# Update on the role of vitamin K in skeletal health

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*A protective role for vitamin K in bone health has been suggested based on its role as an enzymatic cofactor. In observational studies, vitamin K insufficiency is generally associated with lower bone mass and increased hip fracture risk. However, these findings are not supported in randomized controlled trials (RCT) of phylloquinone (vitamin $K_1$) supplementation and bone loss at the hip in the elderly. This suggests that increased vegetable and legume intakes may simultaneously improve measures of vitamin K status and skeletal health, even though the mechanisms underlying these improvements may be independent of each other. Menaquinone-4 (vitamin $K_2$), when given at pharmacological doses, appears to protect against fracture risk and bone loss at the spine. However, there are emerging data that suggest the efficacy of vitamin K supplementation on bone loss is inconclusive.*



## INTRODUCTION

Vitamin K is one of several nutrients that have been implicated in bone health. However, there is an emerging divergence of conclusions regarding the efficacy of vitamin K supplementation in reducing age-related bone loss. This review summarizes the evidence from human studies and emphasizes the differences in dietary forms and doses.

Vitamin K is a fat-soluble vitamin that exists naturally in multiple dietary forms. Phylloquinone (vitamin $K_1$) is a 2-methyl-1,4-napthoquinone ring with a phytyl group at the 3-position. Menaquinones (vitamin $K_2$) are endogenously synthesized and differ in structure from phylloquinone in their 3′-substituted unsaturated multiprenyl group. The primary menaquinones, menaquinone-4 (MK-4) through menaquinone-10 (MK-10), contain 4–10 repeating isoprenoid units on their side chain, respectively. The only known function of vitamin K is to support gamma (γ)-carboxylation of known vitamin K-dependent proteins, which confers calcium binding properties, including those present in bone.

Phylloquinone is the principal dietary form of vitamin K and is found in green leafy vegetables and vegetable oils.[1,2] MK-4 is unique to the menaquinones in that it is alkylated from menadione present in animal feeds or is the product of certain tissue-specific conversion directly from dietary phylloquinone.[3] Menaquinones 4 through 6 are found in low concentrations in animal-based foods, such as chicken meat and certain types of cheese.[4,5] Menaquinone-7 is found in large amounts in legumes, specifically fermented soybeans (commonly called natto), which is a traditional food in eastern Japan.[2,6]

The adequate intake (AI) for vitamin K is established at 90 μg/d for women and 120 μg/d for men, based on median intakes from food, as estimated from NHANES III (1988–1994).[7] The AI may not be adequate to maintain optimal vitamin K status, based on full carboxylation of all vitamin K-dependent proteins,[8,9] but current limited understanding of the physiological implications of changes in vitamin K biomarkers preclude determination of more precise dietary recommendations. As reviewed elsewhere, there are wide ranges of vitamin K intakes across geographic regions and age groups.[10] Certain subgroups appear to be at risk for low phylloquinone intakes, including the elderly. There are population trends towards decreased phylloquinone intake, and it is not known what the long-term implications of these chronic low vitamin K intakes are with respect to skeletal health.

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## VITAMIN K STATUS

No single biomarker of vitamin K status is a robust measure of vitamin K sufficiency and deficiency. Instead, the current practice is to use multiple biomarkers where possible, each of which reflects a different aspect of vitamin K intake, absorption, and transport, or functions as a cofactor for the γ-carboxylation of vitamin K-dependent proteins. Plasma phylloquinone, a static measure of vitamin K status, fluctuates in accordance with recent dietary intakes and is closely correlated with triglyceride concentrations.[11–13] Percent uncarboxylated osteocalcin (%ucOC) is a measure of carboxylation of osteocalcin (OC), a vitamin K-dependent protein found in bone. It varies according to dietary vitamin K intake[11] and is considered to be a sensitive indicator of the vitamin K status of bone.[14,15]

## POTENTIAL ROLE OF VITAMIN K IN BONE HEALTH

Osteocalcin, which is produced by osteoblasts during bone formation, is the primary non-collageneous protein in bone. Although the exact role of OC is not clear, it most likely functions as a regulator of bone mineral maturation.[16,17] The transcription and translation of OC is regulated by 1,25-dihydroxyvitamin D,[18] and its ability to bind calcium is dependent on the vitamin K-dependent γ-carboxylation of 3 glutamic acid residues.[19,20] The γ-carboxylation of osteocalcin is the primary mechanism underlying the hypothesized protective influence of vitamin K on bone.

