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Vitamin K, well known for its role in coagulation, encompasses 2 major subgroups: vitamin K1 is exclusively synthesized by plants, whereas vitamin K2 mostly originates from bacterial synthesis. Vitamin K serves as a cofactor for the enzyme γ-glutamyl carboxylase, which carboxylates and thereby activates various vitamin K–dependent proteins. Several vitamin K–dependent proteins are synthesized in bone, but the role of vitamin K for bone health in chronic kidney disease patients, in particular the prevention of osteoporosis, is still not firmly established. Herein, we focus on another prominent action of vitamin K, in particular vitamin K2 (namely, the activation of matrix γ-carboxyglutamic acid protein, the most potent inhibitor of cardiovascular calcifications). Multiple observational studies link relative vitamin K deficiency or low intake to cardiovascular calcification progress, morbidity, and mortality. Patients with advanced chronic kidney disease are particularly vitamin K deficient, in part because of dietary restrictions but possibly also due to impaired endogenous recycling of vitamin K. At the same time, this population is characterized by markedly accelerated cardiovascular calcifications and mortality. High-dose dietary supplementation with vitamin K2, in particular the most potent form, menaquinone 7, can potently reduce circulating levels of dephosphorylated uncarboxylated (i.e., inactive matrix γ-carboxyglutamic acid protein) in patients with end-stage kidney disease. However, despite this compelling data basis, several randomized controlled trials with high-dose menaquinone 7 supplements in patients with advanced chronic kidney disease have failed to confirm cardiovascular benefits. Herein, we discuss potential reasons and solutions for this.

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Vitamin K is well known for its role in coagulation and the therapeutic interference with it in patients requiring systemic anticoagulation. Less well known are the roles of vitamin K in cardiovascular disease, in particular calcification. In the present review, we discuss the physiology of the vitamin K system and its disturbances in patients with chronic kidney disease (CKD) as well as emerging intervention studies targeting the cardiovascular system in CKD.

Vitamin K sources and subforms

Vitamin K comprises different entities of naphthoquinone derivatives with a variably long phytylic side chain. It was first described in 1935 by the Danish noble laureate, Henrik Dam, as an essential vitamin for blood coagulation ("*K*oagulation"). The 2 major physiologic forms are vitamin K1 (phylloquinone) and vitamin K2 species (menaquinones [MKs] 4–13) (Figure 1[1]).

Vitamin K1 is exclusively synthesized by plants, where it serves as an electron carrier inside the chloroplastic membrane in photosystem I. Photosystem I is a complex of proteins and pigments (carotenes and chlorophyll) and can be described as a light-driven electron pump,[2] where the initial light reaction of photosynthesis takes place. Thus, green, leafy vegetables are the mainstay of nutritional K1 supply.[3] Foods with the highest phylloquinone contents are kale, parsley, spinach, leek, and purslane[4] (Table 1[5–8]). Vitamin K1 levels are higher in fresh frozen compared with canned food, but the highest contents per gram are detected in dried products.[4] Overall, all natural vitamin K forms are stable to heat, and cooking losses are negligible. Steaming or microwaving K-rich food items can even increase the cellular vitamin K release.[9] In contrast, vitamin K is highly sensitive to daylight. For example, exposure of rapeseed or safflower oil to daylight decreased its vitamin K1 content by ≈95% within 2 days of light exposure.[10]

Vitamin K2 mostly originates from bacterial synthesis. One synthesis pathway occurs in lactic acid bacteria, where menaquinones are used for electron transport.[5,11] Lactococcus lactis strains can synthesize MK5 to MK9[12] and are used in food manufacturing to ferment and preserve dairies, such as yogurt or cheese, as well as vegetables (e.g., sauerkraut). In humans, the most relevant forms are MK4 and MK7. Beyond bacterial synthesis, MK4 can also be synthesized endogenously from K1 or menadione by the enzyme

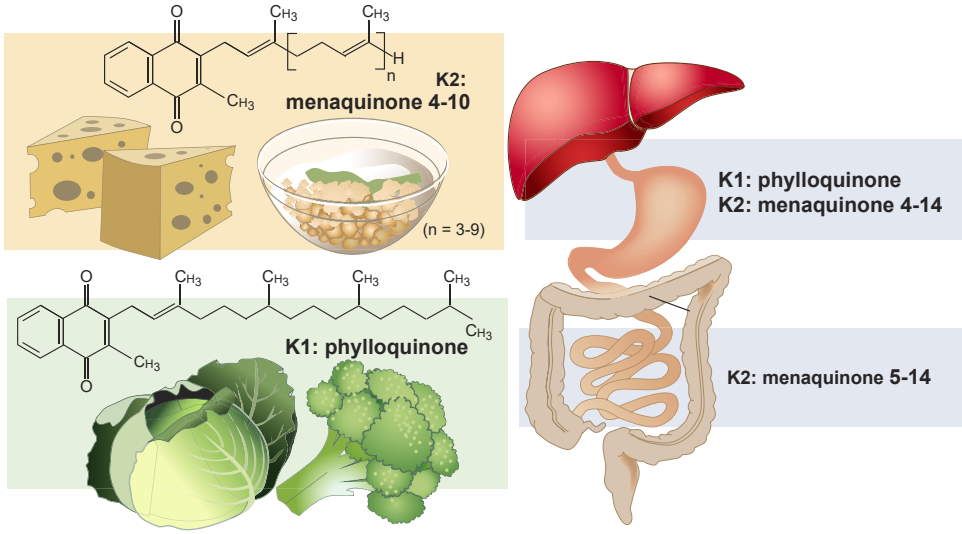

Figure 1 | Exogenous and endogenous sources of vitamin K1 and vitamin K2. Vitamin K1 is present in green leafy vegetables, whereas vitamin K2 can be found in various fermented foods. Vitamin K2 subform menaquinone (MK) 4 can be endogenously synthesized from vitamin K1 by the enzyme UbiA prenyltransferase domain-containing protein 1 (UBIAD1). However, next to vitamin K1, also vitamin K2 derivatives, such as MK4 and MK7, can be converted into menadione to be used by UBIAD1 for conversion to MK4.[1] Indeed, an increase in menadione after vitamin K intake can be detected in urine. In addition, MK5 to MK13 can be synthesized by the microbiome (e.g., by *Lactococcus* species). Whether the latter contributes to the pool, due to low amount of bile acids inside the colon, remains uncertain. Images are from https://pixabay.com/, with special thanks to Lipefontes, AStoKo, Shutterbug75, and Hui Wang.

UbiA prenyltransferase domain-containing protein 1 (UBIAD1; Figure 2).[13] However, next to K1, also vitamin K2 derivatives, such as MK4 itself and MK7, can be used as source of MK4.[1] The intermediate product in this conversion is menadione, which might serve as a substrate for UBIAD1.[1] This observation led to the conclusion that vitamin K is converted in the intestine to menadione and that the prenylation to MK4 takes place in tissues expressing UBIAD1.

