Vitamin K in CKD Bone Disorders

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REVIEW

Vitamin K in CKD Bone Disorders

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Abstract
Vitamin K is principally known because it is involved in blood coagulation. Furthermore, epidemiological studies showed that its deficit was associated with increased fragility fractures, vascular calcification and mortality. There are two main types of vitamin K vitamers: Phylloquinone (or PK) and Menaquinones (MKn). Vitamin K acts both as coenzyme of y-glutamyl carboxylase (GGCX) transforming undercarboxylated in carboxylated vitamin K-dependent proteins (e.g., Osteocalcin and Matrix Gla Protein) and as a ligand of the nuclear steroid and xenobiotic receptor (SXR) (in murine species Pregnane X Receptor: PXR), expressed in osteoblasts. It has been highlighted that the uremic state is a condition of greater vitamin K deficiency than the general population with resulting higher prevalence of bone fractures, vascular calcifications and mortality. The purpose of this literature review is to evaluate the protective role of Vitamin K in bone health in CKD patients.

Keywords Vitamin K · Bone fractures · Osteocalcin · Matrix Gla protein · CKD

Introduction

Henrik Dam, a Danish scientist, in 1926, described an unknown hemorrhagic syndrome as a 'vitamin factor deficiency.' This vitamin was called K, form the word 'Koagulation.' In 1939, the American scientist Edward Adelbert Doisy confirmed Dr. Dam's intuition, announcing the isolation of vitamin K1 (phylloquinone, PK) from vegetable sources. Subsequently, Dam improved our understanding of the vitamin K system, describing a similar 'vitamin factor' in putrefied fish. To distinguish it from the former, he called it vitamin (menaquinone, MKn) [1].

The first publication on vitamin K dates back to 1936, and to date, the number of manuscripts related to it is about 27,000. Compared to the other fat-soluble vitamin D, the first publication of which dates back to 1922, the publications are more than three times the number of vitamin K, about 87,000. However, a more accurate analysis shows that since the year 2000, the publications on vitamin K are almost the same as those made in the previous 64 years, suggesting an increased interest of the scientific community.

The presence of a 2-methyl-1,4-naphthoquinone nucleus implies that a compound can be considered a K vitamer. PK has a phytyl side chain, while MK has a variable number of condensed isoprenic units, both attached at the 3-position of 2-methyl-1,4-naphthoquinone nucleus. When a menaquinone is without side chain, it can be defined as vitamin K3 or Menadione (synthetic form) [1].

Vitamin K: Kinds of Vitamers, Source, Status and Recycling

The term of Vitamin K cumulatively indicates a family of fat-soluble compounds different in origin and function. Although they share a common 2-methyl-1,4-naphthoquinone ring, they are differentiated by the (lipophilic) side

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chains linked at the 3 positions: the three main forms (vitamers) are PK, MKn and menadione according to the side chains linked at 3 positions [2].

Each vitamer recognizes a different food origin, while PK is found in highest concentration in green leafy vegetables such as spinach, cauliflower and cabbage, menaquinones are derived mainly from fermented food and intestinal bacteria [3]. In western diets, MKn can be found in fermented foods such as butter, beef liver, curdled cheese, egg yolk.

The highest source of vitamin K2 (particularly MK-7) is a Japanese food named Natto, which is produced by fermenting soy beans with Bacillus Subtilis.

Furthermore, MKn are also produced by the intestinal bacterial flora: MK-10 and MK-11 are synthesized by Bacteroides, MK-8 by Enterobacteria, MK-7 by Veillonella, and MK-6 by Eubacterium lentum. The only exception is MK-4, which is produced from PK through a side chain removal/addition mechanism in specific tissues (pancreas, testes and vessel wall) [4].

Both forms of vitamin K (Vitamin K1 and Vitamin K2) require a normal pancreatic function and the presence of bile salts for their absorption in the small intestine, thus being transported into the plasma by lipoproteins. Plasma concentrations of both forms of Vitamin k in healthy fasting people are very low as are body storages and therefore without a regular dietary intake, vitamin K stores are rapidly depleted [2].

Consequently, the human body needs a continuous recycling of the vitamin k, in order to offset its poor storage capacity as well as its continuous consumption in the metabolic pathways where vitamin k is involved. The active form of vitamin K is hydroquinone (KH2), produced by a quinone reductase at the expense of NADPH. Following the γ-glutamyl carboxylation, the reduced form of vitamin K (KH2) is oxidized to vitamin K 2,3-epoxide (KO).

