

## Vitamins and bone health: beyond calcium and vitamin D

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*Osteoporosis is a major health disorder associated with an increased risk of fracture. Nutrition is among the modifiable factors that influence the risk of osteoporosis and fracture. Calcium and vitamin D play important roles in improving bone mineral density and reducing the risk of fracture. Other vitamins appear to play a role in bone health as well. In this review, the findings of studies that related the intake and/or the status of vitamins other than vitamin D to bone health in animals and humans are summarized. Studies of vitamin A showed inconsistent results. Excessive, as well as insufficient, levels of retinol intake may be associated with compromised bone health. Deficiencies in vitamin B, along with the consequent elevated homocysteine level, are associated with bone loss, decreased bone strength, and increased risk of fracture. Deficiencies in vitamins C, E, and K are also associated with compromised bone health; this effect may be modified by smoking, estrogen use or hormonal therapy after menopause, calcium intake, and vitamin D. These findings highlight the importance of adequate nutrition in preserving bone mass and reducing the risk of osteoporosis and fractures.*

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### INTRODUCTION

Osteoporosis, a major health problem worldwide, is a skeletal disorder characterized by compromised bone strength, which predisposes those affected to an increased risk of fractures.

The etiology of osteoporosis is complex, as genetic constitution and modifiable factors are both known to influence the risk for low bone mass, bone loss, and fracture development.

Nutrition is one of several important modifiable factors for optimal bone health and prevention of osteoporosis. Indeed, the role of calcium and vitamin D in improving bone mineral density (BMD) and reducing fracture risk has been well established.<sup>1–4</sup> In addition, studies have shown that diets high in fruits and vegetables have positive effects on bone mineral status and that nutrients and vitamins, including vitamin K, vitamin C, phosphorus, potassium, magnesium, protein, and sodium, are important for the maintenance of optimal bone health.<sup>5,6</sup> The findings of many studies have related

the intake and/or serum levels of several vitamins, including A, B complex, C, E, and K, as well as the homocysteine (Hcy) level, to bone health in animals and in humans.

This review examines the available evidence regarding the role of vitamins other than vitamin D in bone health in animals and in humans. Particular attention is given to studies investigating the relationship between vitamins and BMD, and to studies assessing the possible influences of vitamins on reducing the risk of fracture.

### VITAMIN A

Vitamin A is the generic term used for a group of essential fat-soluble dietary compounds, the most important ones being retinol and provitamin A (beta-carotene). Vitamin A is required for growth, reproduction, visual health, and the integrity of the immune system. Since the early 1940s, it has been recognized that vitamin A deficiency has a profound and complex effect on bone, which can lead to different types of bone abnormalities. Mellanby<sup>7</sup> showed that vitamin A deficiency in growing animals altered both

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osteoclastic and osteoblastic activity, which resulted in abnormal growth in the basioccipital bone and the spine, along with serious neurological complications. A few years later, they showed that these bone changes related to vitamin A deficiencies were reversible.<sup>8</sup> Soon after vitamin A was administered to these animals, the osteoclasts and osteoblasts became active again, resulting in the removal of the superfluous bone deposited or not absorbed during the period of vitamin A deficiency; hence, these bones tended to return to normal.<sup>8</sup>

Several studies assessed the activity of retinoids on bone cells in vitro. Dickson and Walls<sup>9</sup> showed that collagen synthesis in embryonic chick calvaria was significantly inhibited after 24 h of culture with retinol. Others showed that in bone rudiments of mouse fetuses grown in a medium containing a high concentration of vitamin A, the terminal cartilage lost its metachromasia, shrank, and finally disintegrated.<sup>10</sup> Studies on the effect of retinoids on osteoclast cultures yielded inconsistent results, with findings ranging from an inhibitory effect to a stimulatory effect, depending on the culture system used, the source of osteoclasts, and the species studied.<sup>11,12</sup> Some studies showed that retinoic acid directly stimulates osteoclastic bone resorption,<sup>11</sup> whereas others showed that retinol and retinoic acid have an inhibitory effect on osteoblastic cell proliferation in vitro.<sup>12</sup> Conversely, studies showed that lycopene, which is a carotenoid, has a protective role because it inhibited basal and parathyroid hormone-stimulated osteoclastogenesis in rat bone marrow cultures and stimulated proliferation and differentiation of osteoblast-like osteosarcoma cells.<sup>13</sup>

Animal studies showed considerable retardation of bone growth in the fetuses of pregnant rats with hypervitaminosis A.<sup>14</sup> Excess feeding of different species of animals with vitamin A or synthetic retinoids was associated with poor bone growth and radiolucency, accelerated bone remodeling with consequent loss of bone mineral content (BMC), and an increased rate of spontaneous fractures.<sup>15–18</sup> In growing rodents, this was attributed to the thinning of long bones, in which radial growth was reduced.<sup>19,20</sup> Kneissel et al.<sup>21</sup> showed that the long bone diameter shrank in adult rodents treated with retinoids, and they attributed this to subperiosteal osteoclastic bone resorption. Moreover, treatment of rats for 15–20 weeks with either all-*trans* retinoic acid or 13-*cis* retinoic acid reduced BMC, BMD, bone diameter, and cortical thickness of the femur, and increased the incidence of spontaneous fractures as compared to values in controls.<sup>22</sup>

The above findings from animal and in vitro studies cannot be extrapolated to humans. Nevertheless, abnormalities of ossification and calcification were described in case series in human subjects who took synthetic retinoids, especially in children who were treated for more

than 4 years. The most pronounced abnormalities described in humans were osteophytic formation of the cervical spine and ossification of the atlanto-occipital ligament. In addition to modeling abnormalities, premature fusion of epiphyses and diminished bone density were observed.<sup>23–25</sup>