So far, all major national nutritional databases lack systematic information on vitamin K2 contents. The food with by far the highest content of vitamin K2 is natto, made by fermenting soy beans (vitamin K2 content, >1000 μg/100 g, mostly MK7).[6] Natto is commonly consumed in Japan, whereas in Western diets, dairy products are the predominant source of vitamin K2.[5] Specific sources are French cheese, hard cheese, or minced meat (Table 1).[6,7] The contribution of K2 to the total vitamin K intake is estimated to be ≈25%.[14]

A third form, vitamin K3 (menadione), lacks the phytylic side chain and is employed in animal nutrition. It remains mainly synthetic but was also shown to be an intermediate in the endogenous conversion from K1 to MK4 by the enzyme UBIAD1.[15] Reportedly, vitamin K3 exerts anti-cancer properties via cytostatic effects.[16,17]

Vitamin K recycling

Vitamin K serves as a cofactor for the enzyme γ-glutamyl carboxylase (GGCX), which catalyzes conversion of the Glu residue of vitamin K–dependent proteins into γ-carboxyglutamic acid (Gla; Figure 2). This process is driven by the oxidation of vitamin K–hydroquinone to vitamin K–epoxide in the vitamin K cycle, generating the possibility to introduce an extra carboxyl group at the γ position of the glutamate residue, thereby adding an extra negative charge to the protein. Vitamin K–oxidoreductase (VKOR) then converts vitamin K–epoxide to vitamin K and back to vitamin K–hydroquinone, generating a recycling process.[18] Vitamin K antagonists (VKAs) inhibit VKOR and thereby the recycling of vitamin K, resulting in a drug-induced vitamin K deficiency.[19] Recent studies have also suggested a role for vitamin K as an antioxidant. This noncanonical function of vitamin K

Table 1 | Selected vitamin K1 and vitamin K2 sources in human diets

Vitamin K1	Amount, μg/100 g	Vitamin K2	Amount, μg/100 g
Vegetables		**Vegetables**	
Kale	713–856	Natto	1096–10,985
Parsley	548	Sauerkraut	5–55
Spinach	380–471	**Dairy**	—
Chive	380	Gouda	473–644
Purslane	381–394	Camembert	681
Broccoli	156–180	Emmentaler	433
Cabbage	80–154	Cheddar	235
Celery	40–41	Brie	125
Leek	47–49	Yogurt	1.0–1.2
Natto	35	**Meat**	—
Sauerkraut	25	Liver (beef)	112
Dairy		Beef	18.9
Emmentaler	3	Minced meat	9-76
Yogurt	0–0.4	Chicken	9–101
Oils		**Fish**	—
Olive oil	54–55	Eel	3–631
		Rainbow trout	3
		Egg	20

Data are from several sources.[5–8]

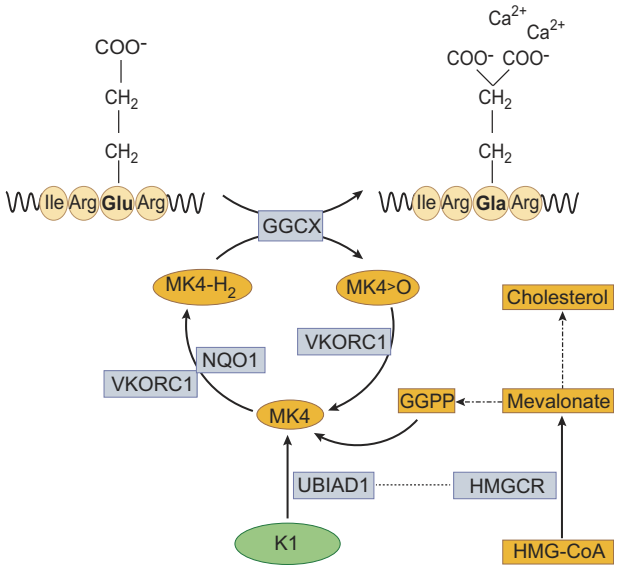

Figure 2 | Vitamin K metabolism and recycling. A vitamin K–dependent protein, such as matrix γ-carboxyglutamic acid protein (MGP), is being γ-glutamyl carboxylated by the γ-glutamyl-carboxylase (GGCX). By introducing a second carboxyl group (COO^-) at the γ c atom, MGP becomes a potent calcium chelator. The reduced form of vitamin K (K-H_2, here shown by MK4-H_2) serves as a cofactor for the GGCX and gets epoxidized (MK4 > 0). In the vitamin K cycle, the epoxidized form gets stepwise recycled by 2 enzymes, vitamin K epoxide reductase complex subunit 1 (VKORC1) and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase–quinone 1 (NQO1). MK4 can be derived from exogenous K1 by the enzyme UbiaA prenyltransferase domain-containing protein 1 (UBIAD1). For the synthesis, UBIAD1 utilizes geranylgeranyl pyrophosphate (GGPP). GGPP itself is derived from mevalonate, an intermediate of the cholesterol synthesis pathway, with hydroxyl-methyl-glutaryl-coenzyme A reductase (HMGCR) as a key enzyme. Thus, HMGCR and UBIAD1 are functionally connected, but they also do physically bind to each other. HMG-CoA, 3-hydroxy-3-methyl-glutaryl–coenzyme A.

relates to the oxidation-reduction potential of vitamin K and scavenging intracellular free radicals.[20] Moreover, we showed that the CKD environment impacts on the activity of GGCX but not the activity of vitamin K epoxide reductase complex subunit 1 (VKORC1).[21] This led to increased vascular calcification in an animal model of CKD.

Vitamin K metabolism

Vitamin K needs to be absorbed from food to fulfill its unequivocal, nonredundant role as cofactor in the carboxylation of vitamin K–dependent proteins. Vitamin K1 is tightly bound to the thylakoid membrane (i.e., parts of the chloroplasts) of leafy green vegetables, thereby limiting its availability for absorption. Ingestion of vitamin K2 results in higher circulating vitamin levels, compared with K1.[6] Because all vitamin K isoforms are fat soluble, they are packaged into chylomicrons during absorption and taken up by enterocytes in the small intestine. Chylomicrons are transported via the lymph, after which they are taken up in the circulation. These chylomicrons are then transformed to chylomicron remnants in the capillaries by lipoprotein lipase before being taken up by the liver.[22] Radiolabeled phylloquinone is thus removed from the circulation within hours.[23] Vitamin K1 is preferentially retained in the liver to support carboxylation of clotting factors. In contrast, vitamin K2, in particular long-chain menaquinones, such as MK7, is redistributed to the circulation and is equally available for extrahepatic tissues, including bone and vasculature.[24]

Both vitamin K1 and K2 are taken up first by the liver via the apolipoprotein E receptor. Long-chain menaquinones, such as MK7, are taken up most efficiently.[24,25] This was shown in healthy volunteers treated with vitamin K antagonist acenocoumerol and supplemented with either K1 or MK7.[25,26] MK7 was shown to interact with anticoagulation several fold stronger than K1, indicating that the efficient uptake of MK7 corresponds with the greater bioactivity. Vitamin K1 and MK4 were readily cleared from the circulation within 2 hours after ingestion, followed by MK7 within 4 hours and MK9 within 7 hours. However, postprandial serum concentrations of long-chain menaquinones were some 10-fold higher than K1.[24] Thus, compared with vitamin K1, long-chain menaquinones are much longer available for extrahepatic tissues, because of their presence in circulating lipoprotein particles.[25]

