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PERSPECTIVES

Non-D Vitamins and Bone Health in Adults

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Abstract

Osteoporosis is a major public health problem. Diet is an important modifiable risk factor for the prevention of osteoporosis. While vitamin D has received significant attention, emerging evidence indicates that other vitamins also play an important role in skeletal health. This *Perspective* highlights current understanding of the non-D vitamins (A, B, C, E, and K) and bone health in adults. Several studies of vitamin A (pre-formed retinol) have reported that excessive vitamin A has negative effects on bone. Yet, pro-vitamin A carotenoids, which can convert into retinol, have shown positive associations with bone mass, protection against bone loss and lower risk of hip fracture. Low levels of B vitamins may influence bone through homocysteine metabolism. While large observational studies have reported negative associations of homocysteine with bone mineral density (BMD) and positive associations with fracture, more controlled trials are needed to clarify the associations. Studies have shown mixed results for vitamin C and bone, indicating complex interactions of vitamin C with smoking, hormone replacement, calcium and vitamin E intake. Vitamin E affects bone formation and remodeling, and appears to be important for the skeleton. However, there is insufficient evidence to conclusively link vitamin E with bone health. Some vitamin K studies have shown that low level intakes increase hip fracture risk, however, intervention trials have been inconsistent. There is little research on multivitamin use and bone health. For most vitamins, prospective studies are needed to explore their mechanisms and pathways. Longer-term controlled trials are needed to determine whether treatment with specific vitamin supplements can improve bone health or reduce fracture risk. *IBMS BoneKEy*. 2010 December;7(12):431-446.

Keywords: Vitamins; Bone health; Osteoporosis; Nutrition; Epidemiology; Hip fracture; Dietary intakes

Introduction

Osteoporosis is a major public health problem, especially as populations of older adults are increasing. An estimated 10 million Americans have osteoporosis (1) whereas low bone mass, or osteopenia, is a major public health threat for almost 44 million people in the U.S. population aged 50 years and older. Similarly, in Europe, an estimated 179,000 men and 611,000 women will suffer a hip fracture each year, and the cost of all osteoporotic fractures in Europe is provisionally €25 billion (2). Diet is an important modifiable risk factor for the prevention of osteoporosis. Over the years, vitamin D has garnered significant attention for its role in preventing bone loss and reducing fractures in intervention trials. However, emerging research suggests that vitamins other than vitamin D also play an important role in skeletal health. For example, vitamin B12 stimulates osteoblast activity and bone formation (3); vitamin C is an essential cofactor for collagen formation and normal bone development (4); and vitamin K is essential for carboxylation and activation of bone proteins (5). On the other hand, too much vitamin A may have a negative impact upon bone (6). This *Perspective* highlights current understanding of the topic of non-D vitamins and bone health in adults and elderly individuals.

Vitamin A

The primary storage form of vitamin A is retinyl esters and the dietary supply of

vitamin A consists of retinoids in animal tissue as well as carotenoids from plants and storage tissue of animals. Retinoids, also called pre-formed vitamin A, can inter-convert and include retinol, retinal, and retinoic acid forms of vitamin A. Some carotenoids such as α-carotene, β–carotene, and β-cryptoxanthin can convert into retinol in the body and therefore are called pro-vitamin A carotenoids. Unlike retinoids, however, most carotenoids can quench singlet oxygen and act as antioxidants.

Retinoids

Retinoic acid, a functional indicator of vitamin A status, is known to stimulate osteoclast formation (7) and consequently inhibit bone formation. Animal studies report that vitamin A may also antagonize the action of vitamin D (8), thereby interfering with calcium absorption. Studies have suggested that a delicate balance exists between vitamin A deficiency and toxicity in elderly individuals because intake of retinol at levels above recommended daily intake (RDI), but well within the upper safe limit, may be associated with osteoporosis (6;9-11). These findings, together with the observation that vitamin A intake is quite high among Americans (6) and Scandinavians (12) due to use of vitamin A-fortified foods, cod liver oil and supplements, has raised concerns regarding the effect of high dosage of vitamin A on bone health. In fact, the constitution of cod liver oil popularly recommended as a valuable source of vitamin A, vitamin D and long-chain omega-3 has been changed in Norwegian countries to prevent excessive vitamin A intakes (13). On the other hand, some studies have found no significant relation between retinol intake or serum retinyl esters and bone markers (14;15) The third National Health and Nutrition Examination Survey (NHANES) (n = 5,790 American men and women aged ≥ 20 years) reported no association between fasting serum retinyl esters (a better measure of vitamin A status) and either bone mineral density (BMD) at multiple bone sites or osteopenia/osteoporosis (14). Furthermore, a 2-year longitudinal study in 78 healthy males (mean age 23 ± 0.7 years) showed a negative association between the bone formation marker osteocalcin with retinol and retinol binding protein 4 (RBP-4). However, the association disappeared after adjusting for abdominal fat mass (16).

