The Role of B-Vitamins in Bone Health and Disease in Older Adults

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Abstract The risk of osteoporosis and bone fractures increases with age. Several other factors are also related to bone disease including gender, race/ethnicity, physical activity, alcohol, smoking, estrogen, and calcium and vitamin D. B-vitamins (folate, B12, and B6) are also emerging dietary factors related to bone health, both individually and through their action on influencing total plasma homocysteine concentrations (tHcy). The primary objective of this review is to summarize the available data on B-vitamins and bone health, highlighting clinical trials and observational data. In populations without folic acid fortification, the totality of evidence suggests that elevated tHcy has a small but significant association with bone fracture risk and bone quality but not on bone mineral density (BMD) or bone turnover biomarkers. Very little supportive evidence exists for a direct role of folate for either BMD or fracture risk; however, the data available are quite limited. Meta-analyses and some cross-sectional and cohort studies suggest a small but significant role of vitamin B12 status on risk of fracture but not on BMD. The mechanism by which tHcy and B12 may influence bone health is not well characterized but may be through modulation of collagen cross-linking or through altering osteoclasts or osteoblasts. Much more data are needed—particularly the role that each vitamin directly has on bone, or whether the vitamins only exert their effect though tHcy concentrations. Nevertheless, consistent findings across different populations with different study designs suggest a role for tHcy and B12 in reducing fracture risk.

Keywords Bone fracture · Homocysteine · B-vitamins · Bone mineral density

Introduction

The risk of osteoporosis and bone fractures increases exponentially with age. One in three women and one in five men over the age of 50 years will experience a bone fracture in their lifetime, and this corresponds to ~9 million individuals affected each year [1]. Hip fracture is a leading cause of disability and is often associated with an increased risk of institutionalization and mortality [2]. Not only is this a public health problem but also bone fracture is associated with extraordinary medical expenditures. Given the rapid global aging of our population, modifiable factors that contribute to maintaining bone health are of upmost importance. Several of the factors that are related to age-related bone disease are well characterized including female gender, race/ethnicity, physical inactivity, excessive alcohol, smoking, loss of estrogen, and inadequate diet—principally calcium and vitamin D. However, the role of B-vitamins is an interesting and emerging area of dietary exposures related to bone health, both individually as vitamins and also through their action on influencing homocysteine concentrations [3].

Vitamin B12 is essential for normal hematological function and DNA synthesis and is involved with cognitive and neuropsychiatric functions. Vitamin B6 is critical for the function of

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more than 100 enzymes in the body and also has roles in cellular and amino acid metabolism, cognitive function, and in the immune system. Folate is also involved with amino acid metabolism and in the synthesis of DNA and RNA. These three B-vitamins, together with riboflavin (vitamin B2), function in one-carbon metabolism and exert an influence on plasma total homocysteine (tHcy) concentrations in the body. Low levels of any of these vitamins can result in elevated tHcy; however, since mandatory folic acid fortification of the food supply was implemented in the USA, the primary nutritional determinant of high tHcy concentrations has been low vitamin B12 [4, 5] because folate status was markedly improved following fortification [6]. However, many of the studies discussed in this review come from countries without mandatory folic acid fortification of food supply, which complicates the summary of the totality of evidence as folate and tHcy status would depend greatly on the amount of folic acid consumed as part of the usual diet.

Serum or plasma levels of B12 directly reflect circulating B12 concentrations, whereas methylmalonic acid (MMA) and tHcy accumulate in the absence of adequate B12 and are considered "functional" indicators of B12 status [7, 8]. All of these biomarkers have limitations with respect to their sensitivity and specificity. To minimize their limitations, a National Institutes of Health-established panel of experts suggested using both a circulating and a functional measure to assess B12 status [9]. It is important to note that most of the earlier studies examining B12 status and bone health only had one measure of B12 status. The primary objective of this review is to summarize the available data on B-vitamins and bone health, highlighting clinical trials and observational data.

Clinical Trials of B-vitamins

A limited number of clinical trials have been published examining the role of B-vitamin interventions on bone health parameters as a primary or secondary outcome. Herrmann et al. [10] examined a high-dose combination of vitamins (2500 µg folic acid, 500 µg B12, and 25 mg B6) vs. placebo in osteoporotic participants (n=41 females, n=6 males; age range 47–82 years). Overall, no significant intervention effect was observed for bone mineral density (BMD) at 1 year or for bone biomarkers (i.e., urinary desoxypyridinoline cross-links and plasma levels of tartrate resistant acid phosphatase, C-terminal cross-links of collagen I, procollagen type I N-terminal peptide, and osteocalcin) or at any time point over the course of a year of supplementation, despite a significant reduction in tHcy concentrations. However, among the small subset with elevated tHcy at baseline (>15 µmol/L; n=8), a significant increase in lumbar spine BMD was observed at the end of the study (mean T-score change from −2.7 to −1.7) [10]. The second Herrmann et al. trial examined the effects of 8 weeks of supplementation (of different doses of folic acid

