Review Article

Interventional Cardiology

The role of vitamin K2 in cardiovascular health

Abstract

Vitamin K naturally occurs as two structurally similar but functionally different vitamins: K1 and K2. Vitamin K2 activates Matrix Gla Protein (MGP) which acts as an inhibitor of vascular calcification. Vitamin K2 plays a role in cardiovascular health. It slows down the progression of coronary artery and aortic valve calcification by inhibiting vascular and valvular calcification. It also has an impact on metabolic syndrome, heart failure, microvascular function, and the progression of arterial stiffness. Vitamin K deficiency was shown to correlate with worse clinical outcomes. Additionally, vitamin K2 supplementation is safe and has been the focus of numerous studies and randomized clinical trials. While some trials have shown no significant effect of supplementation in mitigating coronary artery or valvular calcification, the overall findings remain promising. Many methods and assays to assess vitamin K status and function exist, however, in clinical practice, Protein Induced by Vitamin K Absence/antagonism (PIVKA-II) and vitamin K1 are commonly used together.

Keywords: Cardiovascular health • Metabolic syndrome • Aortic stiffness • Heart failure • Hypertension

Besir Besir, Samir R. Kapadia*

Department of Cardiovascular Medicine, Heart, Vascular and Thoracic Institute, Ohio, United States of America

*Author for correspondence:
Samir R. Kapadia, Department of Cardiovascular Medicine, Heart, Vascular and Thoracic Institute, Ohio, United States of America,
E-mail: kapadis@ccf.org

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Introduction

Vitamin K was discovered through the work of Carl Peter Henrik Dam between 1928 and 1930 [1]. He discovered that chicks fed on cholesterol and fat-free chicken feeds for more than 2-3 weeks were more likely to have a spontaneous hemorrhage [2]. He discovered a new vitamin and called it Vitamin K. In the late 1930s, Edward Albert Doisy was able to isolate vitamin K and received the Nobel Prize jointly with Dam [1]. Vitamin K is a fat-soluble vitamin that exists as 2 compounds that are structurally similar, but functionally different: Vitamin K1 (phylloquinone) and vitamin K2 (menaquinones, MKs) [3]. Vitamins K1 and K2 both have a naphthoquinone ring and a side chain of isoprenoids. The main structural difference between them is the length and saturation of the isoprenoid side chain at the 3rd carbon atom [4]. Vitamin K2 has extrahepatic activity and a longer half-life. Therefore it has an important role in activating γ-carboxyglutamate (Gla) proteins, such as Matrix Gla Protein (MGP), which is an inhibitor of vascular calcification [5]. MGP is synthesized by Vascular Smooth Muscle Cells (VSMCs). To be fully functional, MGP requires vitamin K. Several studies, including randomized clinical trials, have investigated the cardiovascular benefits of vitamin K2. This paper aims to explore the role of MGP and vitamin K2 in cardiovascular health.

Literature Review

Dietary sources of phylloquinone and menaquinones

Vitamin K1 is responsible for coagulation. It is generally present in leafy green vegetables, which contribute around 60% of the total phylloquinone intake [6,7]. Leafy green vegetables with a darker color like collards have higher phylloquinone

concentrations than those with lighter colors such as iceberg lettuce. Phylloquinone can also be found in plant oils such as canola, soybean, olive, and cottonseed [8]. Therefore, spreads and margarines derived from these oils are important dietary sources of phylloquinone [9,10].

Menaquinones (MKs) are characterized by the length of their isoprenoid side chain, but their origins as well as functions are not the same. Their primary origin is bacteria, except MK-4 which is formed in 2 different ways: Either via a realkylation step from menadione, or as a product of tissue-specific conversion directly from phylloquinone [8,11,12]. MK-4 formed from menadione comes from poultry products [13], and that coming from phylloquinone is found in small amounts in dairy products, and in organs such as the kidney [8]. On the other hand, MK-7 is the product of bacterial fermentation and is present in natto, a traditional Japanese soybean-based product [8]. Natto is rich in MK-7, and also contains MK-8, MK-9, and phylloquinone [8]. Figure 1 summarizes the dietary sources of vitamin K1 and K [8,9,14,15].