Intestinal microbiome

The intestinal microflora of humans produces large amounts of long-chain menaquinones, which theoretically could serve as a source of vitamin K, in particular K2. However, by far the largest reservoir of intestinal bacteria is confined to the large intestine. Because the absorption of bile salts and fat-soluble compounds takes place in the duodenum and is completed in the ileum, the contribution of menaquinones produced in the colon to human nutritional needs is still debated. Experiments in vitamin K–deficient rats given vitamin K via either the oral or the colorectal route showed that the bioavailability of colonic vitamin K is ≈50-fold lower than that of oral vitamin K.[27] Moreover, feeding both conventionally housed rats or rats kept under germ-free conditions a vitamin K–deficient diet, vitamin K deficiency became prevalent under both conditions within 3 days, demonstrating that menaquinones synthesized in the large intestine are not utilized sufficiently to prevent vitamin K deficiency.[28] Thus, it seems that relevant nutritional usage of endogenously produced vitamin K2 is confined to those species that exhibit coprophagia (i.e., rodents or monkeys).

In CKD, the colon plays an important role in the production of uremic toxins. CKD-related gut dysbiosis may also lead to decreased microbial synthesis of vitamin K.[29] Because vitamin K is produced by microbiota to support energy production via ATP generation, and support gut microbiota growth,[30] the decreased colonic vitamin K synthesis in CKD can further dysregulate gut microbiota. Drug and vitamin K metabolism interactions in CKD are discussed below.

Vitamin K functions

Coagulation. In the liver, vitamin K functions as a cofactor for vitamin K–dependent coagulation factors,

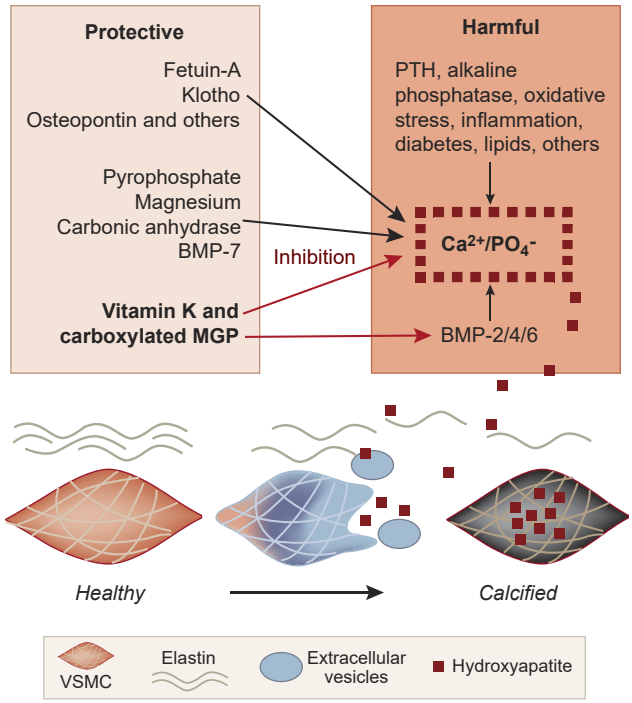

Figure 3 | Processes involved in vascular calcification. Calcification is an active process, resulting from an imbalance of anti-calcific factors (fetuin, klotho, osteopontin, pyrophosphate, magnesium, carbonic anhydrase, bone morphogenic protein [BMP]-7, vitamin K, and carboxylated matrix γ-carboxyglutamic acid protein [cMGP]) and procalcific factors (parathyroid hormone [PTH], alkaline phosphatase, oxidative stress, inflammation, diabetes, lipids, calcium [Ca^{2+}], phosphate [PO_4^-], and BMP-2 and BMP-4). Fetuin (at tissue sites), carbonic anhydrase, pyrophosphate, osteopontin, and cMGP directly capture calcium phosphate crystals and prevent release and precipitation of hydroxyapatite. Klotho, BMP-7, and magnesium target PO_4^- by stimulating its excretion or acting as binding partners. Crystals, elastin breaks, and apoptotic bodies, as wells as matrix vesicles, all activated by the crystals themselves, serve as a nidus for more crystals, contributing to vascular smooth muscle cell (VSMC) death.

including 4 vitamin K–dependent plasma procoagulants ("1972"; i.e., factors II, VII, IX, and X) and 3 anticoagulants (proteins C, S, and Z). After carboxylation in the liver, factors II, VII, IX, and X are essential in the formation of a fibrin clot. Circulating as inactive (or zymogen) forms of serine proteases, their biological activity depends on the ability to bind to negatively charged phospholipid surfaces. Cleavage of these zymogens bound to these surfaces yields the active protease clotting factors. The carboxylation of these vitamin K–dependent coagulation factors is necessary as the resulting Gla residues provide an efficient chelating site for calcium ions that enables phospholipid surface binding.[31] The intricate control of coagulation is illustrated by the fact that protein C, once activated, acts as an anticoagulant by specifically degrading phospholipid-bound, activated factors V and VIII in the presence of calcium. This anticoagulant activity of activated protein C, in turn, is dependent on protein S that acts as a synergistic cofactor by enhancing the binding of activated protein C to negatively charged phospholipids. The consequence of vitamin K deficiency for hemostasis is an inability to synthesize active, carboxylated molecules of factors II, VII, IX, and X, resulting in a hypocoagulable state, which promotes bleeding.

Bone. Several vitamin K–dependent proteins are synthesized in bone. Most extensively studied is osteocalcin, a small protein of 49 amino acid residues with a central domain comprising 3 Gla residues. Osteocalcin is a highly abundant noncollagenous protein of bone extracellular matrix and synthesized exclusively by osteoblasts and odontoblasts. It has a high-affinity binding to hydroxyapatite mineral in bone[32] and appears to be involved in the regulation of bone remodeling and mineralization.[33] In Japan, vitamin K2 has been licensed for the treatment of osteoporosis since 1995, although so far there is insufficient evidence to recommend the routine use of supplemental vitamin K to prevent osteoporosis and fractures in postmenopausal women.[34,35]

Vessels. Matrix Gla protein (MGP) is a vitamin K–dependent protein, mainly synthesized by vascular smooth muscle cells, valvular interstitial cells, and chondrocytes.[36,37] MGP is widely expressed, yet predominantly accumulates in calcified vascular tissue. The importance of carboxylated MGP for cardiovascular health has been recognized for decades.[38] Although originally found in bone, MGP-deficient mice exhibit no prominent bone phenotype, but die within 2 months after birth as a consequence of massively calcified vessels, leading to hemorrhages due to, for example, aortic rupture.[39] Shortly thereafter, the role of vitamin K as a cofactor to activate MGP was shown by inhibition of MGP function using warfarin, a vitamin K antagonist.[40] The role of the Gla domain in inhibiting vascular calcification was revealed by mutagenesis of the protein-bound glutamate residues, resulting in nonfunctional MGP.[41] Although the precise mode of action of MGP has not been unraveled, functions seem to include direct inhibition of calcium crystal growth and blocking of bone morphogenic protein-2 and bone morphogenic protein-4 binding to the bone morphogenic protein receptor[42] (Figure 3).

