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Vitamin K – an update

Part 1: Basic nutritional facts

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Abstract

In the first part of this two-part update on the basic nutritional facts on vitamin K, following an extensive literature search, we discuss the occurrence of this essential micronutrient in food, and its consumption, metabolism, functions and biomarkers. We will focus in particular on the functions that go beyond the vitamin's contribution to blood clotting, on the calcium paradox, and on vitamin K antagonists. According to our findings, it is not necessary to change eating habits with regard to foodstuffs that contain vitamin K when starting anticoagulants.

Keywords: Vitamin K, menaquinone, phylloquinone, vitamin K cycle, matrix gla protein, osteocalcin, vitamin K antagonists

Introduction

Although neonatal prophylaxis for the prevention of vitamin K deficiency-related blood clotting disorders is standard in many countries [1], vitamin K intake is otherwise rarely discussed. This can be attributed to the inconsistent evidence regarding the other functions of vitamin K. Here we discuss the issues surrounding the fact that vitamin K is also involved in the inhibition of calcification of soft tissues (especially blood vessels) as well as in the mineralization of bones, and that due to the activation of extrahepatic vitamin K-dependent (VKD) proteins such as matrix gla protein (matrix gamma-carboxyglutamic acid protein = MGP) and osteocalcin (OC), there seems to be an association between vitamin K status and the risk of developing atherosclerosis and osteoporosis.

It is assumed that the reference values for adequate intake of vitamin K in order to guarantee proper blood clotting according to the German Nutrition Society (DGE), Austrian Nutrition Society (ÖGE), and Swiss Society for Nutrition (SGE) [2], are being attained across all age groups. However, the reference values available at the moment do not take into account studies that investigated the vitamin's functions beyond blood clotting. Given that on April 5th, 2017, the European Food Safety Authority (EFSA) released a draft for "Dietary Reference Values for vitamin K", it is appropriate to update current knowledge regarding this vitamin whose importance could potentially have been underestimated. This first in a series of two articles on vitamin K deals with its occurrence in food, its metabolism in the human body, how well this essential nutrient (whose functions go beyond contributing to blood clotting) is being supplied in the population, and biomarkers for determining vitamin K status. The second part[1] presents some investigations into the effect of vitamin K on atherosclerosis, osteoporosis, and other diseases.

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Research question and methodology

The focus of both articles is on the extrahepatic effects of the K vitamers phylloquinone and menaquinone.

A literature search was carried out (search terms: vitamin K, phylloquinone, menaquinone, matrix gla protein, osteocalcin, atherosclerosis, osteoporosis, osteoarthritis, diabetes, postmenopausal, anticoagulation) in PubMed and in Cochrane database. The literature search included clinical trials – observational and interventional studies (RCTs) – as well as meta-analyses and reviews from the years 1998–2016. The literature cited in these works was also included in the search. In the review, we excluded in vitro and animal studies as well as trials in which other micronutrients were substituted along with vitamin K. Due to the heterogeneous nature of the subjects, biomarkers, and target variables, a review was preferred over a meta-analysis.

Vitamers and their occurrence

The fat-soluble "coagulation vitamin" was isolated by the Danish scientist Dr. Henrik Dam in 1929 [3]. The American Edward A. Doisy [4] identified its structure and synthe-

[1] see next issue of Ernährungs Umschau: 12/2017

sized it in 1939. What we are dealing with here is not a single compound, but rather a "family" of different substances with similar chemical structures and similar properties – known as vitamers. These are derived from vitamin K_3 (menadione), a methylated naphthoquinone (◆ Figure 1), which can be chemically produced from 2-methylnaphthalene, and also represents an intermediate stage in the transformation of phylloquinone into menaquinone-4 (MK-4) in the bodies of both humans and animals. MK-4 occurs in large quantities in animal products – especially in egg yolk, butter, fish, sausages, and cream (◆ Table 1).

Vitamin K_1 (phylloquinone) is substituted at C atom 3 with an aliphatic side chain consisting of 4 isoprenoid residues (5 C atoms each), three of which are saturated (phytyl residues; ◆ Figure 1). It is synthesized in the chloroplasts of seed plants. Good sources include green vegetables and green cruciferous vegetables, especially kale, spinach and broccoli (◆ Table 1). Soybean oil (193 µg/100 g) and rapeseed oil (127 µg/100 g) also contribute to the supply of vitamin K_1 [6].