The apoE genotype, which influences serum cholesterol and triglycerides, has been suggested to influence skeletal health through the transport of vitamin K to bone.[21] More specifically, it has been shown that individuals who carry the apoE4 allele and have a rapid hepatic clearance of chylomicron remnants as well as lower serum cholesterol and triglyceride concentrations, also have lower bone mineral density (BMD) and increased risk of fracture,[22,23] which some have attributed to inadequate vitamin K transport to the skeletal tissue.[21,24] However, the data supporting an association between apoE genotype and BMD or fracture risk are inconsistent.[25]

Alternative roles for vitamin K in skeletal health have been suggested. In vitro studies indicate MK-4 can enhance bone mineralization and decrease bone resorption more effectively than phylloquinone.[26–28] MK-4 and phylloquinone differ structurally in the configuration of the side chain, but they share the same napthoquinone ring, which is the active site for the carboxylation reaction. This suggests that MK-4 may influence bone turnover via a mechanism other than the carboxylation reaction.[27] Vitamin K can modulate certain cytokines involved in bone turnover, such as osteoprotegerin and interleukin-6,[29–31] which may be an additional mechanism by which vitamin K influences bone turnover. Furthermore, MK-4 treatment has been shown to upregulate genes involved in bone turnover[32] and collagen formation[33] through the SXR nuclear receptor in osteosarcoma cells and osteoblast cells, respectively.

## EVIDENCE

### Observational studies – vitamin K intake and bone health

Phylloquinone intake is associated with lower risk of hip fracture in the majority of published studies, as summarized in Table 1. In the Nurses Health Study ($n = 72,327$), Feskanich et al.[34] found an increased risk of hip fracture over 10 years in women aged 30–88 years who were in the lowest quintile of phylloquinone intake. Similarly, Booth et al.[35] observed an increased risk for hip fracture associated with lower phylloquinone intake in men and women participating in the Framingham Heart Study (mean age 75 years) over 7 years of follow-up, with no effect modification by apoE genotype. However, the associations between phylloquinone intake and BMD are less consistent. There were no significant associations between phylloquinone intake and BMD in this elderly cohort.[35] In the younger Framingham offspring cohort, phylloquinone intake was positively associated with BMD cross-sectionally in women, but not in men (29–86 years), while in prospective analyses of the same cohort, there was no association between vitamin K and change in BMD over 5 years.[9]

In a recent population-based study of women aged 45–54 years, which examined the associations between phylloquinone intake and apoE polymorphisms with bone health, phylloquinone intake was positively associated with BMD cross-sectionally but no change in BMD was observed after 5–7 years of follow-up, and there was no effect modification by apoE genotype.[36] Since the primary dietary source of phylloquinone is green leafy vegetables, any positive associations between vitamin K intake and skeletal outcomes are reflective of generally healthier diets, which are also positively associated with bone health in population-based studies.[37]

There are few reports of associations between MK-4 intake and skeletal health, primarily due to the limited food composition data available for this form of vitamin K. However, reports from Japan indicate that consumption of natto, fermented soybeans rich in MK-7, is associated with higher BMD; this was found both in a population study[38] and in a smaller study of premenopausal women ($n = 117$) with a VDR polymorphism that renders them prone to bone loss.[39] In a recent analysis of

*Table 1* **Observational studies assessing associations between vitamin K and bone mass or fracture risk.**