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). It is still unknown if this reductase activity is made by VKOR or by its paralog, the VKORC1-like 1 (VKORC1L1), whose function is not fully defined [5].

Therefore, the activity of vitamin K reductase is required to synthesize KH2, the active coenzyme for the γ-glutamyl carboxylase (GGCX) in the endoplasmic reticulum, as well as to reduce 'new' molecules of vitamin K that entered into the cycle. Therefore, the vitamin K recycling lowers its dietary needs ensuring its availability for all the pathways in which it is involved [6].

Moreover, it must be considered that the evaluation of vitamin K in plasma is difficult due to low circulating vitamin K levels and non-polar characteristics of vitamin K as well as lipid interference. In addition to analytical variability, diet, inflammation and the coexistence of chronic diseases can influence plasma levels of vitamin K subtypes. McCabe et al. highlighted in rats with mild and severe CKD had significantly lower amounts of K1 measured in liver, spleen and heart while they found higher levels of MK-4 measured in kidney cortex and medulla. Moreover, they showed a decrease in the thoracic aorta expression of vitamin K recycling (Vkor) and utilization (Ggcx) enzymes, and a decrease in the kidney level of vitamin K1 to MK-4 bioconversion enzyme UbiA prenyltransferase domain-containing protein 1 in CKD [7].

Mechanism of Vitamin K Actions

Vitamin K acts as a coenzyme of GGCX which catalyzes the carboxylation (and thereby activation) of vitamin-K-dependent proteins (VKDPs). GGCX catalyzes the post-translational γ-carboxylation of glutamic acid (Glu) residues contained within vitamin K-dependent proteins (also known as Gla proteins), to γ-glutamyl carboxylase (Gla) residues [8]. To date, seventeen members of the Gla protein family, involved in various biological processes, have been identified, although the function of some of them has not been fully defined. In particular, the Gla family includes (i) seven proteins belonging to the coagulative cascade: prothrombin, factor VII, factor IX, factor X, protein C, protein S and protein Z; (ii) four proteins that regulate bone and vascular mineralization: matrix Gla protein (MGP), osteocalcin (OC), growth arrest-specific protein 6 (Gas6), Gla-rich protein (GRP); (iii) two proline-rich Gla proteins, two transmembrane Gla proteins, periostin and periostin-like factor [9].

Since some Gla proteins are involved in bone metabolism and vascular health, it has been hypothesized that their reduced carboxylation may lead both to bone metabolism impairment and increase in vascular calcification [8]. Vitamin K deficiency hampers VKDPs to acquire their carboxylated form able to binding 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. Moreover, adequate calcium binding is a critical physiologic step both in regulating bone mineralization and counteracting VC [10].

The role of Vitamin K in bone metabolism does not take place only by the γ-carboxylation pathway since MK-4 binds to the nuclear receptor, the steroid and xenobiotic receptor (SXR) and its murine ortholog, pregnane X receptor (PXR), able to regulate the expression of genes encoding enzymes involved in steroid metabolism and detoxification of xenobiotics and various drugs [11, 12]. SXR is expressed in the liver, intestine and human osteoblastic cells; once bound to a ligand, SXR forms a complex with retinoid X receptor which in turn binds to an SXR-responsive element on the target

gene promoter that rules the transcription. MK-4, by activating SXR pathway, may play a relevant role in bone health by inducing expression of genes coding proteins such as matrilin-2 (Matn2), tsukushi (Tsk) and CD14 which are involved in bone remodeling and in the maintenance of bone quality, this latter through the effect SXR-mediated on collagen content [2, 13, 14]. The bone quality includes the material properties of bone, the degree of mineralization, the crystal size and structure and the microdamage accumulation, and these features are all influenced by collagen cross-link formation. The type-I collagen fibers wired with crosslinks fibers shape the framework that binds matrix proteins and mineral crystals. In CKD, the arrangement of collagen fibers is deranged and the mineral crystals remain immature, thus impairing the bone quality [15].

Furthermore VK2 enhances bone formation and suppresses bone resorption by antagonizing basal and cytokine induced activation of the nuclear factor κB (NF-κB) in both γ-carboxylation-independent and dependent pathways [16].

