Review

Role of vitamin K2 in bone-vascular crosstalk

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Abstract: Vitamin K (VK) is a fat-soluble vitamin that is indispensable for the activation of vitamin K-dependent proteins (VKDPs). It has been shown to play an important role in the proper calcium deposit at the bone level, hindering that on the vascular walls. The deficiency of this vitamin in European populations is frequent and unknown. It is related to several factors, poor dietary intake, altered intestinal absorption or altered production by bacteria, indicating possible dysbiosis. For Vitamin K2 (VK2), there is currently no official reference daily intake (RDI). However, the effects of VK2 on the improvement of health in cardiovascular diseases, on bone metabolism, on chronic kidney diseases have been the subject of research in recent decades. The microbiota in the gastrointestinal tract plays an important role: *Bacteroides* are primarily capable of synthetizing very long chain forms of menaquinones and, in addition to the bacteria present in the intestinal flora, VK2 is also produced by bacteria used in food fermentation processes. This review provides an update on the current literature regarding the origin of VK2 and its implications in what is called the "calcium paradox", namely the lack of calcium in the bone and its storage in the wall of the vessel.

Keywords: Vitamin K2, vascular calcification, cardiovascular disease risk, calcium paradox, Matrix Gla protein, Menaquinone-7, microbiota

Introduction

Vitamin K (VK) is a fat-soluble vitamin that was first identified by Henrik Dam in 1935 for its anti-hemorrhagic activities and physical properties similar to those of vitamin E, but with a different coagulation function than any known vitamin [1]. It occurs in two biologically active forms, phylloquinone (vitamin K-1; VK1) and menaquinone (vitamin K-2; VK2) and synthetic forms, such as menadione (vitamin K3) and menadione esterified (vitamin K4). All types of vitamins share the same 2-methyl-1,4-naphthoquinone ring structure (menadione), but differ for the substituent on the third carbon of the naphthoquinone ring [2]. The characteristics of menaquinones (Mks) depend on the number of isoprenoid residues in the side chain which includes a polymer of repeated prenilic units (Figure 1). They are named according to the number of these prenilic units (hence MK-n) and there are 14 forms. Among the different isoforms of MK, human foods are particularly rich in MK-7 [3]. Phylloquinone is mainly contained in leafy green vegetables, where it is found in quantities between 400–700 g/100 g. The other main source is vegetable oils (e.g. soybean oil, rapeseed and olive oil) which contain 50–200 g/100 g and contribute significantly to VK intake in the diet [4]. Among vegetables, sauerkraut contains a good percentage of both VK1 and VK2 (22.4 g per 100 g of K1, and 5.5 g per 100 g of K2) [5]. Menaquinones are mainly found in animal products such as meat (MK-4) and fermented foods, particularly cheeses and curds (mainly MK-8 and MK-9) [6]. Nutritionally relevant amounts of MK-9 are found in Norwegian Jarlsberg and Swiss Emmental cheeses [7], while, more generally, the cheeses with the highest VK2 content are Munster (80.1 g per 100 g), Camembert (68.1 g per 100 g) [5]. Dairy products are probably the predominant food sources of long-chain MK. Cheese and dairy products are estimated to contribute 54% and 22% respectively of the total MK contribution. Table 1 summarizes the Menaquinone content of foods in the main studies discussed. However, the absence of complete data on food MK content indicates that much more research is needed to accurately quantify the individual and population intake of MK. Although VK is a fat-soluble vitamin, the body stores very little of it and without a regular dietary intake its reserves are quickly depleted. Human cells cannot synthetize VK2 and can only introduce it through nutrition, however, the amount of VK2 in normal

