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Effect of vitamin K in bone metabolism and vascular calcification: a review of mechanisms of action and evidences

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ABSTRACT

Osteoporosis is a public health concern associated with an increased risk of bone fractures and vascular calcification. Vitamin K presents unique benefits on these issues, although understudied. The two main forms of vitamin K are phylloquinone (vitamin K₁) and menaquinone (vitamin K₂). In this study, it was especially investigated the action of vitamin K₂ in bones and vessels. Vitamin K₂ has shown to stimulate bone formation by promoting osteoblast differentiation and carboxylation of osteocalcin, and increasing alkaline phosphatase, insulin-like growth factor-1, growth differentiation factor-15, and stanniocalcin 2 levels. Furthermore, vitamin K₂ reduces the pro-apoptotic proteins Fas and Bax in osteoblasts, and decreases osteoclast differentiation by increasing osteoprotegerin and reducing the receptor activator of nuclear factor kappa-B ligand. In blood vessels, vitamin K₂ reduces the formation of hydroxyapatite, through the carboxylation of matrix Gla protein and Gla rich protein, inhibits the apoptosis of vascular smooth muscle cells, by increasing growth arrest-specific gene 6, and
reduces the transdifferentiation of vascular smooth muscle cells to osteoblasts. The commonly used dosage of vitamin K$_2$ in human studies is 45 mg/day and its application can be an interesting strategy in benefitting bone and vascular health, especially to osteoporotic post-menopausal women.

Keywords
menaquinone; osteoporosis; cardiovascular disease, postmenopause.
INTRODUCTION

Osteoporosis is a serious public health issue throughout the world, especially for postmenopausal women. It is associated with an increased risk of bone fractures and mortality. Scientific evidences indicate that osteoporotic patients have an increased risk of developing cardiovascular diseases, mainly due to vascular calcification (VC) (Tankó et al., 2005; Wong et al., 2005; Lampropoulos et al., 2012). Calcium deposits in arteries can compromise vasomotor responses, by reducing elasticity, increasing the risk of high blood pressure, aortic stenosis, cardiac hypertrophy, heart attack, and ischemia of the lower limbs (Lampropoulos et al., 2012).

The optimal therapy for common physiological changes in postmenopausal women, with or without osteoporosis, has not been established. Anti-catabolic drugs, such as nasal spray calcitonin, raloxifene, strontium ranelate, estrogen, alendronate and risedronate, besides supplements such as calcium and vitamin D, have been used in clinical practice (Riggs and Parfitt, 2005). In this context, vitamin K has been studied. Originally only identified as essential for blood coagulation, vitamin K, especially vitamin K$_2$, has been associated with unique benefits in regulating bone metabolism and soft tissue calcification. However, there are no specific dietary recommendations for this nutrient.

The menaquinone, or vitamin K$_2$, is essential for the activation of vitamin K-dependent proteins (VKDP), acting as a cofactor for $\gamma$-glutamyl carboxylase in the post-translational carboxylation of these proteins, transforming residues of glutamate (Glu) in $\gamma$-carboxyglutamate (Gla) (Gallieni and Fusaro, 2014). Some VKDP may play a role in bone and vascular metabolism. The most studied are osteocalcin (OC) and matrix Gla protein (MGP). After carboxylation, OC is better secreted by osteoblastic cells and can be deposited in a mineralized...
bone matrix to accomplish its physiological role in bone formation (Razzaque, 2011). On the other hand, the higher deposit of OC observed in calcified arteries can indicate the role of this protein in vascular disease (Steitz et al., 2001; Rajamannan et al., 2003). MGP is considered an inhibitor of soft tissue calcification, because it is able to strongly bind and inhibit the growth of calcium crystals (Hauschka et al., 1989; Spronk et al., 2001). Scientific evidences indicates that vitamin K₂ has effects in bone health independent of glutamyl carboxylation. Vitamin K₂ promotes an increase in the number and activity of osteoblasts (Urayama et al., 2000), modulates target genes of bone mineralization in osteoblasts (Ichikawa et al., 2007) and induces osteoclast apoptosis (Kameda et al., 1996).

Since vitamin K₂ is commonly present in low amounts in the diet (Geleijnse et al., 2004; Maas et al., 2007), oral supplementation can be considered an interesting option for improving the vitamin K₂ status, especially in postmenopausal women, a risk group for osteoporosis and vascular complications. The aim of this review was to investigate the effect of vitamin K₂ on bone metabolism and VC, considering the mechanisms involved in these processes.

METHODS

The search was conducted in Medline/Pubmed, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (SciELO) and Science Direct for original articles or reviews to assess the effect of vitamin K₂ on bone and/or cardiovascular health, published between 2005 and 2015. Combinations of the following keywords were used for the search: “vitamin K”, “vitamin K₂”, “menaquinone”, “menatetrenone”, “bone”, “osteoporosis” and “vascular calcification.” A manual search was also conducted in the reference lists of selected articles for relevant studies about the subject. Unpublished dissertations and
theses or conference abstracts were not included. Intervention studies performed with humans or animals, *in vitro* studies, observational studies and literature reviews were included. Each article selected to compose this review was critically analyzed.

**RESULTS**

The search strategy resulted in the selection of 99 articles (Figure 1). The inclusion of different types of study allows synthesizing a wide range of information about the mechanisms of action of vitamin K$_2$. These mechanisms may explain the effects on bone and cardiovascular metabolisms found with the intake of vitamin K$_2$, for which, despite the biological plausibility, there are no specific dietary recommendations.