Matrix Gla Protein (MGP)

GP plays a key role in cardiovascular disease [16]. Mice who had their MGP gene knocked out were found to die prematurely due to arterial calcification and spontaneous aortic rupture [17]. MGP undergoes two post-translational modifications essential for its activation: serine phosphorylation and γ-glutamate carboxylation, the latter being a step that requires vitamin K [18]. Various forms of MGP exist, depending on its phosphorylation and carboxylation status. Since vitamin K is needed for activation of MGP, the unphosphorylated, and uncarboxylated form (dp-ucMGP) can be used as a marker of vitamin K deficiency [19,20]. The active form of MGP plays a role in preventing vascular calcification. In the absence of active MGP, VSMCs produce a matrix that favors calcium deposition, a characteristic of osteoblasts and chondrocytes [21]. Active MGP inhibits the formation of calcium crystals and modulates the transcription factors that prevent VSMCs from differentiating into cells that act similarly to osteoblasts and chondrocytes [22,23]. Active MGP is also an inhibitor of Bone Morphogenic Protein-2 (BMP-2), which induces osteogenic gene expression in VSMCs [5,23]. Moreover, it activates fetuin-A, which is an inhibitor of calcification. Figure 2 summarizes the mechanism of action of vitamin K.

Relationship of vitamin K with cardiovascular health

To investigate the impact of vitamin K2 on cardiovascular health, a proper assessment of vitamin K status is needed. Assays measuring MGP reflect vitamin K bioactivity over a period of weeks to months [18,24-27]. These assays rely on dual antibody ELISA to measure dephosphorylated-uncarboxylated MGP and dephosphorylated-carboxylated MGP, and mono-antibody assays measuring total uncarboxylated MGP and total dephosphorylated MGP. Table 1 summarizes those assays and their reference values. Moreover, the measurement of dp-ucMGP is used to quantify vitamin K deficiency. Figure 3 summarizes the cardiovascular benefits of vitamin K supplementation.

Vascular calcification: Many studies have found that vascular calcification was associated with vitamin K deficiency. Loss-of-function mutations in the MGP gene cause systemic vascular calcification [28]. Studies have also shown that elevated dp-ucMGP levels correlate with coronary and peripheral artery calcifications, and more plaque stability [29-31]. Furthermore, Vitamin K Antagonists (VKA) was found to accelerate calcification of the coronary arteries [32-34].

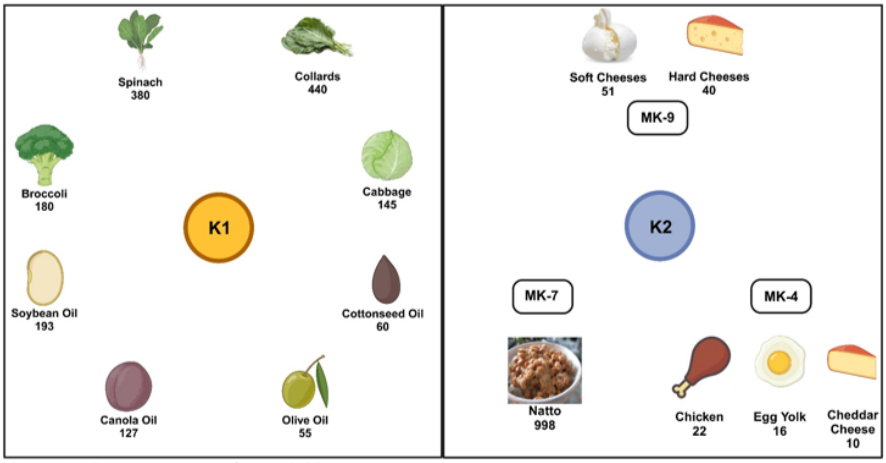

Figure 1: Dietary Sources of vitamin K1 and vitamin K2. **Note:** Concentrations are presented as µg/100g.

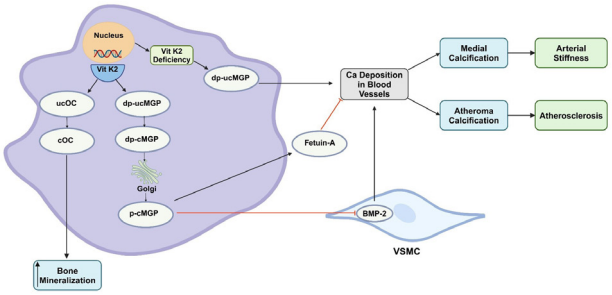

Figure 2: Mechanism of action of vitamin K2. **Note:** Ca: Calcium; dp-uc: Dephospho-Uncarboxylated; MGP: Matrix Gla Protein; OC: Osteocalcin.