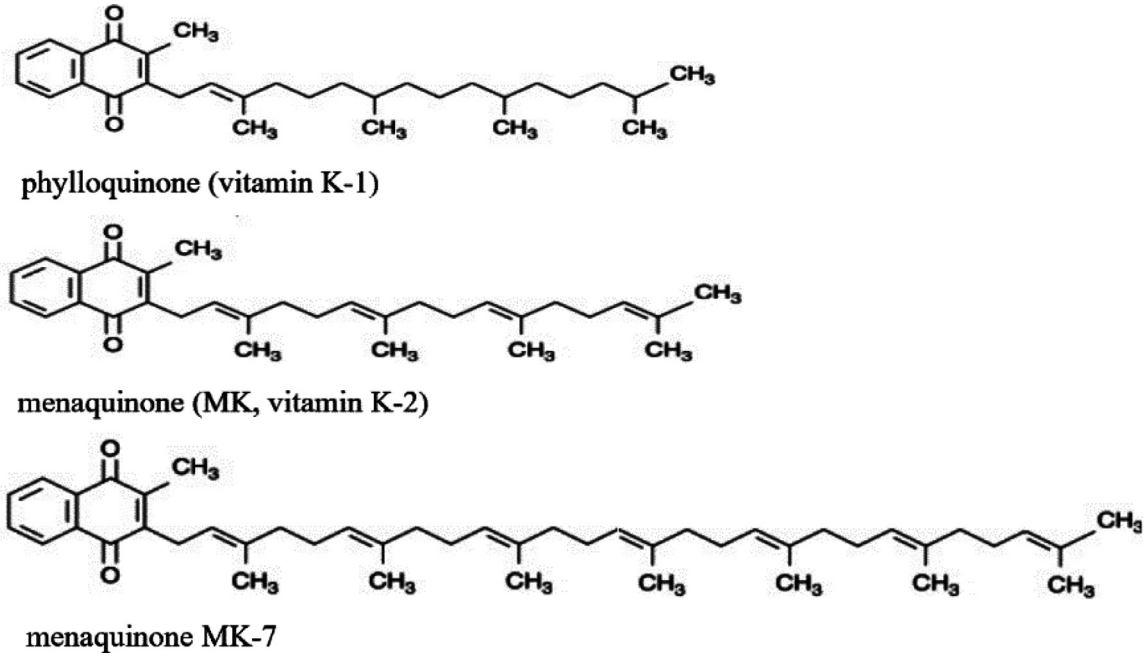

Figure 1. Structural formula of vitamin K-phylloquinone (vitamin K-1), menaquinone (MK, vitamin K-2) and menaquinone mk-7.

Table 1. Menaquinone (vitamin K2) content of foods

Authors, Year, [Ref.]	Vitamin K Analysis	Type of food	Menaquinone content
Vermeer et al. 2018 [5].	Samples were supplemented with the appropriate amount of internal standard VK1(25) (GL Synthesis, Worcester, MA, USA) and extracted with chloroform (Biosolve BV, Valkenswaard, The Netherlands)-methanol (Biosolve BV, Valkenswaard, The Netherlands) (1:1, v/v).	Several Dutch cheeses of different fat content and ripening periods were analyzed for their phylloquinone (VK1) and menaquinones (VK2) content	Menaquinone content dependent on the type of cheese, the time of ripening, the fat content and the geographic area where this is produced. In general, hard cheeses (Gouda 13 weeks, 50% dry weight fat content was around 650 ng/g) contain more menaquinones than soft cheeses (Brie content was 125 ng/g). Raw milk cheese (not industrially prepared but originating from local farms) was rich in menaquinones (approximately between 600 and 790 ng/g).
Hojo et al. 2007 [7].	VK in the cheese was extracted according to the method of Kojima et al., 2004. The MK-9 (4H) concentration was quantified using an HPLC instrument with a fluorescence detector after postcolumn reduction.	This study aimed to determine the MK-9 (4H) concentration in commercial propionibacteria-fermented cheese.	Swiss Emmental and Norwegian Jarlsberg cheeses contain a meaningful amount of VK because of their high MK-9 (4H) concentrations (200–650 ng/g).
Tarvainen et al. 2019 [9].	A rapid ultra-high performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometric (UHPLC-APCI-MS/MS) method was developed for the analysis of VK compounds.	Fermented food products	The highest MK-7 content was detected in natto (902 μg/100 g). Some MK-9 was present in kefir (5 μg/100 g).
Schurgers et al. 2000 [12].	VK was analyzed by HPLC using a C-18 reversed phase column and fluorometric detection after postcolumn electrochemical reduction.	They present a database with VK1 and VK2 contents of a wide variety of food items available on the Dutch market.	It was confirmed that phylloquinone is mainly present in green vegetables, margarins (93 μg/100 g) and some plant oils such as olive oil (53 μg/100 g). MK-4 was present in nearly all animal products like meat (369 μg/100 g), dairy produce, eggs (31 μg/100 g).