Genetics. The enzymes involved in vitamin K–dependent carboxylation, hence activation, are GGCX and VKORC1. Mutations and/or polymorphisms affecting the activity of either enzyme can alter carboxylation, usually resulting in bleeding disorders, but they may also affect vascular calcification[43] via reduced activation of MGP. Genetic screenings identified many naturally occurring mutations in GGCX of patients with vitamin K–related disorders.[44] Nonbleeding phenotypes of these GGCX mutations lead to cardiac, dermatologic, ophthalmologic, and osseous symptoms.[44] In both genetic and CKD-associated reduced activity of GGCX, dietary vitamin K supplementation can rescue the carboxylation of vitamin K–dependent proteins to some extent.[21,45]

Warfarin inhibits recycling of vitamin K by reducing the activity of VKORC1. The presence of particular polymorphisms in the VKORC1 gene is also associated with reduced enzyme expression and activity.[46] Several clinical studies have linked these polymorphisms to an increased risk of vascular calcification[47] or cardiovascular disease.[48]

Table 2 | Assessment of vitamin K status

Methods	Normal range	Remarks	References
Food Frequency Questionaire	70–200 μg intake per day	Includes K1 only	49, 51, 52
		K1 and K2	53
Biomarkers			
Vitamin K1 in serum	>0.15 ng/ml	Highly dependent on short-term supply	54
International normalized ratio	0.8–1.1	Target of vitamin K antagonists but also affected by, for example, hepatic dysfunction	49, 55
PIVKA-II in serum	≤2 μg/L	Interassay variability	54
Osteocalcin in serum			
ucOCN/tOCN			
ucOCN/cOCN	<20%	Experimental	52
dp-ucMGP in plasma	<500 pmol/L		56

cOCN, carboxylated osteocalcin; dp-ucMGP, dephosphorylated-uncarboxylated matrix γ-carboxyglutamic acid protein; PIVKA-II, proteins induced by vitamin K absence or antagonism (factor II); tOCN, total osteocalcin; ucOCN, uncarboxylated osteocalcin.

Vitamin K status: biomarkers

Dietary assessments can provide an indirect tool to monitor nutrient supply (e.g., via validated food frequency questionnaires), but this method is highly time-consuming and requires expertise. Vitamin K1 status can also be assessed using functional coagulation tests, such as the international normalized ratio.[49,50] However, such functional tests are not particularly specific for vitamin K bioavailability. Therefore, direct measurement of circulating vitamin K levels or, better even, of vitamin K–dependent proteins is preferred to assess the vitamin K status, as described below (Table 2[49,51–56]).

Circulating vitamin K levels. Dietary intake or circulating levels of vitamin K can be used as biomarkers of the vitamin K supply. Most of circulating vitamin K is in the form of phylloquinone.[6] After a single phylloquinone ingestion, plasma levels peak 2 to 4 hours later and decrease rapidly thereafter.[57] Thus, circulating vitamin K mainly reflects short-term dietary supply rather than status, and interpretive errors arise from recent dietary intake.[54] Therefore, measurements of phylloquinone need to be performed in fasting individuals. Fasting phylloquinone reference values in healthy adults exhibit large interindividual variations and range from 0.15 to 1.0 μg/L.[58]

Vitamin K1 and K2 concentrations can be quantified in serum or plasma by high-performance liquid chromatography coupled with different fluorescence detection units as well by liquid chromatography/mass spectrometry techniques.[59] The Vitamin K External Quality Assurance Scheme[20] monitors the accuracy of vitamin K analyses. For example, a reliable assay for MK7 in plasma requires 94% recovery.[60] In addition, sensitivity is high, and the methods mentioned above have lower detection limits, ranging from 0.1 μg/ml to 0.03 ng/ml.[61,62] Of note, at present, there is no international gold standard for determining circulating vitamin K levels.

Proteins induced by vitamin K absence or antagonism: factor II. In states of vitamin K insufficiency or deficiency, uncarboxylated vitamin K–dependent proteins are produced at their site of synthesis and released into the bloodstream. The historical collective term for these uncarboxylated proteins is PIVKA (proteins induced by vitamin K absence or antagonism). More recent terms specify the proteins and uncarboxylated species of prothrombin (factor II), for example, and are therefore named PIVKA-II. PIVKA-II is a biomarker for hepatic vitamin K status, as prothrombin is exclusively produced in the liver. The half-life of prothrombin is some 60 hours, and the half-life of PIVKA-II is several days and not impacted by recent dietary intake. Assays for PIVKA-II are useful for monitoring subclinical vitamin K deficiency,[63] especially in at-risk groups such as young infants[64] and CKD patients.[8,65] The most sensitive PIVKA-II assays are enzyme immunoassays with antibodies that recognize uncarboxylated prothrombin but do not cross-react with the native, carboxylated prothrombin. A sensitive immunoassay for PIVKA-II exists that can detect uncarboxylated species of factor II when its circulating concentration is as low as 0.2% of total factor II. This threshold is well below the reduction of ≈50% in circulating levels of active total factor II that is needed to trigger a detectable change in the international normalized ratio.[63]

Osteocalcin. Osteocalcin is released during bone formation and resorption and is thus regarded as a marker for bone turnover. It can be produced as carboxylated, active osteocalcin (in case of vitamin K sufficiency) and uncarboxylated osteocalcin (in case of vitamin K deficiency[66]). Carboxylated osteocalcin has a high affinity for calcium ions and aids in forming a hydroxyapatite lattice preceding mineralization of bone.[67] An increase of total circulating osteocalcin has been demonstrated in CKD due to parathyroid hormone–related bone resorption and retention of intact and fragments of osteocalcin.[68] Osteocalcin carboxylation was shown to be responsive to changes on dietary intake[69] and associates with higher bone mineral content.[70] Given the high prevalence of bone loss in CKD, both uncarboxylated and carboxylated osteocalcin levels are increased, resulting in a normal ratio.[71] Uncarboxylated osteocalcin is not solely a marker of vitamin K deficiency, but also released during bone resorption.[72] Taken together, these CKD-related disturbances render

