Chapter 9

Vitamin K2: Implications for Cardiovascular Health in the Context of Plant-Based Diets, with Applications for Prostate Health

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Additional information is available at the end of the chapter

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Abstract

Vitamin K was originally discovered as a blood coagulation factor. But observations regarding intakes in populations and health outcomes lead to a deeper understanding of the differences between vitamins K1 and K2. Studies of warfarin-treated rats and MGP - deficient mice led to understanding the central role of MGP in controlling calcification of arteries. A sensitive biomarker assay was then developed, based on a particular species of matrix γ–carboxylation protein OR matrix GLA protein (MGP). Warfarin therapy in people, especially those suffering from chronic kidney disease, was found to cause the highest level of this biomarker desphospho-uncarboxylated MGP (dp-ucMGP). Intervention studies with vitamin K2 brought down levels of dp-ucMGP and also led to relief of some disease endpoints. The process of varicose vein formation includes a role for vitamin K, implicating a lack of vitamin K in the development of varicoceles, which leads to benign prostate hyperplasia. It is likely that much good will be accomplished using vitamin K2 in interventions. Complex, multifaceted diseases will not be treated by single-nutrient solutions. The best interventions will be those which combine vitamin K2 treatment with a healthy diet rich in fruits and vegetables, combined with a healthy lifestyle.

Keywords: menaquinone, menaquinone-4 (MK-4), menaquinone-7 (MK-7), cardiovascular, calcification, prostate, plant-based diet

1. Introduction

The story of vitamin K goes back to the 1930s, when Henrik Dam at the University of Copenhagen isolated an antihemorrhagic vitamin that was fat soluble but different from previously isolated vitamins A, D, and E [1]. He found high concentrations of the vitamin in hog liver fat and hemp seed, but found it to be virtually absent in cod liver oil while testing a variety of animal organs, hen eggs, cereals, seeds, vegetables, and various fats and oils including butter fat. The initial quantification was all based on the time required to coagulate blood from a chicken. The term "vitamin K" was used as short for the German term "koagulation." Because of the type of assay used, all vitamin K factors, whether K1 or K2, were thought to be useful only for coagulation of the blood.

At the same time as Henrik Dam was working out the details of the antihemorrhagic vitamin, there was another investigator working in the USA with a vitamin factor not found in cod liver oil, but which worked synergistically with them to promote proper mineralization, bone growth, and to prevent dental caries [2]. Dr. Weston Price found that the amount of the factor in mammalian milk varied with the "nutrition of the animal," with highest levels from milk of cows that were consuming rapidly growing green grass. Because Dr. Price's assay was based on the release of iodine from hydroiodic acid, a test for peroxides, no connection was made between his discovery and any other vitamin activity. What Price found was the activity of vitamin K2, formed in mammary glands from phylloquinone, vitamin K1, found in abundance in rapidly growing green grass [3]. No connection was made between the anticoagulation activity of K1 and the mineral-directing activity of vitamin K2 until the modern research era.

Because of the way vitamin K was discovered for its coagulation function, it was assumed that this was its only function for many years. The Recommended Daily Allowance (RDA) for vitamin K is based on its coagulation function. Though both phylloquinone (phyllo—from plants) and menaquinones support coagulation, as we explore in this chapter, there are clear differences between the functioning of phylloquinone and the menaquinones, and they are not fully interchangeable.

This chapter is a review of vitamin K2 research in the area of cardiovascular health, especially dealing with arterial calcification. It is organized in a loose chronological order, following the themes of the research as the field matured. First, there were observations about dietary intake of K1 and K2 and health outcomes, forming hypotheses to be tested further. Associations were discovered at this stage. Harmful observations with warfarin-type drugs, which are vitamin K antagonists, were seen early on in the research cycle as well. Hypotheses were then explored with animal studies, looking for mechanisms and biomarkers—to help get endpoints that were quicker to develop than mortality and disease. Then came the rise of biomarker studies, using markers for vitamin K status (dp-ucMGP), as well as markers for risk factors, such as arterial calcification, pointing to disease outcomes. As the science matured even further, intervention studies related to K2 and biomarker changes emerged. The final stage is now emerging with intervention studies looking not just at biomarkers but disease outcomes, thus tying all of the research together.

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2. Observational studies of dietary vitamin K intake and health outcomes

Observational studies help form hypotheses by finding associations between factors that may or may not be related to the health outcome of interest. One of the early population-based studies in the 1990s examined the vitamin K status of 113 postmenopausal women [4]. Dietary intake of vitamin K was assessed along with examinations for the presence or absence of aortic calcified lesions. Blood samples were assayed for osteocalcin, a vitamin K-dependent protein that is responsible for proper deposition of minerals in bone tissue. It was thought that some forms of osteocalcin might be a marker for vitamin K status. The first type had low affinity for hydroxyapatite, while the second had high affinity. Women with calcified lesions (n = 34) had a lower intake of total vitamin K as well as a higher amount of low-affinity osteocalcin. Together these results indicated that these women had impaired vitamin K status that might be related to their atherosclerotic lesions.