Vitamin K_2 (menaquinone, MK-n) also has an aliphatic side chain at C atom 3. It consists of 4–14 isoprenyl units, n standing for the number of isoprenyl units (◆ Figure 1). MK-n are mainly formed by facultative or obligate anaerobic bacteria which occur, inter alia, in the intestine [7]. The human intestinal microbiota mainly synthesizes MK-8 *(Enterobacteriaceae)*, MK-10 and MK-11 *(Bacteroides)*, however these can hardly be absorbed in the colon due to a lack of lipases and bile salts [7]. Bacteria that are added to foodstuffs for fermentation purposes mainly form MK-8 (lactobacilli) and MK-9 (propionic acid bacteria), which is why hard cheese, soft cheese, and quark make a significant contribution to the supply. Nattō, a traditional Japanese food made from fermented soybeans *(Bacillus subtilis natto)*, is the most vitamin-K_2 rich food with 1,100 µg MK-n and 1,000 µg MK-7 per 100 g (◆ Table 1).

Foodstuff	MK-5 to MK-9	MK-4	Phylloquinone
Nattō	**1,103**[a]	-	34.7
Hard cheese	**71.6**	4.7	10.4
Soft cheese	**52.8**	3.7	2.6
Sauerkraut	**4.4**	0.4	25.1
Buttermilk	**2.3**	0.2	-
Plaice	**2.0**	0.2	-
Pork steak	**1.6**	2.1	0.3
Buckwheat bread	**1.1**	-	3.0
Egg yolk	0.7	**31.4**	2.1
Butter	-	**15.0**	14.9
Salami	-	**9.0**	2.3
Chicken breast	-	**8.9**	-
Minced meat	-	**6.7**	2.4
Whipped cream	-	**5.4**	5.1
Egg white	-	**0.9**	-
Whole milk	-	**0.8**	-
Salmon	-	**0.5**	0.1
Mackerel	-	**0.4**	2.2
Kale	-	-	**817**
Spinach	-	-	**387**
Broccoli	-	-	**156**
Margarine	-	-	**93.2**
Olive oil	-	-	**53.7**
Chocolate	-	1.5	**6.6**
Apple	-	-	**3.0**
Wheat bread	-	-	**1.1**
Rye bread	-	-	**0.7**
Banana	-	-	**0.3**
Black tea	-	-	**0.3**

Tab. 1: **Vitamin K in foodstuffs in µg/100 g** [5]
[a] 90 % MK-7
Values printed in bold refer to the foodstuffs that contribute the most to the supply of various K vitamers.

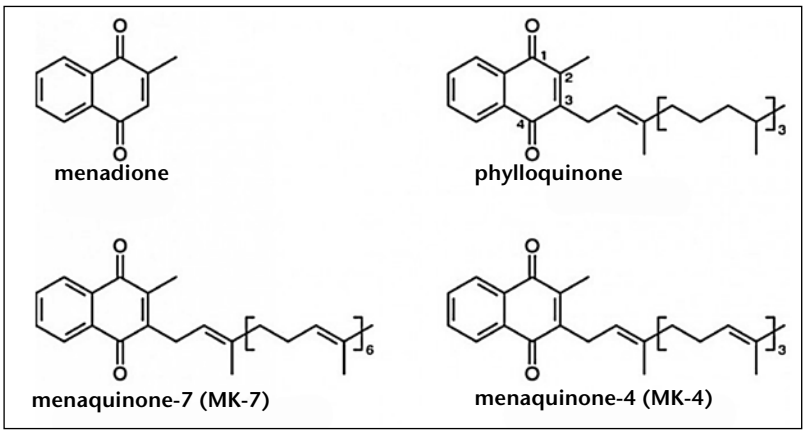

Fig. 1: **Structural formulae of selected K vitamers**

According to an epidemiological study conducted of 11,319 German men between 35 and 64 years of age whose median intake of vitamin K_1 was 94 µg/day and whose intake of vitamin K_2 was 35 µg/day, 62 % of the phylloquinone consumed came from vegetables (of which 42 % was from green vegetables), while 60 % of the menaquinone (MK-4 to MK-14) consumed came from milk products (of which 43 % was from cheese), and 17 % came from meat and sausages [8]. In a study of 38,094 Dutch people (20–70 years old) who consumed 200 ± 98 µg vitamin K_1 and 31 ± 7 µg vitamin K_2 per day, 78 % of the phylloquinone intake was found to be derived from vegetables, while 53 % of the menaquinone came from cheese, 19 % from other milk products, and 17 % from meat [9].

