

Nutraceutical Profile (September 2009)

Vitamin K and Osteoporosis

By Tina Kaczor, ND, FABNO

Introduction

Osteoporosis affects an estimated 75 million people in Europe, the US, and Japan. Fractures are most common in the spine, wrist, and hips with hip fractures having the highest morbidity. Between 1990 and 2000, there was a nearly 25% increase in the number of hip fractures worldwide.¹ By the year 2050, the risk of hip fracture is projected to increase 240% and 310% for women and men, respectively.² The need for interventions to reduce this risk is a worldwide imperative, as the cost to individuals as well as the socioeconomic cost is rapidly advancing.

Ideally, prevention of bone loss through nutritional repletion would begin prior to a person's age of peak bone mass at 18-24 years old, as higher peak mass provides protection from later osteoporosis.³ For those beyond peak bone mass years, age-related bone density decline is expected to be 0.5-1% annually, with an increase to 2-3% in postmenopausal women. Nutritional factors affecting bone structure and turnover include organic minerals (calcium, magnesium, phosphorus, sodium and trace minerals), vitamins (vitamins A, D, E, K, B vitamins) and macronutrients (proteins and fatty acids). Of these, calcium and vitamin D have been most prominent in the promotion of bone health and prevention of osteoporosis.⁴ The role of vitamin K in bone health has been known for several decades. Growing evidence suggests that vitamin K is another modifiable risk factor in the prevention and treatment of osteoporosis.

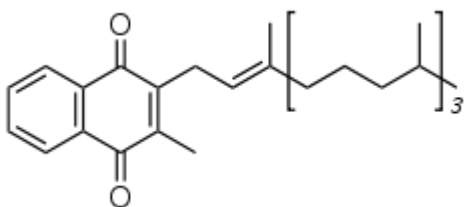
Vitamin K is a term that encompasses several K vitamers, or structurally related compounds conferring similar cofactor activity. Naturally occurring phylloquinone, originally known as vitamin K1, is synthesized by plants and structurally linked to the photosynthetic apparatus. There is also a pharmaceutical form of phylloquinone called phytonadione, which is a synthetic, water-soluble form of the vitamer. Menaquinones, originally known as vitamin K2, are synthesized by various bacteria, and may be formed from dietary phylloquinone in the liver or extrahepatic tissues.⁵ Menadione, originally known as vitamin K3 is synthetic vitamer with a more toxic profile than the biologically derived phylloquinone and menaquinones. While menadione's use as an anti-cancer agent holds promise,⁶ it is not used in the prevention or


treatment of osteoporosis. “Vitamin” K4, K5 and K6 are all synthetically derived compounds used in experimental settings. In regard to osteoporosis, phyloquinone and menaquinones are the most relevant so this review will address only these vitamers’ biological significance in the prevention and treatment of osteoporosis.

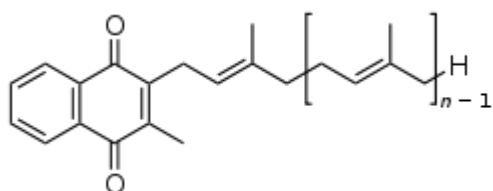
Structures of Vitamin K


Both phyloquinone and menaquinones have a 2-methyl-1,4 naphthoquinone ring backbone with an isoprenoid side chain at the C3 position (Figure 1). Structurally, phyloquinone has a side chain composed of four prenyl units, the first of which is unsaturated. Menaquinones differ in their side chain composition, namely the number of repeating isoprenyl units, which vary in length from 4-13 and are all unsaturated. The family of menaquinones are commonly abbreviated as MK-*n*, where *n* designates the number of repeating prenyl units. The most commonly used form in interventional studies of osteoporosis is MK-4, or menatetrenone, and is available in Japan as a prescription drug. Recently another form of menaquinone, MK-7, is also showing promise in its application to bone health.