Vitamin K and VKDPs in Bone Health

The effect of VK on bone strength mainly takes place through the VK-dependent activation of OC and MGP. OC mainly produced, under the control of vitamin D, by osteoblasts and to a lesser extent by chondrocytes, plays an essential role in the synthesis and regulation of bone matrix (Fig. 1). The carboxylation pathway, vitamin K mediated, is pivotal for the transformation of OC from the undercarboxylated form (ucOC) into the fully functional carboxylated form (c-OC) (Fig. 1) [8, 17].

The ucOC discloses a low calcium and hydroxyapatite binding activity, while the c-OC form rules bone mineralization allowing the interaction between Gla residues with the calcium ions of hydroxyapatite (Fig. 1). This property has been proposed as the main mechanism that enables OC to regulate the formation and the growth of hydroxyapatite crystals [18]. The level of ucOC represents a more sensitive marker of vitamin K status and therefore can reveal subclinical vitamin K deficiency [19].

Since ucOC parallel total OC level, the expression of ucOC as a percentage of the total OC (% ucOC) is considered a more reliable index of vitamin K storage than the absolute value of ucOC. Booth et al. showed that a value of % ucOC > 20% is consistent with subclinical vitamin K deficiency in vitamin K depletion and repletion studies [20].

However, a high ucOC level is not an exclusive index of low vitamin K levels and intake since it can be released during osteoclastic resorption [18] (Fig. 1). OC can also play a mechanical function within the bone matrix since it binds hydroxyapatite and forms a complex with collagen, so acting as a bridge between the matrix and mineral component of bone tissue [21, 22] (Fig. 1).

The involvement of VK on bone health and remodeling is not limited to OC but also involves MGP, a 12-KDa gamma-carboxyglutamic acid-containing protein, synthesized, as well as from vascular smooth muscle cells, endothelial cells, also by osteoblasts, chondrocytes and osteoclasts. MGP considered one of the most effective endogenous inhibitors of vascular calcification in vivo plays a multifaceted effect in bone turnover since it not only promotes bone formation by upregulating Wnt/β-catenin signaling but also inhibits osteoblast mineralization and affects bone mass by regulating the deposition of the bone matrix [11, 23, 24]. Notwithstanding the low MGP expression in mature osteoclast, its role in osteoclastogenesis is relevant although complex. MGP expression is induced significantly by RANKL, and in turn osteoclasts differentiation and bone resorption are enhanced by MGP depletion and blunted by MGP overexpression [25]. This finding is related to the effect of the Nuclear Factor of Activated T cells, cytoplasmic 1 (NFATc1), the leading player in osteoclastogenesis, that is ruled by MGP. This scenario outlines a negative feedback loop to make osteoclast formation under strict control (Fig. 1) [25, 26].

The different effects of MGP can be traced to the complex posttranslational modification of MGP before maturation: (i) phosphorylation of up to three serine residues and (ii) γ-carboxylation of up to five glutamate residues. While the carboxylation determines MGP's bioactivity as a calcification inhibitor, the function of phosphorylation is not yet fully defined. Recent data suggest a role of phosphorylation in regulating MGP secretion into the extracellular fluid [27, 28].

These reactions do not proceed in parallel, and, according to the level of carboxylation and/or phosphorylation, at least four different molecules can be found in circulation: (i) dephosphorylated-carboxylated MGP (dp-cMGP); (ii) dephosphorylated undercarboxylated MGP (dp-ucMGP: vitamin k deficiency is characterized by dp-ucMGP levels higher 500 pmol/L) [29]; (iii) phosphorylated uncarboxylated MGP (p-ucMGP); (iv) phosphorylated carboxylated MGP (p-cMGP). The active form is both phosphorylated and carboxylated (p-cMGP) and its synthesis is stimulated by vitamin D [30].

