The Role of Vitamin K in CKD-MBD

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Abstract

Purpose of Review We describe the mechanism of action of vitamin K, and its implication in cardiovascular disease, bone fractures, and inflammation to underline its protective role, especially in chronic kidney disease (CKD).

Recent Findings Vitamin K acts as a coenzyme of y-glutamyl carboxylase, transforming undercarboxylated in carboxylated vitamin K-dependent proteins. Furthermore, through the binding of the nuclear steroid and xenobiotic receptor, it activates the expression of genes that encode proteins involved in the maintenance of bone quality and bone remodeling. There are three main types of K vitamers: phylloquinone, menaquinones, and menadione.

Summary CKD patients, for several conditions typical of the disease, are characterized by lower levels of vitamin K than the general populations, with a resulting higher prevalence of bone fractures, vascular calcifications, and mortality. Therefore, the definition of vitamin K dosage is an important issue, potentially leading to reduced bone fractures and improved vascular calcifications in the general population and CKD patients.

Keywords Vitamin K · Chronic kidney disease · Inflammation · Cardiovascular disease · Bone fractures

Vitamin K Types of Vitamers, Cycle

The centrality of vitamin K's role has dramatically expanded in recent years thanks to the discovery of vitamin K involvement in vascular calcifications, cardiovascular, and bone disease. In the last two decades, many articles analyzed vitamin K effects, both at physiological and therapeutic levels.

Vitamin K is part of the fat-soluble vitamins family. It is characterized by the presence of a 2-methyl-1,4-naphthoquinone nucleus. Three principal forms (or vitamers) of vitamin K are known, which are differentiated by the (lipophilic) side chains linked at the 3 positions: phylloquinone or PK (vitamin K1) with a phytyl side chain; menaquinone or MKn with a variable number of condensed

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isoprenoid units; and menadione (vitamin K3) without chain, it is a synthetic form (Fig. 1) [1]. All vitamers have different food origins: vitamin K1 is more commonly present in green leafy vegetables, while MKn derives from fermented foods and intestinal bacteria such as Bacteroides and Enterobacteria (Fig. 1) [2]. Menaquinone-4 (MK-4), a type of MKn, is the only one not produced by the intestinal bacterial flora. Still, it is converted from PK through a side chain removal mechanism in specific tissues (pancreas, testes, and vessel wall) into an intermediate molecule, such as menadione (vitamin K3) [3]. The latter molecule is converted into MK-4 by liver enzymes. A normal pancreatic function and the presence of bile salts are the key elements to obtain a good absorption of vitamin K, given its lipophilic structure. Furthermore, vitamin K is transported into plasma by lipoproteins [4, 5]. Among fat-soluble vitamins, vitamin K is the one with the lowest serum levels in humans. For this reason, its metabolic recycling helps to maintain adequate sources of vitamin K [1].

Mechanism of Vitamin K Actions and Status

The primary mechanism of vitamin K action is related to its coenzymatic activity. Indeed it is a substrate for the gamma-glutamyl carboxylase (GGCX) that, adding CO_2 to glutamic acid residues, leads to the active form of different vitamin K–dependent proteins (VKDPs) [5].

To date, we know seventeen types of VKDPs. Many of them are involved in well-defined biological processes, while for others, such as proline-rich Gla protein 1 and 2 (PRGP1 and PRGP2), transmembrane Gla protein 3 and 4 (TMG3 and TMG4), the function is not fully understood. Among the VKDPs, we can observe a group of this protein involved in the coagulative cascade: coagulation factors II, VII, IX, X, and anticoagulation proteins C, S, and Z. They are synthesized in the liver and depend on vitamin K1 activity. Other four proteins, activated outside of the liver, such as matrix Gla protein (MGP), osteocalcin (OC) or bone Gla protein (BGP), growth arrest-specific protein 6 (Gas6), and Gla-rich protein (GRP), are implicated in the bone and vascular mineralization. Finally, there are two transmembrane Gla proteins, periostin and periostin-like factor periostin (PLF), that bind integrins, leading to stimulation of cellular adhesion and migrations [6, 7].