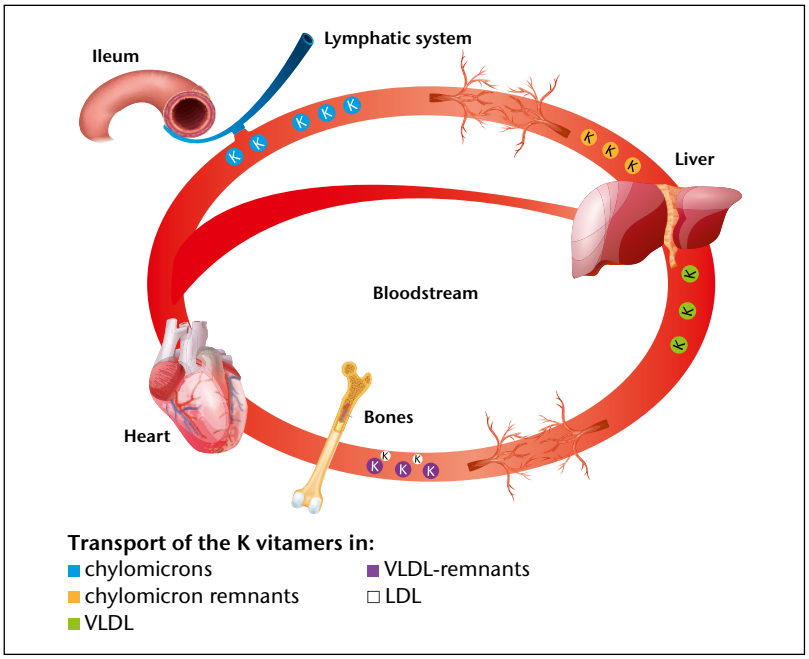

Fig. 2: **Intake and distribution of vitamin K** (according to [14])

It is assumed that the percentage of menaquinone in the overall vitamin K intake from food is only 12–25 % [5, 7], unless nattō is consumed on a regular basis, which is not the case in Europe as this food does not conform to typical European taste preferences. However, because menaquinone has a better bioavailability than phylloquinone [5, 10], which is bound to chloroplasts, animal foodstuffs contribute significantly to the supply of vitamin K.

Metabolism

Absorption of fat-soluble K-vitamers, which takes place in the proximal ileum, requires the presence of pancreatic lipases and bile acids, and depends on the composition of the meal: better availability is found in the case of cooked foods and in the presence of fat [11]. According to estimates, 3–50 % of the phylloquinone that comes from food is absorbed, whereas MK-7 is absorbed to a greater extent, and MK-4 and MK-9 are absorbed to a lesser extent [12]. In about 5–25 % of the intake amounts of phylloquinone and menaquinone, a separation of the aliphatic side chains takes place, which leads to the formation of menadione, which can be (re-)prenylated to MK-4 both in the liver and in the extrahepatic tissues [13].

The transport of the K vitamers from the small intestine – shown in ◆ Figure 2 – takes place (as with vitamin E) in chylomicrons, which enter the bloodstream via the lymphatic system and then progress to the fatty tissue. From there, they are transported onwards in chylomicron remnants to the liver, which is the target tissue for phylloquinone [15, 16]. Embedded in VLDL, VLDL remnants, IDL, LDL, and HDL, the further distribution to the periphery takes place before IDL, LDL, and HDL return to the liver [15]. Vitamin K_1 is the dominant vitamer in the blood, unless vitamin K_2 is taken as a supplement or eaten in the form of nattō [12]. 75–90 % of phylloquinone is transported in the triglyceride-rich lipoproteins, MK-4 mainly in LDL and HDL, and MK-7 and MK-9 mainly in LDL [12].

The highest serum concentrations are reached 4–6 hours after oral intake [12, 17, 18]. MK-4 levels increase quickest, followed by phylloquinone and MK-7 (at about the same rate), and finally MK-9 [10, 15]. Whilst MK-4, phylloquinone, and MK-9 are almost completely eliminated from the serum after 8 hours, MK-7 is still detectable 3–4 days later (biphasic kinetics) [5, 10]. This means that the serum concentration of MK-7 is more stable than that of vitamin K_1, and when equimolar amounts are administered, the concentration values of MK-7 are 7 to 10 times higher [5]. In the case of supply in line with the Dietary Reference Intake (DRI; 120 µg/day for women, 90 µg/day for men) the serum concentration of phylloquinone is around 1 nM [19], whereas in the case of European eating habits, the MK-n concentrations are often below the detection limit when using HPLC in laboratories with standard equipment [7, 18, 20].

As with vitamin E, elimination takes place (after shortening of the side chains and glucuronidation in the liver) in equal parts via the bile and the urine (5C/7C metabolites) [21]. The initial side degradation step consists of a cytochrome P450-dependent ω-oxidation followed by a successive β-oxidative chain shortening. Due to the high turnover, the body's storage capacity is limited to about 1.5 µg/kg body weight [22], which is why a regular intake in food is essential. Reserves are stored mainly in the liver (2.5–

74 % vitamin K_1) and in the pancreas, kidneys, brain, adipose tissue, and reproductive organs (mainly MK-4) [12]. More than three weeks of either a lack of vitamin K or use of oral coumarin-type anticoagulants results in depletion of the body's stores. In the short term, this manifests itself as a longer blood clotting time, and higher susceptibility to hemorrhages. In the long term, there appears to be an association between suboptimal vitamin K status and atherosclerotic/osteoporotic processes (▶ part 2, issue 12/2017).