Excessive intake of dietary vitamin A was negatively associated with BMD in the spine, hip, and total body in a cross-sectional study of 175 women aged 28–74 years. This effect persisted after adjustment for energy intake, level of physical activity, body mass index, smoking status, and use of estrogen.<sup>26</sup> This negative effect of dietary vitamin A intake on BMD was not confirmed in other studies.<sup>27–29</sup> In the Rancho Bernardo Study, which included data from 570 women and 388 men, aged 55–92 years at baseline, there was an inverse U-shaped association of retinol intake, as assessed by food-frequency questionnaires, with baseline BMD, BMD measured 4 years later, and BMD change in the spine, total hip, and femoral neck. This association persisted after adjustment for potential osteoporosis risk factors. This association suggested that excessive as well as insufficient vitamin A intake is associated with compromised bone health.<sup>30</sup>

Several studies showed an association between vitamin A intake or retinol level and osteoporotic fractures.<sup>26,31–33</sup> In a nested case-control study of 247 women aged 40–76 years and 873 age-matched controls, a high intake of dietary retinol was associated with an increased risk of hip fractures. In this study, for every 1-mg increase in the daily intake of retinol, the risk of hip fracture was further increased by 68%.<sup>26</sup> This result was confirmed by the Nurses' Health Study, which included a total of 72,337 postmenopausal women aged 34–77 years in whom long-term intake of a diet high in retinol was associated with an increased risk of hip fracture. This association was attenuated among women on estrogen therapy.<sup>31</sup> In this study, there was a nonsignificant increase in the risk of hip fracture among women using vitamin A supplements compared with women who did not use supplements. Conversely, in the Iowa Women's Health Study, which included 34,703 postmenopausal women who were followed up for 9.5 years, supplemental, but not dietary, vitamin A intake was associated with an increased risk of fracture, but this relationship was not dose dependent.<sup>32</sup>

The relationship between the risk of fracture and vitamin A status, assessed as serum retinol levels, was also evaluated. In a prospective study of 2,322 men aged 49–51 years and followed up for 30 years, the relative risk of hip fracture was 2.4 in the group with the highest serum retinol level (75.62 µg/dL) as compared with that in subjects whose retinol levels were between 62.16 µg/dL and 67.60 µg/dL.<sup>33</sup> Conversely, case-control studies suggested a negative relationship between serum retinol level and

vertebral fractures in women.<sup>34</sup> Interestingly, the association between vitamin A and fracture was attributed mostly to retinol, but beta-carotene intake and/or levels were not associated with fracture risk.<sup>31,33</sup>

On the other hand, some studies evaluated the association between retinol levels and markers of bone turnover. A cross-sectional study of postmenopausal women aged 50–60 years showed that higher lycopene intake, as assessed by dietary records, was associated with lower levels of cross-linked N-telopeptides of type I collagen and other bone turnover markers and hence may have a beneficial effect in reducing the risk of osteoporosis.<sup>35</sup> Unlike vitamin A intake from dietary sources, vitamin A supplementation was not associated with changes in serum markers of bone turnover in a prospective, randomized, single-blinded study of 80 healthy men aged 18–58 years.<sup>29</sup>

In conclusion, studies evaluating the association between serum retinol level or retinol intake and skeletal health in humans showed inconsistent results. This inconsistency may be related to the difficulty in obtaining an accurate assessment of the different methods used to estimate vitamin A intake, and to the use of serum retinol level, which is an unstable marker and a poor indicator of vitamin A status. Rebaya-Mercado and Blumberg<sup>36</sup> recommended the use of retinyl esters rather than retinol in future studies assessing vitamin A status. Further studies are needed to determine the safest dose of vitamin A that does not have a deleterious effect on skeletal health. Until additional evidence is available, it is important to ensure sufficient vitamin A supplementation, especially in children, in whom vitamin A deficiency occurs worldwide (Table 1).

## VITAMIN B COMPLEX

One of the potentially modifiable risk factors for osteoporotic fracture is an elevated Hcy level.<sup>37,38</sup> Increased plasma Hcy concentrations result from defective clearance or from defects in intracellular Hcy metabolism. These metabolic defects can have a genetic or nutritional origin.<sup>39–41</sup> Vitamin B, including B<sub>2</sub> (riboflavin), B<sub>6</sub> (pyridoxine), B<sub>11</sub> (folate), and B<sub>12</sub> (cobalamin), serve as cofactors or substrates for the enzymes involved in Hcy metabolism and are therefore determinants of Hcy status.<sup>40–43</sup>

A link between Hcy level and skeletal abnormality was first noticed in studies of homocysteinuria, a classic metabolic disorder caused by the deficiency of cystathionine beta-synthase, the first enzyme involved in Hcy transsulfuration.<sup>43</sup> In vitro studies attributed the cause of osteoporosis in these patients to impaired cross-linking of collagen.<sup>44</sup> In vitro studies performed later showed that Hcy-thiolactone, a natural metabolite of Hcy, inhibits lysyl

oxidase, an enzyme involved in collagen cross-linking, and that increased concentrations of Hcy itself stimulate osteoclast activity in vitro.<sup>45–47</sup> Moreover, a number of studies showed that cobalamin (vitamin B<sub>12</sub>), an important determinant of Hcy levels, had a stimulatory effect on the expression of alkaline phosphatase in human-rib-derived osteoprogenitors, in rat osteosarcoma cells, and in chicken calvaria-derived osteoblasts.<sup>48,49</sup> Animal studies showed a 40% reduction in bone strength and a drastic 90% removal of spongy bone matrix after 3 months of induced hyperhomocysteinemia in adult rats.<sup>50</sup> Other studies in mice showed that pyridoxine (vitamin B<sub>6</sub>) deficiency, which also leads to increased Hcy levels, resulted in impaired cross-link formation in bone.<sup>51</sup>

Several studies in humans revealed an association between Hcy levels and markers of bone resorption and BMD. In a study of 328 postmenopausal British women, osteoporotic patients had a significantly higher Hcy level compared with nonosteoporotic patients.<sup>52</sup> High Hcy levels were also associated with an increased rate of bone loss in the hip in a cohort of 1,213 women aged 70–85 years who were followed up for 4 years. This effect persisted after accounting for bone size, dietary nutrient intakes, physical activity, and renal function.<sup>53</sup> The correlation between Hcy and BMD in postmenopausal women, however, was not confirmed by others.<sup>54–56</sup>

In addition to investigating the relationship between Hcy levels and BMD, several studies assessed the relationship between BMD and the determinants of Hcy levels, such as riboflavin, pyridoxine, cobalamin, folate, and methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms.