Among the VKDPs, the principal proteins implicated in the bone and vascular function are (1) MGP, a 10.6 kDa protein synthesized and secreted in the extracellular matrix mainly by vascular smooth muscle cells (VSMCs) and chondrocytes, which specifically exerts the role of calcification inhibitor. To be activated, in addition to the carboxylation process, this protein will have to undergo phosphorylation of three serine
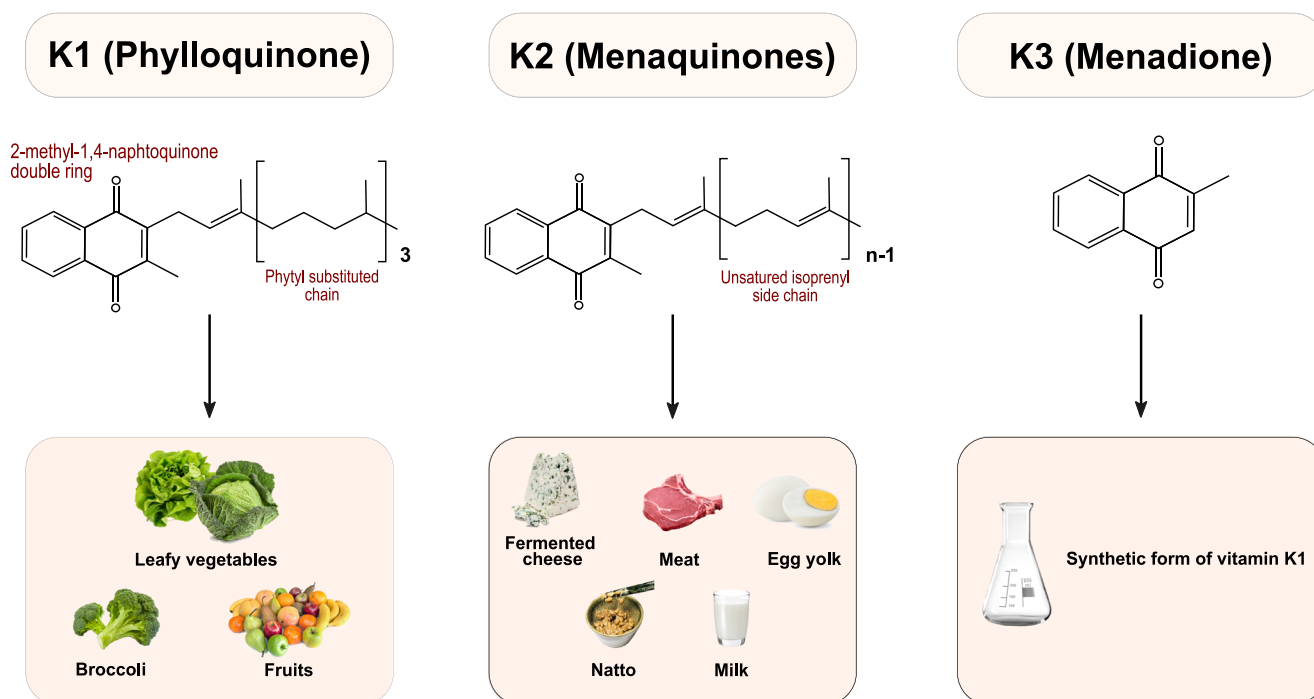
Fig. 1 The sources of vitamin K are different depending on the vitamers. PK can be found mainly in green leafy vegetables (e.g., kale), vegetables in the Brassica genus (e.g., Brussels sprouts, broccoli), fruits (e.g., avocado, kiwi, and green grapes), herbs (e.g., cilantro, parsley), and green and herbal teas. Other dietary sources are plant oils such as soybean, canola, and olive oils. Fermented foods such as fermented butter or cheese, curdled cheese, egg yolk, and beef liver are sources of MKn. Natto, a traditional Japanese soybean-based food produced by fermentation using *Bacillus subtilis*, is a source of menaquinone-7 (MK-7)

sites. Thus, phosphorylation also determines the activity status: p-cMGP: active form; dp-ucMGP: inactive form; (2) OC, which is secreted exclusively in bone by osteoblasts and only a small part of it diffuses in vascular circulation, plays an essential role in the synthesis and regulation of bone matrix, and it is validated as a marker of bone turnover; (3) Gas6, a protein with a molecular weight of 75 kDa, has a characteristic function in multiple physiological activities (cellular homeostasis, cell proliferation to cell survival); (4) GRP, involved in the inhibition of articular and cardiovascular calcification and, similarly to MGP, through its ability to bind large amounts of calcium ions by the Gla residues it determines the inhibition of calcium crystal formation [5, 8].

Another important mechanism of vitamin K action for bone tissue health is the interaction between MK-4 and the steroid and xenobiotic receptor (SXR), which is expressed in several human cells (liver, intestine, and osteoblastic cells). When SXR is activated by its ligand (such as MK-4), it forms a complex with retinoid X receptor, which binds an SXR-responsive element on the target gene promoter that rules the transcription. With this mechanism, MK-4 induce the expression of genes that encode matrilin-2 (Matn2), tsukushi (Tsk), and CD14 proteins, involved in the maintenance of bone quality (regulating the collagen bone content) and in bone remodeling [7].

The status of carboxylation of the Gla proteins is crucial for bone metabolism and vascular health. It is hypothesized that the deficiency of vitamin K and their reduced carboxylation may lead both to bone metabolism impairment and increase in vascular calcification. Indeed, when vitamin K status is deficient, the undercarboxylated (uc) VKDPs are unable to bind calcium ions, thus allowing coagulation factors (protein induced by vitamin K absence II: PIVKA-II), OC, and MGP to interact with negatively charged phospholipid membranes [9].

It follows that the correct dosage of vitamin K is essential for human health. Unfortunately, the assessment of vitamin K concentration in plasma is difficult both for the low circulating levels and for non-polar characteristics of vitamin K and lipid interference. Therefore, there are functional tests that can indirectly indicate the blood levels of vitamin K, such as the prothrombin time (low sensitivity) or measurement of undercarboxylated (uc) proteins, such as OC and MGP (more sensitive for the assessment of subclinical deficit levels) [10, 11].

Vitamin K and Cardiovascular Disease

As previously pointed out, CKD patients are exposed to the risk of subclinical vitamin K deficiency. True deficiency and functional shortage of vitamin K lead to reduced activation of MGP, a potent inhibitor of tissue calcification, and to the enhancement of vascular inflammation. Therefore, vitamin K deficiency may contribute to CKD patient's high vascular calcification (VC) burden [7, 12•, 13].

Several prospective studies assessed the relationship between circulating vitamin K status and coronary calcification or incident CVD. Among type 2 diabetes patients, higher dp-ucMGP levels were associated with incident CVD hazard ratio [14, 15].

As a consequence of poor vitamin K status present in CKD, it is not unexpected that functional vitamin K deficiency may contribute to high vascular calcification (VC) burden in CKD patients [16].