A groundbreaking study took place in the Netherlands which changed the way vitamin K2 was thought of afterward. First, a very careful survey of foods eaten in the Netherlands was carried out by interview-based dietary intakes of over 5400 people, guided by a validated food frequency questionnaire (FFQ). For many animal-based foods, the quantitation of vitamin K2 had not been done, so this was carried out and reported as well. The mean intake of K1 and K2, respectively, varied from 124 and 9.3 μg/day in the lowest quartile to 375 and 45 μg/day in the upper quartile [5]. A report of health outcomes from the Rotterdam Study in 2004 from 4800 of these subjects revealed that even though vitamin K2 was a minor part of the total vitamin K intake, only K2 and not K1 intake was associated with a lower risk of disease [6]. For people in the upper third of intakes of K2 compared to the lowest third, there were decreases in relative risks of coronary heart disease (CHD) mortality (57%), all-cause mortality (26%), and severe aortic calcification (52%). Even though intakes of K2 were only about 10% as much as the amounts of K1, their effect on cardiovascular disease was greater. Cheese was the primary source of menaquinones in this cohort in the Netherlands, not exactly highly regarded as a heart healthy food. This fact made confounding by other "healthy" nutrients less likely and made the results more robust.

Similar results have been seen in a second study, from the Prospect-EPIC cohort, also from the Netherlands [7]. About 16,000 women aged 49–70 were followed for 8 years. Vitamin K1 and K2 intakes were estimated from a FFQ . Vitamin K2 intake varied from <20 to >36 μg/day across quartiles. For every increase of 10 μg of K2, there was a 9% reduction in hazard ratio of risk of CHD, with the effects coming mainly from menaquinone subtypes MK-7, MK-8, and MK-9. There was no association between intake of K1 and CHD, as seen in the Rotterdam Study.

Two large cohort studies have been analyzed for associations between vitamin K intake and CHD. When the Nurses' Health Study (NHS) cohort was analyzed for an association between dietary vitamin K intake and cardiovascular outcomes, it was found that K1 intake was associated with a 21% decrease in multivariate relative risk of total CHD [8]. The association was attenuated by adjustments for other dietary factors and lifestyle patterns, so that it was not apparent to the authors whether the results were due to vitamin K1 or that K1 was just a marker for a lifestyle pattern associated with a high intake of K1. Median intakes of K1 for the

lowest and highest quintiles were 87 and 300 μg/day for the NHS cohort. In another large cohort, the Health Professionals' Follow-up Study, there was a decrease in the relative risk of total CHD across increasing quintiles of vitamin K1 intake. However, when the results were adjusted for lifestyle and other dietary factors, the trend was no longer significant [9]. Neither of these large cohorts reported dietary intakes of K2, perhaps because the database for menaquinone concentrations in the USA was not complete at that time, nor is it fully available at the time of writing this chapter (only partial data are available on MK-4 but none on higher menaquinones), 17 years after such data were obtained for the Rotterdam Study [5]. The negative results from these cohorts for vitamin K1 and CHD only reinforce just how striking the results were from the Dutch studies for vitamin K2. The findings from the Netherlands were not expected or anticipated by many.

The question of whether vitamin K intake is related to arterial calcifications has been probed in two observational studies. In a cross-sectional study of 1689 women, dietary intakes of vitamin K1 and K2 were estimated with an FFQ, and standard screening mammograms were assessed for the presence of breast arterial calcifications [10]. Unadjusted results showed an inverse association between intake of vitamin K2 and breast arterial calcifications, but adjustments for aging, smoking, diabetes, and dietary factors made the association no longer significant. Adjustment for diabetes may have been unwise, as vitamin K2 intake has also been shown to reduce the risk of diabetes among 38,000 Dutch men and women in the Prospect-EPIC cohort mentioned previously [11]. So, this adjustment may have attenuated the results enough to make the association no longer statistically significant.

In another cross-sectional study, 564 postmenopausal women were examined for an association between coronary calcifications and intake of vitamins K1 and K2 [12]. Women were chosen from the Prospect-EPIC cohort study. In this cohort, cheese contributed 54%, milk products contributed 22%, and meat contributed 15% of the K2 intake. The mean intake of K2 ranged from 18.0 ± 4.5 to 48.5 ± 9.0 in the lowest and highest quartiles, respectively. Examinations found that 62% of the women had coronary calcifications. In the model adjusted for age and cardiovascular risk factors, increased menaquinone intakes were associated with a decreased calcification prevalence ratio of 0.80 (95% CI: 0.65–0.98), comparing highest to lowest quartile.