An association between MTHFR gene polymorphisms and BMD was reported in postmenopausal Japanese women. Women who were homozygous for the thermolabile TT variant had a lower BMD in the lumbar spine and total body compared with women with the CC and CT genotypes.<sup>57</sup> Similar findings were reported in the Danish Osteoporosis Prevention Study and the Framingham Osteoporosis Study.<sup>58,59</sup> In the latter study, this association between MTHFR gene polymorphisms and BMD was dependent on folate status.<sup>60</sup>

Several epidemiological studies have shown a positive association between folate and/or cobalamin status and bone endpoints.<sup>56,59–64</sup> In the British study, folate was an independent predictor of BMD after adjustment for age, weight, and height.<sup>52</sup> Conversely, the third US National Health and Nutrition Examination Survey did not show an association between serum or red blood cell folate levels and BMD in 1,550 elderly Americans.<sup>62</sup> This controversy could be related to the older age of the population studied and the higher mean plasma folate concentration in that population. In the US National Health and Nutrition Examination Survey, serum vitamin B<sub>12</sub> was

**Table 1 Relevant studies examining the relationships between vitamin A intake or serum retinol levels and bone health.**

Study design, duration	Study focus	Population	Main results	Reference
Cross-sectional and nested case-control study	Dietary intake of vitamin A and – BMD – Hip fracture	Cross-sectional: 175 women (28–74 years) and Case-control: 247 hip fractures 873 controls	– For every 1-mg increase in retinol intake, the risk of hip fracture increased by 68%. – For intake >1.5 mg/day compared with <0.5 mg/day: risk of hip fracture increased OR 2.1, and the BMD decreased by 10% at the femoral neck, 14% at the spine, and 6% at the total body.	Melhus et al. (1998) <sup>26</sup>
Longitudinal observational, 4 years	Dietary and supplemental vitamin A intake and – BMD	570 women, 388 men (55–92 years)	– Inverse U-shaped association between retinol intake and BMD changes at the femoral neck, total hip, and spine. – Similar associations for retinol from dietary and supplemental sources.	Promislow et al. (2002) <sup>30</sup>
Prospective observational, 18 years	Intake of vitamin A supplements and hip fracture	72,337 women (34–77 years)	– For intake $\geq 3$ mg/day compared with intake <1.25 mg/day, there was an increased risk of hip fracture (RR 1.48).	Feskanich et al. (2002) <sup>31</sup>
Prospective randomized single-blinded, 6 weeks	Vitamin A supplement versus placebo and bone markers	80 men (18–58 years)	– No difference between vitamin A and placebo group on N-telopeptide of type 1 collagen, bone alkaline phosphatase, or osteocalcin.	Kawahara et al. (2002) <sup>29</sup>
Longitudinal population-based, 5 years	Dietary vitamin A intake and – BMD – Fractures	2,016 perimenopausal women	– No association between vitamin A intake and baseline femoral neck or spine BMD. – No association between vitamin A intake and changes in BMD from baseline. – No increased risk of fracture.	Rejnmark et al. (2004) <sup>28</sup>
Prospective observational, 9.5 years	Dietary and supplemental vitamin A intake and – All fractures – Hip fractures	34,703 postmenopausal women	– Small increased risk of hip fracture in users of vitamin A supplements compared with nonusers (RR 1.18).	Lim et al. (2004) <sup>32</sup>
Longitudinal population-based, 30 years	Serum retinol level, serum beta-carotene and – All fractures – Hip fractures	2,322 men (49–51 years)	– No evidence of increased risk of all fractures. – No association between serum beta-carotene and fracture risk. – For serum retinol levels in the highest quintile compared with the middle quintile, there was an increased risk of all fractures (RR 1.64) and of hip fracture (RR 2.47).	Michaëlsson et al. (2003) <sup>33</sup>

Abbreviations: BMD, bone mass density; OR, odds ratio; RR, relative risk.

related to BMD.<sup>62</sup> This correlation between B<sub>12</sub> concentrations and BMD was also reported by others.<sup>59,62,63</sup> In the Framingham Osteoporosis Study, a low vitamin B<sub>12</sub> concentration was associated with lower hip BMD in men and lower spine BMD in women<sup>59</sup>; conversely, marginal or deficient vitamin B<sub>12</sub> levels were associated with an increased risk of osteoporosis in elderly Dutch women but not in men.<sup>63</sup> A longitudinal study showed that low serum vitamin B<sub>12</sub> concentration was associated with an increased annual rate of bone loss in the hip but not in the calcaneum in postmenopausal elderly women.<sup>64</sup> However, the relationship between B<sub>12</sub> and bone density was not consistent in all studies.<sup>52,56</sup>

Just as with vitamin B<sub>12</sub>, a significant correlation between vitamins B<sub>2</sub> and B<sub>6</sub> and BMD has been reported.<sup>65</sup> In a large cohort of subjects aged 55 years and older from the Rotterdam Study, a small but significant correlation between dietary intake of riboflavin and pyridoxine and femoral neck BMD was found, with a decreased risk of nonvertebral fractures. This relationship persisted after adjustment for comorbidities and dietary intake of other vitamins, protein, calcium, and vitamin D.<sup>65</sup> In a longitudinal study of 1,241 Scottish women aged 45–54 years and followed up for a mean of 6.6 years, there was no significant association between BMD and either MTHFR genotype or B complex vitamins when examined separately. However, there was a significant interaction between riboflavin intake, MTHFR TT genotype, and BMD, suggesting that riboflavin intake and the MTHFR genotype might interact to regulate BMD.<sup>66</sup>