**Vitamin K**

Vitamin K is a term used to represent chemically similar liposoluble compounds, which differ in their origin and function (Hamidi et al., 2013). The two main forms of vitamin K are phylloquinone (vitamin K$_1$) and menaquinone (vitamin K$_2$). Menadione, present in both forms, is a ring structure whose side chain may contain a phytol group, producing vitamin K$_1$, or 1 to 14 repetitions of isoprenoid groups, giving rise to different forms of vitamin K$_2$ (Schurgers and Vermeer, 2000).

Menaquinone may be abbreviated as MK-n, where “n” is the number of isoprenoid groups present in the side chain. The most common and most studied dietary supplements of vitamin K$_2$ are MK-4 and MK-7. In a study with healthy women, MK-7 compared to MK-4 was found to have greater bioavailability, contribute more to the increase of vitamin K in serum, and therefore have greater importance to extrahepatic tissues (Sato et al., 2012). MK-4 is the unique menaquinone that is not synthesized by bacteria and it is found mainly in animal products such...
as egg yolk, meat, liver, and butter (Schurgers and Vermeer, 2000). The long chain menaquinones (MK-7 to MK-10), are exclusively synthesized by bacteria and found in fermented foods, particularly in fermented soybean (“natto”), typical in the Japanese diet, cheese, curd and sauerkraut (Schurgers and Vermeer, 2000; Sato et al., 2012). In addition, higher menaquinones (notably MK-10) are also produced by intestinal bacteria in the colon (Shearer, 1996; Schurgers and Vermeer, 2000). In relation to vitamin K₁, its main sources are green leafy vegetables such as kale, spinach and lettuce, and brassicas such as brussel sprouts and broccoli (Schurgers and Vermeer, 2000).

In the intestine, dietary vitamin K and the products of pancreatic hydrolysis of triglycerides (TG) are enveloped in micelles and processed into chylomicron (CM). Mainly in the muscle and adipose tissues, CM are stripped of their TG. The resultant CM remnant retains vitamin K in the lipophilic core. In the liver, the CM remnant lipidis are repackaged into VLDL, that return to circulation. The removal of TG results in IDL, which is subsequently transformed into LDL. Vitamin K is mantained in the lipophilic core. Lipoproteins such as CM remnants and LDL can deliver lipids to osteoblasts by interacting with receptors, as the low-density lipoprotein receptor (LDLR) and low-density lipoprotein receptor-related protein 1 (LRP1) allowing endocytosis of the particles and vitamin K. In osteoblasts, it is believed that vitamin K₁ is obtained mainly through CM remnants and the majority of MK-7 by LDL (Shearer and Newman, 2008).

Vitamin K is recycled in the liver in order to maintain sufficient levels for activating VKDP. In this process, vitamin K hydroquinone (KH₂) is oxidized to vitamin K epoxide (KO), which is in turn converted into vitamin K quinone by the enzyme vitamin K epoxiredutase.
Vitamin K quinone, which is present in the diet, is then converted to KH$_2$ by the vitamin K quinone reductase enzyme, closing the cycle. The two enzymes involved in these reactions are dithiol-dependent. Another enzyme, NADPH-dependent quinone reductase, is also capable of converting vitamin K quinone into KH$_2$. Warfarin, a drug commonly used in dialysis patients, inhibits the activity of dithiol-dependent reductase, but not NADPH-dependent reductase (Gallieni and Fusaro, 2014) (Figure 2a).

In the liver, vitamin K acts as a cofactor for carboxylation and activation of coagulation proteins II, VII, IX, X and proteins C, S and Z (Schurgers and Vermeer, 2002; Cranenburg et al., 2007; Shearer and Newman, 2008). Vitamin K$_2$ is carried by triglyceride-rich lipoproteins, low-density lipoproteins (LDL) or high-density lipoproteins (HDL) (Schurgers and Vermeer, 2002; Shearer and Newman, 2008), including arterial walls, which prefer to accumulate and use vitamin K$_2$ compared to K$_1$ (Schurgers and Vermeer, 2000) (Figure 2a). Schurgers et al. (2007) compared the absorption and efficacy of K$_1$ and MK-7 in healthy volunteers. Both forms were well absorbed, but MK-7 was more effective in catalyzing OC carboxylation in bone. The authors suggest that the greatest effect of vitamin K$_2$ on bone can be attributed to the longer half-life time of MK-7, compared to K$_1$, resulting in more stable serum levels and higher accumulation (Figure 2b).

The dosage commonly used in human studies is 45 mg/day of MK-4, since it was associated with greater benefit to bone and vascular health in Japan, a pioneer in studies in this area (Iwamoto et al., 1999; Shiraki et al., 2000; Ozuru et al., 2002; Ishida and Kawai, 2004; Cockayne et al., 2006). This dose is 500 times higher than the Adequate Intake (AI) of vitamin K (90 μg/day). However the tolerable upper intake level (UL) was not determined and there is no
specific recommendation for vitamins K\textsubscript{1} and K\textsubscript{2} (Trumbo et al., 2001). None of the analyzed studies reported risks associated with the supplementation of vitamin K or concerns about coagulation or thromboembolic events. Furthermore, it is speculated that the AI of 90 μg/day is too low to permit the adequate extrahepatic VKPD carboxylation, since the AI for vitamin K is solely based on the intake of vitamin K\textsubscript{1} and hepatic activation clotting factors (Schurgers et al., 2001).

