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Vitamin K: Nutrition, Metabolism and Current Evidence from Clinical Trials

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Abstract

During the past decades evidence that vitamin K is involved in areas extending far beyond hemostasis has accumulated. Several mechanisms of action involving both direct interaction via receptors such as the steroid and xenobiotic receptor and indirect via post-translational activation of Gla proteins have been proposed. The metabolism of vitamin K is complex and has large interspecies variation. Also, the vitamin K content in various foods and the recommended daily intake are not clearly specified. Notwithstanding, several clinical trials evaluating the effect of different vitamin K species on cardiovascular disease, osteoporosis, metabolic disease and cancer are ongoing or recently completed. The review attempts to summarize the source and metabolism of different forms of vitamin K, as well as give an overview of the current evidence from clinical trials.

Keywords: Atherosclerosis; Cancer; Diabetes; Growth Arrest Specific 6 Protein; Matrix Gla Protein; Osteocalcin; Vitamin K_2

Introduction

During the last two decades, the research focus of vitamin K has shifted from coagulation to a broader range of topics involving cardiovascular disease, osteoporosis, diabetes and cancer. Since its discovery in the 1930s vitamin K has been most recognized for its involvement in post-translational γ-carboxylation of hepatic clotting factors II, VII, IX and X as well as protein C, S and Z. In addition, several vitamin K dependent proteins originating from extra-hepatic tissues have been identified. These are collectively named Gla proteins, referring to the transformation of Glutamate (Glu) residues to γ-carboxyglutamic (Gla) during carboxylation upon which the protein adapts a tertiary structure able to bind calcium ions [1]. While the introduction of new oral anticoagulants may have diminished the research interest of vitamin K in hemostasis, new applications are emerging for vitamin K and the associated extra-hepatic Gla proteins. The most extensively researched Gla proteins are Matrix Gla Protein (MGP), Osteocalcin (OC), Growth Arrest Specific 6 protein (gas6) and Gla Rich Protein (GRP). MGP is a potent calcification inhibitor and high levels of desphospho-uncarboxylated MGP (dp-ucMGP) has been correlated to increased vascular calcification score [2,3] and mortality [4] in at-risk populations. Similar to MGP, GRP is thought to prevent calcification in its carboxylated state [5]. OC is involved in bone metabolism [6] and gas6 has been implicated in proliferative and inflammatory signaling [7]. The activity of the aforementioned Gla proteins is dependent on their γ-carboxylation status. Therefore, an inadequate intake of vitamin K may result in suboptimal function of the Gla proteins. This is supported by large-scale studies demonstrating inverse associations between vitamin K intake and cancer [8] and cardiovascular disease [9]. The recommended daily intake of vitamin K is based on the amount needed to fully carboxylate hepatic coagulation proteins and does not take extra-hepatic Gla proteins into account. Furthermore, different forms of vitamin K seem to have different bioavailability for the extra-hepatic Gla proteins. Previous studies have demonstrated that substantial fractions of MGP and OC are uncarboxylated in healthy subjects [10], suggesting that vitamin K insufficiency might be more common than previously thought. The aim of this review is to summarize the source and metabolism of different forms of vitamin K, as well as give an overview of the current evidence from clinical trials investigating the efficacy of vitamin K supplementation on cardiovascular disease, diabetes, osteoporosis and cancer.

Different Forms of Vitamin K

Vitamin K occurs naturally in two forms, vitamin K_1 (Phylloquinone) and vitamin K_2 (Menaquinone). Together with the other fat-soluble vitamins D, E and A, quinones belong to the large isoprenoid (terpenoid) family, which among others also comprise sterols and carotenoids [11]. All forms of vitamin K share a polar, hydrophilic 2-methyl-1,4-naphthoquinone ring structure (Menadione, vitamin K_3), which is accompanied by a hydrophobic side chain of varying length and saturation. Vitamin K_1 has a phytyl side chain attached to the naphtoquinone head and contains only one unsaturated bond. Vitamin K_2 is subdivided into menaquinone-n (MK-n) where n is the number of prenyl units in the isoprenoid side chain with repeating unsaturated bonds [12]. In addition, synthetic forms vitamin K_3-K_5 exist. The molecular structure of the different forms of vitamin K is shown in (Figure 1).

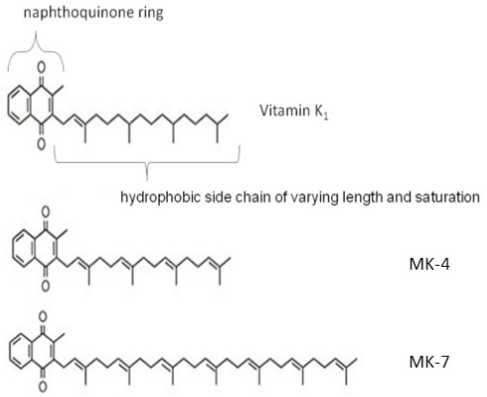

Figure 1: Molecular structures of Vitamin $K_1$and menaquinone-4 and -7.