Reference values and consumption

The D-A-CH reference values for vitamin K are 70 µg/day for men and 60 µg/day for women [2], and the DRIs are 120 µg/day for men and 90 µg/day for women respectively, with these values referring to phylloquinone [23]. The EFSA specifies an estimated value for phylloquinone of 1 µg/kg of body weight/d. This value has been in place since 1993, and it is unlikely to be changed in 2017 in the light of the new studies that have been conducted (a draft is currently available) [12].

Data on vitamin K consumption is not routinely collected. In epidemiological studies, food frequency questionnaires (FFQs) are often used to estimate the vitamin K_1 and K_2 intake, the amounts consumed being calculated by means of nutritional value tables. However, FFQs place high demands on the memory and truthfulness of the study subjects (under-reporting/over-reporting), and must therefore be interpreted cautiously, especially in the case of phylloquinone [18], because in the case of intakes of > 200 µg/day, a linear association between intake and plasma concentration can no longer be detected [24]. Another problem is that the calculated consumption depends on the quality of the nutrition tables used, and these are often incomplete, especially with regard to menaquinone. Furthermore, older tables usually show higher values than those based on newer data sets (e. g. those from the USDA Food Composition Database), which makes it much more difficult to make comparisons between different studies. In some prospective cohort studies conducted in the Netherlands, vitamin K consumption was calculated using an FFQ and the data shown in ◆ Table 1. The results of these studies and comparable studies conducted in Germany and the UK are listed in ◆ Table 2. According to these studies, the vitamin K intake of adults and adolescents ranged from 231 to 374 µg/day, of which 27 to 54 µg/day was apportioned to MK-n.

In the Dutch National Survey [31] and in the German National Nutrition Survey II [32], newer nutritional databases were used, and based on these, the daily consumption of vitamin K among Dutch people was estimated as 128 µg (men) and 111 µg (women), and the estimation for Germans was 75 µg (men) and 70 µg (women). Although the over 25s attained the estimated values for vitamin K intake, this was not the case for the 15 to 25 age group.

Regardless of the nutritional value tables used, vitamin K consumption is generally considered sufficient. As EFSA (2017) highlights [12], the Adequate Intake (AI) for phylloquinone that was determined in 1993 – 1 µg/kg of body weight/day [33] – closely corresponds to the vitamin K consumption for adults recorded in the German National Nutrition Survey II [32]: 75 and 70 µg/day, assuming the reference body weights. And indeed, typical signs of deficiency (blood clotting disorders) are very rare among the population. However, authors of some recent studies consider that only the coagulation factors formed in the liver are typically 100 % carboxylated with vitamin K mediation, whereas 10–40 % of the extrahepatic vitamin K-dependent (VKD) proteins that are detectable in the blood, such as matrix gla protein and osteocalcin, are undercarboxylated – in older people this figure can be as high as 40–50 % – which means that these proteins may be present in an inactive form [16, 24, 34–38].

In the case of a shortage of micronutrients, functions that ensure short-term survival take precedence over less essential functions [37]. It can be assumed that the vitamin K requirement for carboxylation of a larger percentage of all vitamin K-dependent (VKD) proteins is higher than for coagulation factors alone [39]. According to Vermeer's review [16],

Studies	Age [years]	Phylloquinone [µg/day]	Menaquinone [µg/day]
Rotterdam Study [25]	> 55	257 (♂)	31 (♂)
		244 (♀)	27 (♀)
EPIC Niederlande [26]	21–70	200	31
Prospect-EPIC [27]	49–70	213 (♀)	29 (♀)
EsKiMo[a] [28]	13–14	316 (♂)	
		304 (♀)	
	15–17	374 (♂)	
		304 (♀)	
EPIC Heidelberg [8]	40–65		35 (♂)
UK Dietary and Nutrition Surveys[b] ([29], cited in [30])	11–18		54 (♂)
			41 (♀)
	16–64		43 (♂)
			36 (♀)

Tab. 2: **Median phylloquinone and menaquinone consumption in prospective cohort studies**
[a] Nutrition interviews instead of the FFQ
[b] Weighed dietary record instead of the FFQ

daily supplementation of 1 mg phylloquinone or 200 µg of MK-7 would be required to achieve an almost complete carboxylation of all gla proteins. However, it is important to note that there is no scientific evidence that 100 % carboxylation of the extrahepatic gla proteins is desirable.

The "optimal" proportion of carboxylation of extrahepatic vitamin K-dependent (VKD) proteins for proper functioning – and therefore health – is not known [12].

In a study of 42 healthy men and women aged 18 to 45, 12 weeks of supplementation with MK-7 at a dose of 90 µg/day caused a significant increase in the proportion of carboxylation of matrix gla protein and osteocalcin – with no effect on thrombin, which was completely carboxylated at all times [38].

