

# Multi-faceted function of vitamin K with particular consideration of vitamin K2 – literature review

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## ABSTRACT

**Introduction:** Vitamin K, discovered in the 1930s, is a very important compound for the human body, performing many functions. The most well known of them are calcium homeostasis and coagulation. Nowadays it is apparent that many more beneficial multiorgan aspects of vitamin K exist.

The aim of the study was to review the properties of vitamin K and to show its potential therapeutic value.

**Materials and methods:** Medline databases (PubMed) and other scientific sources were searched.

**Results:** Vitamin K shows a multifaceted effect on the proper functioning of the human body: preventing coronary vessel calcification, maintains normal blood pressure, has neuroprotective effects, reduces the risk of myocardial infarction, slows the process of osteoclastogenesis, and influences the production of bone reabsorption factors. In addition, vitamin K supplementation has

been shown to reduce the risk of hepatocellular carcinoma (HCC) by interfering with tumour cells cycle and inducing their apoptosis. The pro-apoptotic activity of menaquinone is not limited to HCC only, but also to other cancers such as glioblastoma multiforme, breast cancer or bladder cancer, which reveals the importance of vitamin K in oncology. Possibly, introduction of vitamin K to the therapy may improve malignancy treatment outcomes. **Conclusions:** Vitamin K derivatives participate in many metabolic pathways of the human body. Their multifaceted activity may be used both in prevention of many diseases and in their potential treatments. However, further multicentre studies are necessary to understand better possible therapeutic properties of vitamin K derivatives.

**Keywords:** vitamin K; menaquinone; *VKORC1*; PIVKA; menadi-one; matrix Gla protein; phyloquinone.

## INTRODUCTION

Henrik Dam, the Danish biochemist, discovered vitamin K in 1930 while studying cholesterol metabolism in chickens. In animals on a low-fat diet, frequent bleeding in subcutaneous tissue were observed, while in chickens fed an optimal fat amount and many leafy vegetables, the bleeding problem did not occur. This substance has been called a vitamin of coagulation [1]. In further studies it was proven that there are compounds of similar structure although they have different properties. A characteristic feature of the chemical structure of vitamins belonging to the K group is the 2-methyl-1,4-naphthoquinone ring with isoprenoid groups attached in the C-3 position. These groups, depending on their number, are used in classification of vitamins K to different families (K1, K2 and K3) [2].

## NATURAL OCCURRENCE AND FUNCTION OF VITAMIN K GROUP COMPOUNDS

Vitamin K1 – phyloquinone, is found naturally in leafy plants such as spinach and kale. It is absorbed in the gut, then transported to the liver and stored there. Its main function is to maintain normal blood clotting. In case of a deficiency, excessive bleeding occurs. Vitamin K2 – menaquinone, is produced by bacteria in the gut and is also found in the food of

animal origin, e.g. egg yolks [3]. It is possible to convert phyloquinone to menaquinone. Unlike phyloquinone, menaquinone is not stored in the liver, but in other tissues, mainly in blood vessels and bones. Vitamin K2 activity in the body is pleiotropic. It has a beneficial effect on bone mineralization, while it removes calcium from areas where its accumulation is harmful – in blood vessels and soft tissues. Vitamin K3 – menadione is a metabolite of vitamin K1 and a precursor of menaquinone – 4 [4].

## VITAMIN K AND THE CIRCULATORY SYSTEM

Vitamin K has beneficial effects on the human circulatory system. It was shown to significantly reduce coronary artery calcification (CAC), which is a subclinical symptom of coronary heart disease. Vitamin K dependent proteins, including a calcification inhibitor called matrix Gla protein (MGP) present in vascular tissue, have a high affinity for calcium ions and act as inhibitors of vascular mineralization. It has been observed that a low level of phyloquinone (vitamin K1) in blood is associated with a higher risk of CAC, especially in older people suffering from hypertension, however a vitamin K status itself is not significantly associated with a general risk of coronary heart disease [5]. The relationship between a deficiency of both vitamin D and K and an increased cardiovascular risk is

more distinct because their deficiency leads to an increase of arterial pressure, both systolic and diastolic, and thus predisposes to hypertension [6].

