57,72–74]. Alternatively, folate may exert a protective effect on bone through its homocysteine lowering effect[54].

Evidence to support an effect of vitamins B_{12} and B_6 on bone

Findings from a small clinical trial showed significantly lower alkaline phosphatase (a marker of bone formation) in vitamin B_{12} deficient patients compared with the controls, and a subsequent rise in alkaline phosphatase following B_{12} supplementation in the deficient patients but not in the controls[75]. Additionally, evidence from an *in vitro* study showed a stimulatory effect of vitamin B_{12} on alkaline phosphatase[76]. Such findings suggest a protective effect of vitamin B_{12} on bone formation. However, a large retrospective study of 9506 pernicious anaemia patients in the UK, which showed a 74 % increased fracture risk in patients *v.* healthy controls, reported that the increased fracture risk remained after correction of the vitamin B_{12} deficiency, thereby suggesting a detrimental effect of pernicious anaemia on bone not related to low vitamin B_{12} status[77]. Low vitamin B_6 status was found to be associated with decreased concentrations of bone formation markers in an animal model[78]. However, trials involving supplementation of folate, vitamins B_{12} and B_6, and thereby lowering homocysteine concentrations, have failed to find a beneficial effect on bone turnover; suggesting that if a benefit of B-vitamin supplementation, or homocysteine lowering, on bone health exists, it is independent of any effect on bone turnover[79,80]. Interestingly, vitamin B_6 acts as an essential co-enzyme for lysyl oxidase, an enzyme required for collagen cross-linking; suggesting that low vitamin B_6 may be detrimental for collagen cross-linking and result in poorer bone mechanical performance[81,82].

It is apparent that the interrelationship between the B-vitamins, homocysteine and bone health is complicated, and probably multi-factorial, with further trials needed to fully elucidate potential mechanisms.

Bone health in coeliac disease

Coeliac disease

Coeliac disease (CD) is a common autoimmune, inflammatory condition in genetically predisposed individuals and is induced by the ingestion of 'gluten', a generic term used to describe the disease activating proteins and peptides found in wheat, barley, rye and sometimes in oats[83].The mainstay treatment of CD is the gluten-free diet. Untreated CD is characterised by villous atrophy, leading to malabsorption of nutrients, and is responsible for many of the traditional clinical manifestations of disease including diarrhoea, weight loss and vitamin deficiencies in iron, folate and calcium. The gold standard diagnosis of CD is based on the presence of villous atrophy (or more mild features of enteropathy) as determined by a well-orientated biopsy of the small intestine, with the presence of autoantibodies to gluten (endomysial antibodies and tissue transglutaminase antibodies) being screened for in the first instance[83].

Osteoporosis in coeliac disease

CD patients are well known to be at a higher risk of osteoporosis compared with the general population. A recent review reported that 38–72 % of CD patients have significantly lower BMD at presentation than non-CD populations matched for age and sex[2,84]. The gluten-free diet is expected to alleviate mucosal damage, and the corresponding malabsorption of calcium and other nutrients, and thereby results in higher BMD[85]. The evidence, however, suggests that the CD patients still fail to achieve the BMD of healthy controls[86]. Additionally, a meta-analysis involving eight studies and approximately 21 000 CD patients reported a 43 % increased fracture risk among patients compared with controls[87]. Although the aetiology of poor bone health in CD is unclear, various factors, including calcium and vitamin D malabsorption, resultant secondary hyperparathyroidism and the inability to reach peak bone mass in young adulthood, are reported to play a role[84]. More recently, a negative impact of pro-inflammatory markers (such as TNF-α and interferon-γ), which are chronically released during gut inflammation and associated with bone loss, are also believed to contribute to poor bone health in CD[88]. Additionally, a lower ratio of osteoprotegerin (known to protect against excessive bone resorption) to receptor activator of NF-κB-ligand (RANKL; associated with bone resorption activity) found in treated CD patients (with recovered mucosa) compared with healthy controls, and a positive correlation between the osteoprotegerin :RANKL ratio and BMD, suggests a damaging effect of a low ratio on BMD even in treated CD patients[89].

It is also possible that nutrients not typically linked to bone health, such as the B-vitamins, contribute a

https://doi.org/10.1017/S0029665114000044 Published online by Cambridge University Press

protective effect on bone in these patients. Low folate and vitamin B_{12} status, accompanied by elevated concentrations of homocysteine, are well described among CD patients[90]. Given the emergence of evidence to support a protective role of B-vitamins and a detrimental effect of homocysteine on bone health, it is possible that B-vitamin inadequacies and/or elevated concentrations of homocysteine, play a role in osteoporosis development in CD. Indeed, given the much higher incidence both of osteoporosis and B-vitamin deficiencies, CD patients comprise a unique 'at-risk' group to investigate in terms of a potential link between B-vitamins and bone health. A 2-year RCT supplementing CD patients with folic acid and vitamin B_{12} and measuring BMD (by dual-energy X-ray absorptiometry scans) pre- and post-intervention is currently underway at our centre with results expected in 2014. This trial will likely shed light as to whether a causal relationship between the B-vitamins and/or homocysteine and BMD exists.

Conclusion and future work

There is consistent observational evidence of an association of homocysteine related B-vitamins with bone health. This epidemiological evidence is further strengthened by genetic studies showing an important association between the common *MTHFR* 677C→T polymorphism and osteoporosis risk. However, confirmation of a causal link between B-vitamins and bone health from RCT is lacking. Further trials to investigate fracture risk or other robust outcomes such as BMD should focus on various 'at-risk' population groups (such as CD patients, post-menopausal women or individuals with the *MTHFR* 677TT genotype). Such evidence will confirm whether low B-vitamin status is causally associated with osteoporosis risk and whether there are beneficial effects of B-vitamin supplementation in bone health.

Acknowledgements

None.

Financial Support

This work was supported by funding from the Northern Ireland Department for Employment and Learning which funded the PhD studentship for M. M. C.

Conflicts of Interest

None.

Authorship

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

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