Vitamin Supplement

Vitamin K: the effect on health beyond coagulation – an overview

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Abstract

Vitamin K is essential for the synthesis of proteins belonging to the Gla-protein family. To the members of this family belong four blood coagulation factors, which all are exclusively formed in the liver. The importance of vitamin K for hemostasis is demonstrated from the fact that vitamin K-deficiency is an acute, life-threatening condition due to excessive bleeding. Other members of the Gla-protein family are osteocalcin, matrix Gla-protein (MGP), and Gas6 that play key functions in maintaining bone strength, arterial calcification inhibition, and cell growth regulation, respectively. In total 17 Gla-proteins have been discovered at this time. Recently, it was observed that the dietary vitamin K requirement for the synthesis of the coagulation factors is much lower than for that of the extra-hepatic Gla-proteins. This forms the basis of the triage theory stating that during poor dietary supply, vitamins are preferentially utilized for functions that are important for immediate survival. This explains why in the healthy population all clotting factors are synthesized in their active form, whereas the synthesis of other Gla-proteins is sub-optimal in non-supplemented subjects. Prolonged sub-clinical vitamin K deficiency is a risk factor for osteoporosis, atherosclerosis, and cancer. Present recommendations for dietary intake are based on the daily dose required to prevent bleeding. Accumulating scientific data suggests that new, higher recommendations for vitamin K intake should be formulated.

Keywords: *phylloquinone*; *menaquinone*; *cardiovascular disease*; *osteoporosis*; *triage*

Vitamin K received its name from the fact that it is required for normal blood coagulation, and during about half a century it was believed that hemostasis was the only metabolic process in which vitamin K is involved. This view is obsolete now. Since the identification of Gla as the result of vitamin K action, it became possible to search for vitamin K-dependent proteins on the basis of their amino acid structure: each protein in which gammacarboxyglutamate, generally referred to as Gla, is found contains the fingerprint of vitamin K action during its biosynthesis (1). In this way a wide variety of proteins were found ranging from osteocalcin (the most abundant non-collagenous protein in bone), to matrix Gla-protein (MGP, the potent calcification inhibitor in our arteries), and the growth-arrest sequence-6 protein (Gas6, a protein involved in cell growth regulation). Whereas the proteins involved in blood coagulation invariably are synthesized in the liver, the newly discovered Gla-proteins have key functions in other metabolic processes and are synthesized in a wide variety of tissues. An overview of the various Gla-proteins and their functions (as far as they are known) is given in Table 1.

The molecular function of vitamin K is precisely known: it serves as a cofactor for the enzyme gammaglutamate carboxylase (GGCX), which is found in the endoplasmic reticulum of a wide variety of mammalian cells and that catalyzes the conversion of the amino acid glutamate into Gla. The oxidation of vitamin K hydroquinone provides the energy required for this carboxylation reaction. All Gla-proteins are secretory proteins found in the extracellular matrix or in body fluids. Gla-residues form strong calcium-binding groups in the proteins to which they are attached and in all cases in which their function is known the Gla-residues are essential for the function of these proteins (2).

All Gla-proteins known today contain multiple Gla-residues per molecule ranging from three in the bone Gla-protein osteocalcin to 10 in prothrombin and as much as 16 in Gla-rich protein (GRP). The position of the Gla-residues within the respective proteins is well-defined, but in the case of vitamin K insufficiency under-carboxylated or even uncarboxylated Gla-proteins are synthesized. Such proteins lack biological activity and may be regarded as biomarkers for vitamin K insufficiency. The most accurate definition of vitamin K is therefore: a compound

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(or a group of compounds) catalyzing the carboxylation of protein-bound glutamate by serving as a coenzyme for the GGCX enzyme.

Vitamin K in our diet

Vitamin K is not a single compound, but a group name for a family of related structures that all share a methylated naphthoquinone ring system substituted with a variable aliphatic side chain (3). In phylloquinone (vitamin K_1) the side chain is composed of four isoprenoid residues, the last three of which are saturated. Menaquinones (vitamin K_2) form a sub-family in which the length of the side chain may range from 1 to 13 isoprene residues, all of which are unsaturated. The various menaquinones are generally denoted as MK-n, where n represents the number of isoprene residues in the aliphatic side chain. Important menaquinones are the short chain MK-4 and the long chain menaquinones MK-7, MK-8, MK-9, and MK-10 that all occur in the human diet, whereas small amounts of MK-6 have also been found in various foods.