The effect of a low dose (1.5 mg) of vitamin K\textsubscript{2} (MK-4) was tested in a randomized, double-blind, placebo-controlled study on the bone health of postmenopausal women. After 6 and 12 months, the concentrations of uncarboxylated OC (ucOC) and pentosidine (marker of glycation endproducts) were significantly lower in the group supplemented with K\textsubscript{2} than in the control group. Furthermore, the bone mineral density (BMD) was lower in the control group at 12 months compared to the sixth month, and there was no significant decrease in BMD in the group supplemented with K\textsubscript{2} throughout the study (Koitaya et al., 2014).

Metabolically, vitamin K\textsubscript{1} can be converted to K\textsubscript{2} (MK-4) to act on reducing the calcium content of soft tissue, such as the kidney where the higher content of vitamin K\textsubscript{2} compared to that of K\textsubscript{1} indicates that K\textsubscript{2} has specific actions in this function (Shearer and Newman, 2008; Rajabi et al., 2012; Kaesler et al., 2014). Furthermore, it was observed that vitamin K\textsubscript{2} has greater efficacy, compared to K\textsubscript{1}, in activating VKDP (Shea and Holden, 2012; Gallieni and Fusaro, 2014), in increasing mineralization, and reducing bone resorption (Kameda et al., 1996; Hara et al., 2002). In human intervention, the effect of vitamin K\textsubscript{2} was tested and, in some cases, compared to placebo or compounds traditionally used for improving bone and/or vascular health, such as calcium and/or vitamin D\textsubscript{3} supplements and some drugs (Tables 1 and 2).
Vitamin K$_2$ and osteoporosis

Mechanisms of action

OC is one of the most abundant matrix proteins found in bones that could affect the growth or maturation of the mineral phase (Glowacki and Lian, 1987; Gundberg et al., 2012). Its retention in bone is dependent on γ-carboxylation promoted by vitamin K$_2$ (Hauschka et al., 1989; Gundberg et al., 2012). OC is secreted by mature osteoblasts, odontoblasts and hypertrophied chondrocytes, and its production is increased in response to administrating vitamin K$_2$ (Shiraki et al., 2000). Gla residues present on the carboxylated OC (cOC) may interact with hydroxyapatite crystals in the mineralization process (Owen et al., 1990). Besides the importance of Gla residues in bone mineralization, glutamate (Glu) has receptors on all mature bone cells and can affect the bone remodeling. Glu also acts as a neuropeptide and may be released in a specific site according to local needs, as cells of bone tissue, and may represent a regulator able to locally control the activity of bone cells (Chenu et al., 1998).

Besides acting on the activation of OC, vitamin K$_2$ also promotes apoptosis of osteoclasts and reduction of their progenitor cells differentiation and bone remodeling (Iwamoto et al., 2006). Furthermore, it was found that vitamin K$_2$ promotes an increase in the number and activity of osteoblasts (Urayama et al., 2000). The addition of vitamin K$_2$ in osteoblast cell cultures resulted in dose-dependent reductions of the expression of apoptotic agents Fas and Bax, and reduction of the cytotoxic effects of Fas ligand (FasL), which mainly occurred after treatment with tumor necrosis factor (TNF)-α, a pro-apoptotic indicator (Urayama et al., 2000). In osteoblasts treated with MK-4, increased expressions of potential promoters of differentiation and proliferation of osteoblasts, growth differentiation factor-15 (GDF-15) and stanniocalcin 2
(STC2), were observed. In this study, these effects were not observed in response to MK-7 or vitamin K\textsubscript{1} (Ichikawa et al., 2007). Vitamin K\textsubscript{2} also promoted increase of insulin-like growth factor-1 (IGF-1) in osteoblasts (Kanellakis et al., 2012), which is associated with increased differentiation of these cells and the bone formation in postmenopausal women (Ghiron et al., 1995). These results indicate that vitamin K\textsubscript{2} has positive effects on osteoblasts and inhibitory effects on osteoclasts, which is beneficial in treating osteoporosis.

In a study with bone marrow cells, which contains both progenitor cells of osteoclasts and osteoblasts, the treatment with MK-4, but not with vitamin K\textsubscript{1}, stimulated the activity of alkaline phosphatase (AP) and OC expression, which are markers of early and late osteoblast differentiation, respectively. In this study it was also found that MK-4 inhibited the expression of RANKL and promoted the reduction in the number of osteoclast-like cells in bone marrow (Takeuchi et al., 2000). Increased AP activity and reduced expressions of RANKL/ODF were also observed in another study of cells treated with MK-4 in the presence or absence of the glucocorticoid dexamethasone, a vitamin K antagonist (VKA). In this study, an increased expression of OPG in stromal cells was also observed (Koshihara et al., 2003).

Scientific evidences

Most studies that relate vitamin K\textsubscript{2} and bone metabolism were conducted with women in the postmenopausal period and the most significant results are observed in the presence of osteoporosis. Table 1 shows that supplementation with vitamin K\textsubscript{2} is effective mainly in VKDP activation, verified by the increase in the carboxylated form and reduction in the uncarboxylated form of OC. Inconsistent findings were observed regarding the role of vitamin K\textsubscript{2} on BMD.
However, a meta-analysis found that vitamin K was effective in increasing BMD at the lumbar spine, but not at the femoral neck (Fang et al., 2012).

A meta-analysis of 19 randomized clinical trials showed that vitamin K2 was effective in maintaining BMD in postmenopausal women with osteoporosis and no effect was found for those without osteoporosis (Huang et al., 2014). In this study, it was found that vitamin K2 reduced the incidence of fractures and ucOC concentration, and increased cOC, suggesting a positive effect on bone metabolism. Higher total OC concentrations were associated with a lower rate of calcification progression of the abdominal aorta and lower mortality in elderly men (Confavreux et al., 2013). However, the association between total OC serum levels and bone health is controversial (Table 1). Cheung et al. (2008) observed a reduction in total OC in women with lower risk of fractures, supplemented with vitamin K1. They suppose that the increase in OC carboxylation and functionality, due to improvement in the vitamin K status, reduces the need and synthesis of OC. Moreover, they suggest that, after carboxylation, the OC will be linked to the bone rather than circulating in the blood.

