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Vitamin K2: an essential protector for cardiovascular health

Growing evidence confirms K2's heart benefits, yet inadequate intakes continue

KEYWORDS: Vitamin K2, menaquinone, MK-7, vitamin K-dependent proteins, calcification inhibitors, coronary heart disease, vitamin K insufficiency.

Abstract Previously, vitamin K was recognized solely as a cofactor for blood clotting. However, the discovery of vitamin K-dependent proteins (VKDPs) led to a more comprehensive understanding of vitamin K's role with respect to bone and cardiovascular health. Research has demonstrated that high intakes of vitamin K2 (but not K1), through its activation of the VKDP Matrix Gla Protein (MGP), is associated with reduced arterial calcification, reduced arterial stiffness, and a reduced incidence of coronary heart disease (CHD). Current data suggests that supplementation with Vitamin K2 may help correct widespread vitamin K insufficiency while improving risk parameters for cardiovascular disease in Western populations.

INTRODUCTION

Discovered in 1929, vitamin K was originally identified for its role as a haemostasiological (coagulation) cofactor. The mechanism by which vitamin K performs this function is via its involvement in several proteins, which play a role in the regulation of blood clotting (1). Vitamin K1 and K2 are the two naturally occurring forms of this fat-soluble vitamin. Plants synthesize phylloquinone (K1), and intestinal microbiota synthesizes a range of K2 forms collectively referred to as menaquinones (2). The menaquinone form of vitamin K2 is designated according to the number of repeating 5-carbon units in the side chain of the molecule. For example, if there are seven repeating 5-carbon units, the designation will be menaquinone-7, or MK-7 (3).

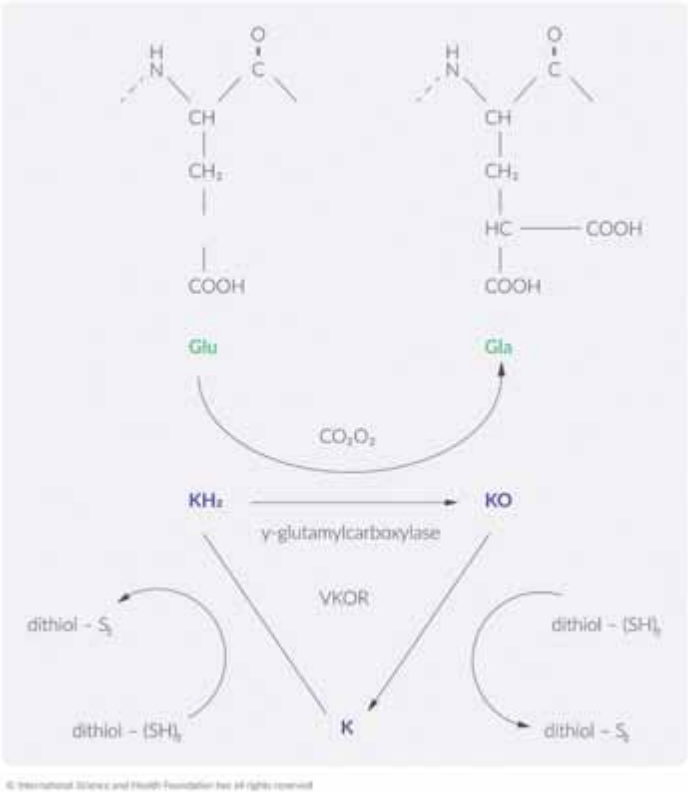

Figure 1. The Vitamin K cycle.