Vitamin K_1 is formed in plants and important sources in our diet are green leafy vegetables such as spinach, broccoli, Brussels sprouts, and kale. It is located in the chloroplasts where it forms part of the electron transport system and about 90% of the total vitamin K in the western diet is formed by K_1. Menaquinones (except MK-4) are of microbial origin and relatively high concentrations are only found in a few food items. *Natto* is a traditional Japanese food consisting of fermented soybeans; *Bacillus subtilis natto* is the source of vitamin K_2 (almost exclusively MK-7) in this food, which may contain up to 1100 μg of K_2 per 100 grams of food. The sharp taste of *natto* is highly appreciated in Japan but not in western societies. Other vitamin K_2-producing bacteria are lactic acid bacteria (mainly MK-8 and MK-9) and propionic acid bacteria (mainly MK-10). Both strains are used in the production of cheese and curd cheese, which form the richest sources of vitamin K_2 in Europe and Northern America. Small amounts of MK-4 may also be obtained from animal products such as meat and egg yolk; the MK-4 originates from menadione (2-methyl naphthoquinone), which is added to the animal food, and which is converted into MK-4 after uptake in the blood stream. It has also been reported that flatfish and eel, living at the bottom of the seas and lakes, contain small amounts of K_2 that were regarded to originate from the bacteria in decomposing organic material serving as their food.

Pharmacokinetics and efficacy of different forms of vitamin K

As can be understood from their molecular structures, the lipophylicity of the various K-vitamins differs substantially with increasing fat-solubility at increasing side chain length. Whereas menadione (no side chain) is water-soluble, phylloquinone and MK-4 are mildly hydrophobic, and the long chain menaquinones (MK-7 and higher) are only soluble in apolar organic solvents like hexane.

Intestinal uptake of K-vitamins is substantially affected by the food matrix in which it is embedded. Vitamin K_1, for instance, is tightly bound to the chloroplasts of green vegetables from which it is hardly liberated in the digestive tract. Uptake from cooked spinach and broccoli is 5–10% only, which can double by concomitant fat intake. K_2-vitamins, on the other hand, are mainly found in the fat fraction of dairy and are absorbed almost completely (4). From these data it can be understood that, although 90% of our vitamin K intake consists of K_1, phylloquinone and menaquinones contribute more or less equally to the human vitamin K status. Also purified forms of vitamin K that may be present in food supplements and functional foods are readily absorbed, especially in combination with a meal (4).

After intestinal uptake, all K-vitamins are incorporated in triglyceride-rich lipoproteins and transported to the liver. A remarkable difference is that most of vitamin K_1 is retained by the liver to be used for clotting factor synthesis, whereas K_2-vitamins are incorporated into low density lipoproteins (LDL) and set free in the blood stream (5). In this way extrahepatic tissues including bone and arteries are supplied with the vitamin K required for extrahepatic Gla-protein carboxylation. The different transport systems for vitamins K_1 and K_2 form the basis for their different target tissues. Since no specific receptors for vitamin K have been discovered at the outer membrane of various cells, it is likely that LDL-bound vitamin K is taken up by the cells via the LDL-receptors and subsequently dissolves in the membrane structures of the cells, notably the endoplasmic reticulum where it displays its cofactor function.

Another difference between K_1 and MK-4 on the one hand and the long-chain menaquinones on the other hand is their biological half-life time in the blood stream (5). Whereas K_1 and MK-4 typically exhibit half-life times of 1–1.5 hour, long chain menaquinones such as MK-7 and MK-9 are characterized by half life times of several days (4, 6). This was demonstrated in a volunteer experiment in which equal amounts of K_1 and MK-7 were ingested; it resulted that K_1 had almost completely disappeared after 8 hours, whereas MK-7 (almost exclusively bound to LDL) remained detectable until more than 4 days. Similar observations were made for MK-9 (LJ Schurgers, unpublished data). It must be concluded, therefore, that the long-chain menaquinones remain available longer for take up by extrahepatic tissues and that more constant circulating levels are generated because the postprandial fluctuations are smaller than in the case of K_1.

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Vitamin K-insufficiency

Frequency

Hepatic vitamin K-insufficiency in healthy adults has never been reported, and it is generally accepted that all vitamin K-dependent coagulation factors are fully carboxylated in the normal adult population. This is in sharp contrast to the extra-hepatic Gla-proteins. Conformation-specific antibodies have been developed for osteocalcin and MGP and it was demonstrated that in non-supplemented adults between 20 and 30% of both proteins occurs in the non-carboxylated state (7, 8). Only after supplementation with >1 mg/day of vitamin K_1 or 200 μg/day of MK-7 extrahepatic Gla-protein carboxylation was near to completeness (8). The consequence of this observation is that, for instance, the arterial vessel wall produces 30% of the MGP in an inactive form, which means that protection against vascular calcification is only 70% of what could be achieved at higher vitamin K intake (9). A general tendency is that under-carboxylation even increases at increasing age. In this perspective it is a reason for concern that the vitamin K consumption in the western society has decreased during the last centuries, especially during the last 50 years (10).