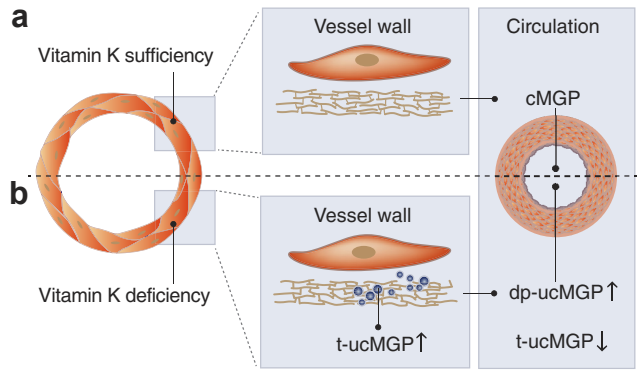

Figure 4 | Matrix γ-carboxyglutamic acid protein (MGP) is produced by vascular smooth muscle cells to prevent unwanted mineralization of the vessel wall. For that function, MGP needs vitamin K as cofactor to become biologically active. (**a**) In case of vitamin K sufficiency, MGP prevents vascular calcification by producing and releasing carboxylated MGP (cMGP). (**b**) In case of vitamin K insufficiency or deficiency, MGP is produced in the uncarboxylated form, unable to prevent or halt vascular calcification. In the vessel wall, total uncarboxylated MGP (t-ucMGP) is accumulating, likely because this fraction binds to hydroxyapatite via the negatively charged phosphorylation sites in MGP. The dephosphorylated-uncarboxylated MGP (dp-ucMGP), lacking all post-translational modification and thus the most inactive form of MGP, does not have affinity for hydroxyapatite in the vessel wall and is thus easily released in the circulation. The dp-ucMGP is thus the fraction that most likely resembles the vitamin K status of the vessel wall.

osteocalcin measurements in CKD patients difficult to interpret and thus osteocalcin carboxylation may not be a useful marker to assess vitamin K status in advanced CKD.[73]

Matrix Gla protein. The development of conformation-specific antibodies against MGP has resulted in the detection and localization of uncarboxylated MGP (ucMGP) versus carboxylated MGP in vascular tissue.[74] Several studies have demonstrated that ucMGP accumulates in and around calcified vascular areas, whereas carboxylated MGP is less abundant in calcified regions. Using these conformation-specific MGP antibodies as biomarkers for detecting vascular calcification or extrahepatic vitamin K deficiency is an attractive possibility.[65] Circulating MGP reflects what is spilled over from the vasculature into the blood stream, which, in turn, depends on MGP synthesis, MGP activity, and binding of MGP to calcified vascular areas. Using antibodies against different epitopes of MGP, significantly lower serum total ucMGP levels were detected in dialysis patients compared with healthy controls.[75] Moreover, total ucMGP levels were inversely associated with aortic stiffness, suggesting that low ucMGP levels reflect the extent of vascular calcification.[75] The development of an assay for dephosphorylated-uncarboxylated MGP (dp-ucMGP), a biomarker for vitamin K bioavailability of the vasculature, was first described in patients with CKD.[76] Plasma dp-ucMGP increased progressively with progression of CKD and was associated with the severity of aortic calcification.[77] Higher dp-ucMGP was also associated with higher all-cause and cardiovascular mortality in a Flemish general population[78,79] as well in patients with coronary heart disease.[80] Moreover, both dp-ucMGP and dephosphorylated carboxylated MGP may serve as predictors of mortality in dialysis patients.[8,76] Today, dp-ucMGP is most often used to measure vascular vitamin K bioavailability in CKD[68] (Figure 4).

Besides CKD, dp-ucMGP concentrations in plasma are also increased in patients with diabetes,[81] in patients with chronic obstructive pulmonary disease,[82] and in hospitalized coronavirus disease 2019 (COVID-19) patients.[83]

Vitamin K status

Status in healthy persons. Reports on vitamin K ingestion mostly focus on vitamin K1 and range from 29 to 398 μg average daily intake.[84] The average American aged ≥55 years consumes 80 to 210 μg of vitamin K1 per day, markedly exceeding that of younger Americans (60–110 μg/d), which seem to relate to a higher green leafy vegetable consumption in older persons.[85,86] In a Norwegian prospective cohort study, the average intake of vitamin K1 varied widely, and the median daily intake was 48 μg for K1 and 7 μg for K2. Intake of both subforms was higher in women, and higher K2 consumption appeared to mitigate the risk for coronary heart disease.[87] A better vitamin K status was also detected in Chinese women compared with men, as evidenced by lower PIVKA-II levels.[88] Above the age of 40 years, dp-ucMGP plasma levels increased gradually and MK7 supplementation was more effective in decreasing dp-ucMGP the more pronounced the vitamin K insufficiency.[89]

Even in healthy individuals, vitamin K–dependent proteins are not fully carboxylated,[90] and subclinical vitamin K deficiency can be present despite normal blood coagulation parameters.[91] A high prevalence of vitamin K deficiency occurs in newborns and during breast-fed early infancy. Vitamin K supplementation is part of standard care to support coagulation in the newborn to prevent vitamin K deficiency bleeding. Other studies show a high prevalence of low vitamin K status in hospitalized patients, particularly in intensive care patients.[92]

Early observational studies revealed that patients with previous femoral neck or spinal fractures had low circulating vitamin K levels.[93,94] More recently, a prospective study in Japanese women followed up for 3 to 4 years also confirmed an association between low vitamin K concentrations and increased incidence of vertebral fractures.[95] *Vice versa*, in the Japanese diet, where natto is popular, MK7 is the predominantly ingested subform; and high natto consumption correlated with a lower fracture risk.[96] Finally, a meta-analysis of 21 articles, including 222,592 participants, found that higher dietary vitamin K consumption was associated with a moderately lower risk of coronary heart disease.[97] Also, in that analysis, vitamin K deficiency, as assessed via plasma dp-ucMGP concentrations, associated with higher all-cause and cardiovascular mortality.[97]

Status in CKD patients. CKD patients commonly exhibit a functional vitamin deficiency, as evidenced using

Phosphorous High (>500 mg/100 g) Medium (100–500 mg/100 g) Low (<100 mg/100 g)		Potassium		
		High >350 mg/100 g	Medium 100–350 mg/100 g	Low <100 mg/100 g
Vitamin K	High (<350 µg/100 g)		Hard cheese Gouda, Emmentaler	
			Celery	
		Parsley, spinach Chive, purslane		
	Medium (20–350 µg/100 g)			Processed soft cheese Brined goat milk cheese
		Pistacho Goose meat	Eggs, nattō	Roquefort
			Chinese cabbage Broccoli	Olive oil, butter
	Low (<20 µg/100 g)	Poppy seeds		
		Peas, crisps Haselnut, peanut Salmon	Mushroom Bun, pork Salami, chicken	Chick peas
		Cocoa, chips Banana Tomato paste	Milk, yogurt Apple Cucumber, onion	Olives Rice, pasta

Figure 5| Classification of selected food items according to their vitamin K (K1 and K2) potassium and phosphorous contents. Data are from several sources.[5–8]

biomarkers.[8,98,99] Although vitamin K1 concentration in serum was low in only 6% of CKD stage 3 to 5 patients,[99] PIVKA-II was increased in 97% of CKD stage 3 to 5 patients and in 64% of dialysis patients.[8] The dp-ucMGP plasma concentrations also increased with the CKD stage and were increased 3.3-fold to 6.5-fold in dialysis patients.[8,76] An increase of uncarboxylated osteocalcin in serum was found in 60% of CKD stage 3 to 5 patients,[99] and there was a highly significantly increase in dialysis patients.[100]

The high prevalence of functional vitamin K deficiency in CKD patients in part relates to dietary recommendations aimed at reducing potassium and phosphate intake.[101] The reduced consumption of potassium-rich leafy green vegetables will result in low vitamin K1 intake, and restriction of phosphate-rich dairy products will decrease vitamin K2 intake.[71] However, in a study in 85 dialysis patients, dietary intake and serum level of MK4, but not K1, were lower compared with healthy controls.[102] Furthermore, at least in rats with adenine-induced CKD, we found reduced activity of vitamin K recycling enzymes, in particular GGCX.[21,103] Via this mechanism, CKD mimics the actions of pharmacologic inhibitors of the vitamin K cycle (i.e., warfarin or phenprocoumon, which inhibit the VKOR by chemical binding).[104] Frequently prescribed medications in CKD might further aggravate the low vitamin K status in CKD (see below).

