

Vitamin K: an old vitamin in a new perspective

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Keywords: bone health, cardiovascular health, matrix GLA protein, menaquinone-7, osteocalcin, phyloquinone, vitamin K

The topic of "Vitamin K" is currently booming on the health products market. Vitamin K is known to be important for blood coagulation. Current research increasingly indicates that the antihemorrhagic vitamin has a considerable benefit in the prevention and treatment of bone and vascular disease. Vitamin K₁ (phyloquinone) is more abundant in foods but less bioactive than the vitamin K₂ menaquinones (especially MK-7, menaquinone-7). Vitamin K compounds undergo oxidation-reduction cycling within the endoplasmic reticulum membrane, donating electrons to activate specific proteins via enzymatic gamma-carboxylation of glutamate groups before being enzymatically reduced. Along with coagulation factors (II, VII, IX, X, and prothrombin), protein C and protein S, osteocalcin (OC), matrix Gla protein (MGP), periostin, Gas6, and other vitamin K-dependent (VKD) proteins support calcium homeostasis, inhibit vessel wall calcification, support endothelial integrity, facilitate bone mineralization, are involved in tissue renewal and cell growth control, and have numerous other effects. The following review describes the history of vitamin K, the physiological significance of the K vitamins, updates skeletal and cardiovascular benefits and important interactions with drugs.

Vitamin K: A Review of its History

The discovery of vitamin K can be traced back to the research of Carl Peter Henrik Dam at the Biochemical Institute of the University of Copenhagen from 1928 to 1930. In his work on cholesterol metabolism, the Danish biochemist observed a spontaneous tendency to hemorrhage in chicks fed for longer than 2 to 3 weeks on cholesterol- and fat-free chicken feed. This coagulation disorder was combined with a lowered prothrombin content (prothrombin = factor II) of the blood.¹⁻³ At that time, as none of the hitherto known vitamins (e.g. vitamins A, C and D) were capable of preventing the coagulation disorder, Dam postulated a new, fat-soluble vitamin, which regulates coagulation. The latter was apparently present in green vegetables and liver, as supplementary feeding with these nutrients resulted in normal blood coagulation in the animals. Moreover, Dam successfully treated the chickens' hemorrhages with an ether extract obtained from lucerne (alfalfa). Dam called the antihemorrhagic vitamin "vitamin K" (after "Koagulation:" coagulation).⁴

In the 1930s, several working groups researched the isolation and identification of vitamin K. At this time, a US American research group working with the biochemist Edward Albert Doisy succeeded in isolating the antihemorrhagic vitamin K and elucidating its chemical naphthoquinone ring structure. In 1943, the 2 researchers, Dam und Doisy, were jointly awarded the Nobel prize for medicine for the discovery and elucidation of the chemical structure of vitamin K.^{5,6}

The precise biochemical function of vitamin K was not finally resolved until the end of the 1970s. As a coenzyme, vitamin K is essential for the γ -carboxylation of specific glutamic acid (Glu) residues in a number of vitamin K-dependent proteins. The resultant γ -carboxyglutamic acid (Gla) compounds can effect complex binding of calcium ions, leading to a protein conformational change, which is a precondition for its physiological function. In this way, e.g., by means of posttranslational modification, the clotting factors II (prothrombin), VII, IX and X develop from precursors.⁷⁻⁹

In this context, the significance of the vitamin K cycle was also recognized: γ -carboxylation is catalyzed by a microsomal carboxylase and requires CO₂ and molecular oxygen. Vitamin K hydroquinone is required as a cofactor. The oxidation of the hydroquinone to vitamin K 2,3-epoxide supplies the energy required for the abstraction of a proton of the gamma carbon of the glutamic acid (Glu) residue, resulting in a carbanion, which is then carboxylated to γ -carboxyglutamic acid (Gla) (Fig. 1). Vitamin K 2,3-epoxide is subsequently regenerated to vitamin K hydroquinone by the enzymes vitamin K epoxide and quinone reductase.¹⁰⁻¹² The γ -carboxylation is thus characterized by a cyclical transformation, in which oxidised and reduced forms of vitamin K are involved as the driving factors. The inhibition of these 2 enzymes by vitamin K antagonists, such as phenprocoumon and warfarin, has considerable medical significance, which is utilised in anticoagulation therapy.¹³ Currently, approximately 14 vitamin K-dependent proteins are known, with broad