Risk factor for cardiovascular morbidity and mortality

After extensive databases had been constructed for the vitamin K_1- and vitamin K_2-content of various food items, population-based studies were initiated to correlate vitamin K intake with cardiovascular disease. In a first survey, Geleijnse et al. (11) demonstrated that vitamin K_2 intake is inversely correlated with cardiovascular disease and mortality. Remarkably, no association was found with the intake of vitamin K_1. The study was a 10-year follow-up of 4,500 elderly subjects (the Rotterdam study cohort) with a highest quartile for K_2 intake of 45 μg/day. These data were confirmed by Gast et al. (12) in over 16,000 participants of the Prospect study. Because of the large number of subjects and the long follow-up period, the effects of individual menaquinones could be tested. Long-chain menaquinones (MK-7 and higher) turned out to have the most beneficial effects on cardiovascular disease, with a mortality risk reduction of 9% for each 10 μg/day of extra intake. These outcomes prompted us to develop a more direct test for vascular vitamin K status and obviously circulating MGP was the target biomarker for such test.

During recent years, monoclonal antibodies specifically recognizing carboxylated MGP (cMGP) and uncarboxylated MGP (ucMGP) have been created (VitaK, Maastricht, the Netherlands). These antibodies were used to build tests based on the ELISA principle with which both conformations could be quantified in blood plasma. The most successful tests thus far have been tests for total ucMGP (t-ucMGP) and desphospho-ucMGP (dp-ucMGP), a fraction of t-ucMGP with very low affinity for vascular calcium precipitates. Circulating t-ucMGP was found to be inversely correlated with arterial calcification in various patient populations (8). Moreover, dp-ucMGP was found to be directly associated with mortality in several patient populations (aorta stenosis, chronic kidney disease) (13, 14). In a number of studies, plasma dp-ucMGP was found to be a strong and independent risk factor for the development of cardiovascular disease and mortality. Also, it was found that increased vitamin K_2 intake (from supplements) quickly decreased dp-ucMGP in a dose-dependent way (C. Vermeer, unpublished data). Whether this also leads to a concomitant cardiovascular risk reduction is subject of investigation at this time.

Table 1. Classification of the 17 Gla-proteins according to their function

Function	Name of protein
Haemostasis (procoagulant activity)	Prothrombin, factors VII, IX, and X
Haemostasis (anticoagulant activity)	Proteins C, S, and Z
Artery calcification inhibition	Matrix Gla-Protein (MGP)
Bone metabolism	Osteocalcin
Cell growth regulation	Growth-arrest sequence 6 protein (Gas6)
Functions unknown	Gla-rich protein (GRP)
	Periostin
	Periostin-like factor
	Four transmembrane Gla-proteins

Risk factor for fractures

Poor vitamin K status was also found to be associated with low bone mass, osteoporosis, and fracture risk. Three different methods were used to assess poor vitamin K status: low circulating vitamin K_1 (15), low vitamin K intake (16), and elevated circulating levels of the bone Gla-protein osteocalcin (17, 18). All three approaches readily showed the inverse association between vitamin K status and fracture risk. Remarkably, throughout the literature the association between vitamin K status and fracture incidence seems to be more evident than effects on bone mineral density (BMD), the golden standard for osteoporotic fracture risk. Moreover, vitamin K intervention studies have shown contradictory results with some studies showing a positive effect on bone health (as measured by BMD) and others with no measurable effect. One explanation for these apparently contradictory results is that BMD is not an appropriate endpoint to monitor effects of vitamin K on bone health. Also, it should be realized that in epidemiological studies only poor vitamin K status is associated with increased fracture risk. It would

be logical, therefore, to investigate the effect of vitamin K supplements on bone health in subjects pre-selected for poor dietary vitamin K status. Such studies have not yet been published today, however.

Risk for age-related diseases

Most vitamins have been identified from life-threatening and acute diseases during periods of deficiencies. For vitamin K this is bleeding, other examples are vitamin C (scurvy) and vitamin D (rickets). Here we will refer to these diseases as the primary deficiency diseases. Recommended daily allowances or dietary reference intakes have been defined as the minimal intake required to prevent the primary deficiency diseases in the majority of the population. During recent years it has become increasingly clear, however, that most vitamins are involved in more metabolic processes than those underlying primary deficiency diseases. Prolonged sub-clinical vitamin insufficiency may not be life-threatening with immediate effect, but forms a risk factor for the development of age-related diseases, which will be referred to as secondary deficiency diseases. Vitamin C is now known for its antioxidant activity, whereas vitamin D is an important factor in the prevention of diseases ranging from neuro-musculatory diseases to cancer. Vitamin K, the blood coagulation vitamin, is now known to be required for the prevention of osteoporotic bone loss, vascular calcification, whereas – via Gas6, a ligand for tyrosine kinase – it is also involved in the regulation of cell growth. The fact that several other Gla-containing proteins of as yet unknown function have been discovered recently suggests that even more vitamin K-dependent metabolic processes may come to light in the near future.