Regarding the action of vitamin K2 on IGF-1, the effect of supplementation of dairy products fortified with calcium, vitamin D3 and Vitamin K1 or K2 (MK-7) was tested for 12 months on bone metabolism variables in postmenopausal women. It was found that the group that received calcium and vitamins D3 and K2 had higher levels of IGF-1 in serum compared to the groups that received only calcium and vitamin D3 or calcium and vitamins D3 and K1. In this study, BMD was increased in both groups supplemented with K1 or K2 (Kanellakis et al., 2012).

Cheung et al. (2008) discussed the fact that having differences in the effects of vitamin K1 and K2 on bone. The authors found that the daily consumption of 5 mg of vitamin K1 by 440
women after menopause, for 4 years, did not protect against the reduction of BMD or reduce bone resorption. However, this supplementation reduced the percentage of ucOC and protected against fractures, compared with the placebo group (hazard ratio [HR] = 0.45, 95% confidence interval [CI] 0.20 to 0.98, \( p = 0.04 \) at 4 y). They suggested that the action of vitamin K\(_1\) in the bone may not be mediated by BMD or bone turnover, but by improvements in bone geometry and quality. In an observational study with 387 patients on hemodialysis, vitamin K\(_1\) deficiency was a stronger predictor of vertebral fractures compared to vitamin K\(_2\) deficiency (odds ratio [OR] = 2.94; 95% CI 1.38 to 6.26) (Fusaro et al., 2012).

Niemeier et al. (2008) demonstrated that, after injection of phylloquinone-enriched CM remnants, the liver was the first and bone was the second most important organ for clearance of CM remnants from the circulation. They observed that this injection resulted in an increase of cOC levels, giving proof that phylloquinone also plays a role in bone formation in vivo. It is noteworthy that vitamin K\(_1\) can be converted to MK-4 in the human body (Shearer and Newman, 2008).

Animal studies

Vitamin K\(_2\) showed, in animal models, to have an effect on bone mass recovery, whose loss was induced by ovariectomy or by the administration of VKAs, such as glucocorticoids, warfarin and phenytoin (Hara et al., 2002; Onodera et al., 2003; Iwamoto et al., 2008; McCabe et al., 2013). These drugs indirectly inhibit the VKDP carboxylation by interfering in the regeneration of vitamin K. Bone loss induced by drugs is a serious problem in clinical practice, and its prevention by the use of vitamin K\(_2\) can be an important and safe strategy for health promotion.
The effect of vitamin K₂ in bone formation was demonstrated after administering prednisolone, a VKA glucocorticoid, in rats. At 4 weeks, BMD was significantly reduced with treatment of 100 mg/kg of prednisolone. At 8 weeks, the dosages of 10 or 30 mg/kg of prednisolone resulted in a reduction of BMD. Vitamin K₂ (15 mg/kg) was given for 8 weeks as a dietary supplement and inhibited the decrease of BMD and the decrease in mineralizing surface and bone formation rate, induced by 30 mg/kg of prednisolone (Hara et al., 2002). The antiepileptic drug phenytoin also resulted in significant reduction in BMD, mainly when the serum and bone levels of vitamin K₂ decreased due to phenytoin administration. The bone loss was prevented by the combined administration of phenytoin and vitamin K₂ (Onodera et al., 2003). In rats with adenine-induced chronic kidney disease (CKD), the treatment with warfarin resulted in significantly greater ucOC and significantly less cOC. In the study, a significant increase in cOC in the high dietary K group was found (McCabe et al., 2013).

**Vitamin K₂ and vascular calcification**

Mechanisms of action

Some studies suggest that the mechanism for VC is similar to skeletal bone formation and involves the calcification of an extracellular matrix mediated by an osteoblast-like phenotype (Rajamannan et al., 2003; Duer et al., 2008). Moreover, it is reported that the process of VC involves other mechanisms, such as the apoptosis of vascular smooth muscle cells (VSMCs), modulated by MGP, the most studied VKDP with role in VC (Proudfoot et al., 2000). Vitamin K₂ acts as a cofactor in the post-translational γ-carboxylation of MGP, which promotes its biological activity in the vasculature as an inhibitor of VC (Schinke et al., 1999). The carboxylated MGP (cMGP) is able to strongly bind and inhibit the growth of calcium crystals.
This effect was observed in human atherosclerotic plaques, where MGP avoided calcium precipitation (Spronk et al., 2001). The cMGP is also a potent bone morphogenetic inhibitor protein-2/4 (BMP-2/4), which promotes VSMCs transdifferentiation to osteoblasts in the vessel wall, which precedes VC and can lead to an accelerated progression of atherosclerosis (Wallin et al., 2008; Yao et al., 2010). In an in vitro study, MGP containing Gla residue, but not the MGP containing Glu, inhibited the transdifferentiation of VSMCs, emphasizing the importance of vitamin K$_2$ in the activation of MGP (Wallin et al., 2008).