MORE THAN A HEMOSTASIOLOGICAL COFACTOR

In the 1970s vitamin K-dependent proteins (VKDPs) were discovered. Essentially, vitamin K functions as a cofactor for the enzyme, γ-glutamylcarboxylase which in turn catalyses the carboxylation of the glutamic acid (Glu) to γ-carboxyglutamic acid (Gla) forming Gla-proteins or VKDPs (1) (Figure 1). Although previously considered primary for its role as a haemostasiological cofactor, this discovery led to a more comprehensive understanding of vitamin K's role with respect to bone and cardiovascular health (4). Some of the 17 VKDPs that have been identified to date act as calcification inhibitors (5). These include osteocalcin (OC),

also known as bone Gla protein (synthesized by osteoblasts), and matrix Gla protein (MGP, found in cartilage, bone, and soft tissue, including blood vessel walls). These proteins are local inhibitors of calcification in the tissues in which they exert their function (6-12). It should be noted that only calcification inhibitors that are small enough to penetrate the collagen and elastin fibrils, i.e., OC and MGP, will be able to move freely into the fibril and prevent mineral growth therein. This explains why the elastin fibrils are prone to calcification, especially during vitamin K insufficiency, and demonstrates the vital importance of vitamin K in the prevention of soft-tissue calcification (13).

Menaquinone and vascular health: epidemiological and observational research

The first evidence for a link between vitamin K status and vascular health was provided by the population-based Rotterdam study (14), which included 4,807 subjects who were analysed for their vitamin K intake as well as its relationship to aortic calcification and coronary heart disease (CHD). The results showed the relative risk of CHD mortality was reduced in the mid and upper tertiles of dietary menaquinone, compared to the lower tertile, and was inversely related to all-cause mortality and severe aortic calcification. Interestingly, phylloquinone intake was not related to any of the outcomes. In similar research, the association between intake of phylloquinone and menaquinone with coronary calcification was examined in a cross-sectional study (15) among 564 post-menopausal women. Results revealed that 62% (n=360) of the women had coronary calcification. Menaquinone intake was associated with decreased relative risk of coronary calcification (p=0.03). As with the Rotterdam cohort, this study showed that high dietary menaquinone intake, but not phylloquinone, was associated with reduced coronary calcification.

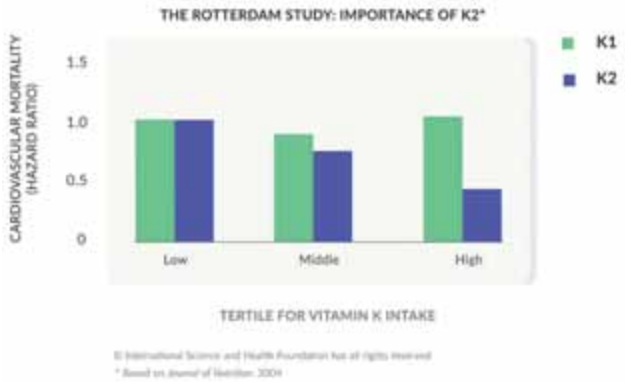

Figure 2. Dietary intake of Vitamin K2 is associated with reduced risk of coronary heart disease.

DIFFERENCES BETWEEN PHYLLOQUINONE AND MENAQUINONE

In addition to the differences between phylloquinone and menaquinone noted in the introduction, there are other noteworthy differences. Transport of phylloquinone is with triacylglycerol-rich fraction which is mainly cleared by the liver. Menaquinones are found in both triacylglycerol-rich lipoprotein and low-density lipoprotein, which transports it to extra-hepatic tissues (16). This is consistent with functions of phylloquinone and menaquinone: phylloquinone predominantly serves as a cofactor for VKDPs in blood coagulation within the liver and menaquinones serve as a cofactor in extra-hepatic tissues such as the vascular wall (17). Also, phylloquinone has a relatively short half-life time(18), whereas research suggests that long-chain menaquinones have a longer half-life(19). Schurgers et al (20) demonstrated that, while phylloquinone and the MK-7 form of menaquinone were absorbed well, the long half-life of MK-7 resulted in much more stable serum levels, and 7- to 8-fold accumulation of higher levels during prolonged intake.