In addition to their relationship with BMD, Hcy levels and B complex deficiencies have also been associated with fracture risk. Van Meurs et al.<sup>37</sup> found a strong relationship between Hcy level and fracture incidence in 2,406 subjects aged 55 years and older who participated in two prospective, population-based studies, the Rotterdam Study and the Longitudinal Aging Study. In these studies, there were three independent cohorts of subjects followed up for a mean duration of 2.7–8.1 years. Although there was no relationship between Hcy level and BMD, an Hcy level in the highest quartile was associated with a double risk of fracture, independent of BMD and other potential risk factors for fracture. The risk was similar in all three cohorts studied, and it was similar in men and women.<sup>37</sup> Similarly, in 1,267 subjects in the Longitudinal Aging Study Amsterdam who were followed up for 3 years, Dhonukshe-Rutten et al.<sup>67</sup> showed that low B<sub>12</sub> concentrations and high Hcy levels were associated with an increased risk of fracture, with a relative risk of 3.8 in men and 2.8 in women. This relationship persisted after adjustment for age, sex, body weight, body height, current smoking habits, mobility, and cognition. These relative risks were similar to those shown in the Framingham Study, which enrolled 825 men and 1,174 women aged

59–91 years. Men and women in the highest quartile of Hcy levels had a greater risk of hip fracture than those in the lowest quartile. The risk was almost four times as high for men and twice as high for women.<sup>38</sup> Important evidence was also obtained from a Japanese intervention study, which showed a 70% reduction in the risk of fracture in stroke patients after Hcy levels were lowered. That study, however, was not extended to other patients with a high fracture risk.<sup>68</sup>

On the other hand, the Danish Osteoporosis Prevention Study showed that the MTHFR gene polymorphism was also associated with an increased risk of fracture. TT individuals had a twofold increased risk of fracture when compared with those with other genotypes.<sup>58</sup> This finding, however, was not confirmed in a study of Chinese men and women.<sup>69</sup> Pyridoxine intake was associated with significantly decreased fracture risk, and this relationship was independent of Hcy levels.<sup>65</sup> Moreover, a correlation was found between decreased circulating pyridoxine concentrations and impaired cross-link formation in bone of human individuals with fracture.<sup>65</sup>

Overall, studies showed evidence of a relationship between beta-complex intake and levels and bone health. Although defects in beta-complex metabolism may have a genetic origin, it is possible that low vitamin B levels may be of nutritional origin. Therefore, an interaction between these low levels/intake and/or the intake/levels of other nutrients that influence bone health cannot be excluded (Table 2).

## VITAMIN C

Ascorbic acid (vitamin C), an essential vitamin that humans are unable to synthesize, is required for the hydroxylation of lysine and proline, which are needed for the formation of stable collagen triple helixes and, therefore, normal bone development.<sup>70</sup>

Studies of different animal and human tissues suggested that ascorbic acid stimulates alkaline phosphatase activity and is required for the formation of type I collagen matrix as well as for the expression of osteoblastic markers and mineralization.<sup>71–73</sup> Scurvy, a disease caused by severe ascorbic acid deficiency, was associated with decreased BMD and BMC in guinea pigs, especially during the phases of skeletal maturation.<sup>74</sup> This negative effect on peak bone mass persisted in these pigs in adult life. Associated bone abnormalities included thinner growth plate, thinner trabeculae, increased bone resorption, decreased differentiation of osteoblasts from mesenchymal cells, and decreased collagen synthesis in the proximal tibial epiphysis.<sup>74</sup> Moreover, a low- or non-ascorbic acid diet resulted in reduced femur calcium and hydroxyproline contents, low collagen formation, low