A more recent observational study reported findings contrary to those found in the Prospect-EPIC cohort and the Rotterdam Study. The PREDIMED cohort is a Spanish study to examine the effect of adoption of the Mediterranean Diet on cardiovascular, cancer, and all-cause mortality. The intakes of vitamins K1 and K2 were estimated by FFQ, and endpoints of cardiovascular, cancer, or all-cause mortality were tracked for a median follow-up of 4.8 years. Energy-adjusted intakes of vitamins K1 and K2, respectively, ranged from 170 and 18.4 μg/day in the lowest quartiles for each vitamin to 626 and 57.5 μg/day in the upper quartiles [13]. People in the upper quartile consumed about twice as many vegetables, especially leafy greens, as those in the lowest quartile. The upper quartile of vitamin K intake in this cohort adopting the Mediterranean Diet was substantially higher than seen in the other observational studies of other European or American cohorts. No protective effects for higher intakes of menaquinones were seen in this cohort for cardiovascular mortality, cancer mortality, or all-cause mortality. However, high intakes of phylloquinone lead to a reduced hazard ratio of 0.54 and

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0.64 for cancer mortality and all-cause mortality. It is possible that enough vitamin K1 and other plant-based protective nutrients such as folate, vitamin C, fiber, potassium, and magnesium were supplied by the diet that even those participants who had low intakes of menaquinones were still not at an elevated risk compared to other subjects in the PREDIMED cohort. Participants who increased their intake of either vitamins K1 or K2 or both during the course of the study experienced decreased risk of cardiovascular mortality (K1 only), cancer mortality, and all-cause mortality. The main conclusion from this observational study perhaps is that eating plants is good for you, and the Mediterranean Diet is generally beneficial, as seen in another report from this cohort [14].

3. Vitamin K antagonist studies

Another line of evidence that led to the discovery of the role of menaquinones was the effect of blood thinning drugs such as warfarin and coumarin. While this class of drugs has been very helpful in preventing strokes in the short term, it has also caused damage long term, as people are often prescribed blood thinners for many years. In 1998, it was reported by Price et al. [15] that warfarin caused calcification of the elastic lamellae in rat arteries and heart valves within a period of 2 weeks, with increasing intensity each week. Vitamin K1 was given concurrently to maintain normal blood coagulation. At the time, menaquinone was not even mentioned in the article, not even in the discussion. The discovery of importance was that warfarin had negative side effects for arterial calcification that were not counteracted by vitamin K1.

This discovery nearly coincided in time with work on an MGP-deficient mouse model. Matrix γ-carboxylation protein, or matrix Gla protein (MGP), was originally discovered in bone tissue but is actually expressed in many tissues of the body, including vascular smooth muscle cells and chondrocytes in cartilage. MGP requires the activation by vitamin K in order to bind calcium ions and prevent crystallization of calcium. These MGP-deficient mice developed normally to term but died within 2 months as a result of extensive arterial calcification, which led to blood vessel rupture [16]. Also seen was the inappropriate calcification of cartilage, including the growth plate of bones. This MGP-deficient mouse model clearly showed that MGP has a central, active role in preventing calcifications of arterial walls and also of cartilage. This research coupled together with the warfarin-caused calcification pointed to a central role for MGP in controlling arterial calcification.

Further work on the interrelationship between vitamin K1 and K2 was spurred on by feeding studies in rats. When rats were made vitamin K deficient, then fed only K2 as MK-4, they accumulated MK-4 especially in the pancreas, aorta, fatty tissues, and brain. Liver and serum levels of MK-4 were low. When vitamin K–deficient rats were fed only K1, they accumulated K1 in the liver, heart, and fatty tissues, and they also accumulated MK-4 in the same way as the rats that were fed MK-4, indicating that there was conversion from phylloquinone to MK-4 [17]. So, with a warfarin-rat model that was able to induce arterial calcification and knowing that there were differences between K1 and K2 distributions in the rats and that K2 prevented heart disease in the Rotterdam Study, Spronk and coworkers set out to see how to prevent

arterial calcification [18]. When warfarin-treated rats were fed K1, the rats got arterial calcification, as shown before [15], even at the highest tested dose of K1. But when the warfarin-treated rats were fed K2 as MK-4 or K1 together with MK-4 simultaneously, the arterial calcification was prevented. The picture was becoming clearer. Further studies have shown that in this rodent model warfarin treatment not only causes arterial calcification but functionally augments aortic peak velocity, aortic valve-peak gradient, and carotid pulse-wave velocity [19].

The work in rats spurred investigators to look at the effect of anticoagulants in people. In one study, aortic heart valves were examined that had been replaced during routine surgery. Some patients received preoperative marcoumar treatment, for between 16 and 35 months, with a mean of 25 months. When compared with patients who did not have any blood thinner treatment, there was about twice as much calcification on the valves from patients who had received the marcoumar [20]. The mean calcified area on the valve went from 16% in the untreated group to 37% in the anticoagulant group. In a cross-sectional study, coronary artery calcium scores and valvular calcium scores were compared between patients on long-term use of anticoagulants and patients without any anticoagulant therapy [21]. The Agaston calcium scores were about double in the anticoagulant treatment group, indicating that the effects of anticoagulants seen in mice and rats are also present in people, even when the treatment was only for a couple of years.