Vitamin K Status and CKD-MBD

CKD patients often show a subclinical vitamin K deficiency [31, 32], and dietary recommendations aimed at reducing potassium and phosphate intake (e.g., reduced consumption of leafy green vegetables and dairy products, rich in vitamin K1 and K2, respectively) may play a relevant role in promoting this deficit [9, 32, 33]. It is presumable that Vitamin K

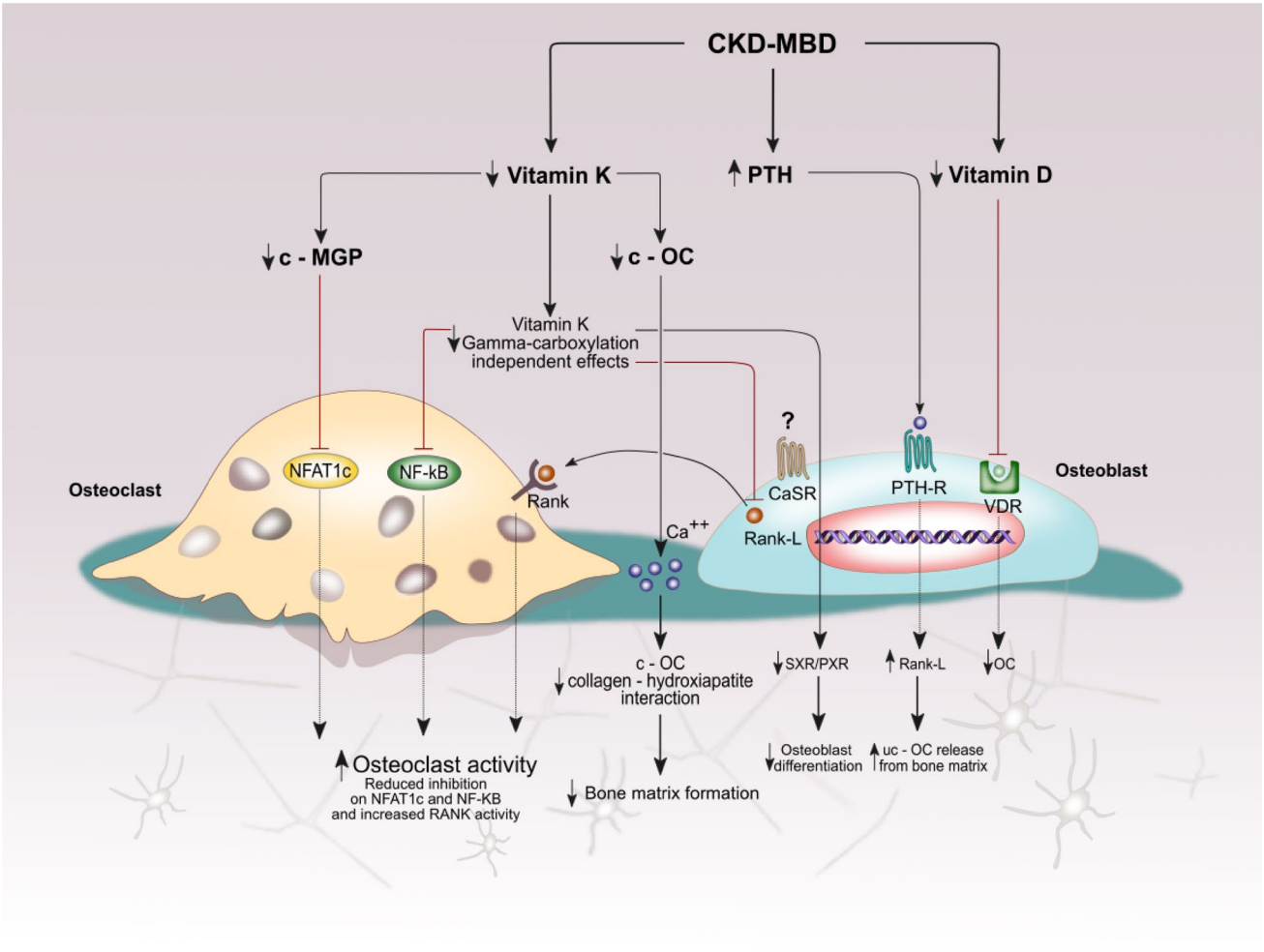

Fig. 1 CKD-MBD influences bone status toward reduced bone formation, through modulation of vitamin K-dependent carboxylated proteins (c-MGP and c-OC), vitamin K gamma-carboxylation-independent effects, PTH and vitamin D activity. CKD-MBD is characterized by low vitamin K levels which in turn lead to reduced MGP and c-OC levels. C-MGP determines a reduction in intracellular calcium flux causing reduction of 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 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 acts also 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 release of ucOC from bone matrix. In addition, in CKD-MBD, lower vitamin D levels leads to a reduced OC synthesis in osteoblasts through low VDR activation

deficiency is also amplified by the uremic milieu, reduction in gut microflora and its depletion which follows the high vitamin K requirement for the carboxylation of VKDPs employed in maintaining bone and vascular health.