Table 1: Summarizes the method and reference values for different assays used to measure MGP.

Assay	dp-ucMGP	dp-cMGP	t-ucMGP	t-dpMGP
Method	Dual-antibody ELISA	Dual-antibody ELISA	Mono-antibody ELISA	Mono-antibody ELISA
Reference Values*	447 ± 188 pM	1763 ± 478 pM	4704 ± 1053 nM	14 ± 3 nM

Note: *Reference values according to Cranenburg et al. "Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species." Thrombosis and haemostasis 104.10 (2010): 811-822. dp-ucMGP: Dephosphorylated-Uncarboxylated MGP, dp-cMGP: Dephosphorylated-Carboxylated MGP, t-ucMGP: total Uncarboxylated MGP, t-dpMGP: total Dephosphorylated MGP.

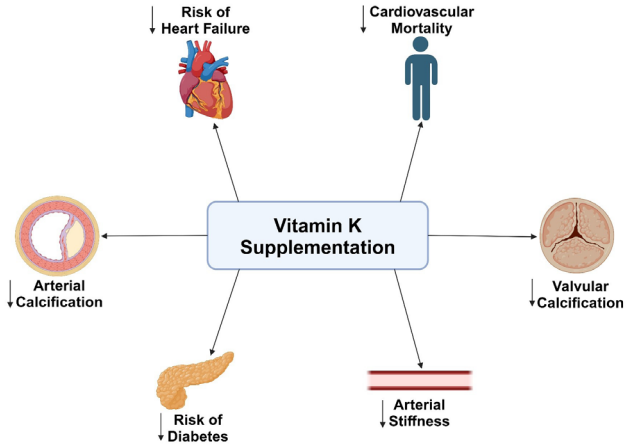

Figure 3: Possible effects of Vitamin K supplementation on cardiovascular health.

Vitamin K is thought to slow the progression of Coronary Artery Calcification (CAC) [35,36]. However, few studies showed that although vitamin K2 supplementation decreased dp-ucMGP levels [37,38], it did not reduce arterial calcification [39,40]. Namely, a multicenter double-blinded randomized controlled trial with 2 years of follow-up concluded that in patients with no prior ischemic heart disease, supplementation with vitamin K2 did not slow the progression of coronary artery calcification [41]. However, this could be attributed to the short follow-up of 2 years. Longer follow-ups could show a significant reduction in mean coronary artery calcification. Moreover, the same trial found a significant reduction in the progression of coronary artery calcification in patients with CAC scores ≥ 400 Agatston Units [41].

Valvular calcification: Vitamin K2 deficiency is thought to correlate with valvular calcification. VKAs like warfarin were linked to aortic and mitral valve calcifications [42,43]. Moreover, a study comparing rivaroxaban with warfarin showed that rivaroxaban was associated with less mitral and aortic valve calcification compared to warfarin [44].

Measuring inactive forms of MGP may be useful in identifying patients at risk for progression of valvular calcification. Valvular calcification is an active process that could be modified. A study by Parker et al. found an association between serum inactive total MGP and mitral annular calcification in non-diabetic patients with CAD [45].

Studies tried to investigate the role of vitamin K2 supplementation in delaying the progression of aortic valve calcification. One randomized clinical trial found that vitamin K1 daily intake slowed the progression of aortic valve calcification [37]. On the other hand, another randomized double-blinded clinical trial concluded that in elderly men with an aortic valve calcification score >300, vitamin K2 supplementation did not affect the progression of AS [46].

Microvascular function: MGP is thought to contribute to the microvascular integrity of the heart, kidneys, and retina [47-49]. Since diastolic dysfunction can be related to inflammation of the coronary microcirculation, MGP can be involved in the disease process [50]. One study correlated dp-ucMGP with diastolic dysfunction assessed by higher Left Ventricular (LV) filling pressures and a higher E/e' ratio [47]. The study also found a higher prevalence of dp-ucMGP in cardiac biopsies of hearts with ischemic or dilated cardiomyopathies than in normal hearts [47]. Therefore, activated MGP, with its role in preventing calcium deposition, protects the heart microcirculation and preserves LV diastolic function [3].