**Table 2 Relevant studies examining the relationships between vitamin B complex and bone health.**

Study design, duration	Study focus	Population	Main results	Reference
Three prospective population-based studies; follow-up 8.1, 5.7, and 2.7 years	Hcy level and – Osteoporotic fractures	Men and women; 562 in cohort 1; 553 in cohort 2; 1,291 in cohort 3; age ≥ 55 years	– Hcy level in the highest quartile was associated with an increased risk of fracture compared with lowest quartile: RR 1.9. – Association was independent of BMD.	Van Meurs et al. (2004) <sup>37</sup>
Cross-sectional	Serum B12, Hcy level, and – BMD	143 women, 51 men; age ≥ 70 years	– Risk was similar in men and women. – 37% of B12-deficient women were osteoporotic, versus 6% of those with normal levels: OR 6.9. – No similar association in men.	Dhonukshe-Rutten et al. (2003) <sup>63</sup>
Longitudinal, 3.5 years (hip) and 5.9 years (calcaneum)	Serum B12, and – Changes in hip BMD – Changes in calcaneal BMD	Women; age ≥ 65 years	– No association between BMD and Hcy. – Increased annual rate of bone loss at the total hip in subjects with B12 levels ≤ 280 pg/mL (–1.6%) versus those with levels ≥ 280 pg/mL (–0.2%). – Similar trend at the femoral neck.	Stone et al. (2004) <sup>64</sup>
Cross-sectional	Serum Hcy, plasma folate, serum B12, and – BMD	737 men, 813 women; mean age 65.9 years	– No association between B12 and calcaneal bone loss.	Morris et al. (2005) <sup>62</sup>
Population-based cohort	Serum B12 and – BMD	1,123 men, 1,453 women; mean age 59 years	– Osteoporotic subjects had lower serum B12 levels and higher Hcy levels compared with nonosteoporotic subjects. – No association between plasma folate and BMD.	Tucker et al. (2005) <sup>59</sup>
Cross-sectional	Serum Hcy, plasma folate, serum B12, serum B6, MTHFR genotype and – Os caldis BMD Hcy level and – BMD – Fractures	328 postmenopausal women  1,213 women; age 70–85 years	– BMDs were 6.2% higher at the spine in women and 5.6% higher at the femoral neck in men with B12 levels >148 pM, compared with subjects with lower B12 levels. – Negative correlation between BMD and Hcy. – Positive correlation between BMD and plasma folate. – No correlation between BMD and B12 or B6. – No difference in BMD between MTHFR genotypes.	Baines et al. (2007) <sup>52</sup>
Prospective, 4 years			– Greater BMD loss in the highest tertile of Hcy level (–2.8%) compared with the middle (–1.6%) and lowest tertiles (–1.2%).	Zhu et al. (2009) <sup>53</sup>
Population-based prospective, 7.5 years	Dietary intake of riboflavin, pyridoxine, plasma folate, and cobalamin and – BMD – Fractures	5,304 men and women; age ≥ 55 years	– No association between Hcy and fracture risk. – Significant association between pyridoxine, riboflavin intake, and femoral neck BMD at baseline. – Decreased fracture risk in the highest quartile of pyridoxine intake compared with the lowest quartile: HR 0.55.	Yazdanpanah et al. (2007) <sup>65</sup>
Cross-sectional	Hcy level and – BMD – Bone markers	143 women; age 57–75 years	– No association between Hcy and spine or hip BMD. – No association between Hcy and osteocalcin, osteoprotegerin, and soluble receptor activator of NF-κB ligand. – Significant association between Hcy and urinary deoxypyridinoline cross-links.	Hermann et al. (2005) <sup>54</sup>

Table 2 Continued

Study design, duration	Study focus	Population	Main results	Reference
Cross-sectional	MTHFR genotypes and BMD	307 women; age 46–91 years	– Lower spine and total body BMD in TT genotype.	Miyao et al. (2000) <sup>7</sup>
Cross-sectional	Hcy level, serum B12, plasma folate and BMD	163 postmenopausal women	– No association between BMD and Hcy or serum B12. – Progressive increase in spine BMD from the lowest to the highest quartile of plasma folate.	Cagnacci et al. (2003) <sup>56</sup>
Cross-sectional	Hcy level, serum B12, plasma folate, MTHFR genotype and BMD	271 women; age 60.8 ± 6.8 years	– BMD of spine and femoral neck correlated negatively with log of Hcy and positively with plasma folate. – Plasma folate explained 3.0% of variance of the femoral neck BMD and 1.7% of variance of the spine BMD.	Golbahar et al. (2004) <sup>61</sup>
Longitudinal, 4 years	Hcy level, serum B12, serum B6, plasma folate and Hip fracture	1,002 men and women; age 74.5 ± 4.5 years	– No association between plasma folate and hip fracture. – B12-deficient subjects had 89% greater hip fracture risk compared with normal group. – B6-deficient subjects had 73% greater hip fracture risk compared with normal group. – Subjects with high Hcy levels had 69% greater hip fracture risk compared with normal group.	McLean et al. (2008) <sup>38</sup>
Cross-sectional	MTHFR genotypes and BMD	425 women, age 55–79 years; 232 men, age 70–79 years	– No association between MTHFR gene polymorphisms and vertebral fractures	Li et al. (2004) <sup>69</sup>
Prospective, 3 years	Hcy level, serum B12 and Bone markers	615 men, 652 women; age 76 ± 6.6 years	– Women with B12 levels <200 pM and Hcy concentrations >15 mM had lower BUA, higher DPD/Cr, and higher osteocalcin concentrations than their normal counterparts. – No similar associations in men.	Dhonukshe-Rutten et al. (2005) <sup>67</sup>
Prospective, 6.6 ± 0.7 years	MTHFR, serum B12, serum B6, riboflavin and BMD	1,241 women; age 45–54 years	– Increased risk of fracture with high Hcy and/or low vitamin B12 concentrations: RR 2.8 in women and 3.8 in men. – No association between BMD and either MTHFR genotype or B complex vitamins when examined separately. – Increasing dietary riboflavin intake correlated with spine and femoral neck BMD in homozygotes for the MTHFR T allele.	Macdonald et al. (2004) <sup>66</sup>

Abbreviations: BMD, bone mass density; BUA, broadband ultrasound attenuation; Cr, creatinine; DPD, deoxypyridinoline; Hcy, homocysteine; MTHFR, methylenetetrahydrofolate reductase; NF-κB, nuclear factor-kappa B.

femoral bone density, and abnormal cartilage growth morphology of the proximal tibial metaphysis of these animals.<sup>75</sup>

In humans, the Postmenopausal Estrogen/Progestin Interventions Trial found a positive association between vitamin C intake and BMD of the spine and hip. This association was modified by the level of dietary calcium intake but was independent of the intake of other nutrients.<sup>76</sup> This association between dietary vitamin C intake and BMD was confirmed by some,<sup>77–79</sup> but not all, studies.<sup>80,81</sup> In the Women's Health Initiative Study, although there was no significant association between vitamin C intake and BMD, the beneficial effect of hormone treatment on BMD at all skeletal sites was stronger with higher intakes of vitamin C.<sup>80</sup> In the Framingham Osteoporosis Study, vitamin C intake was associated with femoral neck BMD among male nonsmokers,<sup>82</sup> but in contrast to the Postmenopausal Estrogen/Progestin Interventions Trial, the significant association was found among men with low calcium intake but not among those with high calcium intake. After 4 years of follow up, both supplemental and dietary vitamin C intake were associated with less BMD loss in the hip, spine, and radial shaft. This association was most evident in men with low calcium and low vitamin E intakes and was greater in those who obtained vitamin C from diet as opposed to supplements.<sup>82</sup>

Thus, there is a positive but complex association between vitamin C intake and bone density, which may be related to the interaction of other factors like smoking, estrogen use or hormonal therapy after menopause, calcium intake, and vitamin E intake (Table 3).