In CKD, the metabolism and the effects of VKDPs on bone remodeling as well as vascular health fit into the complex scenario of chronic kidney disease—mineral bone disorder (CKD-MBD) characterized by changes in mineral metabolism, a high risk of bone fractures and cardiovascular calcification. This syndrome develops early in the course of renal insufficiency and is characterized by abnormal circulating levels of calcium, phosphorus, parathyroid hormone (PTH), vitamin D and FGF23 [34–36]. Recently the pattern of molecules is further expanding also involving the Wnt/β-catenin signaling and activins [37, 38]. Furthermore, some of these factors play a direct and indirect role in regulating the synthesis and release of VKDPs; this effect is added to the metabolic impairment induced by the poor vitamin K status found in these patients.

Vitamin D and K share osteoinductive properties, in particular two studies demonstrated that MKn enhances Vitamin D3-induced mineralization by increasing the OC gene

expression as well as its content in extracellular matrix [2]. An increase of total OC has been demonstrated in CKD due to PTH-related bone resorption and retention of ucOC and OC fragments [9]. PTH also upregulates RANKL that in turn enhances the synthesis of MGP: the latter is able to upregulate Wnt/β-catenin signaling (Fig. 1).

In addition to the complexity of its metabolism, one of the issues that preclude a complete understanding of vitamin K function, in health as well as in pathological conditions, is represented by the troubles in developing adequate assays for the measurement of vitamin K.

Functional tests can be performed to estimate vitamin K blood levels indirectly; in particular, the measurement of undercarboxylated proteins, OC and MGP, has proved to be more sensitive than prothrombin time in detecting subclinical vitamin K deficiency [29]. In CKD patients, several studies assessed a poor vitamin K status either directly by means of low levels of serum phylloquinone or indirectly by high levels of ucOC, ucMGP and dp-ucMGP [29]. The poor Vitamin K status worsens parallel to the CKD progression jointly to the overall poor nutrients intake.

Schurgers et al. evaluated dp-ucMGP levels in a cohort of 107 patients whose kidney function varied from CKD stages 2–5D; they reported that levels of plasma dp-ucMGP increased progressively with CKD worsening [39].

The lipophilic nature of Vitamin K does not allow its removal through the dialysis membrane; nevertheless, several studies show that HD patients disclose a persistence of a poor vitamin K status. Indeed, Westenfeld et al. reported that hemodialysis patients had 4.5-fold higher dp-ucMGP and 8.4-fold higher uncarboxylated osteocalcin levels compared with controls [40]. Poor Vitamin K status is not a prerogative of HD treatment since peritoneal dialysis patients disclose a similar degree of vitamin K deficiency although evaluated exclusively by increased percentage of ucOC [41].

The plasma dp-ucMGP (and likely the vitamin k status) is not influenced by the dialysis technique: in particular, neither the use of high flux membranes nor their composition seems able to influence the vitamin k removal. In a prospective study carried out on 35 patients, Uhlin et al. demonstrated that the change in dialysis technique, from conventional hemodialysis to online Hemodiafiltration has no influence on dp-ucMGP plasma levels [42].

In CKD patients, phosphate binders used for the treatment of hyperphosphatemia can further worsen vitamin K deficiency thus abating the advantage of lowering phosphate blood levels. Studies analyzing this issue are still limited; the results currently available show significant 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, with or without the phosphate addition. 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 [43]. In a cross-sectional study of 387 hemodialysis patients (VItaminKItalian: VIKI study), Fusaro et al. showed that MK-4 deficiency was the strongest predictor of aortic calcification (OR, 2.82; 95% CI, 1.14–7.01) [31]. In a secondary analysis, they found in patients treated with Sevelamer an odds ratio of MK-4 deficiency of 2.64 (95% CI 1.25–5.58, $p = 0.01$) [44]. Furthermore, sevelamer use significantly amplified the effect of total OC levels on the risk of VFs: BGP < 150 μg/L compared to those with total BGP ≥ 150 μg/L (OR 3.15, 95% CI 1.46–6.76, $p = 0.003$) [44].