These initial results have been confirmed by further studies. Rennenberg et al. [22] examined 19 patients younger than 55 years of age who had used coumarins for more than 10 years but did not have other cardiovascular risk factors. These patients were compared with 18 matched healthy controls. When they examined femoral arteries, they found the coumarin users had 8.5 times the chance of having arterial calcification compared to the healthy controls. Fourteen of 19 coumarin users, but only 4 of 18 controls had femoral arterial calcifications. Another cross-section examination of low-risk atrial fibrillation patients found that both age and use of oral anticoagulants were related to increased coronary calcium score [23]. And as length of time using the anticoagulants increased, the coronary calcium score also increased, going from 53 ± 115 for no use to 90 ± 167 for 6–60 months, and to 236 ± 278 for >60 months of use. These findings were also confirmed in a series of 133 oral anticoagulant users matched by age, gender, and Framingham cardiovascular risk score [24]. Agaston calcium scores increased from 79.6 ± 159.8 for use of 2.5 ± 1.5 months, to 142.4 ± 306.0 for 18.7 ± 8.8 months, to 252.5 ± 399.3 for 86.4 ± 47.1 months of use.

In women undergoing screening mammography who took warfarin, breast arterial calcifications were also more common with increasing length of warfarin treatment [25]. Prevalence of breast arterial calcifications increased from 25.0% for <1 year of therapy to 74.4% for >5 years of therapy. So, these calcifications can appear in peripheral tissues as well, not just in the aorta. To show this peripheral effect further, and in men, after completing the breast arterial calcification study, Han and O'Neill examined radiographs of ankles and feet, retrospectively, and checked records for warfarin use prior to the x-ray [26]. They found a significant increase, from 19% to 38% prevalence, in peripheral arterial calcifications in people who had been using warfarin for at least 5 years prior to their x-ray. While these drugs could be termed "anticoa-

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gulants," the preferred term for many of these authors is vitamin K antagonists, for this is their mode of action.

Calcification of arteries had originally been thought of as a one-way process, without reversibility, similar to the thinking about coronary plaque. However, just as regression can be seen of atherosclerotic plaques [27], so calcification of arteries, too, is a dynamic process. Using the warfarin-treated rat as a model for arterial calcification, Schurgers et al. [28] first fed rats for 6 weeks on the diet to induce calcification. Then the warfarin treatment was stopped and rats were fed normal levels of K1, or high levels of K1 or K2 (as MK-4). Normal levels of vitamin K1 continued to progressively increase calcification, but both forms of vitamin K at high doses reversed arterial calcification by about 50%. Vitamin K1 does not work as long as warfarin is present, as it inhibits the conversion of K1 into MK-4. But when the warfarin treatment is stopped, this research clearly showed that this calcification process could be reversed by high doses of vitamin K, especially K2.

4. Biomarker research studies

One difficulty in this field of research is determining the functional vitamin K status of an individual. A blood test of vitamin K levels is not sufficient. The amount of vitamin K in the blood is very small and generally only reflects the vitamin K1 that was consumed within the last 4 hours or so, as K2 levels are too low to assay in blood, and K1 clears from the blood with triglycerides. As research progressed, it became increasingly apparent that there were more functions for vitamin K than originally discovered. Coagulation was only the most immediately obvious function of vitamin K in the liver. But the observation studies and vitamin K antagonist research indicated more functions beyond coagulation, dealing with regulation of calcification throughout the body. McCann and Ames [29] elaborated on this multifunction vitamin, indicating that triage theory helps us understand the distribution of vitamin K to various organs. Triage theory states that the most critical functional needs are met first in the body (coagulation) when there is a shortage of a micronutrient. Then when there is an abundance of the micronutrient, all of the secondary functions important to long-term health are also met.

For these reasons, and possibly others, functional tests for vitamin K status for these secondary functions beyond coagulation were sought. Osteocalcin, a vitamin K–dependent protein found in bone, can be measured in the circulation as well. The ratio of carboxylated to undercarboxylated or uncarboxylated osteocalcin is one biomarker for functional vitamin K status. However, this applies more to the status of vitamin K as it applies to bones. Since MGP is involved in arterial calcification, assays for determining the concentrations of various forms of MGP were developed [30, 31]. Of the various forms of MGP, the dephosphorylated, uncarboxylated form has been most closely related to arterial calcification. Among coumarin users an elevated dp-ucMGP level was found compared to controls (1439 ± 481 pM vs. 299 ± 163 pM, respectively) [22]. In a cohort of 101 chronic kidney disease patients, the level of dp-ucMGP increased with increased severity of the disease [32]. Plasma dp-ucMGP was also

independently associated with aortic calcification, and a concentration greater than 921 pM was a predictor of all-cause mortality in a crude analysis.

What about when people on dialysis are also taking oral anticoagulants? Among 160 hemodialysis patients in Belgium, the 23 who were treated with anticoagulants had much higher circulating concentrations of dp-ucMGP, 5604 pM (interquartile range: 3758, 7836 pM) and 1939 pM (interquartile range: 1419, 2841pM) for the anticoagulant treated and non-treated groups [33].