In the Nurses' Health Study cohort, women consuming more than 3000 μg/d of retinol equivalents (RE) versus less than 1250 μg/d RE were 1.5-times more likely to have a hip fracture, and those consuming more than 2000 μg/d versus less than 500 μg/d of retinol were 1.9-times more likely to have a hip fracture over 18 years. In the same study, vitamin A supplement use alone was associated with a 40% (non-significant) increase in the risk of hip fracture (9). In contrast, in the Women's Health Initiative study of postmenopausal women (n = 75,747, mean age 64 years), no association was observed between vitamin A or retinol intake and the risk of hip or all fractures (15). However, a modest increase in risk of all fractures with high vitamin A and retinol intakes was observed in the low vitamin D intake group. A case-control study from Denmark used two nationwide registries to examine the association of systemic vitamin A analogs (isotretinoin and acitretin) and fracture among 124,655 cases and 373,962 age- and sex-matched controls (mean age 43 ± 27.4 years). No trend in risk of any fracture or of hip, forearm, or spine fractures was present with increasing doses (doses of ≤ 5.25 or 5.26-14.00 or >14.00 mg/d) or durations of treatment with vitamin A analogues. Subdividing vitamin A analogues into isotretinoin and acitretin did not change the results (17). These recent null studies suggest the need for longer-term prospective intervention studies to shed light on the controversy over vitamin A and bone health.

Pro-vitamin A carotenoids

Carotenoids are natural pigments that are synthesized by plants and are responsible for the bright colors of the majority of fruit and vegetables. Pro-vitamin A carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin can convert into retinol in the body. Pro-vitamin A carotenoids may impact

the skeleton via two pathways. First, they may have an impact on the skeleton via their vitamin A activity. Second, they may have a positive effect on the skeleton via their antioxidant activity by which they may reduce oxidative stress and resultant bone resorption. Data from several *in vitro* (18;19) and *in vivo* (18;20) studies suggest that further investigation into the relationship between carotenoids and bone health is warranted.

Few observational studies have reported direct positive association of one or more carotenoids with BMD or bone loss. One observational study conducted in an Anglo-Celtic Australian population (n = 68 men and 137 women, aged 26-86 years) reported a positive association of dietary β-carotene intake with lumbar spine bone mass in postmenopausal women (21). Weak but significant cross-sectional associations were observed between serum β-cryptoxanthin, β-carotene and radial BMD in post-menopausal Japanese women (22). In the Framingham Osteoporosis study, men in the highest tertile of total carotenoids and β-carotene intakes had significantly less BMD loss at the trochanter compared to men in the lowest tertile of these nutrients ($P < 0.05$, respectively) over the 4-year follow-up. (Across the tertiles, P trend = 0.0005 for total carotenoids; for β-carotene, P trend = 0.02) (23). However, the Women's Health Initiative Study (n = 11,068, age = 50-79 years) reported no significant protective associations at any BMD site with five serum carotenoids examined. In fact, a negative association of total β-carotene intake with femoral neck BMD was observed ($P = 0.03$) (24).

In a case-control study, Maggio *et al*. reported that all carotenoids, with the exception of lutein, were consistently lower in osteoporotic than in control women (n = 1200, mean age ~ 70 years, post-menopausal) (25). However, plasma carotenoid levels were not directly associated with femoral neck BMD. Therefore, investigators concluded that there is no direct effect of carotenoids on bone and that pro-vitamin A carotenoids may be linked to bone via their retinol activity. Since then, only one study has examined the association of carotenoids other than β-carotene with hip fracture (26). In the Framingham Osteoporosis Study, subjects in the highest tertile of total carotenoid intake had significantly lower risk of hip fracture compared to the subjects in the lowest tertile of intake (hazard ratio (HR): for tertile 3 versus 1: 0.54; 95% CI: 0.32- 0.90; $P = 0.02$, P trend = 0.02) over 17 years of follow-up (26).

In vivo and *in vitro* studies have suggested that β-cryptoxanthin has a unique anabolic effect on bone calcification (19), which could be because it increases calcium content, alkaline phosphatase activity and DNA content in femoral tissues of rats and it directly stimulates bone formation and inhibits bone resorption (20). This was confirmed in a controlled human trial (n = 21 men and women, aged 23-47 years) where intake of β-cryptoxanthin-fortified juice caused a significant increase in β-carboxylated osteocalcin concentration and a corresponding decrease in serum bone tartrate-resistant acid phosphatase (TRAP) activity (bone-specific alkaline phosphatases) and N-telopeptide of type I collagen, a marker of bone resorption (18). β-carotene supplementation modestly increased lung cancer incidence in cigarette smokers (27) and increased incidence of and total mortality from lung cancer (28). However, most pro-vitamin A carotenoids, when taken as a part of a healthy diet, are nontoxic in humans at almost any dosage, unlike retinols. Therefore, it is important to examine the association of carotenoids with bone health in a prospective cohort study and to further explore the mechanisms via which they operate.