For vertebrate's vitamin K is an essential micronutrient. Vitamin K_1 is synthesized by photosynthetic organisms and plays a vital role as electron carrier in leafy plants, which is the main dietary source of vitamin K_1 for humans [13]. Vitamin K_2 is produced by obligate and facultative anaerobic bacteria and occurs in animal livers and fermented foods such as cheese, curd and the Japanese specialty natto. Bacteria mainly synthesize long-chain vitamin K_2 (MK-7 – MK-13) using distinct pathways reviewed elsewhere [14]. MK-4 may be found in poultry and pork products. This is because animal feed often use synthetic derivative vitamin K_3 as a source of vitamin K [15], which is converted to MK-4 *in vivo*. The conversion of vitamin K_1 and K_3 to MK-4 is described more in detail below. Furthermore, MK-4 is a ligand to the steroid and xenobiotic receptor (SXR) through which it can affect gene transcription [16]. In general, tissue stores of vitamin K are low. Hepatic stores comprise almost exclusively of long-chain vitamin K_2 and the ratio of vitamin K_1 being only around 10%. In extra-hepatic tissues, especially in the brain, kidney and pancreas, MK-4 is the main form [17].

Already in the late 1950s, animal experiments revealed that animals fed vitamin K_3 or vitamin K_1 demonstrated increased levels of MK-4 in several organs [18]. When vitamin K_1 was administered parentally to pigeons the increase of MK-4 was not seen. In a study from 2006, increased urinary excretion of vitamin K_3 was seen after vitamin K_1 or MK-4 intake in healthy male volunteers [19]. The authors concluded that part of the MK-4 tissue stores derives from uptake and prenylation of circulating vitamin K_3. *In vivo* conversion of vitamin K_1 or vitamin K_3 to vitamin K_2 form MK-4 was demonstrated in mice brain when administered orally or enterally [20]. When administered intravenously or intrathecally only vitamin K_3 produced a low increase of cerebral MK-4 concentration. However, in cerebral slice cultures as well as primary cultures both vitamin K_1 and vitamin K_3 were converted to MK-4. This implies that the conversion from vitamin K_1 to MK-4 may happen through two routes, the first being side-chain removal that happens during intestinal absorption and vitamin K_3 is then prenylated to form MK-4 in the target tissue. The second route is both side chain removal and prenylation taking place at tissue level. The same research group identified UbiA prenyltransferase containing 1 (UBIAD1), a human homologue of *Escheria coli* prenyltransferase MenA, as the biosynthetic enzyme responsible for MK-4 synthesis [21]. As UBIAD1 was able to convert both vitamin K_1 and vitamin K_3 to MK-4, authors postulated that UBIAD1 may cleave the side chain to form vitamin K_3 which is then prenylated by geranylgeranyl pyrophosphate to form MK-4. Inhibition of UBIAD1 by drugs such as statins have been postulated to contribute to vascular calcification due to depletion of vascular vitamin K_2 levels [22].

The Vitamin K Cycle

Since the discovery of vitamin K's role in post-translational gamma-carboxylation of proteins in the 1970s there has been extensive research surveying the reactions involved in the transformation from Glu to Gla. This resulted in the identification of the vitamin K cycle, which is a series of enzymatic modifications vitamin K undergoes in order to support in carboxylation (Figure 2).

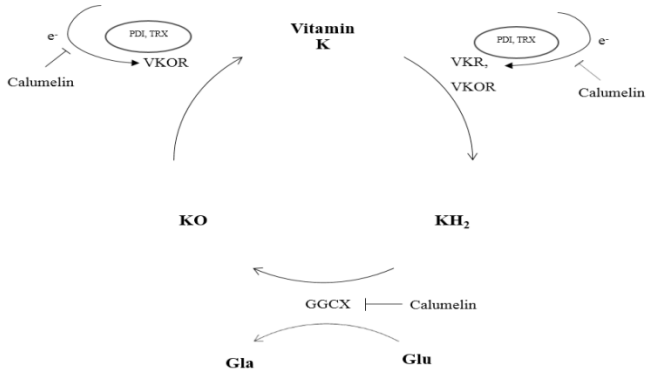

Figure 2: An updated Vitamin K cycle. See text for abbreviations.