Vitamin K epoxide reductase complex (*VKORC1*) is an enzymatic complex that is responsible for a reduction of vitamin K-2,3-epoxide to its active form. The reduction of vitamin K epoxide is responsible for carboxylation of glutamic acid residues in some blood coagulation proteins, including factors VII, IX and X. This complex is of therapeutic importance both due to its role in the high variability of anticoagulant drugs dosing between patients and as a potential participant in vitamin K deficiency disorders [7]. Polymorphism of *VKORC1* (rs9923231) and *CYP2C9* (rs1799853 and rs1057910) are responsible for the low dose phenotype. Enzymatic activity in cytochromes is decreased and the bioavailability of the drug is high. As a result the therapeutic dose should be 3–4 times lower than normal [8]. Warfarin (coumarin derivative) inhibits *VKORC1* activity which leads to a decrease in the concentration of vitamin K available as a co-factor of coagulation factors. An inadequate warfarin dose can result in both large and small haemorrhages [9].

The relationship between *VKORC1* gene polymorphism and the risk of heart attack, ischemic heart disease, and aortic dissection was analysed. People with a C allele in the +2255 locus have been shown to have a double risk of coronary artery disease, while people with CC and CT genotypes were characterized by a lower level of decarboxylated osteocalcin (a vitamin K deficiency marker), probably vascular calcification and lower levels of proteins induced by vitamin K absence (PIVKA) than people with the TT genotype. The results suggest that *VKORC1* haplotype may serve as a genetic marker of a cardiovascular disease risk [10].

## THE IMPORTANCE OF VITAMIN K FOR PROPER BONE STRUCTURE

Vitamin K is of great importance in bone tissue homeostasis. The amount of vitamin K involved in proper bone metabolism is larger than the reserves used for clotting factors production [11]. Vitamin K acts at the protein level, affecting their activity, as well as on genes by regulating their transcription. The main protein dependent on vitamin K presence and actively influencing bone metabolism is osteocalcin (OC) – a known marker of bone turnover [12]. Like other proteins dependent on vitamin K, it is activated by gamma-carboxylation of its glutamine residues – vitamin E is a cofactor of this reaction. The carboxylated form of OC has a high affinity for calcium and attaches to hydroxyapatite of bones [13]. This protein regulates a transformation of osteoblasts into osteocytes and reduces osteoclastogenesis [14]. Additionally, it influences calcium metabolism by reducing calcium urinary excretion and interferes with the production of bone reabsorption factors (prostaglandin E<sub>2</sub> – PGE<sub>2</sub> and interleukin 6 – IL-6) [15]. A low level of the vitamin and thus a high concentration of uncarboxylated osteocalcin (uOC) may result in impaired bone

mineralization and formation, leading to osteoporosis and an increased risk of fractures [16]. On the other hand, increased vitamin K supply promotes activation of a larger OC pool and improves biochemical markers of bone metabolism (decreases uOC, while increasing the pool of carboxylated OC) [17]. Some studies correlate improvement of biochemical profile – proper vitamin K serum level, with increased bone density (BMD) [18].

An analysis of research over the years shows that supplementation of vitamin K improves the skeletal system condition by decreasing bone loss and by protecting against fractures [19]. Vitamin K supply prevents age-related changes in bone structure in postmenopausal women [20]. Therefore in Japan vitamin K2 became one of the drugs registered for osteoporosis treatment [21]. A larger BMD increase and a lower bone mass loss measured using bone turnover markers, such as procollagen type I N-terminal propeptide and tartrate-resistant acid phosphatase isoform 5b, was observed in osteoporosis patients treated with bisphosphonates and vitamin K2, rather than monotherapy with pyrophosphate derivatives [22]. In steroid induced osteoporosis, a significant decrease in the risk of spine fractures was also observed in the combination therapy [23]. Simultaneous vitamin D and K2 supplementation acts synergistically resulting in a reduced loss of bone mass [20].