B Vitamins

B vitamins may influence bone health both through their involvement in the metabolism of homocysteine and through direct effects on bone cells. The B vitamins, which include vitamin B2 (riboflavin), vitamin B6 (pyridoxine), vitamin B11 (folate) and vitamin B12 (cobalamin) function as cofactors and

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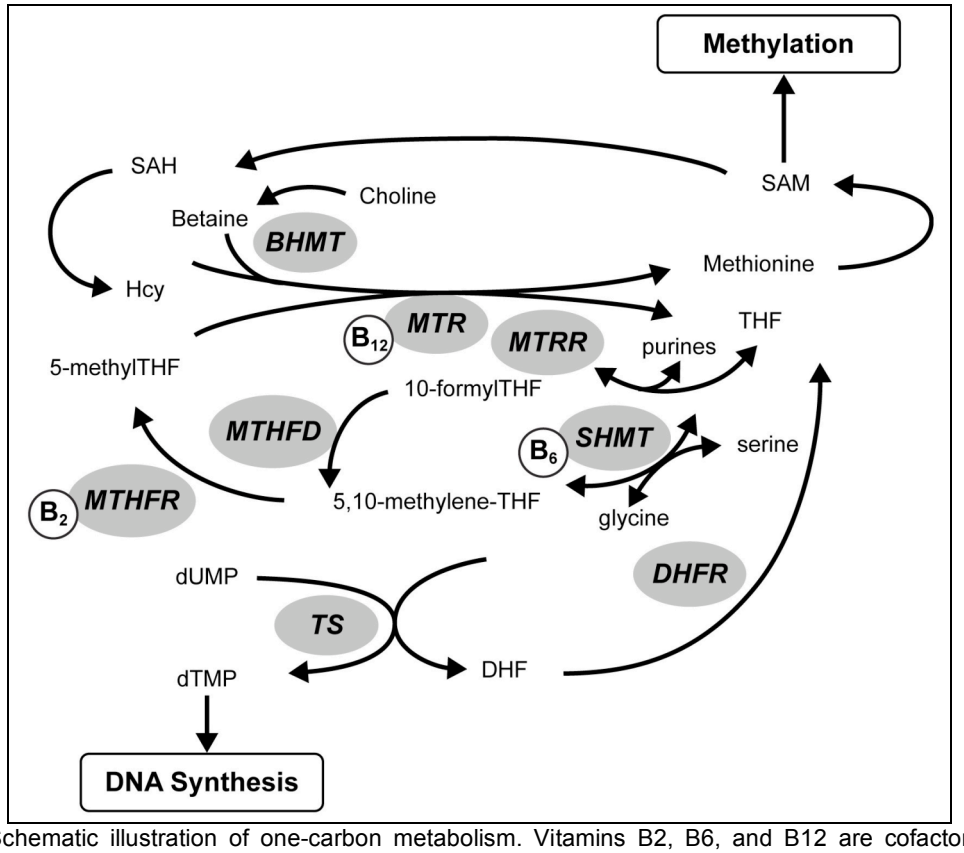

Fig. 1. Schematic illustration of one-carbon metabolism. Vitamins B2, B6, and B12 are cofactors in the pathway. Reprinted from Chen *et al*. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. *Cancer Res*. 2005 Feb 15;65(4):1606-14, with permission of the American Association for Cancer Research (29).

substrates in the metabolism of homocysteine in one-carbon metabolism (29) (Fig. 1).

Low B vitamin status is the primary determinant of increased plasma homocysteine concentrations in older adults (30). Homocysteine is hypothesized to interfere with bone collagen cross-links, which may reduce bone strength and mineralization, thereby increasing susceptibility to fracture (31). In addition, these B vitamins may have a direct effect on bone health. *In vitro* and animal studies have shown that low levels of vitamins B12 and B6 may promote osteoclast activity (32), while higher levels may stimulate bone formation (33;34), while folate may indirectly influence cells involved in bone remodeling as it is important for DNA methylation (35).