(0.4, 1, or 5 mg)) compared to placebo (n=61 males and females, mean age 58 years) among non-osteoporotic adults with healthy levels of bone turnover biomarkers at baseline. Throughout the trial, no significant differences in osteocalcin, procollagen type I N-terminal propeptide, and C-terminal telopeptides of human collagen type I were observed at any dose of folic acid when compared to placebo [11], but the trial was short in duration. The third Herrmann trial examined B-vitamins in combination with vitamin D and calcium among healthy males and females (n=93; >54 years). Participants received 1200 IU vitamin D3, 456 mg calcium together with B-vitamins (500 µg folic acid, 500 µg vitamin B12, and 50 mg vitamin B6) vs. placebo that also provided calcium and vitamin D for a one-year time period [12]. At the conclusion of the study, the treatment group has significantly decreased markers of bone turnover (i.e., bone alkaline phosphatase, tartrate resistant acid phosphatase, and osteocalcin) [12]. It is unclear why the combination of vitamins was not effective on tartrate-resistant acid phosphatase and osteocalcin in the first Hermann trial [10] but was in a subsequent trial [12] as both trials were similar in duration and dose, and the ages of the participants were comparable. The only difference in the two trials was the addition of calcium and vitamin D; however, as both the treatment and placebo group received these, it is unlikely that the effect was due to them.

Green et al. observed no effect on alkaline phosphatase and bone-derived collagen fragments in 135 elderlies aged 65 years and older with an elevated tHcy level (>15 µmol/L) after 2 years of B-vitamin supplementation per day (1 mg folic acid, 1500 µg B12, and 10 mg B6) compared to placebo. A trial in rural Mexico with 132 females aged 20–59 years randomized based on baseline B12 status to receive B12 orally or placebo for 3 months [13] found that B12 status was improved in the treatment group, but there was no change in bone-specific alkaline phosphatase, a bone turnover biomarker of bone formation previously identified as independent risk factors for fracture risk [14]. A randomized, double-blind, placebo-controlled trial of older females in Croatia (n=31; age range 65–93 years; tHcy >10 µmol/L) examined the effect of either a daily folic acid (800 µg) and B12 (1000 µg) supplement or placebo [15]. After 4 months, the treatment group had significantly lower tHcy than the placebo group; however, there were no significant differences observed in serum alkaline phosphatase or C-terminal cross-linking telopeptide of type I collagen [15].

More promising data exist for B-vitamins and the risk of bone fracture. A Japanese trial of older stroke patients (n=628, ≥65 years) recruited through a hospital examined a very high-dose combination (5000 µg folic acid and 1500 µg B12) or placebo [16]. After 2 years of follow-up, among those who remained in the trial (n=559), the intervention group had a significantly lower adjusted relative risk of bone fracture (RR=0.20; 95 % CI 0.08–0.50) [16]. The B-vitamins for the

Prevention of Osteoporotic Fractures (B-PROOF) study was a randomized double-blind placebo-controlled trial (*n*=2919; >65 years with tHcy≥12 μmol/L at baseline) conducted over a two-year time period in the Netherlands. Participants received placebo or 500 μg/day vitamin B12 and 400 μg/day folic acid; both groups also received 600 IU/day vitamin D [17]. Although the B-PROOF clinical trial substantially reduced tHcy concentrations (−4.4 μmol/L) in the treatment group, the overall effect of treatment was not significant for reduction of osteoporotic bone fracture risk [18]. Only treated subjects >80 years of age conferred a lower risk of any fracture (HR 0.26, 95 % CI 0.10–0.71; *p*=0.01) and osteoporotic fracture (HR 0.28, 95 % CI 0.10–0.74; *p*=0.01) [18]. In the HOPE-2 trial, designed to investigate the effect of B-vitamin supplementation (1 mg/day vitamin B12, 2.5 mg/day folic acid, and 50 mg/day vitamin B6) on cardiovascular outcomes, no effect of 5 years of supplementation was observed on fracture incidence among persons with high cardiovascular risk (*n*=5522, mean age 69 years) [19]. In the VITATOPS-trial, also designed to investigate the effect of B-vitamin supplementation (500 μg/day B12, 2 mg/day folic acid, and 25 mg/day B6) on cardiovascular outcomes, no effect of treatment during a mean of 2.8 years on osteoporotic fracture incidence was observed in patients with cerebrovascular disease (*n*=4075; mean age 63 years) [20]. One primary difference in US trials when compared to the European trials is the presence of folic acid fortification of the food supply, which greater enhanced the folate status and decreased the prevalence of elevated tHcy [6]; this may have contributed to the difference in baseline median tHcy levels between the studies (19.9 μmol/L in the Sato trial, 11.5 μmol/L in the HOPE-2 trial, 14.3 μmol/L in VITATOPS, and 14.4 μmol/L in B-PROOF). Furthermore, the secondary prevention trials for cardiovascular disease were looking at bone fracture as a secondary outcome only, and so the studies may not have been powered for bone fracture outcomes. Finally, both HOPE-2 (28 % women) and VITATOPS (36 % women) enrolled a much smaller proportion of women than the trials which were powered to look at bone fracture as the primary outcome.