Active MGP slows the progression of VC in CKD patients by binding hydroxyapatite crystals. This hinders their deposition and promotes macrophage-mediated clearance [17]. Moreover, the interaction between active MGP and bone morphogenetic protein-2 (BMP-2) inhibits VSMC differentiation in osteoblast-like cells, the pivotal step in the development of vascular calcification [18–20].

Some members of the Gla protein family (GRP and MGP) are components of circulating calciprotein particles (CPP) and extracellular vesicles (EVs). Within them, they play an essential role in regulating the calcification process. Circulating CPPs are mainly composed of Ca and P precipitates, fetuin-A, GRP, and probably MGP. They could contribute to the mechanisms by which ectopic mineralization is prevented. Fetuin, GRP, and other soluble proteins contained in CPPs act like mineral carriers with a role in the stabilization, transport, and recycling of water-insoluble minerals in the blood. A pivotal event in the onset of VSMC calcification is the release of extracellular EVs capable of efficiently nucleating hydroxyapatite in the absence of calcification inhibitors like MGP. Likely, γ-carboxylated GRP, fetuin-A, and MGP represent an effective pathway that can both regulate the steps of mineral formation and prevent pathological calcification [21].

Krueger et al. hypothesized that undercarboxylated Gla proteins are released to a lesser extent from the cells in an attempt to prevent inactive proteins from entering the tissues. Reduced phosphorylation of the three serine residues of MGP seems equally crucial as it would result in the lower secretion of MGP [22].

The dp-ucMGP (the wholly inactive form of the protein) does not interact with calcium or BMP-2 and closely reflects the vitamin K status at the vascular wall [23].

Low, rather than high, circulating ucMGP levels are a powerful predictor of cardiovascular calcification, and paradoxically ucMGP serum levels are decreased in CKD patients: this feature could reflect an accumulation of ucMGP within the calcified vessel wall [5, 24–26].

Furthermore, dp-ucMGP levels negatively associate with vitamin K status, hence decreasing with vitamin K supplementation and increasing with the use of vitamin K antagonists [27]. The levels of dp-ucMGP are markedly increased in

CKD, in a stepwise way to the degree of renal failure [8]. The various circulating MGP forms, including plasma dp-ucMGP, associate with CVD outcomes and vascular calcification in the general population as well as in CKD [28]. Moreover, in CKD patients, the association of dp-ucMGP with VC and CVD mortality was found both in cross-sectional and perspectives studies.

Some studies showed that plasma levels of dp-ucMGP increased progressively with CKD stage and were associated with the severity of vascular calcification [29–31]. On the other hand, although confirming that elevated dp-ucMGP levels largely reflect vitamin K deficiency, other studies found no correlation with the extent of vascular calcification [23, 32].

Finally, three prospective studies, two carried out on CKD patients, and one on kidney transplant recipients (KTRs), found an association of dp-ucMGP with CVD mortality [29, 33, 34] (Table 1).

The use of vitamin K antagonists (VKAs) represents a model of functional vitamin K insufficiency, which is added to the pathophysiological and clinical scenario of vitamin K deficiency of CKD. ESRD can be considered an anticoagulant-like condition. Patients on hemodialysis treated with warfarin had a 3.77 odds ratio of developing severe aortic calcifications compared to those not taking the drug [36]. In a multicenter, cross-sectional study with a 3-year follow-up, Fusaro et al. analyzed data from 387 patients on hemodialysis for ≥1 year. The multivariate logistic regression analyses demonstrated that the use of warfarin was associated with increased odds of aortic and iliac calcifications, prevalence of vertebral fractures, and mortality in warfarin-treated hemodialysis patients [37].

Data from the German calciphylaxis registry and a Japanese survey demonstrated that the treatment with warfarin represents a risk factor for calciphylaxis [38, 39].

The effects of long-term vitamin K supplementation on VC have been evaluated in many randomized controlled trials in nonrenal patients. Although some studies showed a clear benefit of vitamin K on VC, especially with MK4 and MK7 supplementation, others with PK supplementation, failed to reveal any improvement of this outcome parameter [40, 41].

On the contrary, while the effects of the commercially available vitamin K1 on the carboxylation status of VKDPs in the HD population have not been studied, several dose-finding studies of MK-7 supplementation have been carried out in dialysis patients, disclosing a consistent decrease in the levels of inactive MGP [42, 43].

De Vries et al. in a multicenter randomized, controlled trial, analyzed for the first time the effects of vitamin K status on the progression of VC in patients on chronic HD. They did not find any difference in the progression of VC over the course of 18 months among patients treated with VKAs, patients in whom the VKA was replaced by rivaroxaban, and patients treated with rivaroxaban that additionally received high-dose vitamin K2 supplements, despite significant differences in dp-ucMGP levels, considered to be the most accurate marker of vascular vitamin K status [44]. A more extensive study should confirm the findings by De Vriese et al. before excluding the possibility of favorable effects of new oral anticoagulants (NOACs) and MK-7 supplements on VC. First, although changes from baseline in dp-ucMGP levels over time in the

Table 1 Epidemiological studies and association of vitamin K status with cardiovascular disease and mortality in CKD patients