A number of large, population-based cohort studies, as well as some cross-sectional studies, of plasma homocysteine have found a negative association with BMD and a positive association with fracture. In a cohort of 1,213 women aged 70-85 years, Zhu *et al*. found that the adjusted 4-year bone loss at the hip was 2.6-times greater in the highest tertile of plasma homocysteine compared to the lowest tertile (P = 0.008) in this study. However, no association was found with clinical fracture and B vitamin levels were not considered (36). Results from the Hordaland Homocysteine Study show that plasma homocysteine was inversely associated with total body BMD in 5,238 middle-aged and older men and women, with stronger associations in women than in men (37). Additionally, Gjesdal *et al*. found that there was a significant increased risk of hip fracture in this same cohort (n = 4,766, 55% female), but only among women, with an adjusted HR of 2.42 (95% CI: 1.43-4.09) for the highest quartile of plasma homocysteine

compared to the lowest quartile over a median of 12.6 years of follow-up (38). Homocysteine was not, however, significantly associated with clinical fractures among older women in the population-based Os des Femmes de Lyon (OFELY) study, nor was it related to femoral neck bone loss in older men and women in the Framingham Osteoporosis Study (39;40). While higher homocysteine was initially associated with increased hip fracture risk in the Framingham Osteoporosis Study, the association was somewhat attenuated after adjustment for vitamin B12, suggesting that the relation between elevated homocysteine and fracture may be due to low B vitamin status (39). In a recent nested case-control study of postmenopausal women within the Women's Health Initiative Observational Study (n = 400 cases, 400 controls), homocysteine was positively associated with hip fracture risk, independent of B vitamins, yet the relation was almost totally attenuated by adjustment for cystatin-C, a measure of renal function. Additionally, women in the highest quartiles of both homocysteine and cystatin-C had a hip fracture odds ratio (OR) of 2.8 (95% CI: 1.61-4.87) compared to all other women, suggesting that the observed association between homocysteine and fracture may be attributed to poor renal function (41). Further studies are needed to determine whether observed relations between elevated homocysteine and increased fracture risk are due to direct effects of homocysteine on bone, or to potential confounding effects of low B vitamin status or renal insufficiency.

The association between folate and bone outcomes has been mostly inconsistent, with many studies finding null results (39;42;43). Even though a significant positive cross-sectional association was reported between folate intake and femoral neck BMD in 1,869 peri-menopausal women from the Danish Osteoporosis Prevention Study, no associations were observed in the longitudinal analyses in this cohort (44). In a smaller cohort study, Cagnacci *et al*. also found that plasma folate levels at baseline were positively associated with the 5-year change in vertebral BMD in 117 postmenopausal women (45). In addition, the lowest quartile of plasma folate was found to be associated with an increased risk of hip fracture among women, but not men, in the Hordaland Homocysteine Study (n = 4766, 55% female) (38).

There is emerging evidence that the other B vitamins involved in homocysteine metabolism may be important for bone health. A few studies have had null findings when examining the effects of vitamin B12 (43;44). However, results from the Framingham Osteoporosis Study suggest that plasma vitamin B12 is positively associated with hip fracture risk among older men and women (n = 581), although these results were somewhat attenuated after controlling for BMD and plasma homocysteine (39). Results from this cohort also suggest that lower plasma vitamin B6 is associated with greater femoral neck bone loss and with increased risk of hip fracture. In the Rotterdam Study (n = 5,304), increased vitamin B6 intake was associated with greater hip and lumbar spine BMD in men and women 55 years of age and older, as well as a decreased risk of non-vertebral fracture (43). In this same cohort, a positive association was also found between intake of riboflavin (vitamin B2) and BMD at the hip and lumbar spine. There is also growing evidence that riboflavin may interact with genes involved in homocysteine metabolism to influence bone health as several association studies suggest that the homozygous form of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism is associated with low BMD and increased fracture risk, but only among those with low dietary riboflavin intake (46;47). Additional research is needed to elucidate the relations of riboflavin, and vitamins B12 and B6, with bone health.

Some recent analyses from randomized controlled trials have begun to examine the effect of B vitamin supplementation on bone health. In the large Heart Outcomes Prevention Evaluation 2 trial (n = 5,522, 28% female), men and women 55 years of age or older with cardiovascular disease or diabetes mellitus were randomized to a supplement of folate, B12, and B6 or

placebo and followed for a mean of 5 years for clinical fracture (48). No significant difference in risk of fracture was found between the two groups. While the majority of evidence from observational studies supports a role for B vitamins and homocysteine in bone health, additional controlled trials are needed to determine whether treatment with B vitamin supplements can maintain or improve BMD or reduce the risk of fractures.

Vitamin C

Bone matrix contains over 90% of protein as collagen and it is well-established that vitamin C is an essential cofactor for collagen formation and synthesis of hydroxyproline (Fig. 2) and hydroxylysine required for the formation of stable triple helices (4). Furthermore, as a powerful water-soluble antioxidant, vitamin C may decrease oxidative stress arising from reactive oxygen intermediates that may be involved in the bone-resorptive process (49). In addition, vitamin C potentiates vitamin E activity in cells by regenerating α-tocopherol from its oxidized derivative (50). Therefore, vitamin C might help in preventing osteoporosis (51). Several epidemiologic studies have examined the association of vitamin C in relation to BMD or fracture (24;52-55). However, results from these studies have been mixed, indicating a complex association involving interaction of vitamin C with smoking (55-57), estrogen use/hormonal therapy after menopause (24;54;55), calcium intake (52;54;57) and vitamin E intake (57;58).