In hemodialysis patients, high serum levels of uncarboxylated osteocalcin associated with a higher incidence of bone fractures.[105] In the Vitamin K Italian (VIKI) dialysis study, vitamin K1 deficiency also independently predicted vertebral fractures in hemodialysis patients.[98]

Dietary recommendations in healthy persons. In 2017, the European food safety authority decided to not yet include menaquinones into the dietary reference intake values for vitamin K. Thus, the current recommended daily allowance for vitamin K is based on its role in blood coagulation. It only includes vitamin K1 and is set in Europe by the European Food Safety Authority for all reference groups to 1 µg/kg body weight.[49] The dietary reference intake by the US Department of Agriculture is set to 90 µg/d for females and to 120 µg/d for males.[106]

Dietary recommendations in CKD patients. There are currently no dietary recommendations for vitamin K intake in CKD patients. In patients with decreased kidney function, dietary management usually targets hyperkalemia, hyperphosphatemia, protein intake, and salt and water load. In advanced CKD, potassium intake should be restricted to <3 g/d[107,108] and dietary phosphorous intake should be restricted to 800 to 1000 mg/d.[109] Overall, by adherence to the dietary recommendations, a vitamin K–rich diet in CKD remains highly challenging as the amount of either potassium (e.g., in spinach) or phosphorous (e.g., in hard cheese) is too high (Figure 5[5–8]). Interestingly, a *Lactococcus* strain was developed to increase the menaquinone synthesis in fermented foods,[12] but introducing genetically modified organisms to food is highly restricted.[110] Vitamin K supplementation in CKD patients is discussed below.

Vitamin K status and cardiovascular disease in the general population and CKD patients. In the Danish general adult population, relative vitamin K deficiency, identified by elevated plasma dp-ucMGP, correlated with obesity and a history of cardiovascular events.[111] Surprisingly, in chronic hemodialysis patients, there was also an inverse relationship between body weight and circulating MK7 levels.[112] The assessment of dietary K1 and K2 intakes in healthy elderly

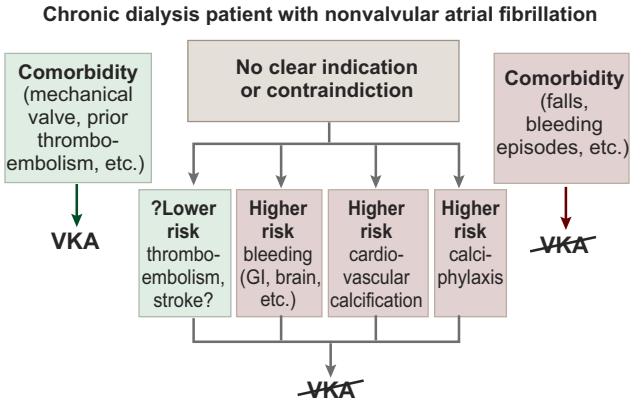

Figure 6 | Decision algorithm pro or contra vitamin K antagonist (VKA) therapy in a chronic dialysis patient with nonvalvular atrial fibrillation. Although there is currently little alternative for patients with particular comorbidity, including mechanical heart valves, prior lung embolism, or other systemic thromboembolism, the risks associated with VKA in end-stage kidney disease seem to outweigh the unproven benefits in most other patients. Modified from Kruger T, Brandenburg V, Schlieper G, et al., Sailing between Scylla and Charybdis: oral long-term anticoagulation in dialysis patients, *Nephrology Dialysis Transplantation*, 2013, volume 28, issue 3, pages 534–541, by permission of Oxford University Press.[132] GI, gastrointestinal.

men and women also revealed a reduced all-cause mortality risk, but not cardiovascular mortality, in those persons with a higher vitamin K consumption.[113] Similarly, a recent meta-analysis of 3 nonrenal cohorts revealed an inverse association between circulating vitamin K levels and all-cause mortality.[114] *Vice versa*, a higher dietary menaquinone intake associated with reduced risk for coronary heart disease[115] and less coronary artery calcification.[116]

The dramatically higher cardiovascular morbidity and mortality in CKD patients at least in part relates to accelerated cardiovascular calcification,[117] which, in turn, is linked to their altered vitamin K metabolism and supply.[99] In diabetic CKD patients as well as in stable kidney transplant patients, higher dp-ucMGP levels in the circulation associated with increased all-cause mortality, cardiovascular mortality, and progression of CKD.[118,119] Similarly, low carboxylation of MGP increased the risk for calcific uremic arteriolopathy (CUA; calciphylaxis; see below) in dialysis patients.[120] As in the general population, in CKD patients, total vitamin K intake above the current recommendations corresponded to a reduced cardiovascular and all-cause mortality.[121] Finally, in end-stage kidney disease patients, poor vitamin K status associated with more systemic inflammation and low bone mineral density,[122] both conditions known to promote cardiovascular damage.

Vitamin K and the bone-vascular axis. Metabolic abnormalities in CKD disturb the balance between mineral resorption and ectopic calcifications by disturbed mineral, parathyroid hormone, and vitamin D metabolism.[123] In this bone-vascular axis, factors like vitamin K, which improve bone quality and allow better mineralization, might at the same time counteract the ectopic cardiovascular calcifications and, for example, allow a more liberal calcium administration in the context of phosphate binding or osteoporosis treatment.[124] However, although appealing, firm evidence for this hypothesis is lacking in CKD patients so far.

Therapeutic vitamin K antagonism

The administration of VKA potently increases circulating markers of vitamin K deficiency in nonrenal and renal patients. For example, a Dutch study compared patients on antithrombotic therapy, who were randomized to aspirin, regular-intensity VKA, or low-intensity VKA.[125] At 1 year, VKA induced uncarboxylation of various Gla-containing proteins, with osteocalcin identified as the most sensitive marker of a poor vitamin K status. Excessively high levels of circulating ucMGP have been observed in chronic dialysis patients receiving VKA.[8]

It is now well established that VKAs promote the development of cardiovascular calcification in many regions, including coronary arteries, aorta, breast arteries, and cardiac valves.[126–128] An extreme form of vascular calcification is CUA (calciphylaxis), a disease mostly manifesting in dialysis patients and characterized by cutaneous necroses associated with bacterial superinfection and high mortality. It is now well established that VKA markedly increases the risk of CUA (≈10-fold).[129] In the EValuation Of Cinacalcet Hydrochloride (HCl) Therapy to Lower CardioVascular Events (EVOLVE) trial, which evaluated a calcimimetic (cinacalcet) in dialysis patients with secondary hyperparathyroidism, we observed that 11 of 24 (45%) of the CUA patients had been on active vitamin K antagonist therapy at the time of CUA manifestation in contrast to 5% to 7% at any one time point in patients in whom CUA was not reported.[130] All these observations plus the lack of proven benefit explain why various guidelines suggest VKAs should not be used in chronic hemodialysis patients with, for example, nonvalvular atrial fibrillation[131] (Figure 6[132]). Indeed, there is some evidence that VKA administration increases morbidity and mortality in chronic dialysis patients,[133] but despite multivariate adjustments, there are concerns about unmeasured confounders that may explain this observation.