Notes. VK1 = vitamin K_1; VK2 = vitamin K_2; VK = vitamin K.

foods is quite low. Studies of European populations have shown that with the western diet 90% of VK introduced with the diet is in the form of VK1 while only 10% is represented by VK2 (about 7.5% from MK-5 to MK-13 and 2.5% of MK-4) [8]. The only people who consume large quantities of it are the Japanese, who consume natto, a food that comes from the fermentation of soya cooked with *Bacillus subtilis* natto and which alone contains about 800–900 μg of VK2 per 100 g of natto in a highly bioavailable form [9]. In healthy Japanese subjects who consumed fermented (natto) menaquinone-rich soya (especially MK-7), serum concentrations of L'MK-7 were significantly increased [10]. Compared to other forms of VK, MK-7 has a much longer half-life in human blood, peak concentration at 2 h and remains in circulation for about 8 h, therefore [11], has attracted researchers to study bioprocess engineering technologies for industrial production of the MK-7.

This review provides an update on the current literature regarding the origin of VK2 and its implications in what is called the "calcium paradox", namely the lack of calcium in the bone and its storage in the wall of the vessel.

Material and method

The literature search was carried out via Pubmed between February 2021 and September 2021 and focused on reviews, systematic reviews, meta-analyses, randomized controlled trial and clinical trial published between 2010 and 2021 that dealt with the topic of VK2 and microbiota. Only articles written in English and published in peer-reviewed journals were considered.

Relevant keywords to term micronutrients were analyzed alone or in association with other terms as "microbiota", "calcium paradox", "Matrix Gla protein", "vascular calcification".

The selection process was carried out by first analyzing the titles, then the abstracts, and finally the full text.

Vitamin MK-7

Of all menaquinones, MK-7 is more efficiently absorbed and has maximum bioavailability. This was demonstrated in a comparative study between VK1 and MK-7 in which it was shown that both VK1 and MK-7 were easily absorbed within 2 hours of ingestion, however postprandial serum concentrations of K2 (MK-7) were 10 times higher than those of VK1 [12]. In many studies MK-7 has been shown to have a long-term protective effect on the development of vascular calcification [13, 14, 15]. In recent decades, much research has attempted to improve the yield of MK-7 production in the fermentations in the solid and liquid state of various species of bacteria. Among these species *B. subtilis natto* appears to be the most promising microorganism. Industrialization of VK2 production began in the 1990s. In 1995, a Japanese company called Honen Corp. studied a solid fermentation process (LSF) with high natural MK-7 content through extraction from food raw materials fermented by *Bacillus natto* [16]. To improve the productivity of MK-7, a liquid fermentation process (LSF) was subsequently proposed, which effectively increases the rate of cell growth and shortens the fermentation period, using *B. subtilis natto* [17]. Under the impetus of these companies in 2008 the US Food and Drug Administration and in 2009 the European Food Safety Authority approved its use as a food and food additive enhancer. In 2009, the European Food Safety Authority also announced that the MK-7 form of VK2 can be claimed as VK with high bioavailability which can help patients maintain bone and cardiovascular health. MK-7 has been shown to improve bone resistance to the femur neck by increasing bone mineral content (BMC) and bone mineral density (BMD) [18, 14]. In addition, MK-7 can be the optimal form for dietary supplementation due to its long half-life. In fact, MK-7, has a half-life of 3 days and among menaquinone is the most hydrophobic form thanks to its longer isoprenoid chain. The chemic-physical properties of this molecule allow its transport by plasma lipoproteins, increase its extrahepatic availability. In addition, MK-7 at the same daily intake produces 5 times higher plasma concentrations than K 1 [11]. However, despite the advances in the development of technologies for the production and extraction of MK-7, this vitamin is not available at an affordable market price for commercial applications. A problem related to nomenclature is arising in recent years. In fact, many industries producing health supplements with VK2, do not specify whether the type of vitamin contained is MK-4 or MK-7. This is especially important for patients taking anticoagulant therapy such as Warfarin. Indeed MK-7, if taken daily, can interfere with anticoagulant therapy [19, 20]. In a study Theuwissen et al. reported that 45 mg/day of supplemented MK-7 was sufficient to significantly reduce the effectiveness of anticoagulant therapy [19]. However, not knowing the bioavailability of MK in various foods, there are no indications to change the dietary habits of patients who use anticoagulants. In addition, it is known that anticoagulant treatment with dicumarols, antagonist of VK, is associated with an increased number of vascular and cartilaginous calcifications and non-traumatic bone fractures [21]. This reinforces the assumption that VK2 in the prevention of ectopic calcifications and bone demineralization.