In addition to cMGP, other forms such as uncarboxylated MGP (ucMGP), phosphorylated MGP (pMGP), and dephosphorylated MGP (dpMGP) can be quantified in serum and plasma, and their use may be dependent on the aim of the study. The total ucMGP may be a more useful VC marker because of its ability to bind to calcium. The dephosphorylated and uncarboxylated MGP (dp-ucMGP) is considered a marker of cardiovascular disease, such as aortic valve disease, aortic stenosis and heart failure, and mortality in patients with cardiovascular risk (Schurgers et al., 2008; Theuwissen et al., 2012; Mayer et al. 2014; Heuvel et al., 2014). Vitamin K$_2$ supplementation has been also associated with the reduction of dp-ucMGP in patients with kidney disease (Schlieper et al., 2011) and healthy individuals (Cranenburg et al., 2007).

The action of vitamin K$_2$ in VC is also associated with activation of other important VKDPs, such as the growth arrest specific gene 6 (Gas-6) and the Gla Rich Protein (GRP). Gas-6 needs $\gamma$-carboxylation to stimulate the anti-apoptotic activity of Bcl-2 and to inhibit the pro-apoptotic protein caspase-3, protecting the VSMCs of apoptosis induced by the starvation of fibroblasts and calcification (Son et al., 2006). Furthermore, Gas-6 acts as a growth promoter of
VSMCs in synergy with other growth factors in VSMCs. These data suggest that Gas-6 prevents atherosclerotic vascular degeneration. GRP is a low studied VKDP, expressed by chondrocytes, chondroblasts, osteoblasts and osteocytes, that has large amounts of Gla residues (Viegas et al., 2008). GRP has shown to be present in the cartilage and bone of rats, as well as in soft tissues of rats and humans (Viegas et al., 2008). Accumulation of GRP was observed in locations affected by pathological calcifications, and the affinity between Gla residues and calcium suggests the association between this protein and CV. GRP has the ability to bind to hydroxyapatite and to be highly accumulated at sites of calcifications in human samples derived from patients diagnosed with skin and vascular calcification pathologies (Viegas et al., 2009).

Scientific evidences

VC is a major risk factor in the development of cardiovascular events such as atherosclerosis and myocardial infarction and its development is a strong negative indicator of cardiovascular morbidity and mortality (Iribarren et al., 2000; Doherty et al. 2003; Abedin et al. 2004; Roijers et al., 2011). Few human studies have investigated the action of vitamin K₂ in VC and mostly evaluated the circulating levels of MGP, which is a less invasive method. Morphological assessments are generally more applicable to animal studies. This fact shows the importance of assessing the different forms of MGP in human studies and the influence of vitamin K₂ on the activity of this protein (Schurgers et al., 2001). Besides being measured in blood and bone, MGP can also be measured in various soft tissues, which was associated with sclerosis of the inner and middle layers in vessels, since their levels were increased in atherosclerosis (Schurgers et al., 2001). Schurgers et al. indicated that ucMGP is a risk factor for VC and that the present RDA values are too low to ensure full carboxylation of MGP (Schurgers
et al., 2001). In a study of patients with type 2 diabetes, it was found that dp-ucMGP, but not total MGP and dpMGP, was associated with an increased risk of cardiovascular disease, high blood pressure and heart failure (Dalmeijer et al., 2013).

Postmenopausal women with calcified lesions in the aorta presented lower intakes of vitamin K (Jie et al., 1995). In Rotterdam Study, the intake of vitamin K₂, but not of vitamin K₁, of 4807 seniors was inversely associated with aortic calcification and mortality from all causes (Geleijnse et al., 2004). In a cross-sectional study with 1689 women 49-70 years old, where the consumption of vitamins K₁ and K₂ was analyzed according to a validated food frequency questionnaire, it was observed that calcification of breast arteries was less common (9% of calcification) in women allocated in the highest quartile of vitamin K₂ intake compared with those in the lowest quartile (13% of calcification). No differences in the prevalence of VC were observed over the K₁ consumption quartiles (Maas et al., 2007). In a prospective cohort European Prospective Investigation Into Cancer and Nutrition (EPIC), composed of 16,057 women aged 49 to 70, the reduction in VC risk was associated with higher intakes of vitamin K₂, but not of vitamin K₁ (Gast et al., 2009). Similar results were observed in a study of 564 women after menopause (Beulens et al., 2009). In the EPIC study, the strongest protective effect against coronary heart disease was attributed to MK-7, MK-8 and MK-9 (Gast et al., 2009).

Patients with CKD, especially those on dialysis, commonly present a low nutritional status of vitamin K (Holden et al., 2010). This occurs because the occurrence of VC in these patients is high, and consequently, the demand for vitamin K to activate the MGP in the vasculature can be high (Schurgers et al., 2010). Poor nutritional status of vitamin K may also occur due to dietary restrictions of potassium (green leafy vegetables, also rich in vitamin K₁)
and phosphate (dairy products, also rich in vitamin K\textsubscript{2}) recommended for these patients (Chatrou et al., 2012). The supplementation of vitamin K\textsubscript{2} for CKD patients seems to be interesting, as shown by the reduction of ucOC after 6 weeks of treatment (Westenfeld et al., 2012). The effect of GRP, another VKDP, was also observed in a group of patients with CDK. GRP was highly accumulated at sites of medial calcification, either in mildly calcified arteries or in extensive and advanced lesions. In contrast, in normal situations the absence of GRP in the extracellular matrix was observed, which reveals that GRP is definitively associated with the processes of abnormal calcification in the vascular system (Viegas et al., 2009).