In a study of 147 patients with symptomatic severe calcific valvular aortic stenosis, the levels of dp-ucMGP were associated with cardiac function and long-term mortality in multivariate analysis [34]. Increasing severity of disease was related to dp-ucMGP concentrations in a study of 179 patients with chronic heart failure [35].

The dp-ucMGP assay was checked for correlation with vitamin K status and coronary artery calcification in a study of older adults without cardiovascular disease [36]. While the assay did correlate well with plasma phylloquinone, uncarboxylated prothrombin, and serum uncarboxylated osteocalcin, there was no association between dp-ucMGP levels and coronary artery calcification. Shea and coworkers [36] presented data that are not consistent with the other reports on this assay. Perhaps the assay works better for much higher levels of dp-ucMGP, such as found in disease states. This study looked at older adults without clinical cardiovascular disease, whose levels of dp-ucMGP were much lower than subjects with cardiovascular disease (CVD). As suggested by the authors, the coronary artery calcification analyzed in this report may have been more in the intimal layer, rather than in the medial layer, where MGP has a greater role [28]. A more recent study involving 200 health women found a borderline statistically significant relationship between dp-ucMGP and coronary artery calcification, as well as a strong relationship between dp-ucMGP and vitamin K status (ratio of carboxylated osteocalcin) [37]. The results in [36] appear to the exception, as there is a consistent relationship between dp-ucMGP, vitamin K status, and health outcomes involving arterial calcification in all of the other studies examined.

Other studies have generally found that the biomarker dp-ucMGP does correlate with vitamin K status and disease outcomes related to arterial calcification. In the EPIC-NL cohort, 518 participants were identified as diabetic at baseline [38]. After 11.2 years of follow-up, incidence of CVD was significantly associated with baseline concentrations of dp-ucMGP, but not other species of MGP. The hazard ratio per standard deviation (HRSD) of dp-ucMGP for all CVD was 1.21 (95% CI 1.06–1.38), for peripheral artery disease HRSD = 1.32 (95% CI 1.07–1.65), and for heart failure HRSD = 1.85 (95% CI 1.42–2.17). The prospective Longitudinal Aging Study, Amsterdam (LASA) examined 577 people aged >55 years who were free of CVD at the baseline [39]. There were 40 incident cases of CVD during the 5.6 years of follow-up. For the highest tertile compared to the lowest tertile of dp-ucMGP, there was a hazard ratio of 2.69 (95% CI 1.09–6.62) for being diagnosed with CVD. The carboxylated form of MGP was not related to risk of CVD.

Two Czech Republic prospective studies have examined the usefulness of the dp-ucMGP as a biomarker to predict cardiovascular mortality. From the EUROASPIRE III and EUROASPIRE-

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stroke surveys, 799 patients were examined who had already experienced a myocardial infarction, coronary revascularization, or first ischemic stroke. After a median follow-up of 5.6 years, 159 patients died. In the fully adjusted model, the patients in the highest quartile of dp-ucMGP (≥977 pM) had higher risk of all-cause and cardiovascular mortality, HRR 1.89 (95% CI 1.32–2.72) and 1.88 (95% CI 1.18–2.61), respectively [40]. For those subjects in the upper quartile of dp-ucMGP who also had heart failure, indicated by an elevated circulating brain natriuretic peptide level >100 ng/L, mortality risk was further increased, HRR 4.86 (95% CI 3.15–7.49) [41]. In a random sample from the general population from the Czech post-MONICA study, Mayer et al. [42] found that aortic stiffness, as measured by pulse wave velocity, was related to vitamin K status. Compared to the lowest quartile, the upper quartile of dp-ucMGP (≥671 pM) has an increased odds ratio of 1.73 (95% CI 1.17–2.5).

5. Intervention studies

One of the first intervention types was to confirm the utility of various species of MGP as biomarkers for vitamin K status. If you improve someone's status by oral supplementation, the biomarker should reflect this improvement in a dose-dependent manner. So, in 2012, Dalmeijer and coworkers [43] reported a randomized, double-blind placebo-controlled trial (RCT) of 60 people taking 0, 180, or 360 μg/day of vitamin K2 as menaquinone 7 (MK-7) for 12 weeks. Assays were performed for three different species of MGP: desphospho-uncarboxylated MGP (dp-ucMGP), desphospho-carboxylated MGP (dp-cMGP), and total uncarboxylated MGP (t-ucMGP). Vitamin K status was also measured using the ratio of uncarboxylated to carboxylated osteocalcin. (Note that the research field on the role of vitamin K2 in bones matured earlier than the field of cardiovascular effects of K2, so osteocalcin was well established as a vitamin K2 marker by this time.) After 12 weeks of the supplements, the osteocalcin ratio decreased significantly, with a 60% drop at 180 μg dose and a 74% drop at the 360 μg dose. The amount of dp-ucMGP decreased significantly and dose-dependently as well, by 31% and 46% at 180 and 360 μg, respectively. There were no changes in the placebo group, as expected. Changes in other species of MGP (dp-cMGP and t-ucMGP) were not different between placebo and the supplement groups. This study was one of the first intervention trials to validate the usefulness of dp-ucMGP as a biomarker for vitamin K status. Observational studies had been carried out, but the intervention studies took the research one more step toward maturity.

