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Vitamin K and bone health

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Vitamin K, originally recognised as a factor required for normal blood coagulation, is now receiving more attention in relation to its role in bone metabolism. Vitamin K is a coenzyme for glutamate carboxylase, which mediates the conversion of glutamate to γ-carboxyglutamate (Gla). Gla residues attract Ca^{2+} and incorporate these ions into the hydroxyapatite crystals. There are at least three Gla proteins associated with bone tissue, of which osteocalcin is the most abundant and best known. Osteocalcin is the major non-collagenous protein incorporated in bone matrix during bone formation. However, approximately 30 % of the newly-produced osteocalcin stays in the circulation where it may be used as an indicator of bone formation. Vitamin K deficiency results in an increase in undercarboxylated osteocalcin, a protein with low biological activity. Several studies have demonstrated that low dietary vitamin K intake is associated with low bone mineral density or increased fractures. Additionally, vitamin K supplementation has been shown to reduce undercarboxylated osteocalcin and improve the bone turnover profile. Some studies have indicated that high levels of undercarboxylated osteocalcin (as a result of low vitamin K intake?) are associated with low bone mineral density and increased hip fracture. The current dietary recommendation for vitamin K is 1 μg/kg body weight per d, based on saturation of the coagulation system. The daily dietary vitamin K intake is estimated to be in the range 124–375 μg/d in a European population. Thus, a deficiency based on the hepatic coagulation system would be unusual, but recent data suggest that the requirement in relation to bone health might be higher.

Vitamin K: Bone health: Man: Osteocalcin

Vitamin K, originally recognised as a factor required for normal blood coagulation, is now receiving more attention for its role in bone metabolism. The vitamin is involved in activation of a number of enzymes, at least three of which are active in bone. There is accumulating evidence that the current intake of vitamin K is too low for optimal bone health, but the optimal requirement is still unknown. The present paper is a brief overview of the current knowledge in relation to vitamin K and bone health.

Structure and function of vitamin K

Vitamin K is a series of structurally-related compounds that can be classified into two groups (Fig. 1): phylloquinone (vitamin K_1), the major form occurring in plants, and the menaquinones (vitamin K_2), which are synthesised by bacteria. The menaquinones are a family of compounds with a varying number of repeating prenyl units of the side chain, the number being given as a suffix, i.e. menaquinone-n.

Vitamin K plays an essential role in the post-translational conversion of specific glutamyl residues to γ-carboxyglutamyl (Gla) residues in a limited number of proteins (Suttie, 1992). These proteins include plasma prothrombin (coagulation factor II) and the plasma pro-coagulants, factors VII, IX and X. Three vitamin K-dependent proteins in bones, osteocalcin, matrix Gla protein and protein S, have received recent attention as proteins with possible roles in the prevention of chronic disease (Vermeer *et al*. 1995). Osteocalcin is exclusively synthesised by the osteoblasts, while matrix Gla protein and protein S are also synthesised in several soft tissues, including chondrocytes, vascular smooth muscle cells and epithelium (Shanahan & Weissberg, 1998; Yagami *et al*. 1999). Osteocalcin appears to be a negative regulator of bone formation, whereas matrix Gla protein seems to be a strong inhibitor of tissue calcification (Luo *et al*. 1997; Karsenty, 1998).

Abbreviations: BMD, bone mineral density; Gla, γ-carboxyglutamate.

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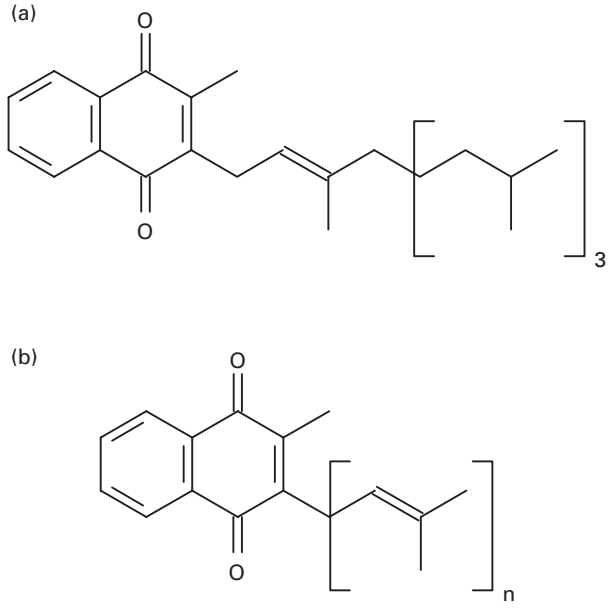

Fig. 1. The structures of (a) phylloquinone and (b) the menaquinones.