| Subjects | Measure of vitamin K status | Primary outcome measure | Association | Reference |
|---|---|---|---|---|
| Women, 38–63 y | Phylloquinone intake | Hip fracture | Inverse | Feskanich et al. (1999)[34] |
| Men and women, mean age 75 y | Phylloquinone intake | Hip fracture | Inverse | Booth et al. (2000)[35] |
| | | BMD | No association | |
| Men and women, 59 ± 9 y | Phylloquinone intake | BMD | Positive (women only) | Booth et al. (2003)[9] |
| Women, 45–54 yrs | Phylloquinone intake | BMD | Positive | Macdonald et al. (2008)[36] |
| Women, 43–58 y | Phylloquinone intake | Hip fracture | No association | Rejnmark et al. (2006)[82] |
| | | BMD | | |
| Men and women, national survey | Phylloquinone and MK-7 intake | Hip fracture | Inverse | Yaogashi et al. (2008)[40] |
| Women, 20–79 y | MK-7 intake | BMD | Positive | Ikeda et al. (2006)[38] |
| Women, 70–97 y | ucOC | Hip fracture | Positive | Szulc et al. (1996)[83] |
| Women, mean age 82 y | ucOC | Hip fracture | Positive | Vergnaud et al. (1997)[43] |
| Men and women, ≥70 y old | ucOC | Hip fracture | Positive | Luukinen et al. (2000)[41] |
| Women, 60–99 y | ucOC | Hip fracture | No association | Liu et al. (1998)[45] |
| Women, mean age 81 y | ucOC | BMD | Inverse | Szulc et al. (1994)[84] |
| Women, 20–90 y | ucOC | BMD | Inverse (only within 10 y of menopause) | Knapen et al. (1998)[85] |
| Women, mean age 61 y | ucOC | BMD | Inverse | Schaafsma et al. (2000)[86] |
| Men and women, 32–86 y | ucOC | BMD | Inverse (men only) | Booth et al. (2004)[87] |
| | Plasma phylloquinone | | Positive (men and postmenopausal women not taking HRT) | |
| Women, 30–88 y | ucOC | Vertebral fracture | No association | Tsugawa et al. (2008)[44] |
| | Plasma MK4 | | No association | |
| | Plasma MK7 phylloquinone | | Inverse | |
| Women, 52–93 y | Plasma MK4, MK7 | Hip fracture | No association | Kawana et al. (2001)[46] |
| Boys and girls, 8–14 y | ucOC | BMC | Inverse | Van Summeren et al. (2008)[49] |
| Girls, 11–12 y | ucOC | BMC | Inverse | O'Connor et al. (2007)[48] |
| Girls, 3–16 y | ucOC, plasma phylloquinone | BMC | No association | Kalkwarf et al. (2004)[51] |
| Boys and girls with cystic fibrosis, 8–12 y | ucOC | BMC | Inverse | Fewtrell et al. (2008)[47] |
| Boys and girls with cystic fibrosis, median age 11 y | ucOC, plasma phylloquinone | BMC, BMD | No association | Conway et al. (2005)[50] |

national survey data from Japan, men and women reporting a higher intake of vitamin K (phylloquinone and MK-7) had a lower incidence of hip fracture. The authors suggest the higher vitamin K intake is attributable to higher intakes of MK-7-rich natto,[40] as suggested by others.[39]

### Observational studies – vitamin K status and bone health

Associations among biochemical measures of vitamin K status and bone health in observational studies are equivocal, as summarized in Table 1. In cross-sectional and prospective analyses, elevated uncarboxylated osteocalcin, which occurs when vitamin K status is low, is a marker of increased risk for hip fracture in the elderly.[41–43] Plasma phylloquinone concentrations were inversely associated with incidence of vertebral fracture in Japanese women aged 30–88 years, whereas ucOC, MK-4, and MK-7 concentrations were not.[44] In case-control analyses, circulating concentrations of ucOC,[45] MK-4, and MK-7[46] did not differ among older women who sustained hip fracture and those who did not.

Outcomes of pediatric studies are also discrepant, as some report an inverse association between vitamin K status and BMC in children (aged 8–14 years),[47–49] while others indicate no associations among vitamin K status and skeletal outcomes.[50,51] The ucOC is associated with sex hormone levels in children,[49] menopausal status,[52,53] and HRT use[54] in postmenopausal women, which suggests that further studies examining the potential influence of estrogen on ucOC, with respect to skeletal health, merit investigation.