In addition, there is a difference in bioavailability between the two most commercially available forms of menaquinone used in the food industry and in dietary supplements, which are MK-4 and MK-7 (Figure 3). A human clinical trial (21) investigated the bioavailability of MK-4 and MK-7, the two most commercially available forms of menaquinone. A single dose administration of MK-4 (420 µg) or MK-7 (420 µg) was given in the morning together with standardized breakfast. Results showed that MK-7 was well absorbed, reaching a maximal serum level at 6 hours and detectable up to 48 hours after intake. By contrast, MK-4 was not detectable in the serum of all subjects at any time point, even after continuing consecutive administration (60 µg) for 7 days. Researchers concluded that MK-4 does not contribute to the vitamin K status as measured by serum vitamin K levels. However, it can be noted that other research has shown that vitamin K status in bone tissue does receive benefit from MK-4 supplementation (22,23), albeit at exponentially higher doses (45 mg/day).

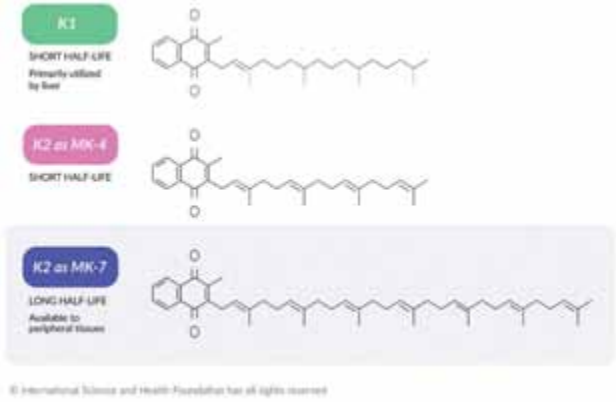

Figure 3. Forms of Vitamin K.

Carboxylation and Gla proteins

Schurgers et al (20) also showed that MK-7 was more effective than phylloquinone in increasing the carboxylation of Gla-proteins. Since 10-40% of OC and MGP remains undercarboxylated, Theuwissen et al (24) studied the dose-response effects of supplemental MK-7 and placebo on the carboxylation of the extra-hepatic Gla-proteins in 42 healthy Dutch men and women aged between 18 and 45 years, using the following daily doses of MK-7 in seven randomised groups: placebo or MK-7 at 10, 20, 45, 90, 180, or 360 µg. Results showed that MK-7 supplementation at doses at or above the RDA (i.e. 75 µg+) significantly improved the carboxylation of circulating OC and MGP, whereas supplementation at doses below the RDA had no significant effects on the circulating levels of both Gla-proteins. The carboxylation of Gla-proteins is particularly significant since this has a positive impact on bone strength (25) and arterial stiffness (26) (described later in this article).

CARDIOPROTECTIVE MECHANISM OF ACTION FOR MENAQUINONE

MGP functions as an important local inhibitor of vascular calcification by binding to crystal nuclei in hydroxyapatite, thereby preventing crystal growth (27). The importance of MGP as an inhibitor of vascular calcification was demonstrated in a study by Luo et al (28) where mice that lacked MGP developed to term, but died within two months as a result of arterial calcification leading to blood-vessel rupture. Since sufficient levels of vitamin K are needed for MGP carboxylation (29-30), this nutrient is necessary in order for MGP to function as a local calcification inhibitor (31).

