Critical Review

Vitamin K Nutrition and Osteoporosis

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ABSTRACT Although the abundance of vitamin K–dependent proteins in bone suggests an important function, the precise role of vitamin K in skeletal health remains to be determined. Serum concentrations of vitamin K are reportedly reduced in older individuals and persons with osteoporotic fracture. Whether this is causally related to vitamin K insufficiency or simply reflects inadequate nutritional status is unclear. Circulating levels of undercarboxylated osteocalcin may be a sensitive marker of vitamin K inadequacy and have been reported to be increased in both postmenopausal women and individuals who sustain hip fracture. It is also possible that vitamin K indirectly affects the skeleton via control of renal calcium excretion. The effect of vitamin K antagonists (oral anticoagulants) on both renal calcium excretion and bone density is controversial. Thus, many of the reports implicating a role for vitamin K insufficiency in the development of osteoporosis are conflicting. This review summarizes current knowledge regarding a possible role of vitamin K insufficiency in the pathogenesis of osteoporosis. J. Nutr. 125: 1812–1821, 1995.

INDEXING KEY WORDS:

- *bone Gla protein (BGP)* • *vitamin K*
- *matrix Gla protein (MGP)*
- *osteocalcin* • *osteoporosis*

Osteoporosis, a disorder of inadequate skeletal strength predisposing to fracture, is one of the most common human conditions associated with advancing age (Riggs and Melton 1986). Both nutritional (Heaney 1993a) and hormonal (Lindsay et al. 1980) insufficiencies are involved in osteoporosis pathogenesis. The role of calcium nutrition in the development of osteoporosis is well recognized (Heaney 1993b). However, calcium-deficient diets are generally nutritionally poor (Barger-Lux et al. 1992); thus other nutritional deficiencies could be involved in the pathogenesis of bone loss (Rico et al. 1992). Inadequate nutrition could also contribute to osteoporotic fractures via reductions in soft tissue padding or potentially through influences on bone remodeling (Heaney 1993b). Although the role of vitamin D status in the maintenance of skeletal health has long been recognized, more recent studies suggest that vitamin K status may also be involved. The role of vitamin K in bone metabolism has previously been reviewed (Price 1988). At that time, it was recognized that vitamin K–dependent proteins were abundant in bone and could possibly be involved in the control of bone remodeling. However, the paradox of apparently normal skeletal development in vitamin K–deficient animals was well appreciated. Since then, much more data have become available and some of the observations have been reviewed (Szulc and Delmas 1995).

Unfortunately, the results of the more recent studies are not consistent, and much of the data is subject to alternate interpretations. This review will summarize the current knowledge regarding the possible role of vitamin K insufficiency in the pathogenesis of osteoporosis and indicate areas where more research is needed.

VITAMIN K NUTRITION

The vitamin K requirement of humans is obtained from two sources (**Fig. 1**). The major dietary form of the vitamin is phylloquinone (vitamin K_1) of plant origin. Menaquinones (MK),[2] originally referred to as vitamin K_2, are produced by bacteria in the bowel and supply an unknown fraction of the daily requirement. Unlike the other fat-soluble vitamins, bodily stores of vitamin K are rapidly depleted (Suttie et al. 1988), and a rapid onset of deficiency is possible.

The classical role of vitamin K has been in the maintenance of normal hemostasis. Vitamin K insufficiency has usually been defined as the presence of

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[2]Abbreviations used: BGP, bone Gla protein; BMD, bone mineral density; Gla, γ-carboxyglutamyl; Glu, glutamyl; MGP, matrix Gla protein; MK, menaquinone; ucOC, the under-γ-carboxylated fraction of osteocalcin.

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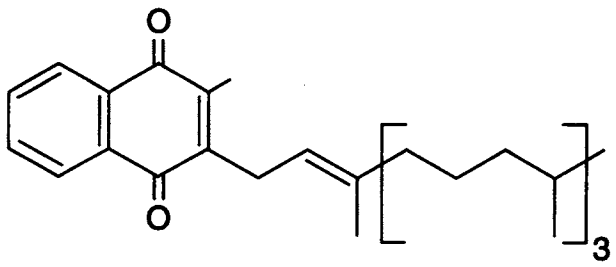
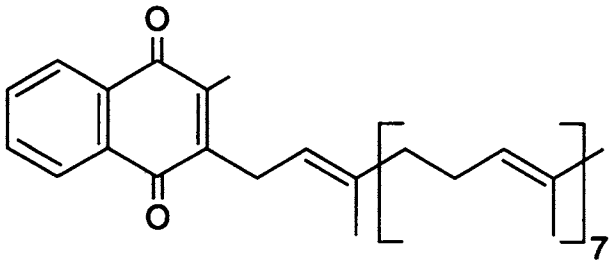
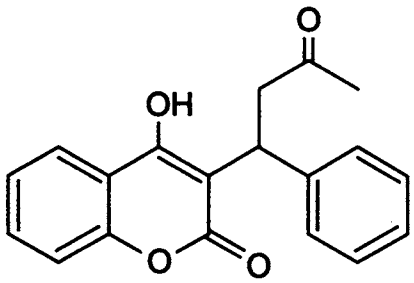