In the initial step, vitamin K is reduced by Vitamin K Reductase (VKR) to form Vitamin K Hydroquinone (KH_2). KH_2 is then utilized by Gamma-Glutamyl Carboxylase (GGCX) which modifies glutamic acid residues to gamma-carboxy glutamic acid in Gla proteins. This reaction also requires presence of oxygen and carbon dioxide. GGCX recognizes its substrates through an amino acid sequence called the propeptide [23]. Despite comparatively high homology among the propeptides the affinity of GGCX for different Gla proteins varies significantly [24]. During the carboxylation process, KH_2 is oxidized to vitamin $K_{2,3}$-epoxide (KO), from which vitamin K is regenerated by the enzyme Vitamin K Epoxide Reductase (VKOR) and re-enters the cycle. VKOR is the target of vitamin K antagonists such as warfarin [25]. The reducing equivalents necessary for the VKOR reaction require a redox partner. The donated electrons have been proposed to derive from Thioredoxin (Trx)-like Protein disulfide reductase (PDI) in the Endoplasmic Reticulum (ER) [26] as well as ER membrane-anchored Trx-like protein TMX [27]. Several factors are involved in the regulation of the vitamin K cycle. Among them, there is the endogenous ER chaperone protein calumenin which has been shown to function as a negative regulator by inhibiting GGCX and VKOR in rats [28]. *In vitro* studies using short interfering RNA (siRNA) silencing calumenin demonstrated a significant increase in functional FIX [29]. Similarly, *in vitro* animal experiments showed that overexpression of VKOR subunit vitamin K epoxide reductase complex subunit 1 ($VKORC_1$) increased production of FIX [30].

Gla Proteins

In 1974, the vitamin K dependent modification where glutamic acid residues are transformed to γ-carboxy-glutamic acid were first described in coagulation proteins [31]. Since then, several vitamin K dependent proteins have been identified and some of them have become subject to extensive research due to their reported involvement in various conditions. Among these proteins, there are OC, MGP, gas6 and GRP. Identified in the 1970s, OC was the first Gla protein discovered that originated from extra-hepatic tissues [32]. OC is mainly produced by osteoblasts and has been suggested to affect bone metabolism by regulating osteoblast activity and bone mineralization [6]. More recently, OC was unexpectedly reported to be involved in glucose metabolism after studies showing that OC-deficient animals demonstrated hyperglycemia, hypoinsulinemia and increased visceral fat as compared to wild type animals [33]. Further animal studies suggested that the uncarboxylated form of OC (ucOC) mediated the endocrine properties by interacting with the pancreatic β-cells through the G protein-coupled receptor class C group 6 member A (Gprc6a) receptor [34]. UcOC was also reported to affect male fertility via Gprc6a in mice. However, some controversy as to whether these results can be applied to humans exists. The current evidence is discussed more in depth in the section about studies on vitamin K supplementation.

MGP was identified in the 1980s from bovine bone matrix [35], and shortly thereafter MGP expression was demonstrated in endothelial cells, fibroblasts, chondrocytes and vascular smooth muscle cells (VSMC). The function of carboxylated MGP as a calcification inhibitor has been confirmed in several preclinical studies [36]. A recent observational study demonstrated that elevated plasma dp-ucMGP was associated with increased cardiovascular mortality [37]. The significance of MGP for vascular health and whether increased carboxylation of MGP could reduce the progression of arterial calcification in risk group populations is currently being investigated in several clinical trials [38]. Arterial calcification may be present in the intimae as obstructing macrophage- and lipid-rich plaques or in the media leading to vessel stiffness. The process of calcification is thought to be regulated by VSMCs, which are plastic in nature and may differentiate into different phenotypes. The characteristics and specific drives for intimal and medial calcification have been reviewed elsewhere [39]. Accumulation of uncarboxylated MGP has been demonstrated in both intimal and medial calcification [40]. Gas6 is structurally similar to protein S and both function as ligands to the Tyro3, Axl and MerTK (TAM) receptor family. TAM receptors activate downstream signalling pathways involved in inflammation, hemostasis, cell cycle regulation and proliferation [41]. Previous studies have suggested that Gas6 is involved in the carcinogenesis [7], diabetes [42] and inflammation [43]. However, results conflict and no clear role has been established. In several studies the degree of γ-carboxylation was not considered. In its uncarboxylated state, Gas6 does not activate TAM receptors [44]. To date, no *in vivo* studies on humans investigating Gas6 carboxylation degree and how it responds to vitamin K supplementation exist. GRP is the most recently identified Gla protein. Previous studies suggest that GRP is involved in cardiovascular calcification, possibly functioning in a complex together with MGP and fetuin-A. Similar to MGP, the calcification inhibition is dependent on γ-carboxylation status [5].

Dietary Sources of Vitamin K

As mentioned above the main source of vitamin K_1 is leafy vegetables, which have been reported to contain 400-700 µg vitamin K_1 per 100 g, with the highest concentrations reported for the vegetables with the darkest green leaves [45]. In addition, vegetable oils provide an important contribution with concentrations ranging from 50-200 µg vitamin K_1 per 100 g. Principal sources of vitamin K_2 in the Western diet are fermented dairy products, with MK-9 and MK-8 being the predominating forms [46]. However, a wide intervariability in the vitamin K_2 content between the different dairy products have been demonstrated. Soft and blue cheese have very high concentrations which might be attributed to their lactic acid bacteria species. The Japanese soy dish natto contains very high levels of MK-7 due to its fermentation with *Bacillus subtilis*, which is also capable of synthesizing MK-7 in the intestines several days after ingestion. As this may result in unpredictable plasma levels of vitamin K, patients undergoing anticoagulant treatment with vitamin K antagonists (VKA) such as warfarin are strongly discouraged from eating natto. However, a study on healthy volunteers demonstrated only mild increases in plasma MK-7 with relatively stable levels up to 48 hours after ingestion when eating boiled natto, compared to markedly increased concentrations with regular natto, most likely since the boiling process eliminated the *Bacillus* species [47]. Another source of vitamin K_2, in the form of MK-4, is pork and poultry products from animals supplemented with vitamin K_3, which as described above can be converted to MK-4 *in vivo*. The dietary sources of different forms of vitamin K are summarized in Table 1.