Oral antiocoagulants are VKAs drugs commonly used in clinical practice for the management of thromboembolic disorders, which are able to reduce blood coagulation, but also causes bone and cardiovascular complications (Chatrou et al., 2012). In low-risk fibrillation atrial patients, the use of VKAs resulted in higher coronary artery calcium scores, compared to patients without VKA treatment. In this study, the mean coronary calcium scores increased significantly with the duration of VKA use (Weijs et al., 2011). Warfarin is a VKA, commonly administered to patients on dialysis, which have a high prevalence of VC due to its use (Schurgers et al., 2004; Koos et al., 2009). Studies that evaluated the concomitant administration of vitamin K\textsubscript{2} and VKAs indicated the benefits of a high intake of vitamin K\textsubscript{2} in these conditions. In a randomized study was verified the positive effect of daily supplementation of vitamin K\textsubscript{2} in 53 hemodialysis patients with vitamin K deficiency, possibly associated to the use of warfarin. The supplementation dose was 45, 135 or 360 μg/day for 6 weeks and the dose-dependent effect on γ-carboxylation of MGP was observed (Westenfeld et al., 2012). Benefits of vitamin K\textsubscript{2} consumption for hemodialysis patients were also found in an observational study of 387
individuals who had significant prevalence of deficiency (below the 5th percentile of the control group) of MK-7 (35.4%), MK-4 (14.5%) and vitamin K$_1$ (23.5%). In these individuals, MK-4 deficiency was a predictor of aortic calcification (OR = 2.82, 95% CI 1.14 to 7.01) and MK-7 deficiency was an iliac calcification predictor (OR = 1.64, 95% CI 1.03 to 2.60) (Fusaro et al., 2012). Despite the use of VKAs being considered a causative factor for VC, overwhelming evidence in humans is still needed. One should also consider that the importance of therapy with anticoagulants may exceed its prejudicial effect on VC in some cases (Brandenburg et al., 2015).

Animal studies

In a study with deficient MGP animals, a smaller stature at birth compared with the control group was observed, due to heavy calcification of chondrocytes in growth plates (Yao et al., 2010). Death of MGP deficient animals was found 8 weeks after birth due to extensive calcification of large and medium-sized arteries (Luo et al., 1997). The administration of warfarin to mice for 6 weeks induced inactivation of MGP and severe VC. However, the replacement of the drug by high doses of vitamin K (K$_1$ or K$_2$) induced significant regression of the arterial calcium content by approximately 50% (Schurgers et al., 2007). Another study demonstrated that animals fed with a diet low in vitamin K showed only 2% of cMGP in the vessel wall (Price et al., 1998). Spronk et al. (Spronk et al., 2003) observed the inhibition of warfarin-induced arterial calcification by the concomitant administration of a high dose of vitamin K$_2$ (30 mg/day of MK-4), which was not observed with administration of vitamin K$_1$.

In animals with adenine-induced renal disease, undergoing therapeutic doses of warfarin, attenuation of the VC development after treatment with high doses of vitamin K$_1$ (100mg/kg diet) was observed. Administration of vitamin K$_1$ resulted in increased renal MK-4
concentration, possibly due to a conversion process of vitamin K$_1$ to K$_2$, described previously by other authors (McCabe et al., 2013).

In rats, GRP was found to be highly expressed in epidermis and dermis. In blood vessels, $\gamma$-carboxylated GRP was highly accumulated in the walls of blood vessels and capillaries, mainly in VSMC of the tunica intima (Viegas et al., 2009). The GRP gene is absent in invertebrate and birds genomes, and was identified in most classes of vertebrates, including mammals, sauropsids, amphibians, bony fish, and jawless fish (Cancela et al., 2012)

CONCLUSIONS

Convincing data of the biological plausibility of the importance of vitamin K for bone and vascular health have been published, especially in recent years. Benefits of vitamin K consumption were demonstrated especially in the occurrence of bone loss in human or animal studies. This revision supports the hypothesis that vitamin K$_2$ acts in the expression and/or synthesis of important biomarkers of bone and vascular metabolism. Vitamin K$_2$ stimulates the carboxylation of VKDP, such as OC, MGP and GRP. Whether carboxylated, these proteins are better secreted by the cells, accumulate more in the tissue, and can accomplish its physiological role. Vitamin K$_2$ is also associated with the reduction of arterial calcium deposits, by promoting the increase of VSMCs, and the inhibition of transdifferentiation of VSMCs to osteoblasts. Furthermore, vitamin K$_2$ promotes bone formation by increasing the differentiation and reducing the apoptosis of osteoblast, and by reducing bone resorption by inhibiting osteoclast differentiation. The effect of vitamin K$_2$ in BMD and bone fractures in humans is controversial and needs to be confirmed in more studies.
Vitamin $K_1$ is shown to have a greater effect in the incidence of bone fractures compared to vitamin $K_2$, but a smaller effect on BMD and bone turnover. This indicates that possibly there are possible differences in the mechanisms of action of these vitamins in the bone. It is important to highlight that vitamin $K_1$ can be converted to MK-4 in the human body.

Most studies were conducted in Japan, where the fermented soybeans *natto*, the most abundant food source of vitamin $K_2$ known, is part of the typical diet. The commonly used dosage of vitamin $K_2$ (MK-4) in human studies is 45 mg/day. Considering that most studies were conducted in Japan and have relatively small sample sizes, we highlight the need of results using larger population samples, mainly from other geographic areas. Faced with literature data, the consumption of natural sources of vitamin $K_2$ and the oral supplementation can be an important strategy in benefitting bone and vascular health, especially in postmenopausal women.

**FUNDING**

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REFERENCES


Iwamoto, J., Takeda, T., and Ichimura, S. (2000). Effect of combined administration of vitamin D3 and vitamin K2 on bone mineral density of the lumbar spine in postmenopausal women


Postmenopausal Women Following a 12-Month Intervention Period Using Dairy Products Enriched with Calcium, Vitamin D, and Phylloquinone (Vitamin K1) or Menaquinone-7 (Vitamin K2): The Postmenopausal Health Study. *Calcif Tissue Int.* **90**: 251–262.


osteoblast function. *Bone.* **43:** 230-237.