The Matrix Gla protein (MGP)

VK is a cofactor for a single microsomial enzyme, namely γ-carboxyglutamyl carboxylase (GGCX). GGCX is needed to catalyse a reaction in which specific glutamate residues of specialized proteins are converted to γ-carboxyglutamate (Gla) [22]. This process proceeds from the oxidation of vitamin K hydroquinone (KH2) to vitamin K epoxide (KO) in the VK cycle. KO is reduced to KH2 by the epoxide reductase of vitamin K (VKOR) [23]. The discovery of carboxylofil-carboxymyl led to the identification of a plethora of Gla proteins. The resulting vitamin K-dependent proteins (VKDP), or Gla proteins, are different in both structure and function and are found in many types of cells and tissues both hepatic and extrahepatic. At least 17 types of protein with glutamate residues have been discovered, designated as vitamin K-dependent Gla proteins [24]. These include factors II, VII, IX, X and anticoagulant proteins C, S and Z, Gla matrix protein (MGP), Gla growth-stopping protein 6 (Gas6), osteocalcin (OC), Gla-rich protein (GRP), periostin (isoforms 1–4), periostin-like factor (PLF), proline-rich Gla protein (PRGP) 1, PRGP2, protein Gla transmembrane (TMG) 3 and TMG4 [25, 26]. Among these the most studied is MGP, which contains five residues of glutamic acid and three residues of serine, and this molecule is activated through two post-translational modifications, namely the carboxylation of glutamate and phosphification of serine [27]. MGP has been identified in the human atherosclerotic plaque, where it can prevent calcium precipitation in a similar way to that of bone. This has been clearly demonstrated in knockout mice for MGP who die due to massive aortic and coronary calcification shortly after birth [28]. When the levels of VK are adequately sufficient in the body, all the MGP synthesised in the vascular smooth muscle cells (VSMCs), at the level of the average tone, is activated (carboxylate) to inhibit calcification, adequate clearance of the vesicles of the matrix (MV) and apoptotic bodies (ApoBD), via phagocytosis, and circulating carboxylate GMP (cMGP) [29]. If there is instead a deficiency of VK (for example due to low intake, the use of warfarin, diseases such as Renal Insufficiency, Diabetes, inflammation), there will be a reduced clearance (phagocytosis) of MV and ApoBD, with increased inactive GMP, non-carboxylated (ucMGP), which will bind to the calcium of the hydroxyapatite of the matrix itself giving rise to the calcification of the vessels. Inactive MGP (ucMGP), acts as a circulating marker for VK. Indeed, the increase in ucMGP levels is widely regarded as one of the best markers of VK2 deficit [29, 30]. However, in a study by Ponziani et al., it has been shown that the intake of VK2 in the diet is not related to serum levels of ucMGP, but it appears to be the gut microbiota responsible for overcoming food shortages under physiological conditions [31]. This opened the door to an important theory that food is not the primary source of VK2, but rather the presence of intestinal dysbiosis, in this specific case SIBO, is the primary cause of vitamin deficit. Murshed and colleagues demonstrated that GMP must be carboxylated to prevent vascular calcification (VC) [32]. The supplementation of phylloquinone and menaquinone substantially reduces the levels of dp-ucMGP, increasing the carboxylation of MGP [33, 34].