Phylloquinone



Menaquinone-8



Warfarin

FIGURE 1 Structure of phylloquinone, menaquinone-8 and warfarin. Phylloquinone (2-Me-3-phytyl-1,4-naphthoquinone) is produced by green plants and is the major dietary form of the vitamin. Menaquinone-8 is one of the major multiprenylmenaquinones produced by bacteria in the gut. The extent to which these forms contribute to satisfying the human vitamin K requirement is not known. Warfarin (3(α-acetonylbenzyl)-4-hydroxycoumarin) is the most commonly used oral anticoagulant in North America.

a vitamin K–responsive hypoprothrombinemia, measured clinically by the prothrombin time, and is uncommon. On the basis of very limited studies utilizing more sensitive coagulation assays, the RDA for vitamin K has been established at ~1 $\mu g \cdot kg^{-1} \cdot d^{-1}$ (NRC 1989). However, even these sensitive functional coagulation assays are not sufficiently sensitive to be accurate measures of tissue vitamin K status (Kindberg and Suttie 1989). Other measures may be found to be more useful, and an increase in the extent of γ-carboxylation of the vitamin K–dependent bone protein, osteocalcin, by vitamin K administration to

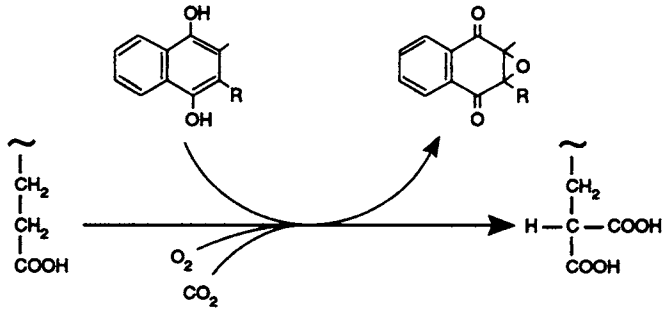


FIGURE 2 The vitamin K–dependent carboxylation reaction. The enzyme is located at the luminal surface of the endoplasmic reticulum, and posttranslationally carboxylates specific Glu residues of a limited number of proteins to γ-carboxyglutamyl (Gla) residues during protein processing. The energy to drive the reaction is obtained from the oxidation of the reduced (hydronaphthoquinone) form of the vitamin to a co-product of the reaction, the 2,3-epoxide of vitamin K.

subjects with no other signs of vitamin K deficiency (Knapen et al. 1989, Plantalech et al. 1990, Bach 1994) suggests that this might be one of the more sensitive criteria of vitamin K status. Bleeding episodes, or prolonged prothrombin times, represent severe manifestation of vitamin K deficiency, and vitamin K insufficiency, as defined by more sensitive measures (Suttie 1992), may be more prevalent in the population than currently appreciated.

VITAMIN K AND BONE PROTEINS

The metabolic role of vitamin K is as a required cofactor for the vitamin K–dependent carboxylase, the microsomal enzyme responsible for the posttranslational conversion of specific glutamyl (Glu) to γ-carboxyglutamyl (Gla) residues (Esmon et al. 1975a) in a limited number of proteins (**Fig. 2**). Coagulation factors II (prothrombin), VII, IX and X and proteins C and S, the vitamin K–dependent plasma proteins involved in hemostasis, have been those most extensively studied. For these proteins, the ability of the Gla residues to bind to Ca^{2+} ions and promote a protein-Ca^{2+}-phospholipid interaction has been shown to be essential to their biological activity (Esmon et al. 1975b). Two of the best characterized vitamin K–dependent proteins not involved in hemostasis are osteocalcin or bone Gla protein (BGP)[3] and matrix Gla protein (MGP), which were initially

[3]The terms osteocalcin and bone Gla protein (BGP) have been used interchangeably as names for the protein. Because osteocalcin is the term most often used in clinical studies, it will be used in this review.

discovered as components of calcified tissue. However, tissue specificity of their expression is not absolute. For example, the mRNA for MGP is expressed in a variety of non-hepatic tissues, including lung, kidney and spleen (Fraser and Price 1988). In addition, the presence of osteocalcin in vascular smooth muscle cells and osteocalcin mRNA production in platelets have recently been reported (Severson et al. 1994, Thiede et al. 1993).