Type of study	Design/patients	Vitamin K assessment	Outcome	Principle results
Cranenburg et al. TH 2009 [35]	Cross-sectional 40 CKD-V	ucMGP	CAC score	Inverse correlation
Schurgers et al. JASN 2010 [29]	Prospective 107 CKD II-V	dp-ucMGP	AOC	Association with AOC
Thamratnopkoon Nephron 2017 [31]	Cross-sectional 83 CKD III-V	dp-ucMGP	Kauppila score	Direct correlation $p=0.004$
Delanaye et al. BMC Neph2014 [30]	Cross-sectional 160 CKD-V	dp-ucMGP	Kauppila score	Direct correlation $P=0.0049$
Schlieper et al. JASN 2011 [23]	188 CKD-V	dp-ucMGP	Vascular calcifications	No association
Meuwes et al. PLoS One 2015 [32]	Cross-sectional 64.9% on dialysis	dp-ucMGP PIVKA-II	CAC score	No association with CAC score
Riphagen et al. Nutrients 2017 [34]	Prospective 712 CKD (PREVEND Study)	dp-ucMGP	All-cause cardiovascular mortality	Curvilinearly association
Keyzer et al. Am J Kidney Dis 2015 [33]	Observational 518 KTRs	dp-ucMGP	All-cause mortality and transplant failure	Direct correlation

CKD, chronic kidney disease; *ucMGP*, uncarboxylated matrix Gla protein; *dp-ucMGP*, plasma dephosphorylated-uncarboxylated matrix Gla protein; *CAC*, coronary artery calcium; *AOC*, aortha calcium score; *KTRs*, kidney transplant recipients

VKA, rivaroxaban, and rivaroxaban + MK-7 groups were significant, the absolute level of the undercarboxylated protein remained elevated, indicating that functional vitamin K deficiency might still have been present despite the dose of 2000 mcg thrice weekly. Moreover, the type of administered MK7 is not specified. Third, the study could be underpowered to exclude a protective effect of NOACs and MK-7 supplements.

Vitamin K and Vitamin D in Synergism

Vitamin D deficiency is common in patients with CKD. Whatever the stage of the disease, it is mainly related to the progressive loss of renal function that involves reduced kidney ability to synthesize active vitamin D. Other reasons for the poor vitamin D status in CKD patients are the reduced nutritional intake associated with dietary restrictions (e.g., low-protein and low-phosphate diets), tubulointerstitial injury together with inadequate sun exposure linked to decreased mobility.

Vitamin D deficiency is associated with several complications in CKD patients, including infection, endothelial dysfunction, impaired myocardial remodeling, and insulin resistance [45]. Recently, growing evidence is emerging about the synergistic interplay between vitamins D and K on both bone and cardiovascular health. Vitamins D and K share osteoinductive properties.

Since vitamin K–dependent gamma-carboxylation proved to be regulated by vitamin D–binding protein-related gene expression, vitamin D supplementation may be a possible therapeutic target for recovering matrix gamma-carboxylation along with vitamin K [46–50]. Gigante et al. demonstrated that vitamin D3 in association with vitamin MK-7 is able to induce osteogenesis by differentiating human mesenchymal stem cells along the osteoblastic lineage [51].

Vitamin D displays an anabolic effect in bone by increasing osteoblasts activity and by reducing osteoclasts reabsorption. Koshihara et al. showed that both MK-4 and vitamin K1 might stimulate osteoblastogenesis in bone marrow cells and regulate osteoclastogenesis, acting through the expression of receptor activator of nuclear factor kappa_B ligand/osteoclast differentiation factor (RANKL/ODF) [52]. Moreover, in obese/diabetic that a combined treatment with vitamins K2 and 1,25 (OH) 2 D3 increased in a time-dependently way (7, 14, and 21 days), the levels of bone anabolic markers and bone formation transcription factors [53].

Transcription and translation of the BGP gene are under the control of 1,25D and PTH [54].

Some studies assessed that menaquinones (MKn) enhance vitamin D3–induced mineralization by increasing BGP gene expression, as well as BGP protein content in the extracellular matrix. BGP has a key role in the synthesis and regulation of bone matrix, in addition to the control bone mineralization [1].

BGP can also carry out a mechanical function within the bone matrix since it binds hydroxyapatite and forms a complex with collagen, acting as a bridge between the matrix and mineral components of bone tissue [55]. Overall, these experimental findings configure a synergistic effect on bone formation and mineralization.

Moreover, vitamin D and vitamin K downregulate inflammatory pathways, in turn, involved not only in osteoporosis but above all, in the progression of vascular calcification [13, 56, 57].

BGP exerts a protective role against vascular calcifications since hemodialysis patients with one or more vertebral fractures have lower serum BGP levels, which are also associated with accelerated abdominal aortic calcification [58].

1,25(OH)2D upregulates matrix Gla protein (MGP) mRNA and raises MGP secretion several folds. The MGP gene promoter contains a vitamin D response element, enhancing MGP expression after vitamin D binding [59]. MGP expression is also amplified by RANKL, whose synthesis in osteoblastic cells is, in turn, stimulated by 1,25 (OH) 2D through a specific vitamin D–responsive element. Therefore, in addition to being considered one of the most effective endogenous inhibitors of VC, MGP also performs a manifold role in bone turnover since it regulates not only bone formation but also osteoclast differentiation and bone resorption [7].

CKD patients are particularly exposed to the risk of a poor vitamin status (D and K). Although all these findings suggest a protective effect in CKD of combined vitamin D and vitamin K supplementation in bone and vascular health, no randomized controlled trials examined the effects of such combined treatment in this population. Until now, most studies have evaluated the combined impact of VK and VD supplementation on bone mineral density and bone fractures, mainly in postmenopausal women with osteoporosis [60]. Anyway, no randomized controlled trials examined the effects of combined VD and VK supplementation in CKD patients.