In the same year, another RCT was reported of 42 Dutch men and women randomized to receive 0, 10, 20, 45, 90, 180, or 360 μg/day of vitamin K2 as MK-7. The ratio of uncarboxylated to carboxylated osteocalcin was determined along with the concentration of dp-ucMGP. The upper three doses (90, 180, and 360 μg/day) increased the carboxylation of osteocalcin and decreased the amount of dp-ucMGP. In these healthy adults aged 18–45, no adverse effects were seen on the generation of thrombin, indicating that coagulation factors were not perturbed by the additional supply of vitamin K2. This is reasonable, for the coagulation factors are generally all carboxylated. Only the extrahepatic vitamin K–dependent proteins seem to suffer when there is a shortage of vitamin K, as explained by the triage theory [29].

Patients with chronic kidney disease and those undergoing dialysis have been shown repeatedly to suffer with high levels of uncarboxylated vitamin K–dependent proteins and have high levels of arterial calcification as well. (The most deficient group is the same patients taking vitamin K antagonist drugs concurrently.) In order to prepare for a RCT with a disease endpoint of stabilizing or reversing arterial calcification in this patient group, a supplement trial was conducted in the Netherlands with 50 hemodialysis patients [44]. An age-matched healthy control group was selected also for comparisons. The hemodialysis patients were randomized into groups taking 45, 135, or 360 μg/day of vitamin K2 as MK-7 for 6 weeks. Measurements were taken for the levels of uncarboxylated osteocalcin (ucOC), dp-ucMGP, and PIVKA-II. PIVKA-II is a prothrombin liver protein that is only seen in the circulation under situations of severe vitamin K deficiency, such as found in hemodialysis patients. At baseline hemodialysis, patients, compared to healthy controls, had 4.5-fold higher dp-ucMGP levels and 8.4-fold higher uncarboxylated osteocalcin levels. PIVKA-II levels were detectable in 49 of the 50 hemodialysis patients. There was a dose-dependent response to the MK-7 treatment, with the 45 μg dose being little different than a placebo, the 135 μg dose giving 37%, 11%, and 34% changes in dp-ucMGP, ucOC, and PIVKA-II, respectively, which was almost significant, and the 360 μg dose yielding statistically significant changes of 61%, 34%, and 42% decreases in dp-ucMGP, ucOC, and PIVKA-II. This short trial showed both the severity of the vitamin K deficiency in this hemodialysis patient group as well as the effectiveness of a relatively high dose (360 μg/day) of MK-7 in bringing down functional markers of vitamin K deficiency.

As a follow-up to the Westenfeld Study [44] just reviewed, another larger study with hemodialysis patients was carried out with slightly higher doses, but administered three times a week by a nurse after dialysis [45]. This method was used to increase patient compliance. Doses for the 200 patients were 360, 720, and 1080 μg of MK-7 three times as week for 8 weeks. This works out to equivalent daily doses of 154, 309, and 463 μg/day. After 8 weeks, levels of dp-ucMGP decreased by 17, 33, and 46% in the three dosage groups, respectively. Results here were similar to those in [44], but with less decrease in relative change in dp-ucMGP at the highest dose (46% vs. 61%). However, the absolute differences before and after intervention were greater in [45]. Absolute changes in dp-upMGP in the Westenfeld Study were −404, −730, and −978 pM at 45, 135, and 360 μg/day [44]. In a study by Caluwé and coworkers [45], absolute changes in dp-ucMGP were −566, −962, and −1487 pM for the equivalent daily doses of 154, 309, and 463 μg/day. So, the outcomes were very similar, especially given that the second trial was 8 weeks long rather than just 6 weeks.

How widespread is extrahepatic vitamin K insufficiency? A cross-sectional sample of 896 healthy individuals showed that dp-ucMGP levels increased with age, staying around 200 pM until about age 40 and then increasing up to over 600 pM for those >70 years of age [46]. Levels of dp-ucMGP decreased in children and adults when given supplemental MK-7, again showing that dp-ucMGP was a biomarker that responded to extrahepatic vitamin K status, and that many adults had less than optimal levels of vitamin K. These levels are not optimal, neither are they as severe as hemodialysis patients, who averaged around 3000 pM but ranged to over 7000 pM in some individuals [44, 45]. Hemodialysis patients who took vitamin K antagonists had a mean dp-ucMGP of about 5600 pM [33]. But the values >600 pM in the >70

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age group are close to the range at which excess heart disease mortality occurred in other studies, >977 pM in [40] and >921 pM in [32]. Calcification of arteries takes time and finally takes a toll on the elderly if not protected against through the years.