Vitamin K2 acts at the transcription level affecting genes associated with bone markers and extracellular matrix. It works by stimulating steroid and xenobiotic receptors *SXR* [24]. This results in activation of genes associated with this receptor, including: tsukushi (small leucine rich proteoglycan), matrilin-2 and CD14 antigen. One of the effects of gene stimulation with vitamin K2 is an increased amount of collagen built into the bones. Vitamin K2 also affects stem cell differentiation into the osteogenic cell line. This process occurs by inhibiting microRNA (miR) 133a, which is an inhibitor of differentiation for these cells. Bone marrow stromal cell exposure to vitamin K results in an increased differentiation into osteoblasts and carboxylation of vitamin K-dependent proteins involved in a bone metabolism [25]. Vitamin K also affects transcription by inhibiting *RANKL* (receptor activator of nuclear factor kappa b ligand). Vitamin K3 analogues used in a breast cancer therapy to reduce bone resorption associated with cancer and osteolytic metastases, have shown therapeutic potential in mice. The vitamin operates by inhibiting *RANKL* and thus the nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells (NF- $\kappa$ B) signalling pathway, which results in a lack of cell differentiation into osteoclasts [26].

Vitamin K is one of the studied factors that may affect osteoarthritis. The study of Neogi et al. showed a correlation between a low phyloquinone (vitamin K1) level and degenerative changes in hands and knees [27]. The study of Shea et al. showed a positive correlation with changes in the knee joint [28]. It was observed that proteins dependent on vitamin K and activated in a similar way to OC are expressed and accumulated in the cartilage. Such proteins include gastrin-releasing peptide (GRP) and MGP. A non-carboxylated inactive form of macromolecules predominates in joints affected

by disease. Matrix Gla protein, after carboxylation, inhibits cartilage mineralization in 2 ways: it binds calcium crystals and inhibits bone morphogenetic protein 2 (BMP2). However the relationship between vitamin K level and the occurrence and progression of degenerative changes requires further research [27].

Menaquinone 4 and 7 (MK-4 and MK-7) could be used in a therapy of rheumatoid arthritis. Menaquinones influence the disease course by activating apoptosis in synovial cells of joints. Treatment with MK-4 and MK-7 results in reduction of: uOC level, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), metalloproteinases and disease activity assessed with the disease activity score (DAS) in 28 joints and OB [29].

## VITAMIN K AND HCC

Vitamin K2 was observed to show an anti-carcinogenic effect on hepatocellular carcinoma (HCC) development. Its supplementation decreases HCC development, can even lead to tumour regression and reduces the risk of non-alcoholic fatty liver disease (NAFLD) [30]. It prevents cancer cell growth and invasion by activating protein kinase A, which is a modulator of several transcription factor activations and an inhibitor of small GTPase Rho, dependent on inhibition of des- $\gamma$ -carboxy prothrombin (DCP). This prothrombin is a serum protein whose increase is observed in the hepatic portal system during a neoplastic cell invasion [29]. Studies have shown that vitamin K2 analogues can prevent HCC recurrence and improve overall survival [31]. A vitamin K analogue – Cpd5, has ability to inhibit tumour cells growth by inducing apoptosis, mainly by a pathway independent from caspase activation [32]. Menatetrenone, a vitamin K2 analogue, may operate by inhibiting extracellular matrix metalloproteinase (MMP) expression through inhibition of NF- $\kappa$ B and mitogen-activated protein (MAP) kinases activity [33]. Vitamin K2 also inhibits primary and 13-acetate-12-O-tetradecanoyl-phorbol (TPA) induced expression of MMP-1 -3 and -7 in a dose dependent manner. It also inhibits the previously mentioned TPA-induced activation of NF- $\kappa$ B and activator protein 1 (AP-1). In this way, it inhibits activity of TPA-induced luciferase which is responsible for MMP-1, -3, and -7 promoter activation, and thus may be useful in HCC treatment. Vitamin K2 activates the receptor for SXR steroids and xenobiotics, which leads to inhibition of HCC tumour cell proliferation and motility [34]. In combination with sorafenib, vitamin K shows an inhibitory effect on cancer cell migration, proliferation and metastasis. The antiangiogenic activity of sorafenib leads to impaired uptake and induction of DCP by vitamin K, which is released by HCC tumour cells [35]. Additionally, inhibition of mitogen-activated kinases (ERK) and depletion of anti-apoptotic Mcl-1 proteins occurs. This leads to inhibition of growth and stimulation of tumour cell apoptosis [36]. The combination of vitamin K2 with angiotensin converting enzyme inhibitors (ACEI) prevents HCC recurrence by suppressing neovascularization mediated by vascular endothelial