Natural sources of vitamin K

Natural sources of phylloquinone are green leafy vegetables and some vegetable oils, while the menaquinones can be found in dairy products such as cheese and fermented soyabean products (Schurgers *et al*. 1999). Recently, databases for vitamin K have been published in the literature (Booth *et al*. 1993; Shearer & Bolton-Smith, 2000). Based on these tables the vitamin K intake for the elderly Dutch population has been calculated to be 124–375 μg/d for phylloquinone and 10–45 μg/d for the menaquinones (Schurgers *et al*. 1999), and for the Framingham cohort study to be 59–262 μg/d for phylloquinone (McKeown *et al*. 2002). As a result of poor extraction of phylloquinone from green leafy vegetables the absorption is only 5–15 % of the total vitamin K ingested, whereas the menaquinones are taken up almost completely (Gijsbers *et al*. 1996). Intestinal bacteria provide some menaquinones in addition to dietary sources (Conly & Stern, 1992), although the actual amounts are not known (Lipsky, 1994). As a result of the differences in absorption, phylloquinone and the menaquinones may contribute equally to the vitamin K status in human subjects (Vermeer & Braam, 2001), despite the high differences in intake. Animal studies indicate that the tissue distributions also differ; the main target organ for phylloquinone seems to be the liver, but some tissues prefer menaquinone and are able to convert phylloquinone to menaquinone when the intake of menaquinone is low (Davidson *et al*. 1998; Ronden *et al*. 1998*a*). What consequences this finding has for bone health is still unknown.

Vitamin K recommendations

The classical sign of vitamin K deficiency has been an increase in prothrombin time (i.e. the coagulation time for plasma after the addition of thrombokinase and Ca^{2+}; if prothrombin activity is low coagulation takes longer) and in severe cases a haemorrhagic event. Various other indicators have been used to assess vitamin K status in human subjects (Booth & Suttie, 1998), but only prothrombin time has been associated with adverse clinical effects. On this basis an average daily intake of 1 μg/kg body weight has been assumed to be sufficient to maintain optimal health (Nordic Council of Ministers, 1996). However, at low vitamin K intakes the liver has been shown to be the principal target tissue for vitamin K, whereas at higher intakes other tissues also accumulate vitamin K (Ronden *et al*. 1998*b*). It is likely, therefore, that intakes well above the recommended dietary allowance are required for adequate carboxylation of extrahepatic Gla proteins (Vermeer & Braam, 2001). Low intakes of vitamin K increase the percentage of undercarboxylated osteocalcin, which has been associated with increasing fracture risk (Feskanich *et al*. 1999). Thus, mild vitamin K deficiency could be defined as the condition in which the circulating osteocalcin is substantially undercarboxylated, but prothrombin is normal, while severe vitamin K deficiency may be regarded as a condition in which descarboxyprothrombin is also detectable in the circulation (Vermeer & Schurgers, 2000).

Vitamin K and bone

The first indications that vitamin K-dependent proteins are involved in bone metabolism were found in the mid-1970s, when serious bone malformation was observed in children born to women who had been treated with vitamin K antagonists during the first trimester of pregnancy (Pettifor & Benson, 1975). Hart *et al*. (1985) reported that patients with osteoporosis who had sustained an acute hip fracture or suffered from a chronic spinal crush fracture had lower serum phylloquinone levels than control subjects. These findings have led to a substantial increase in the research conducted in this field, and many of the studies that have been published in the last 25 years have indicated that long-term low vitamin K intake or the use of vitamin K antagonists in human subjects may lead to impaired bone quality.

Several population studies have shown that low dietary or circulating vitamin K levels or high levels of undercarboxylated osteocalcin are associated with low bone mineral density (BMD) and increased fractures (Hodges *et al*. 1991, 1993; Jie *et al*. 1996; Szulc *et al*. 1996; Knapen *et al*. 1998; Booth *et al*. 2000, 2003; Schoon *et al*. 2001). Other studies have shown that vitamin K reduces the levels of undercarboxylated osteocalcin (Knapen *et al*. 1963; Douglas *et al*. 1995), reduces urinary Ca excretion (Knapen *et al*. 1993) and improves the bone turnover profile (Vermeer *et al*. 1996; Craciun *et al*. 1998). Vitamin K has also been postulated to be sequestered from the circulation for use at fracture sites, as circulating levels of phylloquinone and menaquinones are depressed in elderly women who have sustained hip fractures (Hodges *et al*. 1993).