In one of the first RCT studies to report a disease endpoint, Knapen and coworkers examined arterial stiffness in 244 women after supplementation for 3 years with 180 μg/day of MK-7 [47]. Previous work by this research group had linked increased levels of dp-ucMGP with arterial stiffness [48]. Compared to the placebo group, dp-ucMGP levels dropped by about 50%. The K2 treatment resulted in improvements in the whole group, but the best results for improving stiffness were seen in those women who started with the worse condition, with a stiffness index β above the median of 10.8. For these women, there were improvements in distention, compliance, distensibility, Young's Modulus, and the local carotid pulse wave velocity. Not all became normal, as acute phase markers interleukin-6, C-reactive protein, and tumor necrosis factor-α remained abnormal as well as the markers of endothelial dysfunction vascular cell adhesion molecule and E-selectin.

A Polish RCT study examined disease endpoints for vascular calcification and progression of atherosclerosis in 42 women who had chronic kidney disease but were not undergoing dialysis [49]. The women were split into three groups followed for 270 days: one taking 90 μg/day of MK-7 (K), one group taking 90 μg/day of MK-7 plus 10 μg/day of vitamin D as cholecalciferol (K+D), and one taking only 10 μg/day of vitamin D (D). The results were that the intervention slowed the progression of atherosclerosis but did not significantly slow the increase in coronary artery calcium score. The reason for this lack of success is in the dose of MK-7 used. As noted in the studies by Westenfeld et al. [44] and Caluwé et al. [45], a daily dose of around 360 μg is needed to significantly reduce dp-ucMGP. Changes in dp-ucMGP were reported to be significant in this Polish trial, but actual change was from 1077.1 ± 507.7 to 961.5 ± 506.7, or a change of only 115.6 pM. While this change may have been statistically significant, it was clinically irrelevant. This study also reveals why it is important to develop research stepwise to ensure that interventions will be successful. If the treatment is not expected to yield a large change in biomarker studies, how would it yield a successful clinical result?

A rationale and study protocol has been published for the VitaK-CAC trial [50]. For this 2-year trial, the intervention will be 360 μg/day of MK-7. Patients with coronary artery disease will be monitored for coronary artery calcification as the primary endpoint. Secondary endpoints will be arterial structure and function. This study appears to be well designed to yield good results, as long as the arterial calcification seen in coronary arteries is susceptible to the MGP mechanism of action. Nutrients have a way of working in complimentary fashion, if not synergistically. The best chance for successful intervention is to use all of the known tools available, which is discussed in the section on plant-based diets below.

6. Prostate health and vitamin K2

While the focus of this chapter has been on arterial calcification and cardiovascular health, it has come to the attention of this author that there is evidence that supports a hypothesis that

poor prostate health is not a hormone issue, but a cardiovascular issue [51]. The first piece of evidence comes from interventional radiologists Dr. Gat and coworkers, who were initially working on reversing infertility in men by relieving varicoceles, or varicose veins in the pampiniform venous plexus. They discovered that varicose veins in the internal spermatic vein, which normally returns blood from the testes to the kidneys, prevented normal blood flow. The one-way valves had failed in this vein, possibly causing the varicose vein, or as part of the process of forming varicose veins—the exact mechanism is debated. So instead of normal blood flow, the blood flowed retrograde through the prostatic veins. When testosterone levels were measured near the prostate gland in 12 infertile men with varicocele, the mean concentration was 3632 pmol/l compared to 27.33 pmol/l in the serum, or about 130 times higher [52]. By occluding the internal spermatic vein, Dr. Gat was able to relieve the physical pressure due to the elevated blood pressure caused by the height of the column of blood sitting in the internal spermatic vein and also made a pathway through normal venous pathways for blood to drain away from the testes without retrograde flow past the prostate. This venous occlusion surgery led to relief of benign prostatic hyperplasia (BPH), and possibly prevention of prostate cancer as well [52].

Varicose veins and destruction of the one-way valves in the internal spermatic vein were the direct cause of BPH, but what causes varicose veins? Work by Cario-Toumaniantz and coworkers [53] on the differentially expressed genes and gene products in varicose vein tissue showed an overexpression of genes involved in extracellular remodeling, including matrix Gla protein. Smooth muscle cells were seen proliferating in varicose vein tissue with high expression of MGP, particularly the uncarboxylated form of MGP. Overexpression of MGP and proliferation of smooth muscle cells have been seen before, reported by Price and coworkers investigating the effects of warfarin on arterial calcification in rats [15], and by Schurgers and coworkers reporting the reversal of warfarin-induced arterial calcification in rats [28]. In areas of calcification of arteries, there is proliferation of smooth muscle cells and increased expression of uncarboxylated MGP, similar to what was seen in varicose vein tissue [53]. It is likely then that vitamin K is involved in the mechanism by which varicose veins form, just as it has been implicated in the formation of arterial calcifications. When varicose vein tissue culture was treated with warfarin, mineralization increased, which could be inhibited by the inclusion of vitamin K in the culture media [53], indicating a direct role for vitamin K in the prevention of varicose veins.