## VITAMIN E

Vitamin E is an important fat-soluble vitamin with essential antioxidant properties.<sup>83</sup> There are two types of vitamin E, tocopherol and tocotrienol. Recently, tocotrienol has gained increasing scientific interest because of its high antioxidative activity. Oxidative stress is a state of excess free radical formation. Free radicals are involved in the apoptosis of osteoblasts and osteocytes and in osteoclastogenesis and, therefore, in bone resorption, as shown in different *in vitro* and animal studies.<sup>84</sup> In addition, oxidative stress increases bone resorption through activation of nuclear factor-kappa B, which normally regulates osteoclast differentiation and, thus, bone resorption and remodeling.<sup>85</sup>

An inhibitory effect of vitamin E on collagen production has been reported in different rodent tissues.<sup>86</sup> Other studies, however, found that vitamin E increased hepatic hydroxyproline content in rabbits and partially restored collagen synthesis in primary cultures of avian epiphyseal chondrocytes.<sup>87</sup> In suckling lambs, intramuscular injections

of vitamin E had a protective effect on membranes of chondrocytes during maturation and differentiation.<sup>88</sup> It has been suggested that vitamin E inhibits cartilage resorption and protects chondrocyte membranes through reduction of free radical generation and lipid peroxidation.<sup>89</sup> Schwartz<sup>90</sup> reported that vitamin E has a positive effect on the stabilization of sulfated proteoglycans, which are incorporated into the collagen matrix. Maniam et al.<sup>91</sup> confirmed that vitamin E, especially tocotrienols, had antiosteoporotic activities and improved the trabecular structural and cellular properties in different animal models subjected to ovariectomy, orchidectomy, thyroidectomy, and oxidative stress.

A link between increased oxidative stress and reduced BMD has been documented in humans.<sup>92</sup> Alpha-tocopherol prevented osteoclast formation and bone loss in elderly men by inhibiting both RANK ligand inductions in osteoblasts and c-Fos expression in osteoclast precursors.<sup>93</sup> An observational study of 533 randomly selected nonsmoking postmenopausal women showed that the duration of use of antioxidant supplements, including vitamins C and E, negatively correlated with age-adjusted and weight-adjusted serum C-telopeptide, the marker of bone resorption, thus suggesting that antioxidant vitamins may suppress bone resorption in humans.<sup>94</sup>

Few studies have examined the relationship between antioxidant intake and risk of hip fracture.<sup>94–96</sup> A nested case-control study in the Swedish Mammography Cohort revealed that low intake of vitamins C and E increased the risk of hip fracture in smokers.<sup>94</sup> Similarly, a large case-control study that included data on hip fractures in more than 1,000 male and female patients aged 50 years or older found an inverse dose-response association between intake of vitamin E and risk of hip fracture. This association was found in current and ex-smokers but not in never-smokers.<sup>95</sup> Similarly, Melhus et al.<sup>96</sup> prospectively studied 66,651 women aged 40–76 years. After 64 months, the odds ratio for hip fracture in smokers was 3 for low intake of vitamin E. The odds ratio increased to 4.9 in smokers with low intakes of both vitamins E and C. These relationships were less pronounced in former smokers. The mechanism by which higher intakes of antioxidants were associated with a lower risk of hip fracture in smokers is unclear; however, smoking by itself is a risk factor for bone loss and fracture,<sup>97–99</sup> and this diverse effect of smoking on bone health has been postulated to be due, in part, to the oxidative stress caused by smoking.<sup>86</sup>

In conclusion, vitamin E seems to be associated with increased bone mass and decreased fracture risk in humans. This relationship is modulated by smoking status and is mediated primarily by the antioxidant properties of vitamin E.



Table 3 Relevant studies examining the relationships between antioxidants and bone health.

Study design, duration	Study focus	Population	Main results	Reference
Longitudinal, 4 years	Dietary and supplemental vitamin C and – Bone loss	334 men, 540 women; mean age 75 years	<ul style="list-style-type: none"> <li>– Higher total vitamin C intake was associated with less femoral neck and trochanter BMD loss in men with low calcium or low vitamin E intakes.</li> <li>– Similar trend with dietary vitamin C intake alone, but not with supplemental vitamin C intake alone.</li> <li>– No similar associations in women.</li> <li>– No association between BMD and dietary or supplemental intake of vitamin C in the overall group.</li> <li>– Long-term use of vitamin C supplements was associated with higher BMD in early postmenopausal years and among never-users of estrogen.</li> </ul>	Sahni et al. (2008) <sup>82</sup>
Cross-sectional	Dietary intake of vitamin C and – BMD	1,892 women; age 55–80 years	<ul style="list-style-type: none"> <li>– Significant association between intake of vitamin C and BMD at the total hip and femoral neck.</li> <li>– Similar trend at the spine.</li> </ul>	Leveille et al. (1997) <sup>81</sup>
Cross-sectional	Dietary vitamin C intake and – BMD	Women; age 45–64 years	<ul style="list-style-type: none"> <li>– Increased risk of hip fracture in subjects with low intake of vitamin E compared with those with high intake, OR 3.</li> <li>– Similar results with vitamin C intake.</li> <li>– Low intake of both vitamins C and E increased OR to 5.</li> </ul>	Hall and Greendale (1998) <sup>76</sup>
Case-control prospective	Dietary intake of antioxidants and – Hip fracture	1,120 women with 247 hip fractures, 873 controls; age 40–76 years	<ul style="list-style-type: none"> <li>– Effect was less pronounced in former smokers.</li> <li>– No association between intake of antioxidants and BMD.</li> <li>– Beneficial effect of HRT on BMD was greater at the total hip, femoral neck, and total body in women with higher vitamin C levels.</li> </ul>	Melhus et al. (1999) <sup>96</sup>
Cross-sectional	Dietary intake of antioxidants, serum levels of antioxidants and – BMD	11,068 women; age 50–79 years	<ul style="list-style-type: none"> <li>– Decreased C-telopeptide with increasing duration of exposure.</li> <li>– No pattern observed for serum bone alkaline phosphatase.</li> <li>– No association with total body BMD.</li> </ul>	Wolf et al. (2005) <sup>80</sup>
Observational	Use of antioxidant supplements and – BMD – Bone markers	533 women; age 48–89 years	<ul style="list-style-type: none"> <li>– Highest quintile of vitamin E intake was associated with lower risk of hip fracture compared with the lowest quintile, OR 0.3.</li> <li>– No association between vitamin C intake and hip fracture.</li> <li>– No association in never-smokers.</li> </ul>	Pasco et al. (2006) <sup>94</sup>
Population-based case-control	Intake of antioxidants and – Hip fracture	1,215 patients with hip fractures; 1,349 controls; age ≥50 years		Zhang et al. (2006) <sup>95</sup>