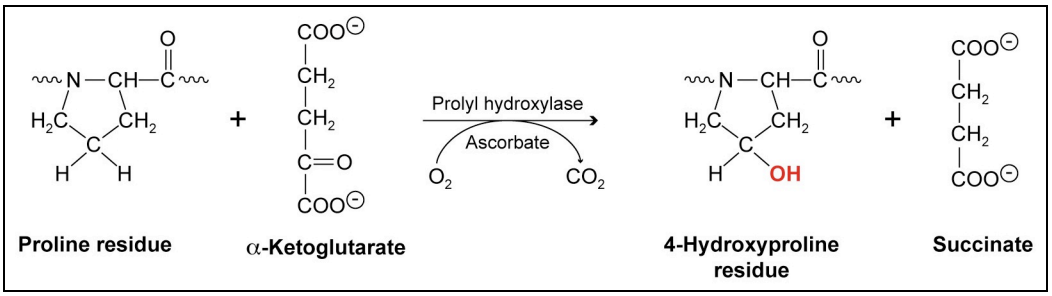

Fig. 2. Ascorbate acts as a reducing agent in the conversion of proline to hydroxyproline, which is required for collagen formation. Image from: http://web.campbell.edu/faculty/nemecz/323_lect/proteins/prot_chapter.html, used with permission.

In the Framingham Osteoporosis Study (n = 334 men and 540 women, mean age ~ 75 years), negative cross-sectional associations were observed between total and supplemental vitamin C intake and trochanter BMD among current male smokers (P-trend = 0.01) (57). Among male nonsmokers, total vitamin C intake was positively associated with femoral neck BMD (P-trend = 0.04). Higher total vitamin C intake was associated with lower femoral neck BMD and trochanteric BMD loss in men with low calcium (all P-trend ≤ 0.03) or vitamin E intakes (all P-trend = 0.03). Higher dietary vitamin C intake tended to be associated with lower femoral neck BMD loss (P-trend = 0.09). These associations were attenuated but retained borderline significance (P-trend < 0.1) after adjusting for potassium intake (a marker of fruit and vegetable intake), suggesting that vitamin C effects may not be separated from other protective factors in fruit and vegetables. Null associations were observed among women. Interaction with calcium intake was also reported in the postmenopausal Estrogen/Progestin Interventions Trial (PEPI). However, in that study dietary vitamin C was positively associated with BMD at the femoral neck (P = 0.002) and total hip (P = 0.01) only in subjects with higher calcium intake (> 500mg/d) (52).

A population-based study (n = 994 postmenopausal women) from the Rancho Bernardo cohort reported that vitamin C supplement users had significantly higher BMD (3%) at the radius, femoral neck, and total hip (P < 0.05) than non-users (54). It also reported that women taking both

estrogen and vitamin C supplements had higher BMD at all sites compared to women not taking estrogen and not taking vitamin C supplements.

Similarly, among women taking vitamin C supplements, those taking calcium supplements plus estrogen had the highest bone mass compared to the other three groups (no estrogen/no calcium supplements, calcium supplements only, estrogen use only). Another study (53) reported that longer duration of vitamin C supplement use was associated with higher BMD in women who had not used estrogen replacement therapy (P-trend = 0.02). Furthermore, among women aged 55-64 years, those who used vitamin C supplements for > 10 years, had higher BMD than non-users (multivariate adjusted mean BMD 0.699 (0.017) g/cm^2 vs. 0.655 (0.007) g/cm^2, P = 0.02). The Women's Health Initiative Study (24) reported no significant association between dietary or total vitamin C and BMD. Yet the beneficial effect of current hormone therapy on BMD at the femoral neck (P = 0.004), total-body (P < 0.04), spine (P = 0.03) and total-hip (P = 0.02) was enhanced with higher intakes of total vitamin C (interaction P < 0.01). Similarly, Leveille *et al*. found no evidence of association of dietary or total vitamin C intake with BMD (53).