Figure 1:



 Phylloquinone (vitamin K1)



 Menaquinone (vitamin K2)

Dietary Sources of Vitamin K

Food concentration of phyloquinone is highest in plants, especially leafy greens, with a lower concentration found in meats, cheeses, fruits and bread (Table 1). Menaquinones are primarily consumed via meats, plant oils and cheeses in Western cultures and via natto, a fermented soy product, in Japan (Table 2). Western societies have higher intakes of phyloquinone⁷ while Japanese have higher intakes of menaquinone, particularly in eastern Japan.⁸

Dihydrophyloquinone, a byproduct of commercial hydrogenation of phyloquinone in oils, is also consumed in small quantities.⁹ This byproduct appears not to have any vitamin K activity.¹⁰

It is postulated that about 50% of the dietary requirement for vitamin K is supplied by the production of endogenous menaquinones by bacterial flora in the gut, however, there is insufficient evidence to support this finding.⁵

Table 1: Approximate Phylloquinone (vitamin K1) content of selected foods

FOOD	PHYLLOQUINONE (ug/100g)
Kale	882
Spinach	493
Brussels Sprouts	192
Green leaf lettuce, raw	174
Broccoli	141
Broccoli, raw	100
Olive oil	60
Tuna, canned	44
Cheese, cheddar	2.86
Wheat Flour, whole grain	1.9
Beef, sirloin	1.6

Adapted for 100 g serving. U.S. Department of Agriculture, Agricultural Research Service. 2004. USDA National Nutrient Database for Standard Reference, Release 17. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>

Table 2: Approximate Menaquinone (vitamin K2) content of selected foods

FOOD	MENAQUINONE (ug/100g)
Natto	1103
Cheddar cheese	10
Meats	1.0-10
Mozzarella cheese	3.6
Whole eggs, cooked	7.0
Whole milk	1.0
Yogurt	1.0

References: Elder, S. J., D. B. Haytowitz, J. Howe, J. W. Peterson, and S. L. Booth (2006, January). Vitamin K contents of meat, dairy, and fast food in the U.S. diet. *Journal of agricultural and food chemistry* 54 (2), 463-467. Vermeer, C., M. H. Knapen, and L. J. Schurgers (1998, June). Vitamin k and metabolic bone disease. *Journal of clinical pathology* 51 (6), 424-426.

Absorption and Transport of K Vitamers

Vitamin K is absorbed in the proximal intestine through bile-mediated uptake.¹¹ As expected, phylloquinone and menaquinones have both shown improved absorption when ingested with fats.^{12,13} Of note, naturally derived phylloquinone, which occurs bound to the chloroplastic membrane, was more poorly absorbed than pharmaceutical preparations of phylloquinone

(phytonadione).^{12,14} Naturally occurring phylloquinone also resulted in lower serum concentrations as well as a longer time until peak concentration compared to phytonadione.¹⁵ When food derived forms of phylloquinone and menaquinones were ingested in equal microgram quantities, menaquinones were found to have a concentration 10 times higher than the phylloquinone.¹⁶

A recent study done by Schurgers et al compared the absorption of pharmaceutical phylloquinone (phytonadione) with natto-derived MK-7. There was adequate absorption of both forms, with peak serum levels at four hours post oral ingestion. MK-7 however, had a dramatically prolonged half-life relative to phylloquinone, providing a more stable serum level over a 24 hour period as well as an accumulated serum concentration that reached up to 8 fold that of phylloquinone.¹⁷

Transport of phylloquinone is primarily via triacylglycerol rich lipoproteins, with a minor fraction carried on LDL and HDL.¹⁸ Menaquinones are also transported via lipoproteins, with LDL and HDL playing a larger role.^{19,20}

Recommendations for Adequate Intake

There is no current recommended dietary allowance (RDA) for vitamin K. The adequate intake (AI) of a nutrient is the median level that is assumed to be adequate based on observation of groups of apparently healthy people. The AI in the U.S. for vitamin K is based on representative dietary intake data from healthy individuals from the Third Nutrition and Health Examination Survey (NHANES III), and is currently set at 120 and 90µg/day, for men and women, respectively. The adequacy of these intakes for promotion of health has not been determined because there are currently no physiological outcomes available that can be reliably used to assess nutritional adequacy.