INADEQUATE VITAMIN K2 INTAKE

According to Theuwissen et al (32) and Cranenburg et al (27), measuring circulating undercarboxylated MGP levels is a more effective method of assessing vitamin K status and an appropriate intake level than using hepatic requirement for clotting factor, the typical method. The reason for this: individuals who are not supplemented with vitamin K have substantial concentrations of undercarboxylated extra-hepatic Gla-proteins in the circulation, while those receiving MK-7 supplementation had much higher levels of carboxylated Gla-proteins (32). In healthy adults, circulating undercarboxylated MGP levels gradually increased with age, with a significant rise was seen above 40 years of age (34). Similarly Cranenburg et al (29) showed a trend of increasing undercarboxylated MGP values with age, and significant higher values for elderly (66-80 years) as compared to young adults (25-40 years). In addition, McCann and Ames (33) presented data from animal and human research suggesting that much of the population and warfarin/coumadin patients may not receive sufficient vitamin K for optimal function of VKDPs that are important to maintain long-term health. Furthermore, research (32) found that circulating MK-7 concentrations only became significant starting with an intake of 90 µg/day, and research by Kalmeijer et al (34) demonstrated that undercarboxylated MGP decreased significantly (P< 0.001) by 31% and 46%, respectively, when subjects received 180 µg and 360 µg/day MK-7, respectively. Also, improvements in arterial stiffness (26) were achieved with a daily intake of 180 µg MK-7, and other randomized trials have also demonstrated improvements in circulating undercarboxylated MGP levels after vitamin K supplementation with 500 µg/day (35) as well as 135 µg and 360 µg/day (36).

While the "Daily Value", a selected amount for each nutrient in adults and children four or more years of age, is utilized for labels of dietary supplements and foods, is set at 80 µg for vitamin K, this appears insufficient. Comparatively, the United States' Adequate Intake (AI) level for vitamin K (there is no RDA for this nutrient) is 120 µg daily for men and 90 µg daily for women. This may be a more appropriate intake range for maintenance purposes, although 180 µg of MK-7 provides great value for cardiovascular health.

HUMAN RESEARCH INVESTIGATING VITAMIN K SUPPLEMENTATION ON VASCULAR PARAMETERS

In the population-based Rotterdam Study (14), 4807 subjects were analysed for their vitamin K intake as well as its relationship to aortic calcification and coronary heart disease (CHD). Results showed that the relative risk (RR) of CHD mortality was reduced in the mid and upper tertiles of dietary menaquinone daily, compared to the lower tertile. Intake of menaquinone was also inversely related to all-cause mortality and severe aortic calcification. Phylloquinone intake was not related to any of the outcomes. Likewise, Gast et al (37) examined the relationship between dietary phylloquinone and menaquinone intake (and subtypes), and the incidence of CHD using data from the Prospect-EPIC cohort consisting of 16,057 women, aged 49-70 years, who were free of cardiovascular diseases at baseline. After adjustment for traditional risk factors and dietary factors, an inverse association between menaquinone and risk of CHD was observed with a Hazard Ratio (HR) of 0.91 per 10 µg/d menaquinone intake. This association was mainly due to menaquinone subtypes MK-7, MK-8 and MK-9. Phylloquinone intake was not significantly related to CHD.

RCT using MK-7

Other clinical research sought to define optimal intake levels of menaquinone intake for various indices of cardiovascular health. Caluwé et al (38) randomly administered 360, 720 or 1080 µg of MK-7 thrice weekly for 8 weeks (≈ 120, 240 and 360 µg/day) to 200 chronic haemodialysis patients to ascertain an appropriate dosage for MGP activation and subsequently the calcification inhibitory activity of MGP. At baseline, desphospho-uncarboxylated matrix Gla-protein (dp-uc-MGP) was not associated with phylloquinone intake (P = 0.92), but correlated inversely with menaquinone intake (P=0.023). Results also demonstrated that MK-7 supplementation dose dependently reduced dp-uc-MGP levels by 17, 33, and 46%, respectively, in haemodialysis patients.

To examine potential efficacy in a normal population, Knapen et al (39) investigated long-term effects of supplementation with 180 µg/day MK-7 (MenaQ7, n=120) or placebo (n=124) on arterial stiffness in healthy postmenopausal women for three years in a double-blind, placebo-controlled trial. Indices of local carotid stiffness, and regional aortic stiffness were measured, as was dp-ucMGP as well as acute phase markers for endothelial dysfunction. After three years of supplementation with MK-7, measures of aortic stiffness significantly decreased. Compared to placebo, MK-7 decreased dp-ucMGP by 50%, but did not influence the markers for acute phase and endothelial dysfunction. Researchers concluded that long-term supplementation with MK-7 improves arterial stiffness in healthy postmenopausal women, especially those with high arterial stiffness (Figure 4).