In a study published in 2000, vitamin K_1 and K_2 contents of a large number of food items were analyzed [48]. Vitamin K_1 occurred most abundantly in kale, spinach and broccoli with concentration ranging from 156-817 µg per 100 grams. Vitamin K_2 was divided into MK-4 - MK-9. The highest MK-4 concentrations were found in goose liver paste (369 µg per 100 gram), goose leg (31 µg per 100 gram) and egg yolk (31.4 µg per 100 gram). MK-8 and MK-9 were mainly present in hard and soft cheeses as well as curd cheese (MK-9), with a peak concentration of 51.1 µg per 100 gram. MK-7 was found in unparallel concentrations in natto (998 µg per 100 gram), which also contained smaller amounts of vitamin K_1, MK-5, MK-6 and MK-8. With the exception of natto, MK-5 and MK-6 were only found in very low concentrations. Another study analyzing vitamin K content in fresh and processed pork products found high concentrations of MK-10 (289-492 µg per 100 gram), as well as MK-4 concentrations ranging from 3 to 27 µg per 100 gram [49]. Authors speculated that these products might be important contributors to overall vitamin K intake and hepatic stores, but since no studies have investigated the bioavailability of MK-10 it is difficult to draw any conclusions on its effect on vitamin K status in extra-hepatic tissues.

Type of Vitamin K	Dietary source	Suggested effects from supplementation trials
Vitamin K_1, phylloquinone	Leafy vegetables (e.g. kale, spinach, broccoli), vegetable oils	Increased insulin sensitivity in prediabetic women [50]. Decreased insulin resistance in nondiabetic adults [51].
Vitamin K_2, Menaquinone (MK) MK-4	Pork and poultry products (e.g. goose liver paste, egg yolk).	Positive effects on bone health in osteoporotic subjects [52].
MK-7	Fermented foods (e.g. cheese, Japanese specialty natto)	Positive effects on cardiovascular health in renal transplant patients [53].
Vitamin K_3, menadione	Synthetic form, used in animal feed.	Cytotoxic, may be used in cancer therapy[54,55].

Table 1: Different forms of Vitamin K.

Vitamin K in the Intestines

As the human gut flora contains bacterial species capable of vitamin K_2 synthesis, it was initially thought these contributed significantly to vitamin K status. This perception was supported by studies reporting antibiotics-induced hypoprothrombinaemia attributed to the elimination of intestinal bacteria [56]. However, a comprehensive review published in 1995 concluded that even though gut microbes provided some contribution to human vitamin K status, it was much less than previously thought. The majority of vitamin K_2 producing bacteria is present in the colon, where gall salts and pancreatic enzymes required for vitamin absorption are missing [57].

The Absorption and Transport of Vitamin K

Similar to other fat-soluble nutrients, vitamin K absorption in the intestines is dependent on pancreatic enzymes and gall salts to form micelles that enterocytes can take up. Previous studies suggest that the bioavailability of vitamin K varies significantly between different foods, for instance the availability from spinach was reported to be only 4% of that from the liquid formulation Konakion [58]. Other studies suggest that vitamin K_1 absorption is significantly greater after consumption of vegetable oil compared

to broccoli [59]. Following intestinal absorption, vitamin K is incorporated into chylomicrons and enters the lymphatic circulation. Chylomicrons are degraded by endothelial lipoprotein lipases to chylomicron remnants, which together with very low-density lipoproteins (VLDL) are referred to as triglyceride-rich lipoproteins (TRL). A smaller but significant fraction of Vitamin K is also transported with low density lipoproteins (LDL) and high-density lipoproteins (HDL). Using deuterium-labeled greens, studies have shown that the plasma vitamin K_1 concentration peaks approximately 6 to 9 hours after ingestion and baseline levels are restored within 24 hours [60]. Vitamin K_2 shows large variation between the subspecies in terms of bioavailability and lipoprotein transport. MK-4 is rapidly cleared from the circulation and distributed equally between TRL, LDL and HDL whereas MK-9 was initially only found in TRL and later in LDL where it remained for up to 48 hours [61]. MK-7 has been shown to be present in circulation for up to 96 hours, suggesting it has higher availability for both hepatic and extra-hepatic Gla proteins [62]. Another factor suggested to impact vitamin K status is polymorphisms in Apolipoprotein E (ApoE), which is a LDL component and related to cellular uptake of lipoproteins. Among the three human alleles E2, E3 and E4, studies have shown that ApoE4 carriers clear vitamin K from the circulation more efficiently which results in lower plasma levels of vitamin K [63]. However, another recent study reported that the ApoE2 allele was associated with markedly decreased urinary Gla excretion indicative of a poor vitamin K status [64].