growth factor (VEGF) [37]. An even more beneficial effect of HCC treatment was observed with vitamin K analogue menatetron, and 5-fluorouracil, since a dose-dependent manner of vitamin K2 administration increased inhibition of tumour cell growth by 5-fluorouracil by blocking G1 cell cycle phase [38]. Vitamin K2 stimulates metabolic maturation of pluripotent stem cells and fetal hepatocytes by activating a PXR receptor (pregnane X receptor) and expression of *CYP3A4* and *CYP2C9* genes [39]. Vitamin K deficiency may result in formation of DCP – a prothrombin precursor produced in HCC, which may be a potential growth factor for HCC cell development and also contribute to tumour angiogenesis by increasing angiogenic factors secretion by HCC cells [40]. In addition, vitamin K5 also plays an anti-cancer role in HCC. It inhibits the growth and proliferation of PLC/PRF/5 (tumour cell line) in HCC, expression of cyclin D1, inhibitor p16INK4a cyclin-dependent kinase (Cdk), stops the G1 phase of cell cycle, which can be used in HCC treatment [41]. Similar effects are shown by vitamin K2 and K3, which also cause inhibition of the tumour cell cycle in G1 phase, cyclin D1, and Cdk4 [42]. Vitamin K2 homologue – menaquinone MK4, may be used in the future in the treatment of HCC, as the prodrug produced from MK4 inhibits liver tumour progression and significantly reduces plasma DCP levels [43].

Furthermore, patients in the early stage of HCC, as well as HCC associated with hepatitis B or cirrhosis, show an increase in PIVKA-II, which is a protein induced by a deficiency in or antagonism of vitamin K2 [44]. Elevated levels of PIVKA-II may be a helpful marker of HCC in monitoring of patients with cirrhosis of the liver with nodular remodelling visible in ultrasonography [45]. The level of PIVKA-II may be used not only as a diagnostic marker of cancer but also for screening HCC high-risk groups [46]. An increasing level of PIVKA-II after HCC treatment may prognose a poor outcome [47]. An increase in HCC relapse within one year after hepatectomy in people with elevated PIVKA-II levels was noted [48]. It can also be used as a prognostic factor in the early stages of HCC, especially in patients with a low number of tumours in the liver. There is probably a correlation between PIVKA-II production and the occurrence of extrahepatic metastases [49]. A decrease in HCC recurrences as well as an increase in 5-year survival was observed in people following liver donor transplant at a PIVKA-II level  $\leq 400$  mAU/mL. In patients below 65 years with a PIVKA-II level  $\geq 200$  mAU/mL, with the presence of a liver tumour  $\geq 5.0$  cm and with undifferentiated HCC, an increase in the risk of vascular microinvasion was observed [50].

## INFLUENCE OF VITAMIN K ON THE LIVER

It has been observed that patients with cystic fibrosis and cirrhosis often have a vitamin K deficiency and a more severe disease course. It turned out that it was not the cirrhosis but the *F508del CFTR* mutation in cystic fibrosis that is an independent risk factor for vitamin K deficiency [51].