Role of the intestinal microbiota

It is evident that most menaquinones are derived from the biosynthesis of human intestinal bacteria [35]. The microbiota in the gastrointestinal tract plays an important role in maintaining well-being and in the genesis of pathological states in the host. It represents an enormous reservoir of microorganisms that is found in the human digestive canal: a biomass of 1014, comprising from 500 to 1000 different species of microorganisms, with wide interindividual variability [36]. It is not a static biosystem but a highly dynamic biosystem. In particular, some of them are resident and stable in the bacterial or autochthonous flora, while other species are transient or foreign and include both microorganisms located at different points of the digestive tract and bacteria ingested through food [37]. Among all types of bacteria there are only two predominant: *Bacteroides* and *Firmicutes*. However, only *Bacteroides* can synthesise menaquinones [38]. *Bacteroides* are primarily capable of synthesising very long chain forms (with more prenilic units) of menaquinones, such as MK-10 and MK-11 [39]. In particular, MK-10 and MK-11 are synthesised by *Bacteroides*, MK-8 by *Enterobacteria*, MK-7 by *Veillonella* and MK-6 by *Eubacterium lentum* [40]. Some lactic bacteria (LAB), including *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Streptococcus*, produce a wider range of MK, including MK-7, MK-8, MK-9 and MK-10 [41]. The Labs are the main organisms used in starter crops to produce meat products and plant foods. In addition to the bacteria present in the intestinal flora, VK2 is also produced by bacteria used in food fermentation processes. An example is the species *Propionibacterium* used in the production of some Swiss type cheeses and which produces MK-9 [41]. MK-4 is an atypical MK which is not [42] but can be synthesised in vivo from VK1 by lateral chain splitting followed by prenylation of the intermediate MD which is performed in target tissues by the multi-functional enzyme UBIAD1 [22, 43]. The MK-4 is also found in small quantities of milk, butter, and cheese, and in the United States is used in poultry feed and some pig feed as a source of VK [44]. Because animal organs that contain high concentrations of MK-4 including kidney, brain, and pancreas, are not

commonly consumed in most regions of the world, the amount taken with the diet is definitely insufficient.

Vascular calcification

Calcification of vessel walls reduces their elasticity, increasing the risk of cardiovascular disease (CVD) [45]. Vascular calcification presents as a complication of atherosclerosis and is characterised by the deposition of calcium phosphate complexes primarily in the form of hydroxyapatite. In fact, following the nucleation of hydroxyapatite (HA) on vesicles bound to the membrane there is the expression of proteins regulating the mineralization associated to the bone [46]. These proteins include some vitamin K2-dependent proteins (VK2DP) that regulate the phenotype of VSMCs and/or inhibit the growth of HA crystals. One of these is protein S [47], which plays an important role in preventing the formation of clots on the internal surface of the wall of the vessel. Growth arrest-specific 6 (Gas6), ligand for the TAM family of tyrosine-receptor kinases, prevents endothelial cells and VSMCs from going into apoptosis and inhibits vascular calcification through its anti-inflammatory effectapoptotic on VSMCs [48]. The most recently identified member of the VKDP family is GRP, named after the large number of Gla residues it contains [49]. It is distributed mainly in bone, cartilage, skin, and vessels, where it plays an important role in inhibiting vascular calcification [52]. Another VK2DP we have already discussed is MGP, which inhibits calcium precipitation in the form of hydroxyapatite crystals at the site of elastic gills [39]. This has been clearly demonstrated in knockout mice for MGP who die from massive aortic and coronary calcification shortly after birth [28]. Therefore, VK2 plays a key role in aortic calcifications (AC) prevention, as it is the only known activator of MGP. Phosphorylated MGP remains attached to growing calcification crystals while dp-MGP is released into circulation due to reduced carboxylation. Levels of dp-MGP in circulation can be measured and the reduction in concentrations of dp-ucMGP can be causally associated with a reduced risk of coronary heart disease (CHD) [51, 52]. Virchow time pathologists have documented the presence of calcium deposits in both the intimate tunic and the mean tunic of sclerotic arteries. Calcification of the intimate is associated with advancing age, hypertension, diabetes, dyslipidemia and smoking and takes the form of atherosclerosis and involves macrophages and VSMCs in lipid-rich regions. Conversely, the calcification of the tunic, or medial sclerosis of Monckeberg, is almost exclusively associated with VSMCs [53]. VSMCs that predominate in these lesions lose expression of calcification inhibitors, such as GMP, and begin to express "late" differentiation markers of osteoblasts (bone scialoprotein; BSP) and chondrocytes (collagen II; COLII) [54]. Initially, MGP was thought to inhibit calcification by merely bonding to HA. However, several observations suggest that MGP can also regulate the differentiation of VSMCs. In fact, over-expression of GMP can inhibit osteo/chondrocytic conversion of mesenchymal stem cell lines [28]. In humans, mutation of the gene for GMP, which leads to an absent or non-functional protein, manifests as Keutel syndrome, transmitted as autosomal recessive character, which is characterised by abnormal calcium deposit in the cartilage of the ears, the nose, larynx, trachea, and ribs [29]. It is now known that most patients who reach the end-stage renal disease (ESRD) and dialysis suffer from VC and therefore have an increased risk of mortality [55, 56]. The problem of VC in patients with Chronic Renal Insufficiency (IRC) is of great importance as the prevalence of VC of the large arteries varies from 30 to 70% [24], to arrive in the hemodialysis even at values of 60–80% [57]. The ucMGP is increasingly taking on the role of a likely biomarker of VC. We need 45 mcg per day to counteract cardiovascular disease, as highlighted in the Rotterdam study, the first study that demonstrates the beneficial effect of VK2. In this study, people who consumed 45 mcg of K2 per day lived seven years longer than people who took only 12 mcg daily. Menaquinone intake was lower in subjects with severe aortic calcification (25.6 mcg/die) than those with moderate or mild calcification (28.6 and 28.8 mcg/die respectively; P 0.001) [58]. However, the International Life Sciences Institute of Europe, in an effort to identify specific dietary reference values for MK, concluded that there is currently no reference value for MK-intake.