From this, it can be concluded that additional oral intake of menaquinone is not associated with increased blood clotting or an increased risk of thrombosis.

Functions

Biologically active vitamin K is present in a reduced form as hydroquinone (= quinol; KH_2) and acts as a cofactor of gamma-glutamyl carboxylase (GGCX). In the course of the post-translational carboxylation of protein-bound glutamic acid residues (glu) to gamma-carboxyglutamic acid residues (gla) (a process that is catalyzed by the aforementioned enzyme), the biologically inactive vitamin K-2,3-epoxide (KO) is formed from vitamin K hydroquinone, and KO is subsequently reduced by vitamin K epoxide reductase (VKOR) to vitamin K-quinone (K). This can be converted back into the biologically active hydroquinone using either vitamin K reductase (VKR) or an NAD(P)H-dependent quinone reductase, after which it is available for another reaction cycle [11, 40]. The vitamin K cycle is depicted in ◆ Figure 3. Coumarin-derivative anticoagulants such as warfarin or phenprocoumon, the active ingredient of the drug Marcoumar®, inhibit the thiol-dependent enzymes vitamin K epoxide reductase and vitamin K reductase [41], thus disrupting the "recycling" of KH_2 (◆ Box "vitamin K antagonists").

The enzyme gamma-glutamyl carboxylase catalyzes the conversion of glu into gla (i. e. the binding of carboxyl groups to the terminal C atoms of glutamic acid residues) in 19 proteins with a very low mass (5–10 kDa) that have been identified thus far. The simultaneous presence of two $–COO^-$ groups at each of the gamma C atoms of gla molecules (◆ Figure 3) is what gives the proteins their biological efficacy, because they enable chelate bonds with calcium ions (Ca^{2+}).

These proteins, known collectively as vitamin K-dependent proteins (VKDP) or gla proteins include – in addition to coagulation factors II (prothrombin), VII (proconvertin), IX (Christmas factor) and X (Stuart factor), as well as anticoagulant proteins C, S and Z, all of which are produced in the liver – MPG (matrix gla protein), osteocalcin (bone gla protein, BGP), GRP (gla-rich protein), Gas-6 (growth-arrest-specific protein 6), periostin, and nephrocalcin-A/-B, which are carboxylated extrahepatically [16, 54]. All K vitamers contribute to carboxylations in the extrahepatic tissues [12].

Out of all the relevant vitamin K-dependent (VKD) proteins, MGP, osteocalcin, and GRP have been the most thoroughly investigated [44, 55]:

- **MGP** (84 amino acids, 4–5 gla residues) counteracts ectopic calcification in soft tissues, e. g. in the intima and media of the arterial walls, thus inhibiting both the development of inflammatory atherosclerosis with focal plaque formation, which is accompanied by vasoconstriction and a risk of thrombosis, as well as generalized arteriosclerosis (Mönckeberg's sclerosis), which is characterized by a loss of elasticity in the vessel walls.
- **Osteocalcin** (46–50 amino acids, 3 gla residues) is active in the

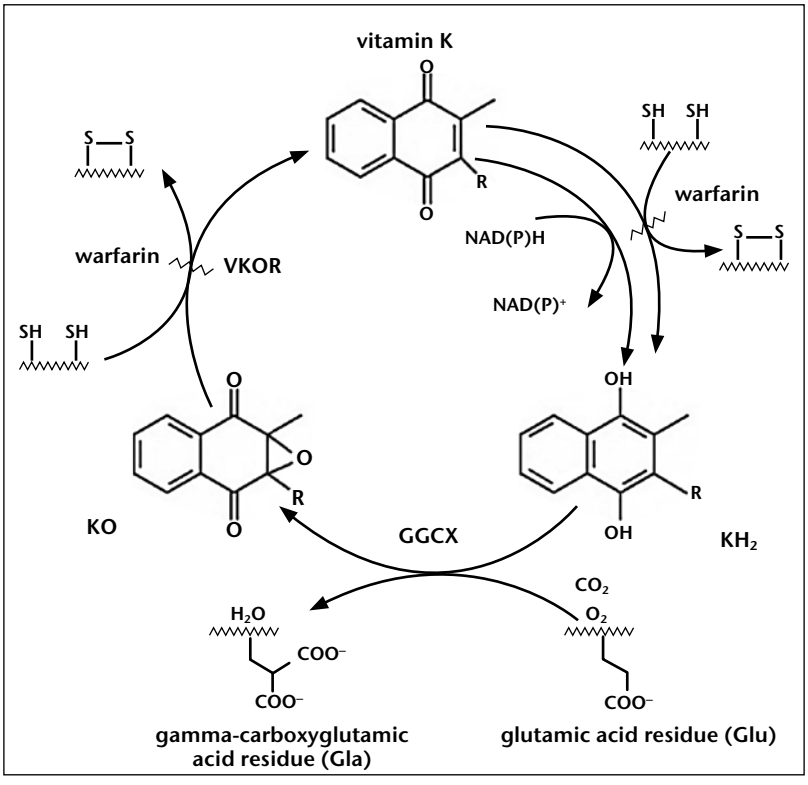

Fig. 3: **The vitamin K cycle** (from [41])
GGCX = gamma-glutamyl carboxylase; KH_2 = vitamin K hydroquinone; KO = vitamin K 2,3-epoxide; R = aliphatic side chain; VKOR = vitamin K 2,3-epoxide reductase