Table 1 Effect of menaquinone (vitamin K$_2$) supplementation on bone metabolism in human studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. patients (K$_2$; C)</th>
<th>Study population; duration</th>
<th>Intervention</th>
<th>Difference in % BMD (K$_2$ vs. C)</th>
<th>Difference in No. Fractures (K$_2$ vs. C)</th>
<th>Difference in % change of biological measurements (K$_2$ vs. C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iwamoto et al., 1999</td>
<td>$n = 19$; $n = 17$</td>
<td>Healthy postmenopausal women; 1 year</td>
<td>45 mg/day menaquinone</td>
<td>+ 3.1 (lumbar spine; p&lt;0.05)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ishida and Kawai, 2004</td>
<td>$n = 66$; $n = 66$</td>
<td>Postmenopausal women, aged 50-75 years; 2 years</td>
<td>45 mg/day menaquinone</td>
<td>+ 1.4 (radius; p = 0.03)</td>
<td>-8 (RR = 0.44 (95% CI 0.20 to 0.99)</td>
<td>+ 20% in OC (p&lt;0.05)</td>
</tr>
<tr>
<td>Shiraki et al., 2000</td>
<td>$n = 120$; $n = 121$</td>
<td>Osteoporotic women; 2 years</td>
<td>45 mg/day menaquinone</td>
<td>+ 3.2 (lumbar vertebrae; p = 0.001)</td>
<td>-21 (RR = 0.43; p = 0.0273)</td>
<td>+ 36.3% in OC (p = 0.0081); - 46.6% ucOC (p&lt;0.0001); no significant</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Change in ucOC/cOC ratio</td>
<td>p-value</td>
<td>Change in ucOC</td>
<td>p-value</td>
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<tr>
<td>Dalmeijer et al., 2012</td>
<td>Healthy men and healthy postmenopausal women, aged 40-65 years; 12 weeks</td>
<td>360 µg/day menaquinone</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-74.5%</td>
</tr>
<tr>
<td>Koitaya et al., 2014</td>
<td>Postmenopausal women, aged 50-65 years; 1 year</td>
<td>1.5 mg/day menaquinone</td>
<td>No significant differences were observed</td>
<td>NA</td>
<td>-30.5% in ucOC</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Koitaya et al., 2009</td>
<td>Postmenopausal women,</td>
<td>1.5 mg/day menaquinone</td>
<td>NA</td>
<td>NA</td>
<td>-28.5% in ucOC</td>
<td>p&lt;0.05</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome</td>
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<tr>
<td>Iwamoto et al. 2000</td>
<td>Postmenopausal women, aged 55-81 years; 2 years</td>
<td>45 mg/day menaquinone</td>
<td>+ 1.69 (lumbar spine; p&lt;0.001)</td>
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<tr>
<td></td>
<td>n = 22; n = 20</td>
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<td>NA</td>
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<td></td>
<td>Postmenopausal women, aged 50 years or more; 3 years</td>
<td>45 mg/day menaquinone + 3 g/day calcium</td>
<td>- 38.7% in incidence of new vertebral fractures (p = 0.029)</td>
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<td></td>
<td>n = 2,016; n = 1,999</td>
<td></td>
<td>NA</td>
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<tr>
<td>Westenfeld et al., 2012</td>
<td>Long-term hemodialysis patients, men and women, aged 18 years or more; 6 weeks</td>
<td>360 μg/day menaquinone</td>
<td>NA</td>
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<td></td>
<td>n = 50; n = 50</td>
<td></td>
<td>- 34.4% in ucOC (p&lt;0.05); no significant difference in AP</td>
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<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Intervention</td>
<td>Outcome Measures</td>
<td>Results</td>
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<tr>
<td>Kurnatowska et al., 2015</td>
<td>n = 28; n = 12</td>
<td>CKD patients, men and women, aged 18-70 years; 270 days</td>
<td>90 μg/day menaquinone e + 10 μg vitamin day D₃</td>
<td>No significant differences were observed</td>
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<tr>
<td>Binkley et al., 2009</td>
<td>n = 126; n = 129</td>
<td>Postmenopausal women without osteoporosis; 1 year</td>
<td>45 mg/day menaquinone e</td>
<td>No significant differences were observed</td>
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<tr>
<td>Emaus et al., 2010</td>
<td>n = 167; n = 167</td>
<td>Women, 1-5 years after menopause, aged 50-60 years; 1 year</td>
<td>360 μg/day menaquinone e (natto capsules)</td>
<td>+7.69% in BAP (p = 0.05); +28.4% in cOC (p&lt;0.001); -40.6% in ucOC (p&lt;0.001)</td>
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<tr>
<td>Kasukawa et al.</td>
<td>n = 26; n = 29</td>
<td>Postmenopausal</td>
<td>45 mg/day menaquinone</td>
<td>+17.9% in OC (p&lt;0.05)</td>
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<tr>
<td>Year</td>
<td>Study Details</td>
<td>Treatment Details</td>
<td>Observed Differences</td>
<td>Results</td>
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<tr>
<td>2014</td>
<td>Osteoporotic women, aged 60 years or more; 1 year</td>
<td>Residronate, e + 17.5 mg/week</td>
<td>Differences were observed</td>
<td>- 25.8% in ucOC/OC (p &lt; 0.001); no significant differences in NTx, BAP and ucOC.</td>
<td></td>
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<tr>
<td></td>
<td>Hirao et al., 2008 n = 21; n = 23</td>
<td>Menaquinone, e + 45 mg/day + 5 mg/day alendronate + 3.9 (femoral neck; p = 0.03)</td>
<td>NA</td>
<td>- 22% in DPD (p = 0.032); - 36% ucOC (p = 0.014); - 43% in ucOC/OC (p = 0.007)</td>
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<tr>
<td></td>
<td>Shiraki and Itabashi 2009 n = 56; n = 53</td>
<td>Menaquinone, e + 45 mg/day</td>
<td>NA</td>
<td>+ 23.5% in OC (p = 0.006); + 33% in cOC (p = 0.001); + 26.5% in urinary NTx (p = 0.019); - 57.1% in ucOC (p = 0.002)</td>
<td></td>
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<tr>
<td>Je et al., 2011</td>
<td>Postmenopausal women, n = 18; n = 27</td>
<td>45 mg/day menaquinone + 400 UI/day vitamin D₃ + 630 mg/day calcium</td>
<td>+ 2.4 (lumbar spine; p = 0.049)</td>
<td>NA</td>
<td>- 49.5% in ucOC (p = 0.008); no significant differences in OC and BAP</td>
<td></td>
</tr>
</tbody>
</table>