Osteocalcin (**Fig. 3**) is a low-molecular-weight protein (49–50 residues, depending upon species) containing three Gla residues that give the protein its mineral-binding properties (Price 1988). It accounts for 15–20% of the non-collagen protein in the bone of most vertebrates and is one of the most abundant proteins in the body. Osteocalcin is produced by osteoblasts during bone matrix formation (Nishimoto and Price 1980) and appears in bone with the onset of hydroxyapatite deposition. Its synthesis is increased by 1,25-dihydroxyvitamin D (Price and Baukol 1980, Weintraub et al. 1987), and its concentration in bone is directly proportional to the amount of calcium in bone (Hauschka and Reid 1978, Lian et al. 1982). A small fraction of newly synthesized osteocalcin is not incorporated into bone but is released into the circulation, where it may be used as a measure of bone formation (Delmas et al. 1985).

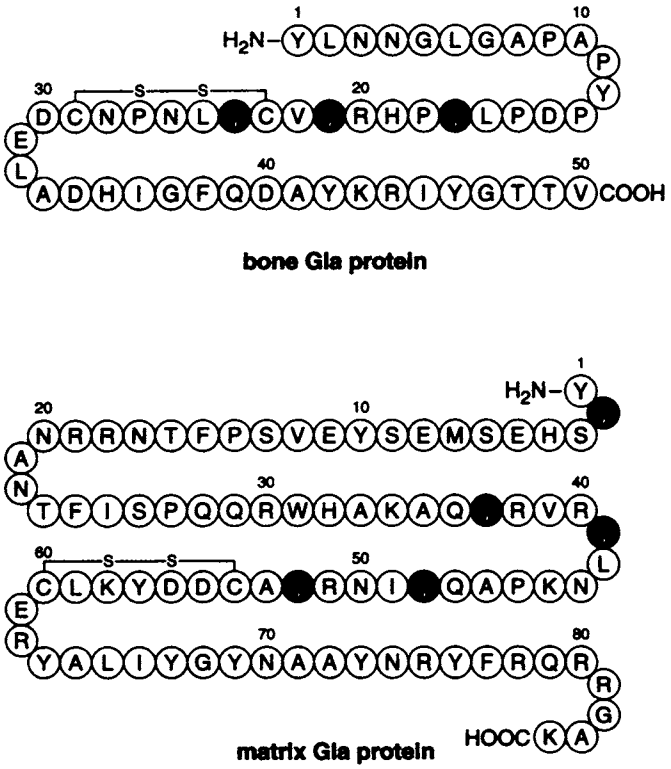


FIGURE 3 Structure of Osteocalcin (BGP) and Matrix Gla-protein (MGP). The amino acid sequence shown is for the rat protein (Price 1988), and there is appreciable sequence identity between the BGP and MGP of most species. There is also sufficient sequence identity between BGP and the carboxyterminal 42 residues of MGP to indicate that this region of MGP must have arisen from a common ancestor. Abbreviations used: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; γ, γ-carboxyglutamic acid.

Many studies have found that circulating osteocalcin concentrations increase with advancing age (Delmas et al. 1983, Johansen et al. 1987, Orwoll and Deftos 1990), although this is not a consistent finding (Vanderschueren et al. 1990). There are now numerous literature reports of circulating osteocalcin concentrations in normal individuals and those in various disease states. Extreme caution should be used in comparing absolute serum osteocalcin concentrations from different laboratories. Masters et al. (1994) compared serum osteocalcin concentrations measured with eight different commercial immunochemically based osteocalcin kits. The mean (range) serum osteocalcin concentration in nine healthy adults varied from 0.6 (0.3–0.9) to 3.4 (2.4–4.5) nmol/L. It is also now well established (Garnero et al. 1994) that the immunochemically based assays that are used to measure osteocalcin also measure proteolytic degradation products of osteocalcin to varying degrees. These fragments may represent the majority of the circulating immunoreactive material and can also be generated by proteolytic action in improperly stored samples.