Observational Data: Homocysteine (tHcy)

An elevated circulating plasma tHcy concentration has been identified as an independent risk factor for osteoporotic fractures in observational studies [21–27], a finding that is consistent with meta-analyses [3, 28] and mechanistic studies [29, 30]. Furthermore, tHcy seems to be associated with low bone mineral density [31–35] and osteoporosis [34–37], but other data are equivocal with respect to tHcy and bone health outcomes [38–40]. Further evidence that tHcy may be related to poor bone health comes from the genetic disease homocystinuria in which brittle bones are seen, even in very young children [41]. A recent meta-analysis of tHcy from 8

observational studies (total *n*=11,511 older adults) showed a small but significant increase in all fracture risk with increasing concentrations of tHcy (RR=1.04; 95 % CI=1.02–1.07) after a period of follow-up time that ranged from 3 to 12 years [3]. Hip (*n*=3) and total fracture (*n*=5) studies were analyzed separately, and a similar but slightly stronger association was found. Three additional observational studies were discussed by van Wijngaarden et al. but were not able to be included in the analysis due to limitations in the data [3]. Individually, these three studies also support a significant impact of high tHcy concentrations on fracture risk [25–27]. Two of the three studies come from the same dataset, the Framingham Osteoporosis cohort, which looked specifically at hip fracture [26, 27], whereas the other study looked at three different cohorts—a sample from the Rotterdam study consisting of two cohorts of subjects and a sample from the Longitudinal Aging Study Amsterdam. In all three cohorts, the subjects in the highest quartile of tHcy had an increase in the risk of fracture such that the risk was twice as high as the risk in each of the lower three quartiles [25]. Further, cross-sectional analysis of B-PROOF and of the Rotterdam Study elderly cohorts I and II (*n*=6040) results indicate that elevated tHcy was associated with poorer measures from quantitative ultrasound of the bone, reflecting bone microarchitecture independent of BMD [33]. The B-PROOF cross-sectional data also describe a small but significant association of tHcy with BMD of the femoral neck and lumbar spine. However, the meta-analysis data indicate no significant effect of tHcy on BMD overall or in the lumbar spine or hip [3]. Of the studies that were not eligible for the meta-analysis, some showed a significant negative relationship between tHcy and BMD of the femur or lumbar spine BMD in Turkish women [34], hip BMD in American males and females [42], or bone loss over time in Australian women [43], whereas no association was observed between tHcy and vertebral BMD decline [44] or lumbar spine BMD in Italian women [45]. The studies not included in the meta-analysis were generally of lower quality, and so, these findings must be interpreted with caution. Nevertheless, the overall association of tHcy appears to be the strongest for risk of fracture, whereas little data support a role for tHcy in BMD.

Observational Data: Vitamin B12

The pooled data from a limited number of studies show no significant relationship between serum or plasma vitamin B12 and BMD at any site in females [3, 32, 38, 46]. Several of the available studies were not included in the van Wijngaarden et al. [3] meta-analysis, and some [34, 47–50] but not all [44, 51] did suggest an association between low serum or plasma B12 and lower BMD or bone loss at various bone sites in older females or males. However, there was a small but significant reduction observed in the meta-analysis of pooled data for reduced risk for fracture for increases in serum vitamin B12

concentrations of 50 pmol/L (RR=0.96; 95 % CI 0.92–1.00) [3]. This is an important finding and comes from four longitudinal studies with an average of 3–16 years of follow-up and indicates an independent 4 % reduction in risk of bone fracture per 50 pmol/L serum B12 concentration increase [3].

