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## **Insights into vitamin K metabolism in chronic kidney disease: more complicated than kale deficiency**

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### **Abstract**

Vascular calcification is a major manifestation of cardiovascular disease in advanced chronic kidney disease and is inhibited by vitamin K dependent proteins. Clinical trials of vitamin K supplementation in chronic kidney disease have failed to demonstrate benefits on vascular calcification. Recent laboratory, human and animal studies have shown that vitamin K handling and metabolism in chronic kidney disease is complex and suggest vitamin K2 subtype supplementation in isolation is unlikely to have significant clinical impact.

The role of vitamin K dependent proteins as a cofactor of normal coagulation and use of vitamin K antagonists (VKAs) as antithrombotic therapy is well known. Recently, there has been interest in the role of vitamin K dependent proteins as mediators of vascular calcification.

Vascular calcification has been widely demonstrated to be a manifestation of accelerated cardiovascular disease in chronic kidney disease (CKD). Although the pathogenesis of vascular calcification is complex, the most potent inhibitor of arterial calcification, matrix Gla protein (MGP) is dependent on vitamin K dependent proteins for its activation from desphosphylated-uncarboxylated matrix Gla protein (dp-ucMGP). Serum dp-ucMGP level is considered the most sensitive marker of functional vitamin K deficiency. Observational data suggest that patients with advanced CKD or on dialysis are relatively deficient in vitamin K with elevated dp-ucMGP, and those patients with highest dp-ucMGP having most severe vascular calcification. High dp-ucMGP has been associated with increased risk of mortality in hemodialysis patients<sup>1</sup>. Calciphylaxis, an extreme manifestation of calcification of small blood vessels associated with cutaneous necrosis, is more common in patients receiving VKAs, with patients with calciphylaxis having higher dp-ucMGP levels than other CKD patients<sup>2</sup>.

Vitamin K in humans is predominantly obtained from the diet. Vitamin K1 (phylloquinone) rich foods include green vegetables, particularly kale, spinach, brussels sprouts and asparagus. Foods rich in vitamin K2 (menaquinone) include animal proteins such as chicken, egg yolk as well as *natto* made from fermented soybeans. It is plausible that patients with advanced CKD may be deficient in vitamin K as dietary restrictions to reduce intake of potassium, phosphate and protein may indirectly lead to reduced consumption of vitamin K rich foods. Estimation of vitamin K

intake is challenging. There are limited data linking estimated vitamin K intake with functional vitamin K status in CKD patients. In one study in kidney transplant patients, vitamin K intake was below recommended intake in 50% of patients based on dietary assessment. Inadequate vitamin K intake was associated with higher dp-ucMGP levels and 80% of patients had elevated dp-ucMGP<sup>3</sup>. Vitamin K supplementation represents an attractive approach for reducing cardiovascular risk by inhibiting vascular calcification by increasing biologically active carboxylated MGP.

Recent randomized controlled trials (RCTs) of vitamin K supplementation targeting vascular calcification in CKD have failed to demonstrate that supplementation with vitamin K subtypes has any effect on vascular calcification, suggesting that insufficient dietary intake is unlikely to be the sole mechanism for vitamin K deficiency. To summarize these RCTs, in the K for Kidneys trial, 159 patients with CKD stage 3b or 4 were randomized to 400  $\mu\text{g}$  daily vitamin K<sub>2</sub> (MK7 subtype) or placebo for 12 months. There was no difference in markers of vascular calcification or associated arterial stiffness such as pulse wave velocity or aortic calcification<sup>4</sup>. In a RCT of 90 kidney transplant recipients randomized 1:1 to menadiol (vitamin K<sub>4</sub>) or placebo, after 12 months there were similar levels of coronary artery calcification in both vitamin K and placebo groups<sup>5</sup>. In 132 hemodialysis patients with atrial fibrillation - randomized to VKA, rivaroxaban or rivaroxaban plus vitamin K<sub>2</sub> (2000  $\mu\text{g}$  menaquinone-7 (MK-7) thrice weekly) for 18 months - there was no demonstrable effect on coronary artery calcification of withdrawal of VKA and vitamin K<sub>2</sub> supplementation compared to VKA alone<sup>6</sup>. Finally, in 48 dialysis patients treated with vitamin K<sub>2</sub> (MK7, 360  $\mu\text{g}$  daily) or placebo, there was no significant between-group difference in abdominal aortic calcification over two years<sup>7</sup>. In these RCTs, subjects allocated to vitamin K had