### Randomized control trials of vitamin K supplementation and bone loss

There are currently four published reports of randomized control trials that have assessed the influence of phylloquinone supplementation on bone loss[55–58] (Table 2). In female endurance athletes who are prone to bone loss, there was no effect of high-dosage (10 mg/day) phylloquinone supplementation on change in BMD at the femoral neck or spine over 2 years.[55] In postmenopausal women, daily supplementation with 1 mg of phylloquinone in combination with calcium and vitamin D was shown to reduce bone loss at the femoral neck, but not the spine, compared to supplementation with calcium and vitamin D alone.[58] In a separate study, 2 years of supplementation with 200 μg/day phylloquinone, also with calcium and vitamin D, increased BMD at the ultra-distal radius. There was no influence of supplementation on bone loss at the femoral neck and mid-radius.[56] Likewise, in a study that examined phylloquinone supplementation in men and postmenopausal women, there were no differences in 3-year change in BMD at the femoral neck, spine, or total body between those who received 500 μg/day phylloquinone with calcium and vitamin D compared to those who received calcium and vitamin D alone.[57] In a study available only in abstract form at the time of this writing, there was no protective effect of 1 mg/day of phylloquinone supplementation on 1-year bone loss in post-menopausal women who were also supplemented with calcium and vitamin D.[59] There was no effect of phylloquinone supplementation on change in biomarkers of bone turnover in any of the aforementioned studies.[56–58] To date, there have been no randomized control trials using phylloquinone in populations at risk of vitamin K deficiency, such as children with cystic fibrosis.

MK-4 in doses of 45 mg/d is used as a pharmacological treatment for osteoporosis in Japan, so there are numerous randomized control studies that have assessed the efficacy of MK-4 supplementation on skeletal health. Such doses cannot be attained from the diet, regardless of the form of vitamin K consumed. Phylloquinone from the diet is converted to MK-4 in certain tissues, including bone, but the proportion of phylloquinone that is converted is not known and no dose-dependent data are available for this conversion.

As reviewed in an earlier volume of this journal,[60] studies indicate a therapeutic dose (45 mg/day) of MK-4 has a beneficial effect on spine or metacarpal BMD and fracture[61–76] (Table 2). There is also improvement in bone turnover, as measured by circulating markers of bone formation and bone resorption, in response to MK-4 supplementation studies.[71,72,76,77] In a separate systematic review and analysis of randomized clinical trials assessing the influence of vitamin K supplementation on hip fracture, Cockayne et al.[78] concluded that supplementation with MK-4 for longer than 6 months reduces risk for hip and vertebral fracture. Included in that analysis were 12 studies that used daily doses of 45 mg/d of MK-4. As discussed by the authors, several of the studies used for the meta-analysis lacked sufficient sample size,[64–66,70,73,79] were non-placebo-controlled intervention trials,[70–74,76,77,80] and/or used concurrent treatment with calcium and/or vitamin D.[62,69,75,76]

It was subsequently disclosed that a large unpublished surveillance study conducted in Japan ($n > 3000$) did not find a protective effect of MK-4 supplementation (45 mg/day) on bone loss and fracture in the elderly, and that inclusion of this study may have altered the results of the meta-analysis.[81] More recently, two placebo-controlled studies with large sample sizes reported no protective effect of 45 mg/d of MK-4 on hip BMD.[59,67] Prior to these two publications, the majority of MK-4 supplementation studies did not report hip BMD as an

*Table 2* **Randomized control trials assessing the effect of vitamin K treatment on bone mineral density.**