Van Ballegooijen et al. demonstrated that both vitamin D deficiency and vitamin K deficiency were associated with higher systolic and diastolic blood pressure [61]. Asemi et al. showed that synergic supplementation of vitamins D and K improved insulin sensitivity and carotid intima-medial thickness in type 2 diabetic patients [62]. Only a cross-sectional study performed on HD patients demonstrated that treatment with vitamin D analogs was associated with a higher percentage of total osteocalcin levels [63].

Further studies are needed to explain the pathways involved by the combined effects of vitamin D and K on bone and vascular health, especially in the CKD population.

Vitamin K and Bone Fractures

The association between CKD and bone fractures has been analyzed in several studies.

The second phase of the Dialysis Outcomes and Practice Patterns Study (DOPPS), which has involved 12 countries, indicated an incidence of 8.9 per 1000 patients-years for new hip fractures and 25.6 per 1000 for any new fracture [64]. In an analysis carried out on 9714 incident dialysis patients from the Swedish Renal Registry, fractures are common and affect 9% of patients starting dialysis [65]. Fusaro et al. demonstrated a high vertebral fracture prevalence (57%) in 86 dialysis-dependent patients [66]. In this population, hip fractures are also common. Alem and colleagues pointed out a higher risk of hip fractures than in the general population, independently of age and gender [67].

In CKD, the metabolism and the effects of VKDPs on bone remodeling and vascular health fit into the complex scenario of CKD-MBD characterized by changes in mineral metabolism, a high risk of bone fractures, and cardiovascular calcification (Fig. 2).

Some studies have investigated the link between poor vitamin K status and bone health in CKD patients. Kohlmeier et al. proposed the first demonstration of an independent and close correlation between vitamin K deficiency and high bone fracture risk in dialysis-dependent patients in 68 hemodialyzed patients. Those without bone fractures had higher phylloquinone and carboxylated osteocalcin serum levels than patients with bone fractures [68]. These results were confirmed in an observational study carried out on 387 prevalent hemodialyzed patients, which found an association between vitamin K status and vertebral fractures, vascular calcification, and survival. In addition, vitamin K1 deficiency (defined as vitamin K1 level lower than 0.21 ng/mL) was the strongest predictor of vertebral fractures, whose prevalence was over 50% [58].

To date, few studies analyzed the issue of vitamin K deficiency in non-dialysis-dependent CKD patients. In these
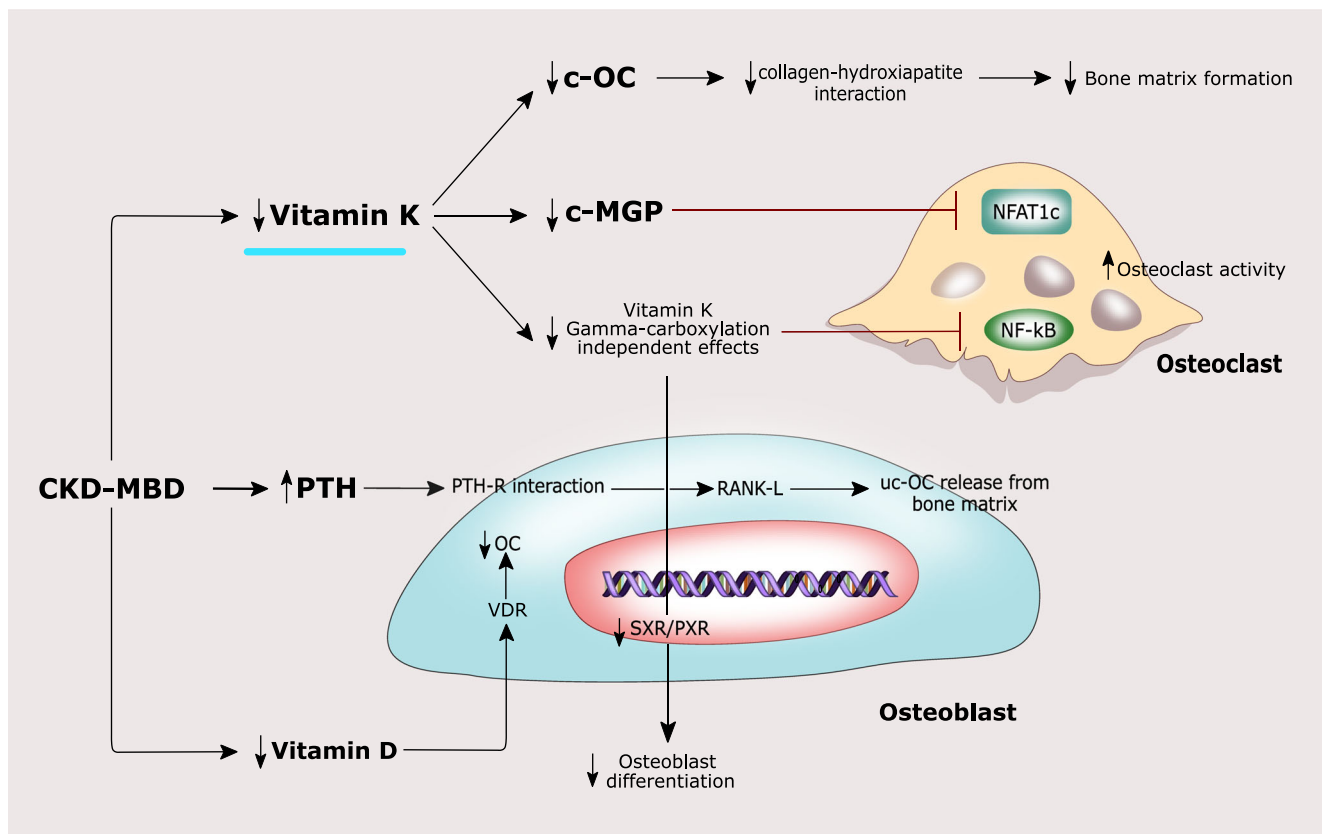
Fig. 2 CKD is characterized by low vitamin K levels, which in turn lead to reduced MGP and c-OC levels. C-MGP determines a reduction in intracellular calcium flux, causing a decreased NFATc1 activity. Low c-MGP leads to a reduced NFATc1 inhibition with increased osteoclast activity. OC, secreted by osteoblasts, plays an essential role in the synthesis and regulation of the bone matrix. The active carboxylated (c-OC) form is mainly involved in bone mineralization allowing the interaction between its calcium-binding Gla residues with hydroxyapatite. OC also acts as an inhibitor of bone mineralization, thus regulating the rate of mineral maturation. Lower vitamin K levels determine a decrease in SXR/PXR activation and a weaker inhibition of NK-kB, leading to reduced osteoblast differentiation and increased osteoclast activity, respectively. Elevated PTH levels contribute to bone loss both by activation of RANKL/RANK axis and by the release of ucOC from the bone matrix. In addition, in CKD-MBD, lower vitamin D levels lead to a reduced OC synthesis in osteoblasts through low VDR activation