The calcium paradox

One of the VK2DPs is the protein osteocalcin (OC) or GLA bone protein (BGP: Bone GLA-Protein), a protein synthesised by osteoblasts during bone formation which favours the mineralization of the trabecular in the bone [59]. The OC has a structural function, in fact, following the g-carboxylation it binds to the hydroxyapatite, depositing calcium on the bone [60, 61]. In particular, the carboxylation of glutamic acid in position 17 is likely to be essential for the spatial and structural conformation of the molecule, allowing it to interact with the hydroxyapatite crystals [62]. G-carboxylation of OC (cOC) occurs with the presence of VK2. Chronically low intake of VK2 causes suboptimal carboxylation of OC, which is thought to lead to a reduction in bone mineralization and an increased risk of fractures and osteoporosis, with increased serum levels of non-carboxylated osteocalcin (ucOC). cOC levels reflect the

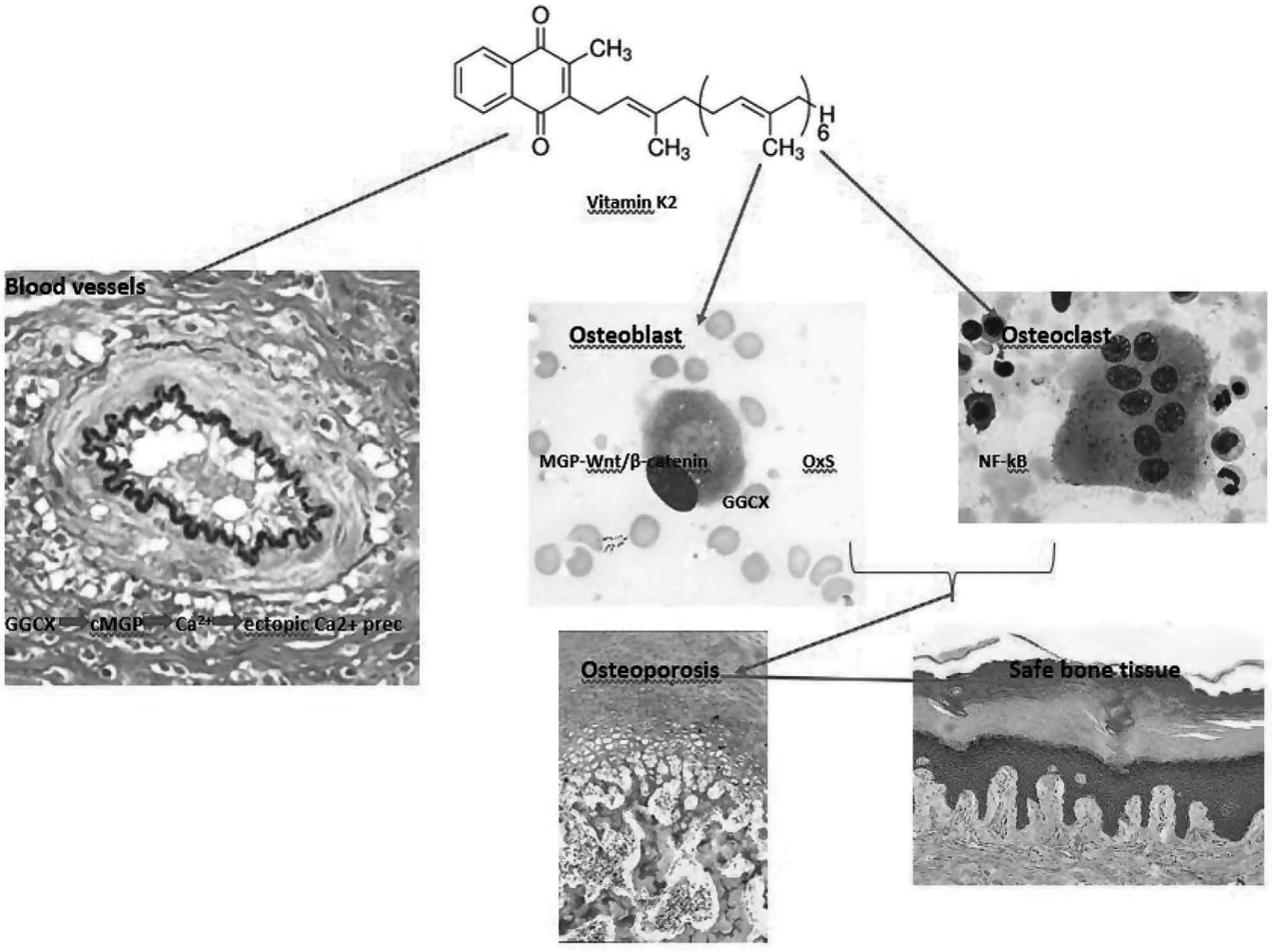

Figure 2. Mechanisms of action of VK2 in "bone and vascular cross-talk". At vascular level, VK2, acting as cofactor for the enzyme GGCX, for conversion in active carboxylated MGP (cMGP). The active cMGP could directly inhibit ectopic Ca2+ precipitation. In bone tissue, VK2 could promote osteoblasts proliferation and activity through MGP and Wnt/β-catenin pathway, control of oxidative stress (Ox-S) imbalance and well-established GGCX-dependent pathway. VK2 may also exert a control of osteoclasts activities through the inhibition of NF-kB.