| Sample | Study duration (months) | Form and dose of vitamin K | Outcome | | Reference |
|---|---|---|---|---|---|
| | | | Hip BMD | BMD at other anatomical site | |
| Women ≥60 y | 24 | 200 μg/d phylloquinone | No effect | Increased BMD ultra-distal radius | Bolton-Smith et al. (2007)[56] |
| Men and women not taking HRT, 60–80 y | 36 | 500 μg/d phylloquinone | No effect | No effect on spine BMD | Booth et al. (2008)[57] |
| Women, mean age 62 y | 12 | 1 mg/day phylloquinone | No effect | No effect on spine BMD | Harke et al. (2005)[59] |
| Women, mean age 62 y | 12 | 45 mg/day MK4 | No effect | No effect on spine BMD | Harke et al. (2005)[59] |
| Women not taking HRT, 50–60 y | 36 | 1 mg/d phylloquinone | Decreased bone loss | No effect on spine BMD | Braam et al. (2003)[58] |
| Female endurance athletes, 15–50 y | 24 | 10 mg/d phylloquinone | No effect | No effect on spine BMD | Braam et al. (2003)[55] |
| Women, mean age 66 y | 36 | 45 mg/d MK4 | No effect | No effect on spine BMD | Knapen et al. (2007)[67] |
| Women with osteoporosis, 60–75 y | 48 | 45 mg/d MK4 | Not reported | Increased spine BMD | Purwosunu et al. (2006)[69] |
| Osteoporotic women, 55–81 y | 24 | 45 mg/d MK4 | Not reported | Increased spine BMD | Iwamoto et al. (2000)[65] |
| Osteoporotic women, 53–78 y | 24 | 45 mg/d MK4 | Not reported | Increased forearm BMD | Iwamoto et al. (2001)[66] |
| Women, mean age 54 y | 12 | 45 mg/d MK4 | Not reported | Decreased bone loss at spine | Iwamoto et al. (1999)[64] |
| Men and women treated with glucocorticoids, mean age 40 y | 12 | 45 mg/d MK4 | Not reported | Decreased bone loss at spine | Sasaki et al. (2005)[70] |
| Women with Parkinson's disease, mean age 72 y | 12 | 45 mg/d MK4 | Not reported | Increased metacarpal BMD | Sato et al. (2002)[71] |
| Women with Alzheimer's, mean age 78 y | 24 | 45 mg/d MK4 | Not reported | Increased metacarpal BMD | Sato et al. (2005)[72] |
| Women with cirrhosis of the liver, 42–72 y | 24 | 45 mg/d MK4 | Not reported | Decreased bone loss at spine | Shiomi et al. (2002)[73] |
| Osteoporotic women, mean age 67 y | 24 | 45 mg/d MK4 | Not reported | Decreased bone loss at spine | Shiraki et al. (2000)[74] |
| Women on leuprolide, mean age 46 y | 6 | 45 mg/d MK4 | Not reported | Decreased bone loss at spine | Somekawa et al. (1999)[75] |
| Osteopenic and osteoporotic women, mean age 54 y | 24 | 45 mg/d MK4 | Not reported | Increased BMD at spine | Ushiroyama et al. (2002)[76] |
| Men and postmenopausal women stroke patients, mean age 66 y | 24 | 45 mg/d MK4 | Not reported | Increased metacarpal BMD on hemiplegic side | Sato et al. (1998)[77] |

*Abbreviations*: BMD, bone mineral density; DM, diabetes mellitus; HRT, hormone replacement therapy; RCT, randomized controlled trial.

outcome (Table 2). Given the heterogeneous quality of the studies used and considering the null findings of more recent, larger, placebo-controlled trials and unpublished surveillance data, prior systematic reviews and meta-analyses may need to be revisited.

## CONCLUSION

While some studies suggest a protective influence of vitamin K on bone health, the current evidence is equivocal. In the United States and Europe, phylloquinone is the form most studied in relation to bone health. In observational studies, inverse associations have been reported between phylloquinone intake and circulating phylloquinone and bone loss in the elderly. However, outcomes of randomized, controlled trials do not support a protective effect of phylloquinone on bone loss at the hip among elderly subjects recruited from the general population, suggesting the positive associations observed in observational studies may reflect the influence of generally healthier diets on bone health. While increasing intakes of vegetables and legumes may simultaneously improve measures of vitamin K status and skeletal health, the mechanisms underlying these improvements may be independent of each other.[37] Although MK-4 is primarily the conversion product of phylloquinone, there appears to be stronger evidence for a protective effect of MK-4 on bone health. The reported outcomes of clinical trials, primarily from Japan, that have assessed the effect of MK-4 treatment on fracture risk and bone loss at the spine are positive overall. However, the doses used in these trials are over 400-fold higher than the current recommended intakes of vitamin K. Recent reports of the lack of a protective effect of MK-4 on bone loss at the hip or on hip fracture risk suggest that a prior meta-analysis needs to be revisited. There is also a lack of data on this putative role of vitamin K on bone health among subgroups that may be at greater risk of vitamin K deficiency than elderly subjects recruited from the general population. The influence of vitamin K supplementation on bone health warrants further investigation using placebo-controlled trials with larger samples of individuals that are designed to help elucidate mechanisms and to explain the divergent outcomes of the available RCTs.


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