Few observational studies have examined the association of vitamin C and risk of hip fracture (56;58) The Swedish Mammography Cohort (n = 66,651 women) reported that OR for hip fracture among current smokers with a low intake of vitamin E was 3.0 (95% CI: 1.6-5.4) and for vitamin C, 3.0 (95% CI: 1.6-5.6). However, in current smokers with a low intake of both vitamin E and C, the OR increased to 4.9 (95% CI: 2.2-11.0) (58). The Utah Study of Nutrition and Bone Health reported a threshold effect for vitamin C intake among elderly men and women who were former or current smokers. The protective association of vitamin C was observed up to 488 mg/d of intake (56). In the Framingham Osteoporosis study, subjects in the highest tertile (median = 313 mg/d) of total vitamin C intake had significantly lower risk of hip fracture over 15 years of follow-up (P-trend = 0.04) compared to subjects in the lowest tertile (median = 94 mg/d). Similar results were observed for total vitamin C intake and the risk of non-vertebral fractures over 17-years of follow-up (P-trend = 0.05). Subjects in the highest category of supplemental vitamin C intake had significantly fewer hip fractures (median = 260 mg/d, P-trend = 0.02) and non-vertebral fractures (median = 260 mg/d, P-trend = 0.07) compared to non-supplement users. Dietary vitamin C intake was not associated with fracture risk (all P > 0.22) (59).

A recent study from NHANES reported that smokers and low-income individuals continue to be at risk of vitamin C deficiency (60). However, if current recommendations to consume five to nine fruit and vegetable servings per day were followed, this consumption could provide the amount of vitamin C associated with lower bone loss and lower fracture risk, while contributing protection against other chronic diseases such as diabetes, cancer, etc.

Vitamin E

In the skeletal system, several reports demonstrated effects of vitamin E on bone and cartilage tissue. Ebina *et al*. reported that iron-induced impairment of bone formation was prevented by dietary vitamin E supplementation in rats (61). In addition, vitamin E stimulated trabecular bone formation in chicks (62) and inhibited differentiation of osteoblasts in rats (63). Therefore, vitamin E is thought to affect bone formation and bone remodeling.

The majority of the studies on this topic have examined vitamin E in relation to vitamin C because these vitamins interact to form a redox system for scavenging free radicals. The Geelong study (n = 533 postmenopausal women) reported that antioxidant vitamin E or C supplements were associated with decreased levels of serum CTx, a marker of bone resorption (64). In contrast, in a study by Macdonald *et al*. it was reported that dietary vitamin E intake appeared to be a negative predictor of femoral neck BMD change over 5 to 7 years

(65). The authors suggested that vitamin E is highly correlated with polyunsaturated fatty acids (PUFA) and may simply be a surrogate marker for them. An alternative explanation for this could be an interaction of vitamin E with vitamin K (66). In the Framingham Osteoporosis Study (n = 334 men and 540 women, mean age ~ 75 years), significant interactions were observed between vitamin E and vitamin C in relation to BMD loss. Higher total vitamin C intake was associated with lower femoral neck BMD and trochanteric BMD loss in men with low vitamin E intakes (all P-trend = 0.03) (57).

There are only a few studies that have examined vitamin E. Most of these studies have focused primarily on fracture as an outcome. In the Utah Study of Nutrition and Bone Health, a case-control study of 1,349 age- and sex-matched controls (aged ≥ 50 years) reported that among persons who had ever smoked the highest quintile of vitamin E intake vs. the lowest quintile (median = 315 vs. 7 mg of α-tocopherol eq./d respectively) had 71% lower risk of hip fracture (OR = 0.29, 95% CI: 0.16-0.52; P-trend < 0.0001) (56). The Swedish Mammography Cohort reported (n = 66,651 women, aged 40-76 years) that the OR for hip fracture among current smokers with a low intake of vitamin E was 3.0 (95% CI: 1.6-5.4) and of vitamin C was 3.0 (95% CI: 1.6-5.6) compared to never smokers with low intake of vitamin E and C, respectively. In contrast, the OR decreased to 1.1 (95% CI: 0.5-2.4) and 1.4 (95% CI: 0.7-3.0) among current smokers with high intakes of vitamin E and C, respectively, compared to never smokers with high intakes of these nutrients. In current smokers with a low intake of both vitamins E and C, the OR increased to 4.9 (95% CI: 2.2-11.0) compared to never smokers (58). Even though animal studies and few epidemiological studies have shown that vitamin E is important for the skeleton, there is insufficient evidence to conclusively link vitamin E with bone health. Furthermore, there is no information on the effect of different forms of vitamin E on the skeleton *i.e.*, α-tocopherol (the most studied tocopherol) versus γ-tocopherol (the major form of tocopherol in the diet in the U.S., but not in Europe, and which has anti-inflammatory properties) (67).

Vitamin K

Vitamin K is of interest for bone health because of its role in the conversion of particular glutamyl (Glu) residues to γ-carboxyglutamyl (Gla) residues in Gla proteins such as osteocalcin, matrix Gla protein, and protein S that are found in bone (68). The reaction is catalyzed by a microsomal enzyme called vitamin K-dependent carboxylase, which in turn is linked to a cyclic salvage pathway known as the vitamin K-epoxide cycle (Fig. 3).