Booth and colleagues have suggested that the current level of intake of 90ug/day for women is not enough to ensure complete gamma carboxylation of osteocalcin.²¹ This corroborates data from more controlled dietary settings, which suggest that changes to serum ucOC begin at levels over 100ug of phylloquinone daily.²² Optimal levels of phylloquinone have been suggested to be 1mg/d,²³ over ten times the recommended adequate intake.

Measurement of Vitamin K Status

As with other lipid soluble vitamins, absorption of vitamin K is dependent upon bile salt mediated uptake, therefore assessment of vitamin K status may be clinically useful in patients with malabsorptive syndromes and/or liver diseases. Direct measurement of phylloquinone in plasma, while associated with dietary vitamin K intake, is difficult due to the nanomolar concentrations and intricacies of sample preparation.^{24,25,26} This explains, at least in part, the large differences in reference intervals for phylloquinone in various populations ranging from a mean concentration of 0.22 to 8.88 nmol/L.²⁷ More recently, several assays have been developed that may lead to a more reproducible and accurate measurement of circulating vitamers of K.^{14,28} To date, however, a reliable assay that directly measures all circulating K vitamers is not clinically available.

The most reliable surrogate marker for overall vitamin K status in bone has been measurement of circulating undercarboxylated osteocalcin (ucOC). Under vitamin K replete conditions, osteocalcin undergoes vitamin K dependent gamma carboxylation of its three glutamate residues.²⁹ When there is insufficient vitamin K, some residues of glutamate remain uncarboxylated. The term “undercarboxylated” accurately conveys that less than all three of the glutamate residues needed for activation of osteocalcin have been carboxylated. There are several variations of serum measurements of undercarboxylated osteocalcin in the literature, including measurement of the absolute concentration of ucOC, the ratio of ucOC to total osteocalcin and the ratio of ucOC to carboxylated osteocalcin(cOC).

There is ample evidence for the use of ucOC as a surrogate marker for vitamin K status in bone.³⁰ In a controlled dietary setting, where either 100ug or 420ug of phylloquinone were ingested daily, vitamin K ingestion inversely correlated with plasma ucOC levels.³¹ Other studies have corroborated the utility of ucOC as a surrogate marker for vitamin K status through correlation with dietary intakes.^{32,33} Furthermore, circulating ucOC has been inversely correlated with low bone mineral density³⁴ and directly correlated with higher fracture risk.^{35,36,37}

In addition to correlation with vitamin K levels in bone, plasma ucOC may have a broader utility as a marker of overall vitamin K status. Since overt vitamin K deficiency is currently defined by coagulation defects, and high levels of plasma ucOC have can be found in subjects without these coagulation defects,³⁸ it is reasonable to conclude ucOC may be a useful marker for subclinical vitamin K deficiency.

Molecular effects of Vitamin K

It is well known that bone turnover involves the dynamic interplay of osteoclastic resorption and osteoblastic formation of bone matrix. Upon absorption of bone, osteoclasts undergo apoptosis, leaving a void that is then repaired by osteoblasts that are recruited to the area. When osteoblastic bone formation cannot keep up with the excavation of bone by osteoclasts, bone mineral density decreases.³⁹ There are three proteins involved in bone formation that depend on vitamin K carboxylation: osteocalcin, matrix Gla protein, and protein S. All of these proteins are produced by osteoblasts and undergo activation via post translational carboxylation.