Abbreviations: BMD, bone mineral density; HRT, hormone replacement therapy.

## VITAMIN K

Vitamin K was originally identified as an essential factor for blood coagulation. However, it was also found to have multiple other functions, and there is emerging evidence that vitamin K may have a protective role against age-related bone loss.<sup>100</sup> This is mediated mainly through the vitamin-K-dependent gamma-carboxylation of osteocalcin. Over 40 years ago, Price et al.<sup>101</sup> and Price and Nishimoto<sup>102</sup> demonstrated that bone Gla protein (osteocalcin) is an excellent marker of bone metabolism. They discovered that bone Gla protein is present in serum and plasma, and they developed a radioimmunoassay to measure it. They also showed that this bone-specific protein synthesized by osteoblasts undergoes vitamin-K-dependent gamma-carboxylation. Undercarboxylated osteocalcin increases with vitamin K insufficiency and with administration of anti-vitamin K because it affects the gamma-carboxylation.<sup>103</sup> Others attributed the antiresorptive effects of vitamin K to its geranylgeranyl side chain via mechanisms independent of gamma-carboxylation. Other suggested mechanisms of action of vitamin K on bone included an enhancement of collagen accumulation by activation of the steroid and xenobiotic receptor.<sup>104</sup> Moreover, vitamin K may influence bone metabolism through its effect on urinary calcium excretion<sup>105</sup> or by inhibiting the production of certain bone-resorbing agents such as prostaglandin E2 and interleukin 6.<sup>106</sup>

Animal data suggested that vitamins K and D work synergistically on bone metabolism.<sup>107,108</sup> Indeed, a study in ovariectomized rats showed that ovariectomy-induced bone loss was reduced in the group that received both vitamins K and D, but not in the groups that received vitamin K alone or vitamin D alone.<sup>108</sup> On the other hand, it has been shown that treatment with vitamin K improved bone strength more than BMD in these animals.<sup>107,108</sup> Indeed, magnesium-insufficient bone is fragile upon mechanical loading, but vitamin K improved both the maximum load and the elastic modulus without influencing BMC in rats fed a low-magnesium diet.<sup>109</sup>

In humans, vitamin K intake and vitamin K status were not related to the biochemical markers of bone metabolism or to BMD in healthy young subjects.<sup>110</sup> In postmenopausal women, vitamin K supplementation increased total and carboxylated osteocalcin, decreased uncarboxylated osteocalcin, and decreased urinary calcium and hydroxyproline.<sup>111</sup> BMD data in postmenopausal women were controversial. Vermeer et al.<sup>112</sup> showed that vitamin K intake was positively associated with BMD at many different sites. Braam et al.<sup>113</sup> randomized 181 postmenopausal women aged 50–60 years to receive a daily placebo, minerals (calcium, magnesium, zinc, and vitamin D) only, or minerals and vitamin D and

phylloquinone (vitamin K1). After 3 years, the group receiving the combination of minerals, vitamin D, and K1 had lower bone loss at the femoral neck but not at the lumbar spine. However, other randomized, double-blind, placebo-controlled trials did not support a role for vitamin K supplementation in osteoporosis prevention in postmenopausal women.<sup>114–116</sup> In a large double-blind placebo-controlled study, 381 North American postmenopausal women were randomized to phylloquinone (vitamin K1), menatetrenone, or placebo for 1 year. At the end of the study, treatment reduced undercarboxylated osteocalcin compared to placebo, but there was no significant effect of K1 or menatetrenone on serum bone alkaline phosphatase, N-telopeptide of type 1 collagen, spine or hip BMD, calcaneal ultrasound parameters, or femur geometry properties as measured by dual-emission X-ray absorptometry.<sup>116</sup>

The first report linking vitamin K serum levels to osteoporotic fractures dates back to 1985. Hart et al.<sup>117</sup> demonstrated that patients who had sustained an acute hip fracture or suffered from vertebral compression fracture had lower circulating vitamin K serum levels compared with control subjects. These findings were confirmed by others.<sup>118–121</sup> The effect of vitamin K intake on fracture risk was assessed in the Nurses' Health Study. In this prospective analysis of data from 72,327 women aged 34–77 years followed up over 10 years, low dietary intake of vitamin K was associated with an increased risk of hip fracture after adjustment for calcium and vitamin D intake.<sup>122</sup> In the Framingham Heart Study, which included 335 men and 553 women aged 75 years, there was no association between dietary phylloquinone intake and BMD in either men or women, but women within the highest quartile of vitamin K intake had a significantly lower risk of hip fracture than those in the lowest quartile of intake.<sup>123</sup>