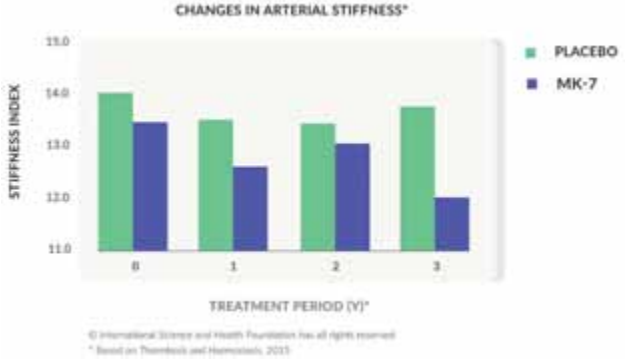

Figure 4. Menaquinone-7 supplementation improves arterial stiffness.

VITAMIN K SAFETY

There is no known toxicity associated with high doses of the vitamin K1 or K2 (although the same is not true for synthetic vitamin K3, menadione). Consequently, no tolerable upper level (UL) of intake has been established for vitamin K1 or K2 by the Food and Nutrition Board, Institute of Medicine (1), or by the World Health Organization (40). Moreover, long-term high dosage therapy with vitamin K is not associated with increased thromboembolic events and no elevated event rate was recorded even with 45 mg vitamin K2 (MK-4) daily for three years in 2,185 postmenopausal osteoporotic women (41). Currently, vitamin K antagonist medications (i.e. coumarins) are the only contraindications for patients using vitamin K2 (420), although novel anticoagulant (NOAC) drugs may be used concomitantly.

CONCLUSION

The role of VKDPs, such as MGP, is understood for their effects as local inhibitors of vascular calcification. The importance of menaquinone the carboxylation and activation of VKDP has also been demonstrated. Convincing data about the biological plausibility of menaquinone, expecially MK-7, and its contribution to vascular health have been published. Ongoing studies will provide further data as to whether these vitamin K-related effects translate into clinical reality. Results from ongoing clinical trials with vitamin K2 will contribute to provide a better understand of how to manage arterial calcification and CVD risk.