Vitamin K antagonists

For 60 years now, vitamin K antagonists have been the most frequently used drugs for inhibition of blood clotting. They belong to the drug class of antithrombotic agents, which are also known as anticoagulants or blood thinners. They are derivatives of coumarin, an aromatic secondary plant substance. Examples of where coumarin can be found include sweet clover, lovage and woodruff, and it is used as a rodenticide (rat poison), for instance.

In medicine, phenprocoumon (Marcoumar®), warfarin (Coumadin®), and acenocoumarol (Sintrom®) are used for the prevention and treatment of thromboembolic diseases of the arteries and veins, such as myocardial infarction, ischemic stroke, deep vein thrombosis, or pulmonary embolism [42]. The anticoagulative effect is produced by reducing the formation of vitamin K-dependent blood coagulation factors II, VII, IX and X. It works by impairing the enzymatic regeneration of biologically active vitamin K (KH_2) in the vitamin K cycle. However, the formation of KH_2 from vitamin K derived from food remains possible because the NAD(P)H-dependent quinone reductase, which is 100 times more active in the liver than in the extrahepatic tissues, is not affected by vitamin K antagonists [42]. The medication dose is determined on an individual basis and must be monitored regularly (prothrombin time, International Normalized Ratio, Quick Value), in order to avoid potentially life-threatening hemorrhages. In the case of an overdose of coumarin derivatives, vitamin K_1 is used as an antidote [42].

People who regularly take vitamin K antagonists exhibit a global undercarboxylation of vitamin K-dependent (VKD) proteins [43, 44]. Case control studies show that both osteoporotic [45, 46] and atherosclerotic processes [47–49] are significantly accelerated. Vascular calcification is also observed more frequently, and this may be accompanied by a loss of elasticity in the vascular walls, and by cardiovascular complications [47]. Theoretically, vitamin K supplements could inhibit these unfavorable effects, but they cannot be used by people who are taking coumarin derivatives because the efficacy of the medication is impaired in these cases. This is why "direct oral anticoagulants", such as thrombin inhibitors (dabigatran) and factor Xa inhibitors (rivaroxaban, apixaban) are currently being tested [42].

A study conducted in 1986 [50] suggested that daily consumption of vitamin K_1-rich green vegetables could make it necessary to increase anticoagulant dosages. Therefore, patients receiving coumarin derivatives have for decades been required to avoid foods rich in vitamin K [51]. Doubts as to the accuracy of this association arose in 2011 based on a cross-over study in which a multivariate correlation analysis was carried out instead of a univariate correlation analysis. The study demonstrated that variation in the medication dose could be explained 52 % by pharmacogenetics, but only 8 % by eating habits, exercise habits, and body weight [52]. A systematic literature review conducted in 2016 concluded the following [51]: It is not necessary to change eating habits when starting anticoagulants. The focus should be less on restricting overall vitamin K intake, and more on avoiding large fluctuations.

But what is the situation when it comes to supplements, in which the vitamin has a higher degree of bioavailability than in foodstuffs [53]? An early study showed that 250 µg vitamin K_1/day prolonged blood clotting time within 1 week, but this was not the case for 100 µg/day [50]. In a dose-response study in healthy subjects taking acenocoumarol, an unfavorable interaction between the supplement and the drug was found at a vitamin K_1 dose of 150 µg/day for 1 week [53]. Compared to vitamin K_1, MK-7 exhibited a three to four times higher potential to reverse the anticoagulative effect of acenocoumarol, leading the authors to recommended < 50 µg MK-7/day of supplementation [10]. Another dose-response study established that as little as 10 µg MK-7/day works as an antidote to acenocoumarol in some people [43].

bone matrix, where it promotes mineralization, which increases bone strength and decreases the risk of fracture.

- **GRP** (74 amino acids, 15 gla residues) appears to act as a calcium modulator in many tissues. It is thought to not only inhibit the calcification of arterial walls, but also inhibit the calcification of the extracellular matrix of cartilage tissue, and bind hydroxyapatite crystals in tumorous cells [44, 56].