Although its abundance, mineral-binding properties and regulation by vitamin D suggest an important role of osteocalcin in bone metabolism, the physiological role of osteocalcin remains unknown (Price 1993). Reduced osteocalcin content of cortical bone (Vanderschueren et al. 1990) and alteration of osteocalcin distribution within osteons (Ingram et al. 1994) are associated with advancing age. It remains unknown whether any of these findings are related to the well-established age-related increased risk of fracture. It is possible that osteocalcin plays a role in the control of bone remodeling because it has been reported to be a chemoattractant for monocytes, the precursors of osteoclasts (Malone et al. 1982, Mundy and Poser 1983). This suggests a possible role for osteocalcin in bone resorption. Supporting this are the observations that osteocalcin-deficient bone particles implanted subcutaneously into rats are poorly resorbed (Glowacki and Lian 1987). In addition, normal bone particles are preferentially resorbed in the presence of osteocalcin-deficient bone particles (Defranco et al. 1991). Thus osteocalcin may be a matrix signal for the recruitment and differentiation of osteoclasts (Glowacki et al. 1991). This suggests that osteoclast recruitment in osteocalcin-deficient bone would be impaired, possibly leading to impaired remodeling. Consistent with this hypothesis is a report of a reduction in bone turnover in warfarin-treated lambs (Pastoureau et al. 1993).

Matrix Gla protein (Fig. 2) is a 79-residue protein containing five Gla residues (Price and Williamson 1985). It is present in high levels in bone and cartilage, and its mRNA is also expressed in a variety of other tissues (Fraser and Price 1988). Because long-term warfarin treatment in rats results in closing of the epiphysial growth plate (Price et al. 1982) and because MGP is present in cartilage, it has been proposed that MGP may inhibit cartilage mineralization (Price 1989) and that impairment of MGP function allows growth plate calcification. Consistent with this, it has been suggested that impairment of MGP function may be in part responsible for the excessive mineralization associated with warfarin embryopathy (Hall et al. 1980, Price 1989). However, given the widespread tissue distribution of MGP, it is unlikely that its physiological role is restricted to mineralized tissue.

Protein S is a vitamin K–dependent plasma protein (DiScipio and Davie 1979) that plays an anticoagulant, rather than a procoagulant, role in blood coagulation (Davie et al. 1991). Two children with typical inherited protein S deficiency have been reported (Pan et al. 1990) to have severe osteopenia, thus raising the possibility that protein S could be important in bone metabolism. Subsequently, the secretion of protein S by osteoblasts in vitro, the reduction of this secretion by warfarin, and the presence of protein S in bone matrix have been reported by Maillard et al. (1992). Therefore protein S also has the potential to be involved in the regulation of bone turnover.

It has generally been assumed that any influence of vitamin K on bone metabolism is mediated through BGP or MGP. However, it is possible that undiscovered vitamin K–dependent proteins in bone might be involved in any response that is eventually verified. It is also possible that an unidentified vitamin K–dependent protein mediating vitamin D or parathyroid hormone action or in some other way altering urinary calcium excretion could be involved in some of the responses that have been reported. An understanding of what specific vitamin K–dependent proteins are involved in any effect of vitamin K status on bone metabolism will not be possible until the responses themselves are more clearly defined.

RELATIONSHIP BETWEEN VITAMIN K STATUS AND BONE HEALTH

Circulating and bone vitamin K concentrations. Supporting a role of vitamin K in bone metabolism, the concentrations of phylloquinone, MK-6, MK-7 and MK-8 in the femoral neck bone, expressed on the basis of dry bone matrix, were recently reported (Hodges et al. 1993b) to be similar to hepatic concentrations. Among the observations suggesting a possible relationship between vitamin K status and osteoporosis have been reports of reduced plasma concentrations of phylloquinone, MK-7 and MK-8 in patients with recent femoral fractures or prior vertebral compression fractures (Hart et al. 1985, Hodges et al. 1991). Serum concentrations of phylloquinone, MK-7 and MK-8 in elderly women after hip fracture were also found to be significantly lower than those in age-matched healthy controls (Hodges et al. 1993a). A reduction in plasma MK-8 concentrations, but not in MK-7 or phylloquinone concentrations, has also been reported in a population of elderly subjects (Hodges et al. 1990) without reference to bone health. Because plasma phylloquinone concentrations reflect recent dietary intake (Ferland et al. 1993), the importance of these observations relative to both tissue vitamin K status and to the pathogenesis of osteoporosis is questionable. Furthermore, the physiologic importance of circulating concentrations of menaquinones in humans is also uncertain. The extent to which menaquinones satisfy the human vitamin K requirement is not known, but menaquinone utilization is clearly inadequate to prevent findings of vitamin K insufficiency in individuals with low dietary phylloquinone intake (Ferland et al. 1993, Suttie et al. 1988).