Markers of vitamin K deficiency are also associated with diabetes, kidney function, adiposity, and inflammation [51]. A randomized clinical trial found that low levels of carboxylated osteocalcin, indicating vitamin K deficiency, were associated with waist circumference and higher fat mass at different sites in the body [51]. More than 60% of patients with Chronic Kidney Disease (CKD) have a deficiency in vitamin K [51]. Markers of vitamin K deficiency, including elevated dp-ucMGP, were associated with a greater risk of developing advanced CKD and a lower Glomerular Filtration Rate (GFR) [52,53]. Functional vitamin K deficiency is also prevalent among kidney transplant recipients and patients on hemodialysis [54]. Although vitamin K levels improve following kidney transplantation, elevated levels of dp-ucMGP in kidney transplant recipients were associated with a greater risk of long-term mortality [55]. Some studies even suggested a correlation between MGP and serum creatinine levels in patients with CKD [56,57].

Vitamin K deficiency is also related to the microcirculation of the retina. Elevated levels of dp-ucMGP were associated with a lower retinal arteriolar diameter [58]. Retinal microvascular diameter narrowing was found to correspond to worse cardiovascular outcomes at 10 years [59].

Metabolic syndrome and diabetes: Studies have shown that supplementation with vitamin K2 decreases the incidence of type 2 diabetes [60]. A randomized clinical trial also showed that vitamin K2 supplementation increases insulin sensitivity [61]. Another clinical trial found that healthy postmenopausal women supplemented with vitamin K2 had a greater reduction in abdominal and visceral fat than those receiving a placebo [62].

Arterial stiffness: Markers of vitamin K deficiency including higher levels of dp-ucMGP have been correlated with aortic stiffness assessed by carotid-femoral Pulse Wave Velocity (PWV), augmentation index, and central pressure [27,63-65]. A significant reduction in arterial stiffness (by brachial-ankle PWV) was noted 3 months after switching warfarin to rivaroxaban [66]. Vitamin K1 and MK-7 were both found to decrease the arterial stiffness of the carotid artery [67]. Supplementation with MK-7 improves arterial stiffness measured by PWV in healthy patients and different subgroups [63,68-70].

Endothelial dysfunction is a predictor of worse outcomes [71]. Vitamin K2 is thought to regulate endothelial function, and some of its protective properties are due to its role in the regulation of endothelial function [72]. MGP inhibits the osteogenic properties of vascular endothelial cells in animal models [73]. Moreover, supplementation with MK-2 improves endothelial function in genetically driven mice models with hypercholesterolemia [74].

Cardiovascular outcomes and heart failure: There is a debate on whether vitamin K deficiency is associated with worse cardiovascular outcomes, with some studies correlating elevated dp-ucMGP levels with cardiovascular morbidity and mortality [16,57,75-79], and others showing no correlation [80,81].

Levels of dp-ucMGP are involved in both the systolic and diastolic functions of the heart. On the cellular level, MGP has a role in cardiac hemodynamics unrelated to calcification inhibition. MGP levels are seen during the rapid myocardial response to pressure overload [82-84], including the setting of acute myocardial infarction even before left ventricular remodeling [83].

Vitamin K deficiency, suggested by elevated dp-ucMGP levels, correlates with unfavorable echocardiographic parameters in patients with heart failure and concomitant severe AS [57,76]. Therefore, dp-ucMGP can be used as a pre-procedural marker for risk assessment in patients undergoing aortic valve replacement [3,76]. In addition, levels of dp-ucMGP correlated with Left Ventricular Ejection Fraction (LVEF), N-terminal pro-brain natriuretic peptide (NT-proBNP), and mortality [46]. Elevated levels of dp-ucMGP also correlated with elevated NT-proBNP, CRP, LVEF, and diastolic dysfunction in patients with chronic heart failure [57].

Vitamin K2 has a significant role in producing mitochondrial ATP. Cardiac muscles are abundant in mitochondria and therefore, vitamin K2 can impact the function of cardiac muscles [85]. One study found that vitamin K supplementation for 8 weeks correlated with increased maximal cardiac output, stroke volume, heart rate, and decreased blood lactate during exercise [86].