patients, Voong et al. showed that subclinical VK deficiency is more frequent with worsening renal function [69]. Evenepoel et al. analyzed 468 adult patients with ESRD referred for a kidney transplant and demonstrated an independent association between vitamin K status (assessed immediately before transplantation) and bone health. A poor vitamin K status, defined by dp-ucMGP greater than 500 nmol/L was independently associated with low aBMD and higher bone incident fractures, while no associations were demonstrated with bone turnover markers [70•].

Several studies identified a poor vitamin K status in KTRs. However, the number of studies that correlate this deficiency with a clinical endpoint (such as bone fractures) is limited [33, 71, 72].

Some randomized controlled trials addressed the hypothesis of a reduction in fracture risk after vitamin K administration, specifically the effects of vitamin K supplementation on fracture risk in the general population [73–75]. In the ECKO trial, 440 postmenopausal women were randomized to either 5 mg of oral vitamin PK or placebo daily for 2 years: in the first group, a lower incidence of clinical fractures was detected [74].

In a smaller study involving 20 patients with chronic glomerulonephritis, Sasaki and colleagues demonstrated the effect of vitamin K supplementation (MK-4) for a year preventing steroid-induced bone loss [76].

The use of warfarin therapy is another crucial variable in the fracture risk profile of dialysis-dependent patients. The mechanism by which warfarin can cause vitamin K deficiency is both direct (inhibition of carboxylation of VKDPs) and indirect (low dietary intake of food rich in vitamin K).

We analyzed and compared patients on warfarin treatment for more than 1 year with untreated patients in a cross-sectional study with a 3-year follow-up: warfarin-treated male patients had more vertebral fractures than controls (77.8% vs. 57.7%, $p<0.04$) [37].

Other studies analyzed the action of novel non-vitamin K antagonist oral anticoagulants (NOACs) on bone health. In an experimental study, dabigatran was compared with warfarin, and the authors demonstrated preservation of femoral and vertebral density and volume in the NOAC treatment group [77]. In three recent clinical trials that compared treatment with VKA versus NOACs for atrial fibrillation on bone health and fracture risks, the authors demonstrated that the use of NOACs was better than VKA in preventing bone fractures [78–80].

Vitamin K Inflammation

Persistent, low-grade inflammation is a hallmark feature of chronic kidney disease (CKD), involved in CKD progression, vascular disease, and all-cause mortality of these patients [32, 81, 82].

Furthermore, inflammation is also a major component of other diseases that are independent risk factors for CKD, such as obesity and diabetes [83].

The causes of inflammation in CKD are multifactorial: changes in the mineral metabolism, increased oxidative stress associated with reduced capacity to repair oxidative DNA damage, increased nonenzymatic glycation, intestinal dysbiosis and increased gut permeability, exposure to bacterial endotoxin, biocompatibility issues during dialysis treatment [84, 85].

Growing evidence shows that nuclear factor erythroid 2–related factor 2 (NRF2)/Kelch-like ECH-associated protein 1 (KEAP1)-dependent antioxidant defense pathway and vitamin K play a crucial role in counteracting oxidative stress, DNA damage, senescence, and inflammation [86]. These processes are involved in vascular calcification and VSMC differentiation in osteoblast-like cells (Fig. 3) [86].

NRF2 target genes encode several antioxidant proteins such as catalase, heme-oxygenase 1, glutathione peroxidases, NAD(P)H quinone dehydrogenase (NQO1), glutathione S-transferases, peroxiredoxins, thioredoxins, thioredoxin reductases, and glutamate-cysteine ligase (He, Ru and Wen, 2020).

Adaptive activation of NRF2 occurs in response to various inducers in phenotypic remodeling of VSMCs, and numerous agonists of NRF2 signaling have proven to be effective in lowering the pathological VSMC osteogenesis in VC [87]. The anti-inflammatory and antioxidant role of vitamin K has been traced to both VKDP carboxylation and independent GGCX effects [88].