Vitamin K Deficiency

In the adult population, hepatic vitamin K deficiency resulting in impaired coagulation is extremely rare. However, studies on perioperative changes in uncarboxylated prothrombin (Proteins induced by vitamin K absence factor II, PIVKA-II) have demonstrated increased PIVKA-II levels preoperatively without bleeding diathesis, suggesting that subclinical hepatic deficiency might be widespread [65,66]. Furthermore, studies on healthy subjects imply a suboptimal vitamin K status in extrahepatic tissues, which is more pronounced in children and adults above 40 years old [67]. Populations at-risk for developing vitamin K deficiency include patients with impaired intestinal absorption [68,69], and patients with chronic kidney disease as it was recently demonstrated that experimentally induced uremia in rats decreases GGCX activity in the liver and kidneys [70]. Substantial evidence from several independent studies suggest that vitamin K deficiency is common in patients with Chronic Kidney Disease (CKD), both in early stages and in those receiving dialysis [71]. CKD patients are also especially at-risk for cardiovascular disease and demonstrate increased vascular calcification. Results from intervention trials with vitamin K in CKD patients are discussed in the section about vitamin K supplementation. In 1996, a study on the dihydro-vitamin K_1 content in various foods was published. Dihydro-vitamin K_1 is a vitamin K_1 derivative which is generated from hydrogenation of vegetable oils and thus occurs in fast foods such as French fries and chicken nuggets. This study demonstrated that in some age groups in the American population dihydro-vitamin K_1 accounts for roughly 30% of the total vitamin K intake [72]. Animal studies have shown that dihydro-vitamin K_1 is able to function as a cofactor in gamma-carboxylation, but it is not converted to MK-4 in extra-hepatic tissues [73,74]. Therefore, groups who consume less vegetables and more processed food, such as socioeconomic disadvantaged groups [75], might be prone to develop extra-hepatic vitamin K deficiency. Another population at risk for developing vitamin K deficiency are neonates as the transplacental passage of vitamin K is limited [76]. A coagulopathy with all the attributes of Vitamin K deficiency bleeding (VKDB) was first described in 1894. Neonatal vitamin K deficiency can be classified as early, classical and late [77]. Early VKDB presents within the first 24 hours of life and is often associated with maternal drugs such as warfarin and anticonvulsants. Classical VKDB manifests during the first week of life, typically between day 2 and 3 due to a natural dip in prothrombin activity [78]. In previous studies, infant vitamin K status has been related to the intake of milk [79], stressing the importance of adequate nutrition. Late VKDB presents after the first week with a peak incidence between 3 and 8 weeks. Late VKDB tend to be more severe and is associated with a high incidence of intracranial hemorrhage. The late form of VKDB may be idiopathic or secondary to insufficient breast feeding or underlying diseases such as biliary atresia or α_1-antitrypsin deficiency [77]. In the 1950s it was demonstrated that antepartum administration of vitamin K to mothers decreased the incidence of neonatal hemorrhage [80]. Initially, water-soluble vitamin K_3 (e.g. Synkavit) was used as prophylaxis but it had rare albeit serious side effects in the form of hematological toxicities such as hemolytic anemia and kernicterus, probably related to erythrocyte glutathione metabolism [81,82]. Therefore, intramuscular administration of vitamin K_1 is the most commonly used prophylactic agent, although vitamin K_3 is still used in developing countries [83,84]. In 1990, a publication claiming intramuscular vitamin K administration to neonates was associated with increased risk of childhood leukemia gained widespread attention [85]. However, several studies since found no evidence for this association [86,87]. Lastly, anticoagulant treatment with VKAs impairs recycling of vitamin K and increases the risk of suboptimal carboxylation of Gla proteins. This is in line with several studies demonstrating negative effects of warfarin treatment of cardiovascular health [88,89].

Recommended Daily intake and Supplementation

Initially, dietary recommendations were based on the amount of vitamin K needed to correct bleeding diathesis in elderly hospitalized men, which was estimated to 0.03 µg per kg