In addition to this evidence from cellular biology, evidence from a prospective observational study showed an association between intake of vitamin K2 and poor prostate health manifested as prostate cancer. There was a significant association between menaquinone intake and advanced prostate cancer in the EPIC-Heidelberg cohort [54]. A nested case-control follow-up study also found an association between the ratio of undercarboxylated osteocalcin to carboxylated osteocalcin and high-grade prostate cancer and advanced prostate cancer [55]. Neither of these reports found a connection with vitamin K1 intake. Further evidence comes from a retrospective study of warfarin use and clinical stage of prostate cancer at diagnosis [56]. While some of the evidence for intermediate or short-term use of warfarin is conflicting, the comparison between those men who had used warfarin for at least 4 years in the 5 years

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before prostate cancer diagnosis and nonusers of warfarin showed an odds ratio of 2.2 (95% CI 1.03–4.81) of poor prognosis disease. This would seem to agree with the hypothesis that vitamin K antagonists would increase the possibility of forming varicoceles, leading to poor prostate health. But there could be other mechanisms of action as well.

At this point, the role of vitamin K2 in prostate health is a good hypothesis but needs further confirmation by interventional radiologists and other researchers before the link between prostate health and cardiovascular function, especially the role of vitamin K2 plays, is certain.

7. Vitamin K2 and a plant-based diet?

Reversal of atherosclerosis by a plant-based diet has already been mentioned in this chapter [27]. Dr. Esselstyn has also shown that a plant-based diet very low in fat can reverse coronary artery disease [57]. Of 198 subjects in the lifestyle intervention study, 177 were adherent to the program while 21 formed a control group of non-adherent comparison subjects. There was reversal of angiographic-verified blockages in 39 adherent subjects. Disease progression occurred in 4 (2.3%) adherent subjects, but in 11 (52.4%) of the non-adherent subjects. Coronary artery calcification was not measured.

There is abundant evidence that increased consumption of fruits and vegetables in conjunction with exercise and a healthy lifestyle is beneficial for cardiovascular health and lower cardiovascular mortality. Healthy diets have been reported to lower cardiovascular mortality risk by about 30–40% [14, 58, 59]. When combined with other lifestyle factors, the risk plummets to about 20% or less compared to the least healthy fraction of the population [60–63].

The point here is that all available resources should be used to counter the disease process and to promote healthy aging. While vitamin K2 is a valuable nutrient that is generally in short supply in the global diet, the context of the entire diet must be kept in mind. The best results will be obtained by a full complement of healthy foods. Population studies have shown that even 40–50 μg/day of menaquinones from the diet is associated with cardioprotection [6, 7] and lower risk of advanced prostate cancer and lung cancer [54, 55, 64].

Is vitamin K2 compatible with a plant-based diet? While most of the common sources of menaquinones are cheese, fermented dairy products, eggs, and meats, there are plant sources as well. While these are animal products, judicial selection could be used to maximize K2 intake without consuming a large amount of any animal-based foods. Natto is a well-known Japanese food that is very rich in MK-7, though not very popular outside of Japan. The menaquinone in natto is made by fermentation with *Bacillus subtilis var. natto*. Fermented vegetables such as sauerkraut contain a small amount of menaquinones as well. By selecting probiotic bacteria based on their production of menaquinones, the amount of vitamin K2 from a serving of fermented vegetables could be significant. More product development is needed in this area.

8. Research directions and priorities

The research on vitamin K2 and cardiovascular health has come a long way, going from observational studies of populations and warfarin studies, to developing biomarkers, finding effective dosage schedules, and begin carrying out RCT studies examining disease endpoints. Very few disease endpoints have been reported at this time, but several should be completed in the next few years. Diseases are multifaceted, so the solutions should likewise be multifaceted. Trying to reverse a complex disease with a single nutrient has generally been unsuccessful. It is likely that the best success will be found when vitamin K2 is used in the context of a whole food plant-based diet that contains some dietary source of vitamin K2, along with supplements to reverse disease damage when appropriate.

One of the major benefits seen by Dr. Weston Price in the 1930s was the prevention and reversal of dental caries using vitamin K2 [2]. This could be a very fruitful area of research that is untapped at this point. Mental health effects of vitamin K2 are likely as well, as the brain contains a significant concentration of K2. Varicose veins in general and prostate health in particular should be studied in light of the K2 research presented here in this chapter. Mechanisms of the function of MGP should be worked out along the way as well. Identification of the function of other vitamin K–dependent proteins is still lacking. There is a gamma-carboxylation–rich protein (GRP) that is quite small yet has 16 carboxylation sites and is highly expressed in cartilage. Its exact function is still unknown. So, there is work that can be done at the molecular level as well as at the public health level in furthering our understanding of vitamin K2.

Conflict of Interest

Michael Donaldson is a research scientist at the Hallelujah Acres Foundation for investigations pertaining to the Hallelujah Diet. Funding for this research has been provided by Hallelujah Acres, Inc.

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