Kidney transplantation does not seem able to restore vitamin K deficiency, the latter derived from circulating dp-ucMGP levels. Evenepoel et al. in 468 de novo renal transplant recipients (KTRs) found that 421 patients had dp-ucMGP levels > 500 nmol/L, indicating a condition of vitamin K deficiency; this finding was associated with incident fractures [45]. Keyzer et al. also found that high circulating levels of dp-ucMGP were associated independently with increased risk of mortality in 518 stable KTRs [46]. These findings could follow the maintenance of their dietary habits before transplantation, therefore contributing to the previous state of vitamin K insufficiency. Furthermore, body mass index, triglyceride level, mycophenolate mofetil use, and smoking have been identified as independent determinants of circulating dp-ucMGP levels in [29].

Vitamin K—Microbiota and Bone Health in CKD

Vitamin K1 (phylloquinone, PK) is mainly found in green leafy vegetables. Vitamin K2 (menaquinone, MK), conversely, is mainly of microbial origin. Fermented dairy and soybean-based (natto) food products and gut microbiota are important sources of vitamin K2 in mammals. To what extent the endogenous gut flora contributes to the overall vitamin K status of the host remains a matter of ongoing debate [47]. Experimental studies on the effect of oral and colorectal administration of vitamin K on circulating prothrombin concentration in vitamin K-deficient rats demonstrated that the bioavailability of colonic vitamin K is more than 50-fold lower than the bioavailability of oral vitamin K [47]. Conversely, data from germ-free rodents, and experimental and clinical studies with broad spectrum antibiotics indicate that gut microbial metabolism may be important to maintain adequate vitamin K stores in the mammalian host [48–50].

Gut dysbiosis is a common finding in CKD [51]. Causes are multiple and include dietary restrictions, polypharmacia

(e.g., antibiotics, phosphate binders), altered gastrointestinal transit and uremia per se [52, 53]. Gut dysbiosis in CKD goes along with a shift from a saccharolytic towards a proteolytic fermentation pattern. Gut dysbiosis may also go along with a decreased production of vitamin K2, and as such contribute to the high prevalence of functional vitamin K deficiency in CKD [54–57].

The gut microbiome is increasingly recognized as a key regulator of bone health [58–62]. The effects of the gut microbiota on bone are mediated in part via effects on the immune system [63]. Another mechanism whereby microbes may influence bone health is through the production of metabolites that diffuse from the gut into the systemic circulation. Protein fermentation metabolites may interfere with parathyroid hormone signaling but may also exert direct toxic effect on bone cells [61]. Recently, short-chain fatty acids (SCFAs), which are generated by fermentation of complex carbohydrates, have emerged as key regulators of bone metabolism, exerting overall bone protecting effects [64]. Finally, MKs produced by the endogenous gut flora may be hypothesized to play an important role in maintaining bone homeostasis. In a recent elegant experimental study, disruption of the gut microbiota by antibiotics resulted in a decreased production of MKs and impaired bone tissue mechanical properties [65].

In aggregate, current evidence indicates that gut dysbiosis and impaired vitamin K2 production may be in the causal pathway between CKD and bone fragility.

Warfarin/DOAC Use and Bone Fractures

For many year, vitamin K-antagonists (VKAs) have been the most widely used anticoagulant drugs for treatment of patients at risk of recurrent arterial and venous thrombosis. VKA have unfavorable pharmacokinetics and are prone to interference with food rich in vitamin K, and thus direct thrombin and factor Xa (FXa) inhibitors (non-vitamin K antagonist oral anticoagulants, NOACs) have been introduced to the clinic as alternative anticoagulants [66].

Both VKA and NOACs are proven effective in reducing thrombotic complications; however, they also influence non-hemostatic activities of coagulation factors. Once it was known that vitamin K was needed for the synthesis of γ-carboxyglutamate (Gla) in prothrombin, the search began for other vitamin K-dependent proteins.