Impact of medication on vitamin K bioavailability

Several studies have investigated the impact of phosphate binders on vitamin K bioavailability. At least *in vitro*, most phosphate binders were found to bind fat-soluble vitamins, including vitamin K.[134,135] In the clinical situation, phosphate binder use versus nonuse did not affect dp-ucMGP levels in dialysis patients,[136] but sevelamer monotherapy, when analyzed separately, was associated with higher dp-ucMGP levels as well as with an altered gut microbial metabolism.[29,136] In the cross-sectional Vitamin K Italian (VIKI) dialysis study in hemodialysis patients, those treated with sevelamer not only exhibited MK4 deficiency but also more aortic calcification and vertebral fractures.[137] This may at least in part explain why it has been difficult to firmly establish

differential effects of calcium-containing versus calcium-free phosphate binders on cardiovascular calcification progress (i.e., the procalcific effect of calcium loading might have been offset by a relative vitamin K depletion using calcium-free phosphate binders, in particular sevelamer). However, a recent meta-analysis nevertheless concluded that sevelamer retards calcification progress compared with calcium-containing binders.[138] Unexpectedly, a pilot study in nondialyzed patients with advanced CKD concluded that phosphate binders promoted cardiovascular calcification, but on closer inspection of the data, only those receiving calcium-acetate but not those randomized to placebo, sevelamer, or lanthanum carbonate did exhibit progressive vascular calcification.[139] Whether, as in preclinical studies, calcium-free phosphate binders, in particular sevelamer, could be "fortified" to reduce vascular calcification and mortality by combining them with vitamin K therapy, remains to be proven in patients.[124]

Beyond phosphate binder therapy, a relative vitamin K deficiency can occur with prolonged antibiotic therapy.[140] Whether there are differential effects of the various antibiotics, whether there are thresholds for the duration of treatment, and whether these effects are similar in nonrenal and CKD patients are largely unknown. Another common class of drugs potentially affecting vitamin K homeostasis are proton pump inhibitors. Although no data are available for CKD patients, in healthy volunteers on a vitamin K restricted diet, proton pump inhibitors unexpectedly increased vitamin K levels in the circulation and reduced levels of uncarboxylated proteins (i.e., partially corrected the vitamin K deficiency).[141] This was related to intestinal bacterial overgrowth with subsequent production of menaquinones in the small intestine.

Finally, statins, frequently prescribed to inhibit 3-hydroxy-3-methyl-glutaryl coenzyme reductase, limit endogenous cholesterol synthesis and, as shown in Figure 2, 3-hydroxy-3-methyl-glutaryl–coenzyme A reductase and the vitamin K conversion enzyme UBIAD1 are connected with each other. *In vitro* lipophilic statins are known to directly inhibit UBIAD1,[142] thus reducing endogenous MK4 levels.[15] Moreover, it has been shown that vitamin K is converted to K3 (menadione), which is prenylated in target tissues to MK4.[1] This conversion in target tissues, such as the vascular wall (vascular smooth muscle cells), is inhibited by statins,[143] thereby compromising vascular vitamin K status.

Vitamin K supplementation

Vitamin K, even if given at excessively high doses of up to 20,000 μg per day to Japanese pregnant women,[144] has no known human toxicity.[145] This markedly facilitates the assessment, whether even intense therapy affects cardiovascular disease manifestation or progression, in particular that of calcifications. Only menadione, sometimes referred to as vitamin K3, in large doses has been shown to cause allergic reactions, hemolytic anemia, and cytotoxicity in liver cells.[146,147]

Studies in patients with normal or mildly reduced glomerular filtration rate. With respect to vitamin K1, its supplementation (500 μg/d) for 3 years increased serum phylloquinone levels and reduced ucMGP in older adults.[148] High-dose vitamin K1 supplementation at 2 mg per day orally for 1 year also significantly attenuated the progress of aortic valve calcification, with some 57% reduction compared with placebo in patients with mild to moderate aortic stenosis.[149]

Vitamin K2 trials so far have mostly been performed with MK7. A Dutch double-blind randomized controlled trial tested the effects of 360 μg/d MK7 or placebo given for 6 months in 68 diabetics with concomitant cardiovascular disease.[150,151] MK7 supplementation reduced dp-ucMGP by ≈200 pmol/L compared with placebo. Unexpectedly, active, ongoing calcification in the femoral artery, as detected by ^{18}F-positron emission tomography, tended to increase in the MK7 group ($P = 0.06$ vs. placebo), whereas no difference was detected by conventional computed tomography scanning. The ongoing Dutch Vitamin K–Coronary artery calcification (VitaK-CAC) trial investigates progression of coronary artery calcification in patients with coronary artery disease treated with either placebo or 360 μg of MK7 daily for 2 years.[152]

Data on circulating dp-ucMGP and vascular stiffness and cardiovascular mortality in the general population are less consistent. A meta-analysis of 13 controlled trials and 14 interventional, longitudinal studies confirmed a reduction of vascular calcification but not vascular stiffness with vitamin K supplementation (K1 or K2) for >4 weeks in adults.[153]

Studies in patients with markedly reduced glomerular filtration rate or end-stage kidney disease. Most published intervention trials in CKD patients so far have focused on vitamin K2, usually employing MK7. In a randomized trial with 3 parallel groups, we assessed the effects of MK7 given orally and daily at 45, 135, or 360 μg daily for 6 weeks to 53 chronic hemodialysis patients versus 50 healthy age-matched individuals.[154] Vitamin K deficiency was confirmed in the hemodialysis patients, given 4.5-fold higher ucMGP and 8.4-fold higher uncarboxylated osteocalcin serum levels compared with controls. In the dialysis patients, MK7 supplementation reduced uncarboxylated MGP levels by 77% and 93% in the groups receiving 135 and 360 μg of MK7, respectively. Similarly, a Belgian group performed a pilot trial in 200 hemodialysis patients randomized to receive 360, 720, or 1080 μg of MK7 thrice weekly for 8 weeks. MK7 supplementation reduced plasma dp-ucMGP by 17%, 33%, and 46%, respectively.[155]