body weight daily [90]. This has been revised multiple times and currently global guidelines vary between 50 to 120 µg per day [91] for adults. For infants aged 0-6 months a reference daily intake of 10 µg has been suggested, and is satisfied either by a single intramuscular dose of 1 mg vitamin K_1 at birth or repeated oral doses during the first 6-8 weeks [92]. These recommendations are based on vitamin K_1, which is the main form in Western diets. However, studies suggest that an intake between 200 to 500 µg per day may be needed to ensure optimal carboxylation of the extra-hepatic Gla proteins [93]. Vitamin K_2 has better bioavailability for extra-hepatic Gla proteins. When administered daily, 25 µg MK-7 daily has been shown to be more efficacious than 100 µg Vitamin K_1, and 50 µg MK-7 has been suggested to interfere with anticoagulant therapy [62]. Currently, there are no dietary reference values for vitamin K_2, and challenges in establishing reference limits include lack of accurate data on food content and intake of different MK species [94]. A complicating factor in establishing adequate reference intervals for vitamin K intake is the lack of a gold standard method for assessing vitamin K status. Measuring vitamin K in plasma is difficult due to the low circulating levels and interference from triglycerides [95]. However, in recent studies determination of vitamin K_1, MK-4 and MK-7 were performed using liquid chromatography tandem mass spectrometry [96]. Another approach is to measure the carboxylation degree of Gla proteins. In a previous review, authors proposed a combination of biomarkers as this might better reflect vitamin K status [97]. Several vitamin K supplements are available today. However, there is a significant cost difference between the different vitamin K forms. Whereas supplementation with vitamin K_1 has been estimated to cost $199.8, vitamin K_2 yields costs estimated to be $865.2 annually [98]. The different strategies to produce MK species include liquid and solid state fermentation, and research on how to improve efficacy and reduce cost in MK production is ongoing [99].

Studies on Vitamin K Supplementation

Cardiovascular Disease

The discovery of MGP and the accumulating evidence from preclinical studies that improved vitamin K status may prevent progression of or even reduce existing vascular calcification have motivated several clinical trials.

In 2004, a randomized case-control study showed that postmenopausal women who were supplemented with vitamin K_1 during three years demonstrated unchanged elastic properties of the common carotid artery whereas these properties were decreased in the other study groups [100]. In 2009, the results of a 3-year double-blind Randomized Controlled Trial (RCT) where 388 healthy older adults were given a daily multivitamin with or without 500 µg vitamin K_1 were published. In unadjusted results there were no differences in the progression of Coronary Artery Calcification (CAC) between the groups, but in secondary analyzes restricted to participants who were > 85% adherent, the vitamin K_1 supplemented group has 6% less progression compared to controls [101]. However, a recently published RCT on 80 older adults with established vascular disease who were given 100 µg MK-7 or placebo daily during six months failed to demonstrate any significant improvement of markers of vascular health. A modest non-significant improvement of pulse-wave velocity was seen in the vitamin K supplemented group. Authors speculated that the lack of significant effects could be related to insufficient doses or too short observation time [102]. Currently, trials evaluating the efficacy of vitamin K_1 supplements in reducing progression of vascular calcification in patients with severe kidney disease are ongoing. In the VitaVasK trial 348 hemodialysis patients are randomized to receive either 5 mg of vitamin K_1 thrice weekly or stay on standard care. Using Multi-Slice Computed Tomography (MSCT) thoracic aortic and coronary artery calcification will be evaluated after 12 and 18 months and compared to baseline scans [103]. The iPACK-HD Randomized Controlled Trial (RCT) has a similar aim and enrolls end stage kidney disease patients who receive either 10 mg vitamin K_1 or placebo three times weekly after dialysis. High-Resolution Computed Tomography (HRCT) is performed at baseline and after 12 months to evaluate progression of CAC [104]. In 2014, a randomized, single-blinded dose-finding study evaluated the effect of MK-7 on dp-ucMGP. Two hundred hemodialysis patients received 360, 720 or 1080 µg MK-7 thrice weekly during 8 weeks. MK-7 dose-dependently reduced circulating levels of dp-ucMGP [105]. Similar results were repeated by another study including 50 hemodialysis patients who received 360 µg MK-7 daily during four weeks, with a resulting drop of dp-ucMGP levels of 86% [106]. This has motivated further research with larger study populations and increased trial length. A three-year double-blind RCT investigating the effect of daily supplementation with 180 µg MK-7 in healthy post-menopausal women demonstrated decreased arterial stiffness in the supplemented group [107]. Currently, several clinical trials investigating the effect of vitamin K_2 (MK-7) on progression of vascular calcification are ongoing. Among them, there is the vitaK-CAC trial in which patients with pre-existing CAC will be randomized to receive 360 µg MK-7 or placebo during 24 months after which vascular health will be evaluated to determine whether MK-7 slows down the rate of progression [108]. As mentioned before, patients suffering from CKD are of particular interest as several studies suggest that they have a high prevalence of both vitamin K deficiency and cardiovascular disease. Some of the aforementioned clinical trials specifically include subjects with severe kidney disease or dialysis. In a randomized trial including 53 long-term hemodialysis patients receiving placebo or MK-7 (doses 45 µg, 135 µg or 360 µg) daily for 6 weeks a dose- and

time-dependent reduction of dp-ucMGP, ucOC and PIVKA-II was demonstrated [109]. In the recently published KING trial, comprising 60 renal transplant recipients supplemented with 360 µg MK-7 daily for 8 weeks, supplementation resulted in decreased dp-ucMGP levels and a 14.2 % reduction in mean carotid-femoral pulse wave velocity [53].