VKA interfere with the carboxylation of vitamin K-dependent coagulation proteins (increase PIVKA-II), Matrix Gla Protein (resulting in an increase in vascular calcifications up to calciphylaxis or calcific uremic arteriolopathy associated with cutaneous necrosis) and thus also with osteocalcin. The first direct evidence that vitamin K metabolism plays an important role in bone comes from skeletal abnormalities that may occur in the developing bone of animals and humans exposed in utero to maternal VKA. Such exposure to VKA may result in a condition termed warfarin embryopathy or chondrodysplasia punctata, which is characterized by pathological skeletal calcification, including the growth plate [67]. The impact of warfarin on osteopenia and remodeling of bone was demonstrated in lambs in which the role of carboxylated osteocalcin was put forward to be importance for maintenance of normal bone mass [68].

Particularly in the elderly, osteoporotic fractures are associated with high mortality and reduced quality of life [69, 70]. In a cross-sectional study that evaluated 387 patients on hemodialysis for more than 1 year, more vertebral fractures were recorded among those treated with warfarin, even if it was not true for females (42.1% vs. 48.4%, $p = 0.6$). Moreover, authors found that total BGP was significantly reduced (82.35 vs. 202 µg/L, $p < 0.0001$), with lower levels in treated men (69.5 vs. women 117.0 µg/L, $p = 0.03$). Furthermore in univariate Cox regression analysis, subjects treated with warfarin had a higher all-cause mortality risk (HR 2.42, 95% CI 1.42–4.16, $p = 0.001$) and data adjustment for confounders attenuated but confirmed the significant warfarin-mortality link (HR: 1.97, 95% CI 1.02–3.84, $P = 0.046$) [71].

Since NOAC lack the direct effect on the vitamin K cycle and on osteocalcin synthesis, they offer an opportunity to assess the real extent of effects of VKAs on bone health. Three recent clinical trials compared the treatment of atrial fibrillation (AF) patients with VKA versus NOACs on fracture risk [72] (Table 1).

In all three trials, the use of NOAC was favorable in preventing bone fractures compared to the use of VKA. This suggests the involvement of VKA on bone via impairing osteocalcin, with no direct effect of NOAC [75]. Indeed, patients who were treated with for more than 12 month with warfarin for persistent or chronic AF and switched to NOAC (rivaroxaban) had increased bone markers and decreased bone resorption markers and a significant decrease in uncarboxylated osteocalcin was noted 6 month after switching to NOAC [76].

In conclusion, NOACs appear to be preferable compared to VKAs to preserve bone health. This adds to the many other advantages of the NOACs, including convenience,

Table 1 Recent clinical trials in patients with AF: comparing the treatment between VKA versus NOACs on fracture risk

	Patients included (n)	Follow-up (months)	Hazard ratio (fracture risk)
Binding et al. [72]	37,350	24	0.85 (0.74–0.97)
Huang et al. [73]	19,414	28	0.84 (0.77–0.93)
Lutsey et al. [74]	167,275	17	0.93 (0.88–0.98)

AF Atrial fibrillation, *VKA* Vitamin K antagonist, *NOACs* Non-vitamin K antagonist oral anticoagulants

lower risk of intracranial hemorrhage, fewer drug and food interactions than the VKAs—probably as the result of some of these pulses-reduced mortality [75].

Vitamin K and Bone Health: Experimental Studies

The effects of vitamin K supplementation on bone health have been studied in animal models. Vitamin K2 supplementation inhibited loss of bone mass density and trabecular bone, improved osteoblast function, and enhanced serum level of bone anabolic markers in ovariectomized rat model [77]. In high-fat diet mice, Vitamin K1 and K2 administration increased serum level of OC as well as of bone formation indexes and reduced the indexes of bone resorption [78].

Combined therapeutic strategy with vitamin K and anti-osteoporotic drug have given interesting results. Vitamin K2 plus teriparatide ameliorated osteoblast activity and increased OC serum level and such effects were higher in combined use [79]. In addition, Vitamin K2 in combination with bisphosphonates showed a reduction in suppressive effect of bisphosphonates on bone turnover and increased bone volume as well as bone formation parameters in rat models [15].

Vitamin K and Bone Health: Clinical Studies

Although the incidence of fractures in CKD patients is demonstrated to be high and represents a major target of renal osteodystrophy therapy, the number of studies evaluating bone fractures in such patients is disappointingly low. Even fewer studies investigated vitamin k involvement in bone fractures in CKD patients.

Kohlmeier et al. were the first to demonstrate an independent association between poor vitamin K status and risk of bone fracture in patients in hemodialysis [80]. Later, Fusaro et al. performed an observational study on 387 hemodialysis patients, finding a prevalence of vertebral fractures of more than 50%. Moreover, authors found that vitamin K1 deficiency was the strongest predictor for vertebral fracture (with an adjusted odds ratio approaching 3) [31] (Table 1).