The triage theory – as formulated by McCann and Ames (19) – implies that during evolution, living organisms have developed systems ensuring preferential transport of vitamins and trace elements to the tissues important for primary deficiency disease prevention. Since bleeding to death is the major and acute threat of vitamin K deficiency, vitamin K entering our blood stream is preferentially transported to the liver, the place where the coagulation factors are synthesized. Only after hepatic vitamin K requirement has been met, the excess vitamin K is transported to extra-hepatic tissues. Secondary (or sub-clinical) vitamin K deficiency would then form an increased risk factor for accelerated bone loss, vascular calcification, and cancer. Indeed all these associations have been reported in the literature (8, 17, 20). The accumulation of risk factors for age-related diseases would be the mechanism underlying the shorter life-expectancy for subjects experiencing long-lasting poor vitamin K status that was observed by several independent groups.

Supplementation

Strategies

The fact that under-carboxylation of key Gla-proteins is common in the general population suggests that increased vitamin K intake may be an important factor in improving public health. The following groups need our specific attention, however:

1) Children. Osteocalcin is one of the most abundant proteins in the human body, and during growth its synthesis is at least 10-fold higher than after peak bone mass has been reached. This means that the vitamin K requirement of children is much larger than that of adults (21). Regrettably, the increasing consumption of fast food and snacks has resulted in declining vitamin K consumption by children year after year. Several studies have demonstrated that – as decided from the extent of Gla-protein carboxylation – the vitamin K status of children during growth is extremely poor. On the basis of the data presently available, we cannot conclude whether sub-clinical vitamin K-deficiency during the first 20 years of life will have health implications at later ages (as predicted by the triage theory), but certainly this is a point of concern that needs our immediate attention.
2) Pregnant women. Vitamin K is a fat-soluble vitamin, which implies that it is not freely transported across the placenta. Nevertheless, the growing fetus is in need of vitamin K that is extracted from the mother's blood stream. It has been demonstrated that especially during the last trimester of pregnancy the vitamin K status of the mother decreases, and that supplemented vitamin K results in improvement of osteocalcin carboxylation both in the mothers and the newborn (cord blood measurement) (22). The vitamin K status of the newborn is extremely low, which is the reason why vitamin K-supplementation immediately after birth is mandatory in most countries worldwide. Hemorrhagic disease of the newborn (HDN) is a serious complication in non-supplemented babies and during the entire period of breast-feeding. It is noteworthy that humans are the only mammals in which breast milk is so low in vitamin K that the child needs supplementation to prevent life-threatening complications. The fact that breast milk can be rapidly enriched by increasing the mother's vitamin K intake illustrates once more that during the last centuries changed dietary habits have resulted in a decline of vitamin K intake to dangerously low levels (23).
3) Elderly. It has been reported that osteocalcin carboxylation (as a marker for general vitamin K

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status) decreases after the age of 50 (24). This may be related to less food intake, decreased intestinal absorption, or increased requirement. Unfortunately, this coincides with the onset of age-related phenomena like increased rate of bone loss and vascular calcification. Of course these processes are multifactorial, but the accumulation of risk factors generally multiplies the risk. If sub-clinical vitamin K-deficiency may be regarded as one independent risk factor, annihilating this single risk factor may contribute substantially to postponing the disease. A 3-year placebo-controlled randomized clinical trial for the effect of vitamin K_2 (MK-7) supplements in this age group is therefore in progress in our institute.

4) Patients with impaired intestinal absorption. Crohn's disease, cystic fibrosis, and galactosemia are chronic diseases, all of which have been reported to be associated with poor vitamin K status (25). Without doubt subjects suffering from these diseases need vitamin K-containing supplements, but these diseases are out of the scope of this paper.

Safety

Since the primary deficiency disease associated with vitamin K is bleeding due to impaired blood clotting, it is often thought that high intake of vitamin K may increase thrombosis risk. This is evidently not true. Full carboxylation (and thus: maximal procoagulant activity) of the vitamin K-dependent clotting factors is essential, and vitamin K metabolism has been designed to meet that goal with highest priority. Excess vitamin K intake cannot result in more clotting factor carboxylation. This has also been demonstrated within our institute in thousands of subjects taking high doses of vitamin K during several years. Even when monitored with the most sensitive techniques (endogenous thrombin potential, ETP), an increased thrombosis tendency was not found in any of the participants. An exception is formed by patients receiving oral anticoagulants like warfarin or acenocoumarol that act as vitamin K-antagonists. Obviously excess vitamin K intake will interfere with this medication. On the other hand it is becoming increasingly clear that the long-term use of these drugs is associated with accelerated bone loss, low bone mass, and widespread valvular and arterial calcifications (26), thus demonstrating once more the importance of vitamin K for bone and vascular health.

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