Overall, although these data raise the possibility that vitamin K insufficiency might be involved in the pathogenesis of osteoporosis or in the development of bone fragility, they may simply be indicative of the general nutritional insufficiency of osteoporotic individuals (Delmi et al. 1990, Older et al. 1980, Rico et al. 1993). These possibilities are, of course, not mutually exclusive.

Under-γ-carboxylated osteocalcin. Early studies of circulating osteocalcin demonstrated that its ability to be adsorbed to a calcium phosphate (hydroxyapatite) gel depended on vitamin K status of the animal. Serum osteocalcin both in experimental animals treated with the vitamin K antagonist warfarin (Price et al. 1980) and in patients treated with oral anticoagulant (coumadin) (Van Haarlem et al. 1988) is less readily adsorbed to hydroxyapatite. On the basis of these observations it is apparently assumed by all investigators that the fraction of serum osteocalcin not adsorbed to hydroxyapatite is under-γ-carboxylated (ucOC).[4] However, there is currently no information that would define the degree of under-γ-carboxylation (1, 2 or 3 Glu rather than Gla residues) needed to render the molecule incapable of being adsorbed. By utilizing specific monoclonal antibodies, assays for both the fully γ-carboxylated (Koyama et al. 1991) and the under-γ-carboxylated

[4]The abbreviation ucOC is a functional definition and does not imply knowledge of the degree of under-γ-carboxylation of the osteocalcin pool.

form (Vergnaud et al. 1993) of osteocalcin have been developed. Although there is still some question of their specificity and they are not yet widely used, the availability of these techniques will aid in assessing the relationship between vitamin K status and bone health.

It has generally been assumed that the osteocalcin pool in normal vitamin K–sufficient subjects is fully γ-carboxylated. This may not be the case. Cairns and Price (1994) recently reported that γ-carboxylation in the osteocalcin isolated from the bone of 20 individuals was not complete. Undercarboxylation averaged 33% at residue 17, 12% at residue 21, and 7% at residue 24. These observations will have to be considered as more detailed information on the influence of vitamin K status on osteocalcin carboxylation becomes available.

The concentration of circulating ucOC has been reported to increase with advancing age in women (Knapen et al. 1989, Pantalech et al. 1991), and administration of vitamin K has been reported to return the concentration of ucOC to normal (Knapen et al. 1989, Plantalech et al. 1990). This occurred in the absence of any coagulation defect, which suggests that the under-γ-carboxylation of osteocalcin may be a more sensitive measure of vitamin K sufficiency. It also implies that osteoblast vitamin K supply or utilization is impaired and that a subclinical vitamin K insufficiency may be relatively common in older women. The corollary to this, that a dietary restriction of vitamin K results in an increased fraction of ucOC in the serum osteocalcin pool, has not yet been reported. This correlation of ucOC with age may be gender based, as a more recent study (Bach 1994) has reported a lower, rather than higher, fraction of ucOC in the serum of an older male (55- to 75-y-old) population than in a younger male (20- to 28-y-old) population. Serum osteocalcin and the extent of its carboxylation have also been proposed as a marker of vitamin K status in pregnant women and their newborn babies (Kon-Siong et al. 1992).

Serum ucOC concentration has been reported to be negatively correlated with bone mineral density (Szulc et al. 1994), and elevated concentrations of ucOC have been associated with an increased risk of hip fracture (Szulc et al. 1993). These observations indicate an association between vitamin K insufficiency and reductions of bone density and (possibly) bone strength. Furthermore, an inverse correlation between serum concentrations of 25-hydroxyvitamin D and ucOC was recently noted (Szulc et al. 1993). It has previously been established at 1,25-dihydroxyvitamin D elicits a prompt increase in osteoblast osteocalcin synthesis (Price and Baukol 1980). If elevated concentrations of ucOC are in fact indicative of vitamin K insufficiency, it is unclear how vitamin D supplementation would reduce circulating concentrations of ucOC, and this potential interaction of vitamins K and D requires further study. These reports of a relationship between ucOC and bone health are of obvious interest; however, they must be substantiated by further epidemiological studies and by intervention protocols before the association between ucOC and bone health can be considered firmly established.

There is no satisfactory explanation at this time for the reported association between elevated ucOC concentrations and hip fracture. However, it has been suggested that accumulation of bone fatigue microdamage may lead to increased fracture risk (Heaney 1993a). The initial step in microdamage repair is osteoclastic resorption (Mori and Burr 1993), which potentially involves osteocalcin. Thus it could be hypothesized that osteocalcin-deficient bone might have an impairment of microdamage repair, leading to increased fragility and increased fracture risk. Although there are no data to support this hypothesis, this is an area that is open to investigation.