Because undercarboxylated vitamin K-dependent (VKD) proteins do not perform the functions of carboxylated gla proteins, some authors conclude that there is an association between the proportion of carboxylation and a possible undersupply of vitamin K [16, 35, 37, 39]. Measuring the concentrations of undercarboxylated osteocalcin and dephosphorylated undercarboxylated MGP in the blood of healthy adults may indicate whether the vitamin K status is suboptimal. However, there are no generally accepted limit values that would be suitable for the early identification of persons at risk of a (sub-)clinical vitamin K deficiency. EFSA [12] also highlights the need for such cut-off values in order to make it possible to establish an estimated value for menaquinone intake.

Vitamin K deficiency can have various causes. These include inadequate nutrition, for example in the case of long-term fasting or dialysis [14], malabsorption syndromes, e. g. those associated with inflammatory bowel disease (Morbus Crohn [57, 58]), or long-term, high-dose vitamin E supplementation (e. g. 1,000 IU RRR-α-tocopherol over 12 weeks [59]). Regular use of oral coumarin-type anticoagulants can also have a negative effect on vitamin K status [43, 56]

(◆ Box "Vitamin K antagonists"), but this should by no means discourage those affected from taking their prescribed medication.

Biomarkers for the determination of vitamin K status

Unlike FFQs, which only allow the estimates of nutrient intake rather than precise values because they depend on the memory of the study subjects and on the completeness of nutritional value tables etc., biomarkers allow concrete conclusions to be drawn about nutrient intake, absorption, and metabolism. However, they can be influenced by variations in health, circadian rhythms, or the time since food was last consumed, which must be taken into account in studies accordingly [18].

There is no gold standard for determining vitamin K status. This is why a combination of several biomarkers, or a combination of FFQs and biomarker(s) should be used for this purpose:

- Urine tests, e. g. for menadione, gamma-carboxyglutamic acid, or 5C/7C metabolites of the K vitamers, provide a similar level of sensitivity, yet they are difficult to implement because they require 24-hour urine collection. Hence, they have not yet been used in large-scale studies [18].
- Measurement of undercarboxylated prothrombin (PIVKA-II) is not recommended because this marker only refers to liver metabolism [67] and can give only an inaccurate picture of differences in vitamin K intake within the context

The calcium paradox

The calcium paradox is the phenomenon whereby increasing calcification of (coronary) arteries and concurrent decreasing of bone density is observed with older age, especially in women who have reached menopause (estrogen levels dropping), but also in chronically ill patients, such as dialysis patients or diabetics [60, 61]. It is still unclear whether there is a causal association between reduced calcium incorporation into the bones and increased calcium deposits in the vessels in the sense of a "bone-vascular cross talk", or whether this is a pathophysiological artifact. However, a common pathogenic factor could be a (sub-)clinical menaquinone deficit.

It appears sensible to recommend that people who regularly take vitamin D_3 (cholecalciferol) supplements also take vitamin K [62, 63]. The reason is that vitamin D_3 (after being converted to calcitriol – vitamin D hormone) promotes the mobilization of calcium from the bones by stimulating the maturation of bone-degrading cells on the one hand, and induces the synthesis of inactive osteocalcin on the other, while vitamin K causes calcium to be incorporated into the bone matrix (via the activation of osteocalcin, MGP and GRP), and not into the vascular walls [64].

People who regularly take calcium supplements (with or without vitamin D_3) are also likely to benefit from a vitamin K supplement because calcium doses that exceed the recommended nutrient intake increase the risk of heart attack by an average of 30 % according to a meta-analysis [65].

As was demonstrated by KANNELAKIS et al. [66], a combination of calcium, vitamin D_3, and vitamin K have a stronger effect on bone density than calcium and vitamin D_3 alone. 173 healthy women aged 54 to 73 took part in the study, in which they were required to consume a given amount of enriched skimmed milk and yogurt over a period of 12 months. Various parameters measuring bone metabolism and bone density throughout the entire body and the lumbar spine were determined at the beginning and end of the study. 121 of these female subjects with high compliance (= 70 %) were included in the statistical evaluation, however this selection could have distorted the evaluation results. 26 women received 800 mg of calcium and 10 µg of vitamin D_3 per day, and two groups consisting of 26 and 24 women respectively received the same amounts of calcium and vitamin D_3, with the first of these two groups also receiving 100 µg phylloquinone, and the second also receiving 100 µg MK-7. The control group, which received no supplement, consisted of 39 women. The study demonstrated that over the course of the 12-month intervention, bone density throughout the entire body increased significantly among the three supplement-receiving trial groups, whereas it remained unchanged in the control group. The addition of vitamin K_1/K_2 also resulted in a decrease in the ratio of undercarboxylated to carboxylated osteocalcin in the plasma, and in an increase in the bone density of the lumbar spine. Calcium plus vitamin D_3 did not lead to an improvement in the bone density of the lumbar spine, and in the control group it even decreased over time. Unfortunately, the effect that the addition of vitamin K_1/K_2 may have on the plasma concentration of dephosphorylated undercarboxylated matrix gla protein and on arterial calcification was not investigated.

of the typical Western diet [18].