biochemical evidence of greater repletion of vitamin K, indicated by lower dp-ucMGP compared to placebo.

There are several possibly explanations why vitamin K subtypes were unsuccessful in reducing calcification in these RCTs. The trials may have been performed too late in the stages of CKD where calcification was not amenable to intervention. By not assessing vitamin K status prior to randomization, it is possible that some subjects were vitamin K replete and further supplementation would not effect clinical benefit<sup>5</sup>. The vitamin K supplement or subtype provided supra-physiological doses of vitamin K in all trials, but it may be that the dose or duration of treatment was insufficient to impart clinical benefit.

In an elegant series of laboratory experiments, animal models and clinical studies, Kaesler *et al* expand on our understanding of vitamin K metabolism in advanced CKD<sup>8,9</sup>. Briefly, in a small clinical study following a dose of oral combined vitamin K1, MK4 and MK7 administered in 10 dialysis patients and 9 healthy controls, there was significant incorporation of MK4 into LDL- and HDL-cholesterol in dialysis patients compared to this being minimal in controls. Uptake of MK7 into LDL-cholesterol was markedly reduced compared to controls, but dialysis patients incorporated more MK4 into both HDL and LDL-cholesterol. This potentially represents a major advance in understanding of vitamin K transport in uremia. However, a larger definitive study replicating these observations is required. Further experiments show that HDL particles from dialysis patients spiked with MK7 were unable to exert biological effect and failed to reduce dp-ucMGP levels in human vascular smooth muscle cells unlike spiked HDL particles from controls. Therefore, they highlight differences in handling of

vitamin K subtypes between dialysis patients and controls and align to this reduction of biological effect on vitamin K on activation of dp-ucMGP, hence demonstrating *in vivo* and *in vitro* loss of vitamin K dependent inhibition of vascular calcification. To assess the role of lipid metabolism on vitamin K status, samples were analyzed from the 4D RCT where hemodialysis patients were randomized to hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibition with atorvastatin or placebo. There were no significant differences in serum protein induced by vitamin K absence-II (PIVKA-II) levels between the atorvastatin and placebo groups. There was a trend of higher serum PIVKA-II levels in patients receiving combined atorvastatin and proton pump inhibition. Plasma dp-ucMGP were not reported making comparison between this and other trials in dialysis patients challenging<sup>6</sup>.

Kaesler *et al* performed animal studies to explore whether there are fundamental differences in vitamin K metabolism in CKD. In a 5/6 nephrectomy murine model of CKD, they demonstrate mice fed a diet rich in vitamin K1, MK4 and MK7 over 8 weeks, had higher serum levels of MK4 and MK7 with lower MK4 content (but not other vitamin K subtypes) in kidney tissue, in CKD compared to control mice. In non-renal tissue, concentrations of all vitamin K subtypes were similar in 5/6 nephrectomy and sham treated mice. They were unable to demonstrate differences in the kidney of UbiA prenyltransferase domain-containing protein 1 (UBIAD1): the enzyme regulating the conversion of vitamin K1 into K2 (MK4) in another rodent model of CKD<sup>8</sup>. Intriguingly, there was significantly less HMG-CoA reductase protein expression in the CKD kidney, which correlated with calcification demonstrated by von Kossa staining, supporting the notion that differences in lipid metabolism exist in CKD and these associate with calcification. They provide supportive data of intra-renal abnormalities

of vitamin K metabolism, using single cell RNA sequencing data to demonstrate reduced *VKORC1* mRNA expression (a key protein in vitamin K activation) in podocytes of CKD patients compared to kidneys from patients with without CKD.