Finally, the effect of supplemental vitamin K on reducing fracture risk was addressed in several randomized trials. In a randomized open-label trial that included 241 osteoporotic patients, treatment with vitamin K2 was associated with reduced fracture occurrence, although there was no change in lumbar spine BMD or in urinary deoxypyridinoline excretion in the treated group.<sup>124</sup> Similarly, small, randomized controlled trials and post-hoc analyses of a large randomized controlled trial in Japanese patients indicated that a large daily dose of vitamin K (45 mg/day given as menaquinone-4) for 12–24 months had a beneficial effect on reducing hip, vertebral, and nonvertebral fractures.<sup>125</sup>

In conclusion, although studies have shown that low circulating levels and/or low dietary intake was associated with low bone density and with increased fracture risk in humans, a protective effect of vitamin K supplementation was not confirmed in randomized con-

Table 4 Relevant studies examining relationships between vitamin K and bone health parameters.

Study design, duration	Study focus	Population	Main results	Reference
Randomized double-blind placebo-controlled, 4 years	Vitamin K1 versus placebo and – BMD changes – Bone markers – Fracture risk	440 women, mean age 40–82 years	– No significant difference in BMD changes. – Serum osteocalcin decreased more in vitamin K group than in placebo group. – Fewer women in the vitamin K group had clinical fractures compared with placebo group.	Cheung et al. (2008) <sup>115</sup>
Randomized double-blind placebo-controlled, 2 years	Vitamin K1 versus vitamin D + calcium, versus calcium, and vitamins K and D versus placebo and – BMD – Bone markers	244 women, mean age ≥60 years	– No significant difference in BMD changes between groups. – No significant difference in changes in serum osteocalcin, bone alkaline phosphatase, and urinary cross-linked N-telopeptides of type I collagen between treatment groups.	Bolton-Smith et al. (2007) <sup>114</sup>
Case-control	Vitamin K1 and K2 level and – BMD – HRT use	95 postmenopausal women	– Lower K1 and K2 levels in women with low BMD compared with those with normal BMD. – Levels of vitamin K1 and K2 did not change with HRT.	Kanai et al. (1997) <sup>119</sup>
Cross-sectional	Vitamin K1 level and – Hip fracture	Women, mean age 81 years	– Lower circulating levels of vitamin K, MK-7, and MK-8 in patients with hip fractures compared with healthy controls.	Hodges et al. (1993) <sup>120</sup>
Prospective, 10 years	Vitamin K intake and – Hip fracture	Women, mean age 38–63 years	– Subjects in quintiles 2–5 of vitamin K intake had lower risk of hip fracture compared with subjects in the lowest quintile: RR 0.7.	Feskanich et al. (1999) <sup>122</sup>
Population-based cohort, longitudinal, 5 years	Vitamin K intake and – BMD – Hip fracture	335 men and 553 women, mean age 75 years	– No association between vitamin K intake and BMD in either men or women. – Reduced risk of hip fracture in the highest quartile compared with the lowest quartile of vitamin K intake: RR 0.35.	Booth et al. (2000) <sup>123</sup>
Randomized double-blind placebo-controlled, 12 months	Vitamin K1 versus MK4 versus placebo and – BMD – Bone markers – Hip geometry	381 women, mean age 62 years	– Reduced undercarboxylated osteocalcin in the treatment groups compared with placebo group. – No significant effect on bone markers. – No significant effect on BMD. – No significant effect on femur geometry.	Binkley et al. (2009) <sup>116</sup>
Randomized, double-blind, placebo-controlled, 3 years	Vitamin K1 supplementation versus placebo versus minerals and – Bone loss	181 women, mean age 50–60 years	– Lower bone loss at the femoral neck with vitamin K: 1.7% lower than placebo group and 1.3% lower than the other group. – No similar difference with respect to changes in spine BMD.	Braam et al. (2003) <sup>113</sup>
Randomized-open label, 2 years	Vitamin K2 and – Bone loss – Vertebral fractures	241 women, mean age 66 years	– Higher incidence of clinical fractures and lower rate of BMD loss at the spine in the vitamin K2-treated group compared with controls.	Shiraki et al. (2000) <sup>124</sup>

Abbreviations: BMD, bone mineral density; HRT, hormone replacement therapy; MK4, menatetrenone.

trolled trials. These discrepancies may be due, in part, to the collinearity between intake of vitamin K and intake of other nutrients. Although vitamin K level is an excellent biochemical marker of vitamin K status, it does not necessarily reflect a subject's overall nutritional status. Dietary and nutritional patterns may be more important than the intake or level of individual nutrients (Table 4).

## CONCLUSION

The available data provide clear evidence that the effects of nutrition on bone health are not limited to those resulting from calcium and vitamin D intake. This review highlights the importance of nutritional factors in preventing and reducing the risk of osteoporosis and fractures.

Both excessive and insufficient intake of retinol may be associated with compromised bone health; however, data on vitamin A intake from dietary sources and from supplemental intake, as well as data on vitamin A status determined by serum retinol levels, showed inconsistent results. Most, but not all, studies showed that vitamin B complex and vitamins C, E, and K correlated positively with BMD at multiple skeletal sites and/or were associated with reduced risk of fracture, independent of BMD. Therefore, nutrients may have adverse effects on bone strength in a variety of different ways, not just by affecting bone mass.

The relationship between vitamins other than vitamin D on bone is complex and seems to be affected by genetic factors, gender, menopausal status, hormonal therapy, smoking, and calcium intake. It is possible that nutrient patterns, and not individual foods or vitamins, are important in bone health, thus explaining some of the paradoxical results related to individual nutrients. Indeed, all tissues need all nutrients, and bone should not be an exception. Therefore, well-balanced and adequate nutrition should be ensured in order to prevent adverse effects on bone health.

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