Vitamin K1 reduces vascular inflammation by antagonizing basal and cytokine-induced activation of the nuclear factor κB (NF-κB) in both γ-carboxylation-independent and dependent pathways [13].

The continuous recycling of vitamin K is ultimately responsible for antioxidant and anti-inflammatory effects. While VKORC1-like 1 has been found to reduce intracellular oxidative damage [89], KH2 acts as a strong radical scavenger.

NQO1, one of the antioxidant proteins encoded by an NRF2 target gene, could link NRF2 signaling and the vitamin K cycle [87, 89, 90].

Experimental and clinical data also show an inverse association between vitamin K status and circulating inflammatory markers [91, 92]. Intestinal malabsorption or the administration of drugs (anticoagulant or prolonged antibiotic therapy) inducing vitamin K deficiency status can increase the levels of inflammatory cytokines, including IL-6 and C-reactive protein [93].

Vitamin K deficiency arising during the early phases of the COVID-19 infection may contribute to the activation of the Th2 storm with increased production of IL-6, which is involved in the building up of the inflammatory response
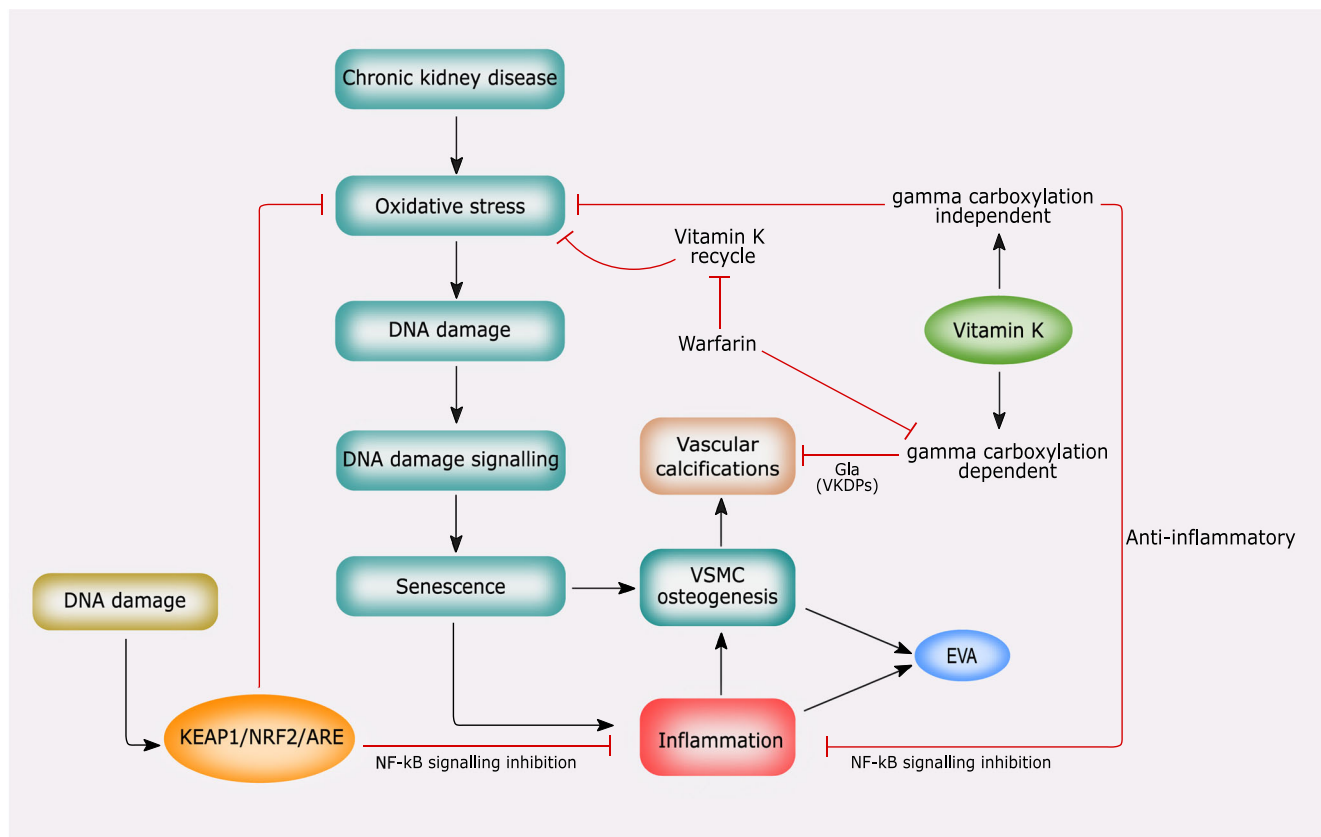
Fig. 3 The role of NRF2 signaling and vitamin K in mediating oxidative stress, DNA damage, senescence, and vascular calcification (see the text for the details)

through the recruitment of both cellular and humoral components [94].

The effects of vitamin K deficiency on the COVID-19 infection take place regardless of its link with inflammation. The active form of vitamin K is hydroquinone (KH2), produced by a quinone reductase at the expense of NADPH. Following γ-glutamyl carboxylation, the reduced form of vitamin K (KH2) is oxidized to vitamin K 2,3-epoxide (KO). In the so-called vitamin K cycle, KH2 is resynthesized by KO reduction through two reductase activities: vitamin K epoxide reductase (VKOR), which first transforms KO into K quinone, and then a vitamin K reductase reduces K quinone to the K hydroquinone (KH2) [7].

The rate of vitamin K recycling depends on a polymorphism in the promoter region of the VKORC1 gene [95].