Many studies explored the impact of vitamin K intake on cardiovascular outcomes. A lower incidence of CAD was found in patients with more intake of food rich in vitamin K1 [16,87]. Prospective cohort studies found that intake of vitamin K2 and not K1 decreased the incidence of severe aortic valve calcification, coronary artery disease, and mortality [35,88,89]. Another study found that patients who increased their vitamin K1 or K2 intake over time had lower mortality rates [90].

Discussion

Laboratory assessment of Vitamin K status

Different approaches are used to evaluate vitamin K status and function.

Global coagulation assays: Measurement of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) only reveals a gross deficiency and thus should not be used [91,92]. The PT begins to rise above the reference range only when the concentration of the fully carboxylated factor II falls below ~50% [91,92].

Coagulation factor assays: If the concentration of factor V is normal, and that of factors II, VII, IX, and X is low, this suggests deficiency of a specific vitamin K-dependent clotting factor [91]. The half-lives of procoagulant Vitamin K-Dependent Proteins (VKDP) range from 6 to 50 hours, and thus, in the setting of vitamin K deficiency, the onset of abnormal coagulation is delayed [91].

Direct measurement: The most commonly used marker of vitamin K status is the direct measurement of serum K1 concentrations. Although this method can assess the levels of vitamin K in general, it does not accurately reflect the actual utilization within target tissues [91]. Vitamin K has a low endogenous concentration of around 1 part per billion and it can be measured either by liquid chromatography-mass spectrometry or high-performance liquid chromatography fluorescence detection [93,94]. Vitamin K2 can also be separated and quantified with the same chromatographic run as vitamin K1, but the clinical relevance of vitamin K2 concentrations is less well understood [91].

Other assays used clinically are those that assess the metabolites of vitamin K. High-performance liquid chromatography electrochemical detection and liquid chromatography-mass spectrometry can be used to measure levels of the 5C- and 7C-aglycone vitamin K metabolites [95,96]. The advantage of this method lies in the ability to assess the status of both vitamin K1 and K2 since they share common metabolites [91,95].

Hepatic VKDPs: Protein Induced by Vitamin K Absence/antagonism (PIVKA-II) is the most commonly used method to evaluate vitamin K function. Multiple assays exist and they include ELISA, liquid chromatography-mass spectrometry, and automated immunoassay [97-100]. When vitamin K stores are low enough to prevent effective carboxylation of factor II, PIVKA-II levels increase, even before the rise in PT [91]. Additionally, PIVKA-II can be used clinically to estimate the status of vitamin K during the preceding 3-4 days [101]. It is also used as a marker of hepatocellular carcinoma together with α-fetoprotein, to detect exposure to VKAs [102,103].

Extrahepatic VKDPs: Assessment of the utilization of vitamin K by extrahepatic tissues is possible. This is mainly done by determining the degree of carboxylation of osteocalcin and MGP [26].

As previously discussed, many methods and assays to assess vitamin K status and function exist. However, the common practice relies on using PIVKA-II and vitamin K1 in tandem [91]. Low concentrations of vitamin K1 in the serum indicate inadequate tissue stores in general. On the other hand, PIVKA-II indicates whether tissue stores are not enough for the hepatic carboxylation of factor II. When there is a low serum vitamin K1 concentration with normal PIVKA-II levels, it could be due to the susceptibility of extrahepatic to low vitamin K status [91]. Additionally, elevated PIVKA-II with normal vitamin K1 levels could be found in patients with hepatocellular carcinoma [91].

Conclusion

The role of vitamin K in cardiovascular health is an area of growing interest. While vitamin K2 is known for its role in vascular calcification, recent studies have shown an impact on heart failure, endothelial dysfunction, metabolic syndrome, and the progression of arterial stiffness. Vitamin K deficiency is associated with worse outcomes. Additionally, vitamin K2 supplementation is safe and has been the focus of numerous studies and randomized clinical trials. While some trials have shown no significant effect of supplementation in mitigating coronary artery or valvular calcification, the overall findings remain promising. Vitamin K1 levels are utilized in tandem with PIVKA-II to assess the status and function of vitamin K. Further research is needed to increase our understanding of the additional roles of vitamin K2 on cardiovascular health, and the benefits of vitamin K2 supplementation on cardiovascular outcomes.

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