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Submitted: 06/30/2014; Accepted: 09/07/2014

<http://dx.doi.org/10.4161/19381972.2014.968490>

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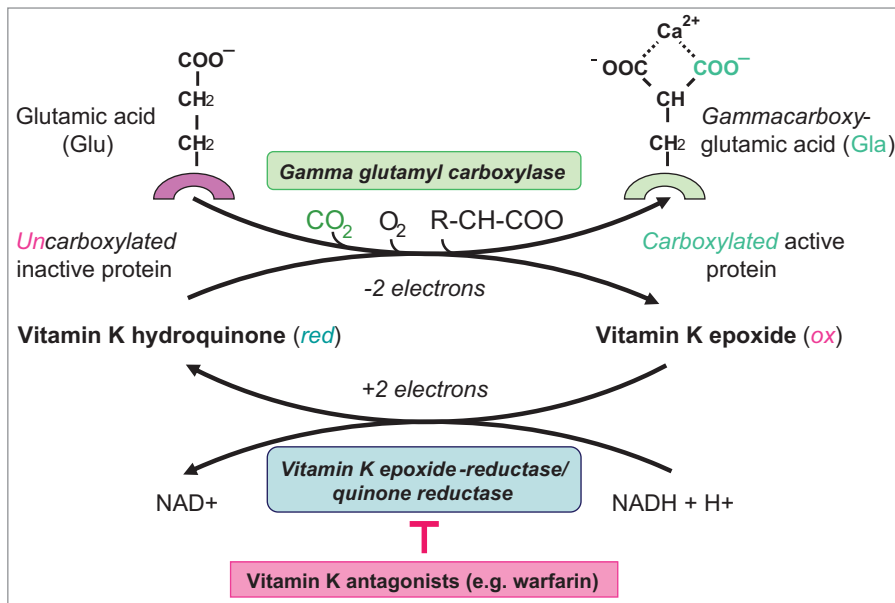


Figure 1. In the vitamin K cycle, vitamin K-dependent gamma-carboxyglutamic acid (Gla) proteins are carboxylated and activated.

spectrum efficacy on haemostasis, calcium metabolism, control of cell growth, apoptosis and signal transduction (Table 1).¹⁴⁻¹⁷ Following elucidation of the vitamin's haemostasiological significance and research into further vitamin K-dependent Gla proteins, such as osteocalcin (BGP = bone Gla protein) and matrix Gla protein (MGP), current research is focusing on the vitamin's effect on bone and vascular health.^{10,13,18}

The K Vitamers: Nomenclature, Structure and Occurrence

Vitamin K is not a single unified substance, but rather a group of closely related derivatives with a 2-methyl-1,4-naphthoquinone structure as a common framework. All vitamin K derivatives contain this framework, which is also called menadione. Menadione is not a naturally occurring substance, but it can be manufactured synthetically and it is also known as *vitamin K₃*. The individual substances from the K vitamin group are also known as K vitamers. They differ

from each other mainly with regard to the length and saturation of the isoprenoid side chain at C3.¹⁶

The most important naturally occurring K vitamins are phyloquinone (2-methyl-3-phytyl-1,4-naphthoquinone, phytonadione), which is contained in green plants and also known as *vitamin K₁*, and menaquinone, with side chains of varying length, which is formed from intestinal bacteria (e.g., Bacteroides) and is also known as *vitamin K₂*. Vitamin K₁ contains a phytyl side chain with 20 C atoms, i.e. a monounsaturated, lipophilic side chain with 4 isoprene units. In plants, for example, phyloquinone is a functional and structural component of photosynthesis. Vitamin K₁ is synthesized by plants and algae. It thus occurs mainly in green leafy vegetables, such as kale (145 µg/100 g), Brussels sprouts (177 µg/100 g), broccoli (180 µg/100 g) and spinach (380 µg/100 g), accounting for approximately 90% of the vitamin K ingested with the diet. In addition, vitamin K is found in

some vegetable oils (e.g., soya oil: 193 µg/100 g, rapeseed oil: 127 µg/100 g) and in foods of animal origin (e.g., liver: 5 µg/100 g and eggs: 2 µg/100 g).¹⁹⁻²¹