Effects of oral anticoagulants on bone. The commonly prescribed oral anticoagulants are vitamin K antagonists of the 4-hydroxycoumarin type (Fig. 1). They inhibit Gla synthesis by an inhibition of the reduced thiol-linked vitamin K epoxide reductase and quinone reductase activities that are necessary for the regeneration of reduced vitamin K from vitamin K epoxide. They therefore create a functional deficiency of vitamin K (Suttie 1990) in the presence of adequate amounts of vitamin. The large anticoagulant-treated patient population should provide important information on the role of vitamin K in bone metabolism. However, studies of the effects of vitamin K antagonists on bone density in adult humans have reported mixed results. Houvenagel et al. (1989) found no difference in spine or hip bone mineral density in 12 patients who were administered warfarin, and Piro et al. (1982) reported normal radial bone mass in a multiracial group of 17 patients treated with coumadin. Rosen et al. (1993) also measured bone mineral density of the spine and hip in a group of 50 patients receiving warfarin for >1 y and found no effect on bone mineral density.

In contrast to these reports, adverse effects have been observed. Resch et al. (1991) measured radial bone mineral density in 78 patients treated with phenprocoumon for various indications, primarily pulmonary embolism and deep venous thrombosis, and found a significant decrease in bone mineral density compared with a control population. In a preliminary report, Monreal et al. (1991) found a decrease in both lumbar spine and femur bone mineral density over a 3-mo period in patients receiving coumadin for deep vein thrombosis of the legs. Finally, Fiore et al. (1990) reported a decrease in radial bone mineral density in 56 women who had been treated with acenocumarol after cardiac valve replacement.

On the basis of these reports, which are summarized in **Table 1**, the effect, if any, of vitamin K

TABLE 1

Studies of the influence of anticoagulant therapy on bone mineral density (BMD)

Author	No. of patients	Length of therapy	Method[1]	Site	Anticoagulant	Result
Houvenagel et al. 1989	12	8.3 y	DPA	Spine and hip	Warfarin	No effect
Rosen et al. 1993	50	7.7 y	DXA	Spine and hip	Warfarin	No effect
Piro et al. 1982	17	8.9 y	SPA	Radius	Warfarin	No effect
Resch et al. 1991	78	1 y	SPA	Radius	Phenprocoumon	Reduced BMD
Monreal et al. 1991	28	3 mo	DXA	Spine and hip	Warfarin	Reduced BMD
Fiore et al. 1990	56	1–5 y	SPA	Radius	Acenocumarol	Reduced BMD

[1]Abbreviations used: DPA, dual photon absorptiometry; DXA, dual energy X-ray absorptiometry; SPA, single photon absorptiometry.

antagonists on bone mineral content is not resolved. All of these studies are compromised by the fact that vitamin K antagonists are often utilized clinically in individuals with chronic cardiac disease. A diagnosis of chronic cardiac disease likely to influence physical activity, and reductions in physical activity adversely affect bone density (LeBlanc et al. 1990). It may be important that the length of anticoagulant therapy in the population in which decreased bone mineral density was observed was much shorter than in the studies that reported no effect. Perhaps these individuals had more severe illnesses. Currently, there are no data that address an alteration in the fracture risk in patients receiving vitamin K antagonists.

Oral anticoagulant therapy has, however, been reported to have other effects on calcium metabolism and mineralization. A retrospective study (Buschbacher et al. 1992) of 227 patients at risk of hetero-topic ossification because of spinal cord injury found that none of the 33 patients who had developed hetero-topic ossification were in the subgroup of 34 patients who had also received warfarin for other indications. Warfarin has also been reported to effectively treat calcinosis in a patient with systemic sclerosis (Yoshida and Torikai 1993). The mechanism of these reported effects is unknown.

Vitamin K–dependent carboxylase enzymes are present in renal tubule cells (Friedman et al. 1982) and are warfarin sensitive (Karl and Friedman 1985). Furthermore, γ-carboxylated proteins such as nephrocalcin are present in the kidney (Nakagawa et al. 1984). Thus, vitamin K status could potentially have effects on urinary calcium excretion. In this regard, experimental vitamin K deficiency in rats has been reported by Roberts et al. (1985) to produce hypercalciuria. Kon-Siong et al. (1993) investigated the effect of vitamin K supplementation or warfarin treatment on urinary calcium excretion in human subjects. They found that supplementation of a group of postmenopausal women with 1 mg phylloquinone/d decreased urinary calcium excretion in the subpopulation that had pre-existing enhanced Ca excretion ("fast losers") but had no influence on the rest of the population. Acenocoumarol administration increased urinary calcium excretion in young males only and had no effect in a group of elderly females in which the pre-existing incidence of "fast losers" was >30%. However, the association of warfarin with calcium loss in male subjects was not noted in a recent report of Worcester et al. (1993), who found no increase in urinary calcium excretion in men receiving warfarin. Thus, although it may be prudent to consider oral anticoagulant use as a potential risk factor in the development of osteoporosis, no firm conclusion can be drawn from the available data, and further study is needed to clarify this issue.