C: control; BMD: bone mineral density; OC: osteocalcin; ucOC: uncarboxylated OC; cOC: carboxylated OC; DPD: deoxypyridinoline; CKD: chronic kidney disease; OPG: osteoprotegerin; AP: alkaline phosphatase; BAP: bone specific alkaline phosphatase; NTx: N-telopeptide of type I collagen; NA: not applicable
Table 2 Effect of menaquinone (vitamin K\textsubscript{2}) supplementation on vascular calcification in human studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. patients (K\textsubscript{2}; C)</th>
<th>Study population; duration</th>
<th>Intervention</th>
<th>Difference in % change of biological measurements (K\textsubscript{2} vs. C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalmeijer et al., 2012</td>
<td>n = 18; n = 20</td>
<td>Healthy men and healthy postmenopausal women, aged 40-65 years; 12 weeks</td>
<td>360 µg/day menaquinone</td>
<td>-46.2% in dp-ucMGP (p&lt;0.001); no significant differences in dp-cMGP and ucMGP</td>
</tr>
<tr>
<td>Westenfeld et al., 2012</td>
<td>n = 50; n = 50</td>
<td>Long-term hemodialysis patients, men and women, aged 18 years or more; 6 weeks</td>
<td>360 µg/day menaquinone</td>
<td>-41.7% in PIVKA-II (p&lt;0.01); no significant difference in fetuin-A.</td>
</tr>
<tr>
<td>Caluwé et al., 2014</td>
<td>n = 53; n = 59</td>
<td>Long-term hemodialysis patients, men and women, aged 18 years or more; 8 weeks</td>
<td>1080 µg menaquinone, 3x/week</td>
<td>-29% dp-ucMGP (p&lt;0.001)</td>
</tr>
<tr>
<td>Kurnatowska et al., 2015</td>
<td>n = 28; n = 12</td>
<td>CKD patients, men and women, aged 18-70 years; 270 days</td>
<td>90 μg/day menaquinone + 10 μg vitamin D$_3$/day</td>
<td>- 57.1% in carotid intima-media thickness (p&lt;0.003); - 14.1% in dp-ucMGP (p = 0.05); no significant difference in coronary artery calcification score.</td>
</tr>
</tbody>
</table>

C: control; MGP: matrix Gla protein; cMGP: carboxylated MGP; ucMGP: uncarboxylated MGP; dp-ucMGP: desphosphorylated uncarboxylated MGP; PIVKA-II: protein induced by vitamin K absence; CKD: chronic kidney disease.
Figure 1 Search and selection of articles
Figure 2 (a) Food sources, absorption and transport of vitamins K₁ and K₂ to the liver and extrahepatic tissues. Vitamin K₁ is mainly carried by triglyceride-rich lipoproteins and vitamin K₂ by low-density lipoproteins (LDL), high density lipoproteins (HDL) and triglyceride-rich lipoproteins. In the liver, vitamin K acts in the activation of clotting factors and is regenerated in a cyclic process. Vitamin K hydroquinone (KH₂) is oxidized to vitamin K epoxide (KO), which
is converted to vitamin K quinone. Then, vitamin K quinone is reconverted to KH$_2$, closing the cycle. The two enzymes involved in the aforementioned reactions are dependent of dithiol and inhibited by warfarin. The quinone reductase enzyme, NADPH-dependent, is also capable of converting vitamin K quinone in KH$_2$. Vitamin K$_1$ can be metabolically converted into menaquinone-4 (MK-4). (b) In extrahepatic tissues such as bones and vessels, vitamin K$_2$ is shown to have a greater effect compared to vitamin K$_1$ in the reduction of bone resorption, increasing bone formation and reducing vascular calcification. ODF: osteoclast differentiation factor; RANKL: receptor activator of nuclear factor κB ligand; OPG: osteoprotegerin; OCIF: osteoclastogenesis inhibitory factor; AP: alkaline phosphatase; cOC: carboxylated osteocalcin; IGF-1: insulin-like growth factor 1; GDF-15: growth differentiation factor-15; STC2: stanniocalcin 2; cGRP: carboxylated Gla rich protein; Gas-6: growth arrest specific gene 6; VSMCs: vascular smooth muscle cells; cMGP: carboxylated matrix Gla protein; BMP: Bone morphogenetic protein