These studies have elucidated novel insights into functional vitamin K deficiency in CKD. By investigating multiple pathways implicated in vitamin K metabolism, Kaesler *et al* show that vitamin K2 transport and tissue effects are altered in CKD and this has implications for its subsequent role in inhibition of vascular calcification. This proposed modification of vitamin K handling gives insights into possible mechanisms explaining the lack of significant effects of vitamin K2 supplementation in recent clinical trials, despite being tested at a variety of doses in different CKD populations with all trials demonstrating meaningful reduction in circulating dp-ucMGP in response to vitamin K<sup>4-7</sup>. Unified approaches to assessment of serum (or plasma) vitamin K activity with dp-ucMGP and/or PIVKA-II levels would allow direct comparison to be made between clinical studies, whilst more widespread use of plasma dp-ucMGP levels would permit greater appreciation of the degree of functional vitamin K deficiency in CKD and permit targeted intervention, akin to routine measurement of LDL-cholesterol to guide statin therapy.

Whilst this report should reinvigorate interest in future RCTs of vitamin K supplementation in CKD, many further questions remain. We eagerly anticipate the results of RCTs of vitamin K supplementation in dialysis including the VitaVask (vitamin K1; NCT01742273), iPACK-HD (vitamin K1; NCT01528800) and Trevasc-HDK (vitamin K2-MK7; NCT02870829) trials targeting coronary calcification in haemodialysis patients. Larger RCTs are needed to address if vitamin K



supplementation is a valid approach to tackling vascular calcification to reduce excess cardiovascular mortality in advanced CKD. We note these are all open label trials, and if neutral, will raise the question if combined vitamin K1 and K2 supplementation is required, as was used in the clinical study of Kaesler *et al.* Irrespective of the findings of the ongoing clinical trials of vitamin K supplementation in dialysis, a further cardiovascular outcome study will be required to demonstrate that reduction in coronary calcification with vitamin K translates to meaningful differences in cardiovascular survival.

## Legend

Figure 1. Considerations to be taken into account when planning future clinical trials of vitamin K therapy in patients with CKD in order to achieve potentially positive outcomes. Additional abbreviations- AXR- abdominal X-ray, CT- computerized tomography, DOAC- direct oral anticoagulant, MRI- magnetic resonance imaging, PPI- proton pump inhibitor

	<p><b>Patient</b></p> <ul style="list-style-type: none"> <li>• Dietary intake</li> <li>• Vitamin K status</li> <li>• CKD stage and duration</li> <li>• Baseline calcification</li> <li>• Concomitant medication (PPI, statin, VKA, DOAC)</li> </ul>	<p><b>Assessment of response</b></p> <ul style="list-style-type: none"> <li>• dp-ucMCP (serum)</li> <li>• PIVKA-II (plasma)</li> <li>• Serum K1</li> <li>• Assay for routine clinical use</li> </ul>	
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## CONSIDERATIONS FOR CLINICAL EVIDENCE OF VITAMIN K EFFICACY IN CKD

	<p><b>Vitamin K supplementation</b></p> <ul style="list-style-type: none"> <li>• Diet</li> <li>• K1, K2, both or other (Menadiol)</li> <li>• Dose and frequency</li> <li>• Route (oral or intravenous)</li> <li>• Medical grade rather than vitamin supplement</li> <li>• Synergistic treatments (vitamin D)</li> </ul>	<p><b>Clinical assessment of efficacy</b></p> <ul style="list-style-type: none"> <li>• CT coronary calcification</li> <li>• Vascular calcification (CT, AXR, MRI)</li> <li>• Valvular calcification (Echo)</li> <li>• Major adverse cardiovascular event</li> <li>• Survival</li> </ul>	
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## Disclosure

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