Studies of anticoagulant administration to experimental animals have also yielded conflicting skeletal results. Warfarin has been reported to have either no effect (Einhorn et al. 1988, Price and Williamson 1981) or an adverse effect (Dodds et al. 1984) on fracture healing. In early studies of the potential role of osteocalcin in bone (Price et al. 1980), young rabbits were treated with warfarin for 50 d and given an intravenous clotting factor preparation to prevent hemorrhage. Although the concentration of osteocalcin in bone was reduced to 5% of normal, no clinical or morphological alterations of bone were observed.

Price and Williamson (1981) subsequently demonstrated that concurrent administration of warfarin and phylloquinone to rats effectively inhibits carboxylation of osteocalcin, but allows normal synthesis of the vitamin K–dependent clotting factors. This finding allows long-term studies of the role of osteocalcin without the complications of hemorrhagic episodes. Price and Kaneda (1987) have suggested that osteoblasts might lack the coumarin-insensitive pathway for vitamin K quinone reduction present in liver, and this would explain the effectiveness of this protocol. A confirmation of this hypothesis by Ulrich et al. (1988) has been contradicted by a second study (Wallin et al. 1990), and the basis for the response is not yet clear. Utilizing this protocol, Price and Williamson (1981) found that warfarin had effect on bone

size, morphology, mineralization or fracture healing. However, in a subsequent study (Price et al. 1982), an adverse mineralization effect, premature closing of the epiphysial growth plate, was demonstrated in warfarin-treated rats. Adverse effects of warfarin treatment on bone have also been observed in other species. Pastoreau et al. (1993) reported that concomitant administration of warfarin and vitamin K to growing lambs led to reductions in cancellous bone area, eroded surfaces, and bone formation rate of iliac crest biopsies. Bone mineral density was not measured in this study, but bone turnover, as assessed by activation frequency, was also reduced. These authors postulated that these responses were caused by the depletion of the vitamin K–dependent proteins from bone. If osteocalcin is important in osteoclast recruitment, reduction of osteoclast-mediated resorption might be expected to lead to a reduction in bone formation by the well-recognized coupling phenomenon (Parfitt 1982).

The effect of vitamin K antagonists on circulating concentrations of osteocalcin seems to be species specific. In rats, vitamin K antagonists do not reduce serum osteocalcin concentrations (Price and Williamson 1981). In contrast, administration of vitamin K antagonists does reduce circulating osteocalcin concentrations in both humans and sheep (Van Haarlem et al. 1988). Because circulating osteocalcin is known to be representative of bone-forming activity, this could be indicative of a reduction in osteoblast function. It is unclear why these species differences are seen. However, normal concentrations of osteocalcin in the bone and plasma of humans are only ~5% of those of rats, and concentrations in sheep are nearer to those in humans than to those in rats. At this time, utilization of animal models has not adequately defined the effects of vitamin K insufficiency on the skeleton, and further work in this area will be required.

Vitamin K supplementation and bone. Supplementation with 1 mg/d of phylloquinone has been reported to return serum concentration of undercarboxylated osteocalcin to normal in a postmenopausal (55- to 75-y-old) population (Knapen et al. 1989). A subgroup of 11 of the 50 women in this study had elevated ratios of urinary hydroxyproline:creatinine and urinary calcium:creatinine, consistent with rapid bone turnover. Vitamin K supplementation led to significant reductions in both urinary calcium and hydroxyproline excretion in this subgroup, suggesting a decrease in bone resorption. However, when this entire population was considered, there was no significant effect of vitamin K supplementation. Assessment of effect of an intervention on a subgroup of the study population is appropriate given the heterogeneity of the osteoporosis syndrome.