Bone Disease

Due to the aforementioned relationship between Vitamin K and bone health several clinical trials have evaluated the effect of vitamin K supplementation on bone turnover. In 2006 a systematic review comprising 13 studies concluded that both vitamin K_1 and vitamin K_2 had a protective effect by reducing bone loss as well as a strong decreasing effect of vitamin K_2 on bone fracture incident rate in Japanese patients [110]. Another review published in 2009 included seven RCTs where postmenopausal women were supplemented with vitamin K_1 or K_2 for at least two years. Supplementation with high doses (5 mg vitamin K_1 and 45 mg vitamin K_2, MK-4 daily) consistently decreased ucOC levels and albeit bone resorption markers were unaffected the bone strength measured as femoral neck width was improved and fracture incidence was reduced [111]. The modest effect on bone resorption was explained by the fact that OC and MK-4-mediated activation of SXR rather stimulates osteoblast differentiation. However, the largest RCT included in this review, comprising around 4000 women with osteoporosis supplemented with 45 mg MK-4, only demonstrated beneficial effects in women with advanced osteoporosis. The cumulative 2-year incident rate was lower among supplemented subjects but the difference was not statistically significant. However, vitamin K-substituted subjects experienced improvement of several activities of daily living (ADL) parameters during the first 12 months of the study compared to controls [112]. In 2015 a systematic review of nineteen RCTs enrolling 6759 participants was performed. Results showed that medium and long-term treatment with vitamin K_2 improved vertebral bone mineral density (BMD) in osteoporotic women, but did not affect BMD in the non-osteoporotic subgroup. Fracture incidence was decreased in the vitamin K_2-treated subjects after sensitivity analysis excluded one study, however the remaining studies had limiting factors such as small sample sizes and lack of critical data such as fracture incidence rate. Nevertheless, authors concluded that osteoporotic women had encouraging results, and that vitamin K_2 may reduce fracture incidence [52]. In summary, many RCTs evaluating the efficacy of vitamin K on bone loss have been performed. However, due to different limitations such as small study samples, it is difficult to confirm the role of vitamin K and to what extent it may affect bone pathology. Currently, studies on concurrent therapy with vitamin K_2 and bisphosphonates are ongoing comprising roughly 1000 study subjects in each group [113].

Cancer

Vitamin K has gained attention in cancer research after several preclinical studies have shown anti-tumour responses produced by different vitamin K forms [114]. Also, large population studies have demonstrated inverse correlations between vitamin K intake and cancer incidence and mortality [8,115]. Few clinical trials exist, and have mainly focused on whether MK-4 supplementation prevents recurrence of hepatocellular carcinoma (HCC) after curative resection. A systematic review analyzing these trials found no beneficial effect on overall survival or improved tumour recurrence with MK-4 [116]. Vitamin K_3 has the ability to generate ROS, therefore it has been of interest in cancer research [117]. Vitamin K_3 administration to patients with advanced HCC yielded reduction of tumour size in 17% of the patient population. These patients also had increased mean survival time, even though overall mortality remained unchanged [54]. In 2008 a clinical trial comprising 17 patients suffering from therapy-resistant prostate cancer demonstrated significant decrease in prostate specific antigen (PSA) increase rate after receiving concomitant vitamin K_3 and vitamin C treatment during 12 weeks. However, no decrease of absolute PSA was observed [55].

Metabolic Disease

Not many large-scale studies on the effect of vitamin K on diabetes exist. In a prospective cohort study encompassing 38 094 Dutch men and women, dietary intake of both vitamin K_1 and vitamin K_2 was inversely associated with type 2 diabetes. Furthermore, vitamin K_2 intake was correlated to a favorable lipid profile and lower CRP levels [118]. Cross-sectional analyses from the PREDIMED study showed that dietary vitamin K_1 estimated from a food frequency questionnaire was associated with reduced risk of type 2 diabetes [119]. Data from the National Health and Nutrition Examination Survey (NHANES) study comprising 5800 US adults suggested that vitamin K_1 intake had a positive influence on the metabolic syndrome and that the highest quartile of intake was associated with significantly reduced risk of low HDL level, hypertriglyceridemia, and hyperglycemia compared to the lowest quartile [120]. Similarly, a high vitamin K_2 intake has been associated with lower occurrence of the metabolic syndrome in a 10-year follow-up study on adults [121].