REFERENCES

1. Food and Nutrition Board, Institute of Medicine: *Vitamin K*, Chapter 5, in *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press, Washington, District of Columbia, USA (2001).
2. Shearer, M.J. "Vitamin K", Lancet, 345, 229-234 (1995).
3. Booth, S.L. "Vitamin K: food composition and dietary intakes", Food Nutr. Res., 56, 5505 (2012).
4. Ferland, G. "The discovery of vitamin K and its clinical applications", Ann. Nutr. Metab., 61, 213-218 (2012).
5. Shearer, M.J., Newman, P. "Metabolism and cell biology of vitamin K", Thromb. Haemost., 100, 530–547 (2008).
6. Ducy, P., Desbois, C., Boyce, B., et al., "Increased bone formation in osteocalcin-deficient mice", Nature, 382, 448–452 (1996).
7. Luo, G., Ducy, P., McKee, M.D., et al. "Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein", Nature, 386,78-81 (1997).
8. Gundberg, C.M., Lian, J.B., Booth, S.L. "Vitamin K-dependent carboxylation of osteocalcin: friend or foe?", Adv. Nutr., 3,149-157 (2012).
9. Booth, S.L. "Roles for vitamin K beyond coagulation", Annu. Rev. Nutr., 29, 89-110 (2009).
10. Boraldi, F., Annovi, G., Guerra, D., et al. "Fibroblast protein profile analysis highlights the role of oxidative stress and vitamin K recycling in the pathogenesis of pseudoxanthoma elasticum", Proteomics. Clin. Appl., 3, 1084-1098 (2009).
11. Boraldi, F., Garcia-Fernandez, M., Paolinelli-Devincenzi, C., et al. "Ectopic calcification in β-thalassemia patients is associated with increased oxidative stress and lower MGP carboxylation", Biochim. Biophys. Acta., 1832, 2077-2084 (2013).
12. Viegas, C.S., Simes, D.C., Laize, V., et al. "Gla-rich protein (GRP), a new vitamin K-dependent protein identified from sturgeon cartilage and highly conserved in vertebrates", J. Biol. Chem., 283, 36655-36664 (2008).
13. Theuwissen, E., Smit, E., Vermeer, C. "The Role of Vitamin K in Soft-Tissue Calcification", Adv. Nutr., 3, 166–173 (2012).
14. Geleijnse, J.M., Vermeer, C., Grobbee, D.E., et al. "Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: The Rotterdam Study", J. Nutr., 134, 3100-3105 (2004).
15. Beulens JW, Bots ML, Atsma F, et al. "High dietary menaquinone intake is associated with reduced coronary calcification", Atherosclerosis;203:489-493 (2009)
16. Schurgers, L.J., Vermeer, C. "Differential lipoprotein transport pathways of K-vitamins in healthy subjects", Biochim. Biophys. Acta., 1570, 27–32 (2002).
17. Cranenburg, EC, Schurgers LJ, Vermeer C. "Vitamin K: the coagulation vitamin that became omnipotent", Thromb Haemost., 98, 120–125, (2007).
18. Novotny, J.A., Kurilich, A.C., Britz, S.J., et al. "Vitamin K absorption and kinetics in human subjects after consumption of 13C-labelled phylloquinone from kale", Br. J. Nutr., 104, 858-862 (2010).
19. Will, B.H., Suttie, J.W. "Comparative metabolism of phylloquinone and menaquinone-9 in rat liver", J. Nutr., 122:953–958 (1992).
20. Schurgers, L.J., Teunissen, K.J., Hamulyák, K., et al. "Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7", Blood, 109, 3279-3283 (2007).
21. Sato, T., Schurgers, L.J., Uenishi, K. "Comparison of menaquinone-4 and menaquinone-7 bioavailability in healthy women", Nutr. J., 11, 93 (2012).
22. Koitaya N, Ezaki J, Nishimuta M, et al." Effect of Low Dose Vitamin K2 (MK-4) Supplementation on Bio-Indices in Postmenopausal Japanese Women". Journal of Nutritional Science and Vitaminology.55:15-21 (2009).
23. Koitaya N, Sekiguchi M, Tousen Y, et al." 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.1-9 (2013).
24. Theuwissen, E.C., Cranenburg, M.H., Knapen, E. J., et al. "Low-dose menaquinone-7 supplementation improved extra-hepatic vitamin K status, but had no effect on thrombin generation in healthy subjects", Br.J. Nutr., 108, 1652-1657 (2012).
25. Knapen, M.H., Drummen, N.E., Smit, E., et al. "Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women", Osteoporos Int., 24, 2499-507 (2013).
26. Knapen, M.H., Braam, L.A., Drummen, N.E., et al. "Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women: double-blind randomised clinical trial", Thromb. Haemost., 113, Epub ahead of print (2015).
27. Cranenburg, E.C., Koos, R., Schurgers, L.J, et al. "Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species", Thromb. Haemost., 104, 811-822 (2010).
28. Luo, P. Ducy, M.D. McKee, G.J., et al., "Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein", Nature, 386, 78-81 (1997).
29. Hristova, M., van Beek, C., Schurgers, L.J., et al. "Rapidly progressive severe vascular calcification sparing the kidney allograft following warfarin initiation", Am. J. Kidney Dis., 56,1158-62 (2010).
30. Schurgers, J.L. Uitto, J., Reutelingsperger, C.P. Vitamin K-dependent carboxylation of matrix Gla-protein: a crucial switch to control ectopic mineralization", Trends Mol. Med., 19, 217-226 (2013).

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