In general, vitamin K acts as a cofactor for the enzyme carboxylase, which catalyzes carboxylation of glutamic acid(Glu) residues to gamma carboxyglutamic acid in vitamin K dependent proteins. This reaction allows the protein to bind calcium ions with high affinity. In the case of osteocalcin, vitamin K dependent carboxylation allows the protein to bind to hydroxyapatite.⁴⁰ While we know that calcium binding is conferred to these proteins after vitamin K dependent carboxylation, and we have identified three vitamin K dependent proteins in bone, the precise molecular interactions at the interface of bone formation remain to be elucidated.

Beyond the role of cofactor in carboxylation of bone proteins, vitamers of K may have more direct actions on cells involved in bone formation. It was demonstrated that menaquinone, but not phylloquinone, was able to induce apoptosis in osteoclastic cells, thereby reducing the lifespan of osteoclasts and their ensuing lytic activity.⁴¹ There is also growing understanding of the role of vitamin K in the transcriptional regulation of proteins involved in bone formation. Over a decade ago, Koshihara found an increase in the amount of osteocalcin produced by

osteoblasts exposed to menaquinone, which implied the vitamin was capable of upregulation of a gene product.⁴² In 2003, Tabb and colleagues suggested that the steroid and xenobiotic nuclear receptor *SXR*, whose gene products include CYP3A4 and MDR1, is involved in bone homeostasis. They went on to demonstrate that menaquinone is a ligand for the *SXR* receptor and that binding leads to an increase in osteoblastic markers including alkaline phosphatase, osteopontin and matrix Gla protein.⁴³ At a transcriptional level, menaquinone binding to the *SXR* domain may work in concert with estrogen receptor alpha to affect the differentiation of osteoblastic cells.⁴⁴ It appears the effect of the menaquinone ligand to the *SXR* receptor results in formation of extracellular matrix proteins as well. Lastly, there may be transcriptional modifications favoring bone formation that are completely independent of the *SXR* receptor region.⁴⁵

Osteoporosis and Dietary Vitamin K Intake

Studies demonstrate that low dietary intake of vitamin K results in undercarboxylation of osteocalcin and higher fracture risk, with equivocal results on measures of bone mineral density (BMD). Booth et al assessed consumption of phylloquinone through food questionnaire on 553 women and 335 men in the Framingham Heart Study from 1988-1989. Bone mineral density at the hip, spine and arms was measured at baseline and again at four years. While there was no association between BMD and vitamin K intake, there was a significantly lower relative risk of hip fractures in the highest (254ug/d) versus the lowest (56ug/d) quintiles.⁴⁶

A ten-year, prospective analysis of 72327 women ages 38-63 in the Nurses' Health Study cohort indicated that dietary intake of vitamin K below 109mcg/day was associated with the highest age adjusted, relative risk of hip fracture.⁴⁷ They also found hip fractures were inversely associated with lettuce consumption, the food that contributed the most to overall vitamin K intake, with a relative risk reduction of 45% between the once or more daily eaters of lettuce versus the once or less weekly consumers.

Limitations of these epidemiologic studies include the potential confounding effect of overall poor diet and unhealthy lifestyle as vitamin K is primarily found in foods associated with a healthy diet.

Osteoporosis and High Dose Vitamin K Intervention

High doses of menaquinones, specifically MK-4, have been used as an approved treatment for osteoporosis in Japan since 1995. There have been many prospective trials in the past decade designed to determine the utility of high doses of phytonadione or menaquinones in preventing a decrease in bone mineral density (BMD) as well as reducing fracture risk. To date, there has not been a large-scale randomized control trial that reaches across cultures and ethnicities to render a definitive recommendation for vitamin K intervention.

A systematic review of clinical trials published in 2006 by Cockayne et al. concluded the clinical data to date "suggests that supplementation with phytonadione and menaquinone-4 reduces bone loss" as measured by BMD.⁴⁸ Most of these trials used a high dose of MK-4, 45 mg daily. His group also found a reduction in overall fracture incidence, although this inference was admittedly not conclusive, as all of the populations with reduced fracture risk were of Japanese descent, many of the studies were not looking at fracture risk as a primary endpoint and many were

underpowered and/or poorly designed with fracture incidences being too low to rule out the element of chance.