The VKORC1-1639A allele is associated with low rates of vitamin K recycling and may be regarded as a natural status analogous to low-dose VKA treatment. Considering the high incidence of VKORC1-1639A in East Asia, this feature may be an explanation for the lower rates of COVID-19 related morbidity and mortality [96]. The increase of dp-ucMGP mediated by vitamin K insufficiency and derangement of TAM signaling can predispose to thrombosis and disseminate

intravascular coagulation, which characterizes the microvascular damage observed in COVID patients [97, 98].

The randomized controlled clinical trial to investigate effects of vitamin K2 in COVID-19 (KOVIT) is an ongoing study designed to evaluate if the improvement of vitamin K status by vitamin K supplementation could have favorable effects on pulmonary damage and coagulopathy in COVID-19 [99].

CKD-MBD Treatment Effects on Vitamin K Status

Few studies so far have explored the effects of these long-standing treatments on vitamin K deficency. This feature is critical when considering that CKD-MBD therapy and vitamin K deficiency act on bone and vascular health.

We carried out a secondary analysis of the VIKI (VItamin K Italian) study, an observational study designed to assess the prevalence of vitamin K deficiency in hemodialysis patients and to investigate the effects of ongoing treatment for CKD-MBD on OC and MGP levels [58]. We showed that vitamin D analogs increase the levels of OC and MGP, while calcimimetics, alone or combined with calcium acetate, increase only MGP levels. In addition, the combination of

vitamin D analogs and calcimimetics proved to be most effective in inducing a further increase of total OC [63].

Equally promising for future developments are the studies that have analyzed the effects of phosphate binders on the absorption and action of vitamin K and VKDP. Studies looking at this issue are still limited, and, most importantly, the results show noticeable differences in the association between various phosphate binders and vitamin K deficiency. Neradova et al. evaluated the interaction of vitamin K2 (menaquinone-7; MK-7) with five different phosphate binders in the presence or absence of phosphate. In this in vitro study, sucroferric-oxyhydroxide and sevelamer carbonate were the only binders that did not interact with vitamin K2. Instead, calcium acetate/magnesium carbonate bound vitamin K2 strongly, both in the absence and presence of phosphate. The binding of lanthanum carbonate and vitamin K2 depended on the absence of phosphate, suggesting a competitive interaction between phosphate and vitamin K2 for this compound [100]. Westenfeld and colleagues analyzed the effect of vitamin K2 supplementation on functional vitamin K deficiency in HD patients and did not observe any relationship between sevelamer use and circulating vitamin K (menaquinone) levels. However, their study was not drawn nor powered to explore this association [42]. In a further analysis of the VIKI study, multivariate logistic regression showed that MK4 deficiency was associated with sevelamer use and aortic calcification [101]. This finding is of considerable importance since low MK4 levels have been recognized as a predictor of aortic calcification [58]. In the same logistic model, sevelamer enhanced the effect of total BGP levels on the odds of vertebral fractures (OR = 3.15; 95% CI 1.46–6.76; $p = .003$) in patients with total BGP <150 μg / L compared to those with total BGP ≥150 μg/L.

Future Perspectives

Based on the previous observations, it is possible to evaluate the possibility of introducing vitamin K supplementation to contrast cardiovascular disease and bone alterations in CKD patients.

In the literature, many ongoing clinical trials and studies analyzed vitamin K supplementation in CKD patients, both dialysis-dependent and non-dialysis-dependent [7].

One of the most important issues is represented by the definition of vitamin K dosage able to reduce bone fractures and improve vascular calcifications both in the general population and in CKD patients. A dosage of 10 mg for PK and a 360 mcg/die until over 1080 mcg/die for MK-7 is considered adequate in CKD patients [7].

The main ongoing trials are reported in Table 2, considering both cardiovascular and bone as primary or secondary outcomes.

Table 2 Description of the main ongoing trials to evaluate the protective role of vitamin K to prevent both cardiovascular and bone health

Study	Participants	Study design, follow-up	Interventions	Bone fracture endpoint	Condition of the study
NCT01528800 iPACK-HD	ESRD on HD, CAC score ≥30 AUs	Phase 2 RCT, DB, 12 m	PK (10 mg three times a week) VS placebo	Secondary Endpoint	Active
NCT03871322 The Vitamin K2 and D3 intervention Trial in Children and Adolescents with the Low-energy Fractures [102]	Age < 18 years, low-energy fracture, vitamin D serum level < 30 ng/m	RCT, DB, 3 m	VitD3 (2000 IU/d) plus MK7 (90 μg/d) or MK7 alone VS placebo	Primary Endpoint	Recruiting
NCT02976246 Effect of vitamin K2 (MK7) on cardiovascular and bone disease in dialysis patients (RENAKVIT) [103••]	HD or PD > 3 months	Phase 4 RCT, DB, 2 y	MK7 (360 mcg/d) vs. placebo	Secondary: bone fracture incidence, thromboembolic events, biomarkers changes	Complete CKJ https://doi.org/10.1093/ckj/sfab017.
NCT04145492 Effect of vitamin K2 on vascular calcification in hemodialysis patients [104]	HD patients	Phase 2, RCT	90 μg of vitamin K2 + 10 μg of inactive vitamin D + combination of 90 μg + 10 μg	Not applicable	Recruiting

CAC, coronary artery calcium; HD, hemodialysis; RCT, randomized controlled trials; ESRD, end-stage renal disease; PK, phylloquinone; PD, peritoneal dialysis

Declarations

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