- Due to the high level of fluctuation of the relevant values within individuals and between individuals [67], serum concentration of phylloquinone is primarily suited for ranking vitamin K status in larger populations [18]. In order to gain a clearer picture of supply status, the measurement should be done in a fasted state and the determined value should be adjusted to account for the triglyceride concentration [24].
- Circulating menaquinone is not a reliable yardstick for vitamin K intake because with the quantities that are usually consumed, the concentrations are often too low to be determined reliably by HPLC [18]. The situation is different in the case of supplementation or nattō consumption [18]: MK-7 plasma concentrations of 5–10 nM were recorded in healthy Japanese women [68–70]. As with phylloquinone, the measurement should be done in a fasted state and the result should be adjusted to account for triglycerides [18].
- Undercarboxylated osteocalcin (ucOC) is a relatively good indicator of vitamin K status. The results of direct determination by ELISA, the antibodies of which also detect carboxylated osteocalcin (cOC), correlate strongly with the total concentration of osteocalcin in the serum (R_2 = 0.687), which is not true for semi-quantitative determination using the older hydroxyapatite adsorption method, which measures the ucOC fraction of the total osteocalcin (R_2 = 0.148) [71]. The ucOC is expressed either as a ratio of undercarboxylated to total osteocalcin (% ucOC) or as a ratio of undercarboxylated to carboxylated osteocalcin (ucOC/cOC) [18]. ◆ Tables 3 and 4[2] summarize the results of some case control and interventional studies that used ucOC/cOC as a marker for vitamin K status. It should be noted that ucOC/cOC was elevated due to disease in the studies shown in ◆ Table 3. Epidemiological studies on the association between ucOC and bone health yielded contradictory results ([18], overview in [36]).
- Dephosphorylated undercarboxylated matrix gla protein (dp-ucMGP), which is determined by ELISA, is regarded as a conclusive marker of vitamin K status [14, 18, 35, 36, 72], and has already been used in some case control and interventional studies, the results of which are summarized in ◆ Tables 3 and 4. As with ucOC/cOC, in the studies listed in ◆ Table 3, dp-ucMGP was elevated due to disease. The results of epidemiological studies on the association between dp-ucMGP and cardiovascular health contradict each other (overview in [18]).

A cross-over study in healthy subjects from all age groups shows that dp-ucMGP concentrations continuously increase from the age of 40 [39]. A prospective cohort study in people aged 55 and over without vascular disease suggests an association between elevated dp-ucMGP concentrations and an increased risk of cardiovascular diseases [42]. Another prospective cohort study in people aged 65 years on average with existing atherosclerotic diseases shows that the dp-ucMGP level is associated with the total/cardiovascular mortality risk [73]. However, it should not be concluded from these results that increased dp-ucMGP values are the cause of an increased risk of atherosclerosis, because the dp-ucMGP level depends on the total circulating amount of MGP, which increases with increasing age regardless of vitamin K intake, and this age factor is not taken into account in most experimental studies [18]. If we assume that MGP is expressed more frequently in the context of the age-related development of cardiovascular diseases, increased dp-ucMGP values cannot be interpreted as a cause – rather, they must be interpreted as a result of atherosclerotic changes [18].

In summary, the investigations shown in ◆ Table 3 and Table 4 clearly show that:

- People who suffer from cardiovascular diseases and osteoporosis, or who have an increased risk of developing these diseases (e. g. dialysis patients or diabetics), have significantly higher concentrations of dp-ucMGP or ucOC/cOC than healthy subjects, and these elevated concentrations (i. e. suboptimal vitamin K status) are more likely a consequence of the disease process than its cause – for example in the sense of inadequate vitamin K intake.
- In both healthy subjects and dialysis patients, daily supplementation with 45–450 µg MK-7 (compared to placebo) leads to a significant increase in the proportion of carboxylation of both biomarkers, as other authors have also stated [7, 38]. However, this cannot justify routine therapeutic or prophylactic use of vitamin K supplements (both menaquinone and phylloquinone) while there is still no evidence that reducing dp-ucMGP and ucOC concentrations also has a positive effect on the incidence of disease (▮▮▮ part 2 of the article in Ernährungs Umschau 12/2017).

This article will be continued in the next issue of Ernährungs Umschau (issue 12/2017). The references for parts 1 and 2 can be found at:
→ www.ernaehrungs-umschau.de

Conflict of Interest
The author declares no conflict of interest.

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[2] ▮▮▮ Tables 3 and 4 in the online supplement