Serum B12 is not the most sensitive or specific indicator of vitamin B12 status; thus, it is not entirely surprising that the results do not indicate a strong relationship between status and bone. Limited data are available on other markers of B12 status; MMA is a specific indicator of vitamin B12 status. BMD and risk of osteoporosis of the hip were associated with elevated MMA concentrations in a nationally representative cross-sectional sample of older adults in the USA (>55 years; NHANES III, 2nd wave 1990–1994) [42]. Similarly, holotranscobalamin, the active form of B12 in the blood, was associated with an increased risk of fracture among Swedish men (n=790; age range 70–81 years) after ~6 years of follow-up [52]. Risk of fracture was the greatest among males in the lowest holotranscobalamin quartile (HR=1.74; 95 % CI 1.12–2.69) [52]. In summary, the data for a role of serum B12 with BMD or fracture risk is lacking; however, more data are needed on functional indicators of B12 status like MMA and holotranscobalamin.

Observational Data: Folate

The pooled data from a moderate number of quality studies show no significant relationship between serum or plasma folate and BMD at any bone region among older females [3, 31, 38, 46, 53, 54]. Of the studies not included in the meta-analysis [3], the majority also showed no relationship between biomarkers of folate status and BMD or change in BMD [34, 42, 47, 51, 55], whereas three studies supported a significant positive association in lumbar/vertebral [45] [44] or hip [31] BMD. The data on folate and risk of bone fracture are more complicated to synthesize because of the manner in which the researchers categorized folate status. As a continuous variable, no association was observed between folate status and risk for incident hip fracture in an elderly US cohort prior to folic acid fortification [26]. When examined as quartiles of serum folate, Norwegian females in the lowest quartile had more than twice the increased risk of hip fracture when compared to women in the highest quartile (HR=2.40; 95 % CI 1.50–3.84); no such association was found among males in this cohort [24]. In a cohort of older Italian males and females, serum folate as a continuous variable was not associated with an increased fracture risk, but when expressed as quartiles, it was significantly associated with an increased risk of incident fractures (HR= 2.06; 95 % CI 1.02–4.18; quartile 1 vs 2–4 quartiles combined) [39]. Holstein et al. examined the femoral heads and blood of adults undergoing hip surgery via DXA, biomechanical testing (i.e., indentation method), and histomorphometry based on B-vitamin status (n=94, 52–83 years). No

differences were observed for any B-vitamins in terms of DXA or biomechanical testing; however, when compared to individuals with recommended folate concentrations, those with low folate had lower trabecular thickness and trabecular area [56]. No differences were observed for any parameters by B12 status, whereas those with low B6 status had a significantly lower trabecular number compared to individuals with normal B6 status [56].

Potential Mechanisms

The mechanism by which tHcy and B12 may influence bone health is not well characterized although preliminary data suggest the role does not appear to be through methylation alone [57]. Elevated tHcy has been associated with poorer measures from quantitative ultrasound of the bone, reflecting microarchitecture of bone independent of BMD [33]. Additionally, tHcy could exert an influence on bone health through modulation of collagen cross-linking, which is critical to bone stability, independent of BMD [30]. Vitamin B6 is also thought to play an important role in collagen formation, but current data in human models is lacking [58]. Low B12 and/or elevated tHcy has been shown to increase osteoclast formation [29, 59], alter osteoblast function [60, 61], and alter rates of bone turnover [23] that all could ultimately reduce bone mass. All of these potential mechanisms are aligned with the data that BMD is not the mechanism by which B-vitamin status can alter bone fracture risk.

Summary

Due to the limited number of clinical trials and the different doses and combinations of B-vitamins provided in these trials and the differences in baseline B-vitamin and tHcy status as well as the ages of the populations being studied, understanding the relationship between B-vitamins and bone health parameters is not clear. The limited number of existing clinical trials examined B12 and folic acid in combination (with or without B6); thus, the independent effect that each vitamin has on bone health is largely unknown. The significant associations for folic acid and B12 supplementation from clinical trials are either only in sub-group analysis (i.e., not the overall trial effect) [10, 18] or in populations that are at high risk for bone disease (i.e., stroke patients) [16]. Therefore, the burden of evidence at this point in time is from a large number of observational trials, which cannot infer causality. In unfortified populations, the totality of evidence suggests that elevated tHcy has a small but significant association with bone fracture risk and bone quality but not BMD or bone turnover biomarkers. Very little supportive evidence exists for a direct role of folate for either BMD or fracture risk; however, the data

available on folate status are quite limited. Meta-analysis and some cross-sectional and cohort studies suggest a small but significant role of vitamin B12 status on risk of fracture but not on BMD. Much more data are needed—particularly the role of each vitamin directly on bone, or whether the vitamins only exert their effect through tHcy concentrations. Nevertheless, consistent findings across different populations with different study designs suggest a role for tHcy and B12 in reducing fracture risk.

Compliance with Ethics Guidelines

Conflict of Interest RL Bailey and J van Wijngaarden both declare no conflicts of interest.

Human and Animal Rights and Informed Consent All studies by J van Wijngaarden involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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