degree of bone formation, which increases to a peak at 12 years in females and 15 years in males, after which they decrease to menopausal age to increase in the event of increased bone turnover [63, 64]. Observational studies in Japan showed that the fracture frequency was inversely correlated with high levels of natto consumption, which contains large amounts of menaquinone-7 (MK-7) produced by *Bacillus subtilis* [65, 66]. The ratio of cOC to ucOC is widely used as a marker of bone metabolism and circulating ucOC is used as a clinical marker of VK deficiency and is a predictive factor of fracture risks regardless of BMD [67]. It has been suggested that the minimum dose of MK-7 to improve bone metabolism is 100 mg, in addition to the usual diet [68], while the minimum effective dose of MK-4 to improve bone parameters is 45 mg [69]. There are controversial findings regarding MK-4 in improving bone health. The results of the larger and longer MK-4 study found no beneficial effects on the vertebral fracture, except in women with advanced osteoporosis [70]. In different pathological conditions the calcium mineral content in the bones decreases, while it increases on extra-osseous sites. This has been called the "calcium paradox" which describes the paradoxical correlation between the decline of calcium-mineral content in bones and a parallel increase in arterial calcification [71]. This is particularly true for post-menopausal women and osteoporotic patients with chronic kidney disease (CKD), where bone loss and simultaneous vascular calcification has been demonstrated [3, 72]. Several studies have been published that highlight the presence of a "bone-vascular crosstalk" and the association between CVD-related mortality and osteoporotic bone fractures [73, 74]. A common mechanism of pathogenesis between VC and osteoporosis has been identified and is represented by the subclinical deficiency of VK2, which could play an important role in the development of the "calcium paradox" [8]. As just described, it is deductible that a deficit of