Effects of vitamin K2 on surrogate cardiovascular outcomes have been studied in 4 randomized controlled trials (Table 3[43,126,156–160]). A small Polish study in 42 patients with CKD stages 3 to 5 compared 90 μg MK7 together with 10 μg of cholecalciferol with cholecalciferol alone.[157] Nine months later, the MK7 group exhibited a lower increase in carotid intima-media thickness, whereas coronary artery calcification progress was not slowed down. A Greek study randomized 102 patients on hemodialysis to 200 μg MK7 orally every day for 1 year or no treatment.[158] MK7 reduced ucMGP serum levels by 47%. After 1 year of follow-up, 52 patients were available for the analysis. Aortic calcification progress did not differ between the 2 groups. In the British K4Kidneys trial,

Table 3 | Intervention trials evaluating effects of vitamin K supplementation in patients with advanced CKD

Patients/trial	Intervention	Duration of follow-up, mo	Relative reduction in ucMGP plasma levels at study end	Effect on calcification in vitamin K group	Effect on other outcomes in vitamin K group
Hemodialysis patients with atrial fibrillation (VALKYRIE)[126]	MK7, 2 mg, thrice weekly	18	47%	None	None on pulse-wave velocity, all-cause death, stroke, and cardiovascular event rates
CKD stage 3b–4 patients (K4Kidneys)[156]	MK7, 0.4 mg, daily	12	Uncertain[a]	None	None on pulse-wave velocity, augmentation index, blood pressure, B-type natriuretic peptide, or physical function
CKD stage 3–5 ND patients[157]	MK7, 0.09 mg, daily	9	19%	None	Reduced progression of common carotid artery intima-media thickness
Hemodialysis patients[158]	MK7, 0.2 mg, daily	12	47%	None	—
Hemodialysis patients[159]	MK7, 0.36 mg, daily	24	39% after 1 yr, 8% after 2 yr	None	None on pulse-wave velocity and blood pressure
Ongoing trials with vitamin K1					
Hemodialysis patients (VitaVasK)[160]	K1, 5 mg, thrice weekly	18	>70%	To be determined	To be determined
Hemodialysis patients (iPACK-HD)[43]	K1, 10 mg, thrice weekly	12	To be determined	To be determined	To be determined

CKD, chronic kidney disease; iPACK-HD, Inhibiting the progression of arterial calcification with vitamin K in HemoDialysis patients; MK, menaquinone; ucMGP, uncarboxylated matrix γ-carboxyglutamic acid protein; VALKYRIE, The Effect of Replacement of Vitamin K Antagonist by Rivaroxaban With or Without Vitamin K2 Supplementation on Vascular Calcifications in Chronic Hemodialysis Patients: A Randomized Controlled Trial; VitaVasK, Vitamin K1 to slow vascular calcification in hemodialysis patients.
[a]No numbers given; the text states "Mean log-transformed dp-ucMGP results fell between baseline and 12 months with vitamin K treatment (7.08 versus 6.89) but not in the placebo group (7.01 versus 7.06)."[156(p2437)]

159 patients with an estimated glomerular filtration rate of 15 to 45 ml/min per 1.73 m^2 were randomized to receive 400 μg oral MK7 or placebo once daily for 1 year.[156] The primary outcome, carotid-femoral pulse wave velocity at 12 months, did not differ between the groups, nor did augmentation index, blood pressure, B-type natriuretic peptide, or physical function. Finally, a Belgian randomized trial investigated the effect of VKA, rivaroxaban, 10 mg daily, or rivaroxaban, 10 mg daily, plus MK7, 2000 μg, thrice weekly during 18 months on vascular calcification progression in 132 hemodialysis patients with atrial fibrillation.[126] The ucMGP levels decreased by ≈50% in the rivaroxaban plus MK7 group. Cardiovascular calcification progress, changes in pulse-wave velocity, and cardiovascular event rates did not differ at the end of follow-up. Bleeding outcomes were not significantly different, except for a lower number of life-threatening and major bleeding episodes in the rivaroxaban arms versus the VKA arm. Finally, the RenaKvit trial lowered dp-ucMGP serum levels in dialysis patients by up to 39% via the administration of 360 μg MK7 daily, but failed to observe a significant impact on pulse-wave velocity, coronary artery calcification, or abdominal aortic calcification.[159] A Singaporian ongoing trial also assesses the cardiovascular effects of MK7, 360 μg, given 3 times weekly for a total duration of 18 months to hemodialysis patients.[161] None of the above studies reported any adverse events related to vitamin K supplementation.

One issue that so far has affected all vitamin K2 trials is that MK7 has not been available in a drug-grade formulation, even if provided in a synthetic form. At least in Germany, regulatory bodies have argued that in clinical trials, where the daily recommended dose of vitamin K is markedly exceeded, only drug-grade formulations can be used, which has restricted our choice to vitamin K1. At present, 2 similar studies (Inhibiting the progression of arterial calcification with vitamin K in HemoDialysis patients [iPACK-HD], NCT 01528800 in Canada; and Vitamin K1 to slow vascular calcification in hemodialysis patients [VitaVasK], EudraCT No. 2010-021264-14 in Europe), both testing high-dose vitamin K1 therapy at 15 to 30 mg per week, and both investigating progression of cardiovascular calcification, are close to publication.[43,160]

A study in the United Kingdom (ViKTORIES) will investigate the effects of vitamin K3 (menadiol diphosphate) in renal transplant recipients.[162] The primary end point is aortic distensibility, assessed by magnetic resonance imaging; and secondary end points include cardiovascular calcifications.

So far, little information is available on the effect of supplemental vitamin K on bone fractures or density in dialysis patients. In the ongoing iPACK-HD and RenaKvit studies, the impact of vitamin K1 (NCT01528800) and MK7 (NCT02976246) supplementation on the incidence of vertebral fractures and bone mineral density is evaluated in hemodialysis patients.[43]

Conclusion

There is a solid biochemical and experimental basis to implicate vitamin K, in particular K2, in the pathogenesis of cardiovascular disease, especially calcifications. A multitude of clinical observations also supports this association. A

particular high-risk population in this respect are patients with end-stage kidney disease, because they not only exhibit (in part iatrogenic) low vitamin K intake but also probably have a defect in vitamin K recycling, which would mimic the action of VKAs. Dietary supplementation of such high-risk patients with physiological or highly supraphysiological doses predictably corrects the vitamin K deficiency at the biochemical level and exhibits no toxic effects. Against this background, it is frustrating to see yet another easy and safe approach tackling cardiovascular disease in CKD failing in clinical trials. It is tempting to speculate that this is one more situation where, in the highly complex and comorbid patients with advanced CKD, a monotherapeutic intervention simply is not effective enough to yield measurable effects. However, of note, complete correction of vitamin K deficiency by MK7 was never achieved in the interventional trials so far. In addition, vitamin K supplementation may only improve cardiovascular calcifications, if at the same time calcium and phosphate are well controlled. However, before setting up such more complex trials, we suggest waiting for the outcome of the 2 trials using vitamin K1 instead of K2. Why? Because once again, we have extrapolated from findings in nonrenal patients to dialysis patients. Considering the massive alterations of the uremic high-density lipoprotein–lipoprotein particle,[163] we have detected prominent disturbances of vitamin K transport (Kaesler N, Schurgers L, Floege J, unpublished data, 2021) that indicate that not all vitamin K is created equal in the uremic situation. So, the story is not over (yet).

DISCLOSURE

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