Osteocalcin, the most ample of these proteins in bone, is believed to regulate bone mineralization, binding to bone hydroxyapatite at the Gla residues that is dependent on γ-carboxylation (68). Undercarboxylated osteocalcin is considered a marker for vitamin K status and studies have found that high levels of undercarboxylated osteocalcin are associated with fracture and low BMD (69;70).

There is strong epidemiological evidence that low phylloquinone (vitamin K_1 of plant origin) intakes are associated with a higher risk of hip fracture (71;72). Despite the protective associations observed in these observational studies, recent studies of the association between vitamin K and bone health have been inconsistent. Much of the discrepancy appears to be due to the type of study conducted, with randomized clinical trials generally resulting in null findings and observational studies finding a somewhat protective effect of vitamin K. The association of vitamin K and bone health also appears to vary by the specific outcome of interest that is chosen as an indication of bone health, with typically null findings for BMD, but mixed findings on bone mineral content (BMC) and fracture. In addition, studies of vitamin K differentiate between vitamin K_1 (phylloquinone) and K_2 (menaquinone).

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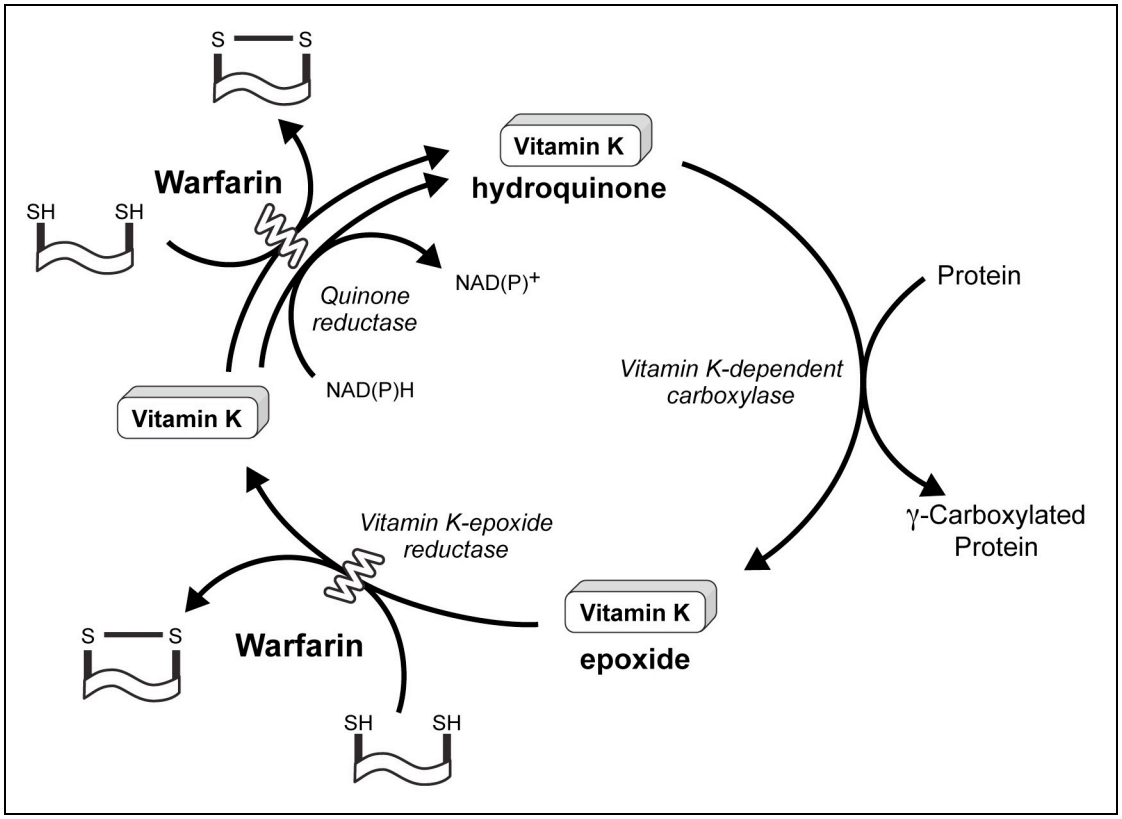

Fig. 3. Vitamin K epoxide cycle showing formation of γ-carboxyglutamyl (Gla) protein. Image from Jane Higdon (copyright 2010, Linus Pauling Institute, Oregon State University, used with permission). (http://lpi.oregonstate.edu/infocenter/vitamins/vitaminK/kcycle.html)