A secondary analysis of the aforementioned study investigated the effects of ongoing treatment for CKD-MBD on OC and MGP levels showing that vitamin D analogs increased OC and MGP levels, while calcimimetics, alone or combined with calcium acetate, increased only MGP levels. Moreover, authors found that the combination of vitamin D analogs and calcimimetics was the most effective in inducing a further increase of total OC, while increased total MGP levels are found only in patients treated with calcimimetics, alone or combined with calcium acetate [81, 82].

Two ongoing clinical trials are evaluating vitamin K supplementation at pharmacological doses in end-stage renal disease adult patients. The iPACK-HD study, in Canada, randomizes incident hemodialysis patients to placebo or to a higher dose (10 mg) of vitamin K1 thrice weekly for 12 months [83]. The primary endpoint is the progression of coronary artery calcification, while the secondary endpoint is the incidence of vertebral fractures. The RenaKvit includes patients in peritoneal or hemodialysis treated with one tablet of vitamin K2 (MK-7) 360 μg given once daily. The study has two primary endpoints: 1. changes in arterial stiffness assessed by pulse wave examination reflecting vascular calcification at 2 years; 2. changes in bone mineral density (BMD) in the distal radial bone (unit T-score) at 2 years. The secondary aim is to determine bone fractures [84] (Table 2).

According to the literature data, our suggestion about a potential dosage of Vitamin K in General Population (GP) or Chronic Kidney Disease (CKD) to prevent bone fractures has been shown in Table 3.

Conclusions

The efficacy of vitamin K on bone fractures and bone quality needs to be ascertained in future large trials. More evidence is needed about the effects of vitamin K supplementation at physiologic and pharmacologic doses and what is the required dose of Vitamin K to ensure the bone and vascular health and finally if CKD patients need a higher dosage than the general population.

Table 2 Published and ongoing randomized controlled trials on the relationship between vitamin K and bone fractures having fractures as pre-specified outcome

Published clinical trials				
Study	Participants, n	Follow-up	Pre-specified outcome	Results
Evenepoel et al. [45]	Prospective observational cohort studies in 468 de novo renal transplant recipients	5 y	The primary outcome was major fragility fracture, which was defined as a composite of hip, leg, ankle, forearm, proximal humerus, rib, and clinical vertebral fractures occurring in the absence of major trauma	dp-ucMGP above median associated with incident fractures: [HR 2.21 (95% CI, 1.00 to 4.91)]
Fusaro et al. [31]	Observational study in 387 patients on hemodialysis	3 y	The secondary objective was to assess the impact of vitamin K status on vitamin K-dependent proteins related to vertebral fractures	Vitamin K1 deficiency was the strongest predictor of vertebral fractures: [OR 2.94 (95% confidence interval CI, 1.38–6.26)]
Ongoing clinical trials				
Study	Participants	Study design, follow-up	Intervention	Endpoint
NCT01528800 iPACK-HD [83]	ESRD on HD, CAC score ≥ 30 AUs	Phase 2 RCT, DB, 12 m	K1 (10 mg three times a week) vs. placebo	*Primary*: compliance *Secondary*: vertebral and lumbar fractures incidence, coronary artery calcification progression, CV events
NCT02976246 RenaKvit [84]	HD or PD > 3 months	Phase 4 RCT, DB, 2 y	MK-7 (360 mcg/d) vs. placebo	*Primary:* arterial stiffness assessed by pulse wave, BMD change in the distal radial bone *Secondary:* bone fracture incidence, thromboembolic events, biomarkers changes

Table 3 Potential dosage of vitamin K to prevent bone fractures both for General Population and CKD patients

	GP	CKD
MK-4	45 mg/day	No data
PK	5 mg/day	10 mg in CKD with ESRD
MK-7	360 mcg/day	Increase in according to stage CKD until over 1080 mcg/die in ESRD

GP general population, *CKD* chronic kidney disease, *MK* menaquinone, *PK* phylloquinone

Compliance with Ethical Etandards

Conflict of interest M. Fusaro, G. Cianciolo, P. Evenepoel, L. Schurgers, M. Plebani declare that they have no confict of interest.

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