In patients with established osteoporosis, Orimo et al. (1992) found that treatment with MK-4 (45 mg of menatetrenone daily) for 48 wk resulted in an increase in metacarpal bone mineral density, increased serum osteocalcin, and reduced urinary calcium excretion. In this report, the lumbar spine bone mineral density was not affected by vitamin K treatment. This amount of vitamin K represents a pharmacological dose rather than a nutritional supplement. In an extension of this study, it was reported that there was a more favorable response in metacarpal bone mineral density in a subpopulation of individuals with high bone turnover as assessed by urinary hydroxyproline: creatinine ratio (Orimo et al. 1993). A hypothetical mechanism to explain these findings, which are currently available only in abstract form, would be the presence of a renal vitamin K–dependent protein whose activity decreased if sufficient vitamin K was not present, thus leading to excessive calcium loss in the urine. This would lead to secondary hyperparathyroidism, increased bone turnover, and ultimately to increased urinary hydroxyproline excretion that would be corrected by vitamin K supplementation. However, Orimo did not find that vitamin K administration reduced serum parathyroid hormone concentration. These preliminary human studies suggest that pharmacologic doses of MK-4 may have an anti-resorptive effect on bone, but substantial further investigation in this area is warranted.

Vitamin K treatment has been reported to be of value in prevention of osteoporosis in an animal model. In rats, pharmacological oral doses (~20–30 mg/kg body wt) of MK-4 have been reported to reduce the loss of both bone mineral density and bone strength in estrogen depletion or corticosteroid-induced bone loss models (Akiyama et al. 1993, Hara et al. 1993). These are straightforward experimental protocols, and the validity of these initial reports should be easily verified or rejected by additional studies.

In vitro studies have also suggested an effect of vitamin K supplementation upon osteoblasts and osteoblast-like cells. Nishimoto and Price (1985) have shown that secretion of osteocalcin by cultured osteoblasts is decreased 50% in the presence of warfarin. Akedo et al. (1992) found that MK-4 administration suppressed proliferation and increased alkaline phosphatase activity of cultured osteoblast-like cells. Pre-treatment with warfarin partially abolished these effects (Hosoi et al. 1993). These authors concluded that vitamin K has an anabolic effect upon osteoblasts rather than a mitogenic effect and suggested that γ-carboxylation is involved in the mechanism of action. Supplementation with MK-4 has also been reported to have a positive effect in an in vitro bone mineralization system (Koshihara et al. 1992).

SUMMARY

Although a number of reports suggest that vitamin K could be involved in the pathogenesis of bone mineral loss and skeletal fragility, few definite conclusions can be drawn at this time. The observations

TABLE 2

Observations relating vitamin K status to osteoporosis

1. Low concentrations of circulating vitamin K in patients with bone fractures
2. Concentration of circulating under-γ-carboxylated osteocalcin associated with age, low bone mineral density, and hip fracture risk
3. Anticoagulant therapy associated with decreased bone density
4. Vitamin K supplementation decreases bone loss and calcium excretion

that support this hypothesis are summarized in **Table 2**. At the present time there is a definite lack of agreement among published studies, and some of the positive reports have not been substantiated by further studies.

Some studies have reported that serum concentrations of vitamin K are reduced in populations at risk of osteoporosis or in individuals with fractures. These findings may simply reflect generalized inadequate nutritional status; however, there have been no studies comparing the vitamin K status of the osteoporotic fracture population relative to other traumatized patients.

A possible association between subclinical vitamin K deficiency and the development of osteoporosis cannot be excluded by the current data. Elevated concentrations of undercarboxylated osteocalcin (indicating a functional osteoblast vitamin K insufficiency) have been reported with advancing age in females and have been associated with a reduction in bone mineral density and increased risk of hip fracture in some studies. Thus vitamin K deficiency might be associated with increased skeletal fragility. There is no doubt that the decreased extent of osteocalcin carboxylation can be reversed by vitamin K supplementation, but as of yet there are no studies that have established a causal relationship between vitamin K deficiency and reduced bone mineral density or increased fracture rate. Studies of the large anticoagulant-treated patient population should answer this question, but from the available reports it remains unclear whether patients receiving vitamin K antagonists experience adverse skeletal consequences. The widespread clinical utilization of anticoagulant therapy and the possibility of adverse skeletal effects require that this issue be resolved.

Studies of both human subjects and animals have suggested that increased vitamin K intake can influence measures of bone mineral density or calcium excretion. Interpretation of these studies is complicated by the pharmacological doses of the vitamin utilized in some studies and by the restriction of positive effects to subgroups of the population in others. It may be that vitamin K in pharmacologic doses has an anti-resorptive effect and as such could potentially be utilized in the treatment or prevention of osteoporosis in some individuals.

Much in the area of vitamin K and bone health remains unclear. It is plausible that vitamin K insufficiency is much more common than is currently thought and that it does contribute to the pathogenesis of osteoporosis and fracture. However, the role of vitamin K in promoting skeletal health, if there is one, remains undefined, and further research is definitely indicated.

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