A randomized controlled trial published in 2008, Vitamin K Supplementation in Postmenopausal Women with Osteopenia (ECKO Trial), looked at supplementing high dose phytonadione (vitamin K1) in a population of postmenopausal osteopenic women. 440 women were randomized to receive either 5mg of supplemental phytonadione or placebo over a 2-4 year period with bone mineral density the primary endpoint. Vitamin D was monitored for repletion in both arms with a serum concentration mean of 77nmol/L at baseline. While serum phytonadione increased 10 fold and circulating uncarboxylated osteocalcin decreased, urinary C-telopeptide was unchanged and BMD at the lumbar spine and hip were unchanged in the two year and four year periods. However, fewer women in the phytonadione-supplemented group had clinical fractures (9 versus 20, $p=0.04$) and there were fewer incidences of cancer in the intervention arm (3 versus 12, $p=0.02$).⁴⁹

A three-year clinical trial published in 2008 by Booth et al., examined the effect of phylloquinone supplementation on BMD of the femoral neck in men and women ages 60-80, who were calcium and vitamin D replete. 452 men and women were randomized equally to receive either a multivitamin with 500µg/day phylloquinone or no phylloquinone plus a daily calcium (600 mg elemental calcium) and vitamin D (400 IU) supplement. There were no differences in changes in BMD measurements at any of the anatomical sites measured between the two groups. The conclusion drawn from this study was that phylloquinone supplementation in a dose attainable in the diet does not confer any additional benefit for bone health at the spine or hip when taken with recommended amounts of calcium and vitamin D.⁵⁰

A review published in 2009 on the effect of vitamin K supplementation on postmenopausal women was undertaken by Iwamoto and colleagues. Search terms "vitamin K(1) or vitamin K(2)," "bone," and "postmenopausal women" were used via PubMed and studies with greater than 50 participants and a minimum of two years duration were used. Seven RCT's met the criteria for inclusion. The authors concluded "Despite the lack of a significant change or the occurrence of only a modest increase in bone mineral density, high-dose vitamin K(1) and vitamin K(2) supplementation improved indices of bone strength in the femoral neck and reduced the incidence of clinical fractures. The review of the reliable literature confirmed the effect of vitamin K(1) and vitamin K(2) supplementation on the skeleton of postmenopausal women mediated by mechanisms other than bone mineral density and bone turnover."⁵¹

The mechanisms of vitamin K's apparent reduction of fracture risk despite conflicting data on BMD is postulated by Vermeer and colleagues to be the result of the inadequacy of BMD as a surrogate measure of bone strength. Bone strength, he argues, is a result of bone geometry and mineral content in addition to its overall density. A recent study by his group suggested that bone strength is preserved with high dose MK-4 (45mg/d) despite no measurable difference in DXA-BMD. Measurements of femoral neck width (FNW) and bone mineral content (BMC) showed improvement in the arm receiving MK-4. Fracture incidence was not tracked in this healthy population of 325 postmenopausal women.⁵² These results, however, were contradicted by a recent randomized control trial of 381 non-osteoporotic postmenopausal women that showed no change in density or geometry at the proximal femur with intervention of

phylloquinone (1mg/d) or MK-4 (45mg/d).⁵³ Regardless, the contribution of vitamin K to the geometry of the bone is an intriguing postulation that deserves further studies.

It should be noted that inconsistencies in the trial outcomes may be due to differences in controlling for other nutrient factors such as calcium and vitamin D. In two interventional studies using MK-4, there appeared to be synergistic effect when vitamin K, calcium and vitamin D were concurrently supplemented.^{54,55} Japanese data suggests that there is greater benefit of MK-4 intervention when vitamin D is adequate.⁵⁶ This suggests underlying vitamin D deficiencies may be a confounding factor when reviewing clinical trials to date. As the role of vitamin D in bone health has become better established, trials going forward will likely account for vitamin D status.