## THE ROLE OF VITAMIN K IN THE NERVOUS SYSTEM

Vitamin K significantly influences the functioning of the nervous system. It has been shown that vitamin K is involved in the synthesis of sphingolipids which are elements of cell membranes and myelin. The role of vitamin K in proper function of the nervous system is also associated with the activity of Gas6 protein, which depends on it. Activity of this protein leads to cell growth acceleration. Furthermore, interaction between Gas6 protein and its receptor counteracts neuronal apoptosis. The described properties of vitamin K are supportive of its use in prevention of neurodegenerative diseases, such as Alzheimer's disease [52]. Vitamin K also has a significant impact on improvement in metabolic and nutritional status in people with autism. A randomized, double-blind trial of vitamin administration conducted for 3 months showed an improvement in the level of oxidative stress, methylation and adenosine-5'-triphosphate (ATP), reduced form of nicotinamide adenine dinucleotide (NADH), reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) in the study group, when compared to the controls. The greatest impact on improvement was attributed to the initial level of vitamin K and biotin. An interesting discovery was also made about the neuroprotective potential of vitamin K against the harmful effects of mercury on neurons [53].

## ANTI-CANCER PROPERTIES OF VITAMIN K

An experimental study was done on the effects of menadione (vitamin K<sub>3</sub>) in combination with sodium orthovanadate on the survival of glioblastoma multiforme cells. During a 10-day exposure to the cytotoxic effect of these substances, no re-growth of cells during re-incubation after the removal of active substances were observed. The obtained results encourage further research on the application of vitamin K in the treatment of glioblastoma multiforme and create hope for better therapeutic effects [54, 55].

Clinical trials demonstrated a pro-apoptotic effect of vitamin K on cancer cells. An experiment carried out in Japan analysed the impact of vitamin K<sub>2</sub> on growth inhibition and induction of apoptosis in human lymphoma and multiple myeloma. Neoplastic cells were cultured in various concentrations of menaquinone with or without dexamethasone or allopurinol. The experiment revealed that myeloma cells and B-cell lymphoma cells were sensitive to vitamin K<sub>2</sub>. The effect of allopurinol on growth inhibition has been demonstrated in myeloma cells, but not in lymphoma cells [56].

The interest in vitamin K in oncology is not limited to hematological cancers, HCC or glioblastoma multiforme. Menaquinone has pro-apoptotic properties and also stopped the cell cycle in a gastric cancer, a breast cancer and a bladder cancer [57]. On the basis of the conducted research, it can be concluded that vitamin K<sub>2</sub> has a high potential in oncology and may in future improve the curability of malignancy.

## CONCLUSIONS

Vitamin K acts on many systems of the body through its dependent proteins, or regulation of gene transcription, thus it plays a much larger role than previously assumed. Beyond haemostasis, where vitamin K is a key element in coagulation factor production, it also affects the circulatory system. Vitamin K deficiency correlates with an increase in the risk of ischemic heart disease, as well as arterial hypertension from vascular calcification. Vitamin K is also an essential element of bone metabolism and a deficiency can contribute to fractures. Vitamin K supplementation can enhance the therapeutic effect of other drugs used in the treatment of osteoporosis, such as vitamin D<sub>3</sub>. A link between vitamin K and the beneficial effect of vitamin K in treating osteoarthritis and rheumatoid arthritis was also noted. The vitamin also shows neuroprotective effects on the nervous system, which can be applied to treat neurodegenerative diseases. Anticancer properties of vitamin K have also been observed in treatments of many types of cancer, including glioblastoma multiforme, multiple myeloma, B-lymphoma of stomach, breast, and bladder. In HCC vitamin K can contribute to tumour regression, prevent cancer cell growth and invasion, and may contribute to the prevention of metastasis and recurrence of the disease.

In conclusion, vitamin K shows beneficial effects on the functioning of many organs and systems in the human body, however its effects require a closer look by conducting additional randomized multicentre clinical trials.

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