Recent randomized controlled trials of vitamin K_1 at both low and high dosages and with consideration for supplementation of vitamin D/calcium in healthy older men and women found no association between vitamin K_1 and change in BMD and BMC at numerous sites over follow-up (73-76). However, when looking at clinical fractures as the outcome among postmenopausal women with osteopenia (n = 440), Cheung *et al*. found that women in the vitamin K_1 intervention group had significantly fewer fractures (P = 0.04), although the study was not powered to examine this outcome and few fractures were experienced as only a subset of the initial participants were followed beyond two years (73). Similarly, in a 3-year prospective, observational study of women aged 30-88 years (n = 379, mean age 63 years), women with low plasma vitamin K_1 concentrations had 3.58-times (95% CI: 3.26-3.93) the risk of vertebral fracture as compared to women with high concentrations, although there was no control for the intake of other nutrients (77). A large cross-sectional study of early postmenopausal women (n = 3,199) also found that women in the lowest quartiles of energy-adjusted vitamin K_1 intake, as assessed by a food frequency questionnaire, had significantly lower BMD at the femoral neck only, yet there was also no control for the intake of other nutrients (78).

Accordant with the findings of vitamin K_1 studies, recent randomized controlled trials of vitamin K_2 (both MK-4 and MK-7), at levels exceedingly higher than what can be achieved through diet, also found that there was no association with change in BMD over follow-up ranging from 1 to 3 years in postmenopausal women (74;79;80). However, Knapen *et al*. reported that in the vitamin K_2 intervention group, BMC at the femoral neck decreased at a significantly lower rate and femoral neck width was significantly increased compared to placebo

in postmenopausal women (n = 325) (79). However, a 3-year prospective, observational study (n = 379, mean age 63 years) found no association between vitamin K_2 intake and vertebral fracture, although an association had been found with K_1 in this cohort (77). The results from these studies suggest that long-term prospective intervention studies are needed to further examine the direct effect of vitamin K on bone loss and the risk of fracture.

Multivitamins

As per the National Institutes of Health, Americans spend more than $23 billion per year on supplements, and among this supplement-using population, the multi-vitamin/mineral supplement is the major category of supplement, used by about one-third of Americans (81). The Swedish Mammography Cohort reported that 23.4% of women in the study were using multivitamins with minerals (82). Individual nutrients within multivitamins may be beneficial for bone health. However, it is unclear what their effects might be when taken together in mega-doses. For example, pro-vitamin A carotenoids, when taken as a part of a healthy diet, are nontoxic in humans in almost any dosage. However, β-carotene supplementation at pharmacologic levels modestly increased lung cancer incidence in cigarette smokers in the α-Tocopherol and β-Carotene cancer prevention trial (ATBC Study) (27). Similarly, the β-Carotene and Retinol Efficacy Trial (CARET) halted its daily intervention of β-carotene (30 mg) combined with retinyl palmitate (25,000 IU) following the observation of increased incidence of and total mortality from lung cancer (28). Correspondingly, an increased risk of all-cause mortality has been reported with vitamin E supplementation (83). It has been suggested that high doses of vitamin E may result in a possible pro-oxidant effect. Furthermore, mega-doses of vitamin E have been found to antagonize vitamin K (66). Furthermore, it is well-established that high doses of vitamin K can reduce the effectiveness of anticoagulant drugs such as warfarin (Coumadin), which is used to prevent blood clotting (84). Despite the widespread use of multivitamins in the United States and Europe, there is a lack of research on multivitamin usage and bone health. One small randomized, double-blind, controlled trial of multivitamin supplementation in a group of Australian, aged care residents reported a greater increase in quantitative heel ultrasound (QUS) in the multivitamin group compared to the placebo group (3.0 ± 2.0 dB MHz $^{-1}$ vs. -2.9 ± 2.1 dB MHz $^{-1}$, respectively, $P = 0.041$) (85). More research should be conducted to examine the association of multivitamin use with markers of bone health.

Conclusion

Diet is an important modifiable risk factor for the prevention of osteoporosis and vitamin intake may be an important consideration. Emerging evidence indicates that vitamins, in addition to vitamin D, play an important role in skeletal health. This review presented our current understanding of the non-D vitamins (A, B, C, E, K) and bone health in adults. Several studies report that excessive vitamin A may have a negative impact on bone; yet, pro-vitamin A carotenoids have shown a positive association with bone health. Observational studies support a role for B vitamins and homocysteine in bone health, however, more controlled trials are needed. Vitamin C has had mixed results with regard to bone outcomes, and typically involves complex interactions. There is insufficient evidence to conclusively link vitamin E with bone health. Recent studies of vitamin K and bone have been inconsistent.

For most of the non-D vitamins, prospective studies are needed to explore the mechanisms by which they operate and to make recommendations for bone health. Work comparing vitamins from dietary sources to supplement use would advance our understanding. Controlled trials are needed to determine whether treatment with specific vitamin supplements can improve BMD or reduce fracture risk. Observational studies have both supported and shown disagreement with controlled trials. Longer-term prospective intervention studies are

needed to further examine the effect of vitamins on bone loss and the risk of fracture.

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