Interactions/Contraindications

Phylloquinone is not toxic when consumed orally via food or when taken as the pharmaceutical form, phytonadione. Dosages of up to 20mg of phytonadione in an acute setting are indicated for blood coagulation defects.⁵⁷ Menaquinones have also demonstrated no toxicity even at high levels. The Institute of Medicine at the National Academy of Sciences has indicated there is no tolerable upper limit (UL) that needs to be recommended for either form of vitamin K.⁵⁸ A common misconception is that excessive vitamin K will result in overcoagulation. However, the vitamin K-dependent proteins have a limited number of Glu residues capable of γ -carboxylation per molecule, beyond which there can be no further γ -carboxylation or excessive coagulation.

It has been postulated that the slight blood thinning effect of vitamin E is due to interference with vitamin K. This is supported by evidence that vitamin E may lead to excess bleeding in patients receiving warfarin or its derivatives.⁵⁹ There is also evidence that high dose vitamin E (1000IU/d) may interfere with vitamin K status even in patients not on anticoagulant therapy.⁶⁰ The mechanism for this interaction is not clear.⁶¹ As the interactions are poorly understood, vitamin E should be used with discretion in patients on anticoagulants and those suspected of having inadequate vitamin K intake.

Synergy with vitamin D has been demonstrated in clinical trials mentioned in this review. The mechanism by which this is conferred likely involves mutual potentiation of each vitamin's effects on the production and activation of osteocalcin.⁶² In light of the evidence, vitamins D and K should be used in conjunction to maximize the possible beneficial effects of each on bone health.

High vitamin A intake has been linked to increased risk of hip fractures in postmenopausal women.⁶³ It appears likely that the mechanism by which vitamin A may be affecting bone is through upregulation of osteoclastic activity. While one can suspect that there is some molecular interplay between vitamin A and vitamin K, indirectly at least, there have been no studies elucidating these details.

Regarding drug interactions, vitamin K is generally accepted as contraindicated in patients receiving vitamin K antagonistic anticoagulant therapies (i.e., warfarin). Logically, consumption and supplementation of vitamin K in all forms was assumed to interfere with the drug action.

However, in a study of healthy subjects given anticoagulant medication, INR levels were unchanged at consumption of less than 150ug/d of phylloquinone, implying low levels of phylloquinone in the diet may be tolerated in those taking anticoagulants. Furthermore, a select group of patients already taking anticoagulants but unable to stabilize their INR may derive particular benefit with the addition of 150ug/d of phylloquinone to their diets.⁶⁴ It is essential to note that this data cannot be extrapolated to include the safe usage of forms of menaquinone in the same manner. Menaquinones, including MK-4 and MK-7, have longer half-lives, thus reaching much higher serum values than phylloquinone. Menaquinones are expected to interfere with the anticoagulation effects of vitamin K antagonists, perhaps even at low doses.

Summary

Osteoporosis is a growing problem that is expected to take a large toll on individuals and society around the globe in the next several decades. Dietary intakes of vitamin K may not be adequate for prevention of bone loss with aging in Western societies in particular. Increased consumption of foods containing both phylloquinone and menaquinone would likely result in lowering the prevalence of osteoporotic fractures. Intervention with high doses of phylloquinone (>1mg/d) or menaquinone (>45mg/d), while having equivocal data on BMD, has shown promise in reducing the risk of fractures. Indeed, menaquinone (45mg/d) is an approved therapy in Japan for patients with osteoporosis/osteopenia. Menaquinone is offered as a nutritional supplement in the States and while there is no definitive recommendation on the use of vitamin K, the low toxicity profile of the agent lends itself to feasible adaptation in clinical practice. In addition, it would be prudent to encourage all patients to increase their consumption of foods high in vitamin K and perhaps monitor the status of ucOC in patients at high risk of osteoporosis.

For a thorough review of this topic, the reader is referred to an excellent reference work on vitamin K, authored by Suttie.⁶⁵

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