Supplementation trials investigating the relationship between vitamin K and diabetes are scarce. In a study where prediabetic women were randomized to receive either 1000 µg Vitamin K_1 or placebo once daily during four weeks, the supplemented group demonstrated increased insulin sensitivity and glycemic status. However, insulin resistance was not affected [50]. Another study found that daily supplementation with 500 µg vitamin K_1 to older non-diabetic adults during three years, decreased insulin

resistance defined as the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [51]. However, a meta-analysis published in 2017 comprising eight RCTs with a total of 1077 participants found no effect of vitamin K supplementation on insulin sensitivity. The authors suggested that larger, well-designed RCTs are needed to establish whether any association exists and if so determine its clinical relevance [122]. The relationships between OC and glucose homeostasis observed in animal studies have warranted several studies aiming to determine if the preclinical results can be extrapolated to humans. Variations of the gene bone gamma carboxyglutamate protein (BGLAP),which encodes OC, have been linked to alterations in body mass index (BMI) in an European population [123]. In 2015, several meta-analyses were published. Two of them looked specifically at type 2 diabetes and found that type 2 diabetes patients had significantly lower plasma levels of OC. Furthermore, an inverse association between OC levels and risk of developing type 2 diabetes were demonstrated in these studies [124,125]. Another meta-analysis showed inverse correlations between both total OC and ucOC with fasting plasma glucose (FPG) and glycated hemoglobin A1c (HbA1c) [126]. However, in a 3-year prospective study of high-risk individuals OC did not predict diabetes [127] and in a nested case-control study monitoring the risk of type 2 diabetes over a 10-year follow-up OC and ucOC were not associated with increased risk of type 2 diabetes [128].

Other Diseases

In addition to the aforementioned areas, vitamin K has been suggested to be involved in other diseases. One of these is osteoarthritis where elevated levels of dp-ucMGP have been associated with presence of osteophytes, meniscal damage and subcondral cysts, but not with progression of the disease [129]. Subjects in this study with low plasma vitamin K_1 were more likely to have progression of articular cartilage damage. Genome-wide association and functional studies with low expression of MGP are linked to an increased risk for hand osteoarthritis [130]. Other studies have identified vitamin K deficiency as a risk factor for osteoarthritis and have suggested supplementation studies to evaluate the effect of vitamin K on osteoarthritis [131,132]. To date only one such study has been published. In that study subjects received vitamin K_1 supplementation during three years and hand x-rays were performed at baseline and after the supplementation period. No effect of vitamin K_1 supplementation was seen [133]. Possible reasons include insufficient doses and observation period, underpowered study or wrong vitamin K subspecies as vitamin K_2 has a better bioavailability for extra-hepatic Gla proteins. Another research area where vitamin K supplementation has been suggested is neurodegenerative diseases such as Alzheimer's disease. Possible mechanisms of action involving sphingolipid metabolism, Gas6-mediated Axl stimulation and apolipoprotein E genotypes are discussed in a previously published review article [134]. Furthermore, vitamin K_3 analogues have demonstrated inhibition of Aβ aggregation *in vitro* and in silico, and might be potential anti-amyloidogenic drug targets [135]. Another possible area of application for vitamin K_3 is as an antimicrobial agent as *in vitro* studies have demonstrated growth inhibition and decreased exotoxin production in both gram-positive and gram-negative bacteria exposed to vitamin K_3 [136]. An additional antimicrobial strategy, could be inhibition of menaquinone biosynthesis, as several bacteria require menaquinones for their respiratory chain. *In vitro* studies have demonstrated that inhibition of menaquinone synthesis resulted in inhibition of both replicating and non-replicating as well as drug-resistant strains of *Mycobacterium tuberculosis* [137].

Conclusions

During the past decades evidence that vitamin K is involved in areas extending far beyond hemostasis has accumulated. Several mechanisms of action involving both direct interaction via receptors such as the SXR and indirect via post-translational modification of Gla proteins have been proposed. The metabolism of vitamin K is complex and has large interspecies variation. Furthermore, limited knowledge about bioavailability and the lack of a gold standard biomarker complicate creating optimal vitamin K intake guidelines for different populations.

Pre-clinical studies have suggested involvement of vitamin K and Gla proteins in cardiovascular disease, diabetes, osteoporosis and cancer, which has motivated several clinical trials evaluating the effect of supplementation with different forms of vitamin K. In general, the bulk of the trials considered in this review comprise a small number of subjects making it hard to draw definitive conclusions. The involvement of vitamin K and MGP in cardiovascular disease is among the most extensively studied and the limited number of completed studies shows promising results for the vitamin K_2 form MK-7. Larger, well-designed trials are ongoing and results are expected within the next few years. Studies on whether vitamin K supplementation is beneficial for patients with osteoporosis have shown reduced fracture incidence and improved bone health in patients with advanced disease. The least explored area in terms of clinical trials is whether vitamin K can be used in cancer therapy. MK-4 was initially reported to have anti-tumour effects on HCC, but this was not confirmed in a recent systematic review. Vitamin K_3 produces ROS which have been utilized in clinical trials but not enough data exist on this issue. The involvement of vitamin K in metabolic disease has been investigated after large population studies suggested an inverse relationship between vitamin K intake and type 2 diabetes, but so far results have been inconclusive. The latest debated topic is whether uncarboxylated OC affects glucose homeostasis, but no consensus has been reached and studies show conflicting results.

In conclusion, clinical trials indicate that supplementation with vitamin K has beneficial effects on various diseases with the most compelling evidence for cardiovascular disease.

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