The epidemiological data used to support the association between dietary vitamin K and fracture risk have been criticised for the potential confounding effect of overall poor nutritional status (Binkley & Suttie, 1995). Thus, well-designed placebo-controlled intervention studies were and

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are still needed to confirm the interaction between vitamin K and fracture risk.

A number of intervention studies have already confirmed the observations from the population studies. Supplementation with phylloquinone in doses ranging from 80 µg/d to 10 mg/d reduced the levels of undercarboxylated osteocalcin (Plantalech *et al*. 1991; Douglas *et al*. 1995; Sokoll *et al*. 1997; Craciun *et al*. 1998; Binkley *et al*. 2000; Schaafsma *et al*. 2000). The response, in terms of undercarboxylated osteocalcin concentration, is observed within a few days of vitamin K supplementation. One study found an increase in bone-specific alkaline phosphatase (Knapen *et al*. 1989), a bone formation marker, and other studies have found that the addition of vitamin K to the habitual diet also results in decreases in urinary Ca and hydroxyproline excretion (Plantalech *et al*. 1991; Craciun *et al*. 1998).

Most long-term studies investigating the effect of vitamin K on bone strength have used high doses of menaquinones and were carried out in Asian populations (for review, see Weber, 2001). Supplementation with menaquinones alone maintained BMD in post-menopausal women, while supplementation with menaquinones and cholecalciferol together increased BMD over a 2-year period (Ushiroyama *et al*. 2002). So far, only one study has investigated the effect of phylloquinone on BMD in post-menopausal women at an intake that is achievable from the diet (Braam *et al*. 2003). Supplementation with phylloquinone for 2 years protected against bone loss (Braam *et al*. 2003).

Vitamin K–vitamin D interactions

Although the percentage of undercarboxylated osteocalcin is a sensitive biochemical indicator of vitamin K nutritional status, it has been suggested that the relationship with hip fracture reflects a combination of both poor vitamin K status and poor vitamin D status (Szulc *et al*. 1993; Booth *et al*. 1999). In women with low BMD there was a greater increase in the percentage of carboxylated osteocalcin and in BMD when they received supplements of both vitamins D and K compared with vitamin K alone (Schaafsma *et al*. 2000; Ushiroyama *et al*. 2002).

1,25-Dihydroxyvitamin D directly induces the synthesis of osteocalcin by promoting transcription of its gene (Lian *et al*. 1989). Animal and cell studies have shown that vitamin D induces osteocalcin synthesis and vitamin K is responsible for post-translational activation. One study showed that menaquinones induce *in vitro* mineralisation, but in the presence of vitamin D, they enhance vitamin D-induced mineralisation and thereby facilitate Ca deposition, followed by mineralisation (Koshihara *et al*. 1996). Menaquinones have been shown to enhance osteocalcin mRNA expression and production in the presence of 1,25-dihydroxycholecalciferol, whereas alone they have no effect on mRNA expression, although a small amount of osteocalcin is released into the cell-culture medium (Koshihara *et al*. 1996). In a study using ovariectomised rats vitamins K and D also seemed to have a synergistic effect in reducing bone loss (Matsunaga *et al*. 1999). Feskanich *et al*. (1999) found an increased risk of hip fracture in women who had a low vitamin K intake but a high vitamin D intake. Theoretically, the effects of the two vitamins on Ca homeostasis may explain this finding. Vitamin D acts as an inducer of bone resorption and thus higher intakes may result in increased bone turnover and increased urinary Ca excretion. On the other hand, results from some animal (Scholz-Ahrens *et al*. 1996) and human (Jie *et al*. 1993) studies indicate that vitamin K decreases urinary Ca excretion. Thus, despite high intakes of vitamin D there may be an increased risk of hip fractures when vitamin K intake is low (Feskanich *et al*. 1999). Furthermore, vitamin D induces synthesis of osteocalcin, but if vitamin K intake is low the osteocalcin remains undercarboxylated, thereby increasing the levels of undercarboxylated osteocalcin in blood and bone.

Osteoporosis is a multifactorial chronic disease and a number of macro- and micronutrients are involved in the maintenance of bone health. Recently, it has become evident that vitamin K has an important role to play in this context. Most of the present data has been derived from studies using high doses of menaquinones in Asian populations. However, the few intervention studies performed with lower doses of phylloquinone have shown that vitamin K given together with vitamin D increases BMD, probably through activation of the bone Gla proteins. However, information on the exact role of the bone Gla protein is still scarce. Furthermore, the possibility that different tissues have preferences for specific vitamers of vitamin K needs to be investigated. So far, there is no information on the form or the levels of vitamin K required to achieve optimal bone health. There are a number of ongoing trials that may answer some of the questions relating to vitamin K and bone health that remain unanswered.

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