Table 2. Clinical evidence linking vitamin K2 supplementation and bone health

Study	Type of the Study	Number of Patients Enrolled	Outcomes
Kanellakis, S.; Moschonis, G. Changes in parameters of bone metabolism in postmenopausal women following a 12-month intervention period using dairy products enriched with calcium, vitamin D, and phylloquinone (vitamin K(1)) or menaquinone-7 (vitamin K (2)): The Postmenopausal Health Study II. Calcif. Tissue Int. 2012, 90, 251–62 [75].	Randomized controlled trials	219	BMD increase following one year of vitamin K2 supplementation (100 μg/day)
Knapen, M.H.; Drummen, N.E. Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women. Osteoporos. Int. 2013, 24, 2499–2507 [14].	Randomized controlled trials	244	Decrease bone loss following three years MK-7 supplement (180 μg/day)
Huang, Z.B.; Wan, S.L. Does vitamin K2 play a role in the prevention and treatment of osteoporosis for postmenopausal women: A meta-analysis of randomized controlled trials. Osteoporos. Int. 2015, 26, 1175–86 [70].	Meta-analysis	6759	BMD improvement and low incidence of fracture in osteoporotic subjects following K2 treatment
Mott, A.; Bradley, T. Effect of vitamin K on bone mineral density and fractures in adults: An updated systematic review and meta-analysis of randomised controlled trials. Osteoporos. Int. 2019, 30, 1543–59 [76].	Meta-analysis	11.122	Vitamin K2 treatment (MK-4: 45 mg/day) reduce fracture, increase cOC and decrease ucOC serum concentration
van Summeren, M.J.; Braam, L.A. The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children. Br. J. Nutr. 2009, 102, 1171–78 [77].	Randomized controlled trials	55	8 weeks MK-7 supplementation increase cOC serum concentration
Nakamura, E.; Aoki, M. Low-dose menaquinone-4 improves γ-carboxylation of osteocalcin in young males: A non-placebocontrolled dose-response study. Nutr. J. 2014, 13, 85 [78].	Non-placebo-controlled dose-examination study	55	MK-4 supplementation (600 and 900 μg/day) decrease ucOC and increase cOC level respectively
Koitaya, N.; Sekiguchi, M. Low-dose vitamin K2 (MK-4) supplementation for 12 months improves bone metabolism and prevents forearm bone loss in postmenopausal Japanese women. J. Bone Miner. Metab. 2014, 32, 142–50 [79].	Randomized controlled trials	48	Serum ucOC concentrations were significantly lower following 6–12 months MK-4 treatment (1.5 mg/day)
Inaba, N.; Sato, T. Low-Dose Daily Intake of Vitamin K (2) (Menaquinone-7) Improves Osteocalcin γ-carboxylation: A DoubleBlind, Randomized Controlled Trials. J. Nutr. Sci. Vitaminol. 2015, 61, 471–80 [68].	Randomized controlled trials	60	MK-7 treatment (100 μg/day) significantly decrease ucOC and increase cOC/ucOC ratio

VK in terms of clinical impact leads, in addition to an increase in vascular calcification to decrease cMGP and increase in the share of ucMGP, also to an increase of the fracture pathology for the decrease of the active cOC and increase of the ucOC quota (Figure 2).

Conclusions

We have come to the conclusion that VK plays a role in the storage of calcium in bones and at the same time provides protection against vascular calcification. Therefore, it is tempting to hypothesise that the calcium paradox actually reflects a VK deficiency. Supplementation with VK, to the most common therapy with calcium and vitamin D, could therefore serve as protection against the increased risk of calcification. Recent studies have highlighted how VK2 is important for the bone and vascular health of patients with CKD and in menopausal women thus creating a window of opportunity for integrate that vitamin into this group of patients frequently lacking in VK. However, there are no internationally recognised guidelines on the use of VK2 supplements, we can certainly conclude that proper dietary supplementation and correction of any intestinal dysbiosis can be the right strategy to follow. The identification of VC promoters and inhibitors will allow future therapies to modulate these agents to inhibit progression or even induce regression of VC. vascular thus allowing a safer treatment of osteoporosis. Table 2 summarize the principal studies on supplementation of VK.

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