Vitamin K₂ consists of a group of menaquinones, which are characterized by the length of their isoprenoid side chain, a lipophilic, polyunsaturated side chain of variable length (Fig. 2). A menaquinone with 7 isoprenoid units was formerly called vitamin K₃₅, as one isoprenoid unit contains 5 C atoms. Today, menaquinones are generally called MK-n, where n signifies the number of isoprenoid units. With regard to preventive and therapeutic aspects, menaquinone-4 (MK-4) and menaquinone-7 (MK-7) are among the most important forms of vitamin K₂ with 4 and 7 isoprenoid units, respectively.^{16,19,22} Menaquinones are mainly found in foods of animal origin, such as bovine liver and in bacterially fermented foods, such as yoghurt and some types of cheese (e.g., MK-8 and MK-9: 5–20 µg/100 g). The richest source of MK-7 at 10 µg/g is a Japanese dish called natto, which has a long nutritional tradition and is made from bacterially fermented soya beans.

Table 1. Vitamin K-dependent gamma carboxyglutamate (Gla) proteins (e.g., prothrombin, osteocalcin)

Vitamin K dependent Gla protein	Function
Liver	Hepatic carboxylation
Clotting factors II, VII, X and XII	Haemostasis (procoagulant activity)
Protein C, S and Z	Haemostasi (anticoagulant activity)
Various tissues	Extra hepatic carboxylation
Osteocalcin	Calcium and bone metabolism
Matrix-Gla-Protein	Inhibitor of vascular calcification (cartilaginous tissue, vascular wall of the vascular smooth muscle cells)
Growth-arrest specific gene 6 (Gas6)	Cell growth (endothelium, smooth muscle cells), apoptosis, phagocytosis (?)
Transmembrane GLA-protein	Signal transduction to phosphatidylserine (?)
Periostin	Bone metabolism, cell migration, angiogenesis (?)
Other: carboxylase, transthyretin, Gla-rich-Protein (GRP)	To date mainly unknown

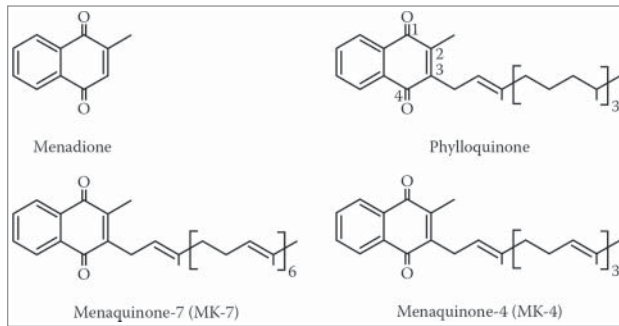


Figure 2. Structural formulae of biologically active K vitamins.

The bacterium that produces MK-7 in soya is called *Bacillus subtilis natto*. The earliest written documentation on natto can be found in the Japanese book “Shin Sarugakki” by Fujiwara no Aki-hira, who lived from 989–1066 BC.^{23–25}

The Vitamin K Requirement

There are no precise recommendations for the vitamin K requirement and those of the nutritional associations are usually based on the hepatic requirement for the formation of blood clotting factors. Taking the plasma thrombin level into consideration, a daily vitamin K intake of 1 µg per kg body weight is recommended as being adequate for all age groups beyond the neonatal phase.

In a recent study by the University of Maastricht, however, in which 896 blood samples from healthy subjects were analyzed, it was shown that, although all coagulation proteins were completely carboxylated by vitamin K, a high concentration of uncarboxylated Gla proteins (osteocalcin, matrix Gla protein) was present in the majority of the subjects investigated. Uncarboxylated osteocalcin (ucOC) and uncarboxylated matrix Gla protein (ucMGP) are functional laboratory parameters for a vitamin K deficiency and are associated with an increased risk of bone fractures and vascular complications. Based on the results of this study, it must be assumed that the majority of the population has an inadequate supply of vitamin K.²⁶

Effect of Vitamin K on the Bones and Vascular System

As a result of vitamin K-mediated γ -carboxylation, the various Gla proteins can bind calcium ions and are activated in this way. Carboxylated osteocalcin (cOC) binds calcium in the bone tissue, which is incorporated into the hydroxylapatite of the bone with the help of the osteoblasts. A low dietary vitamin K intake and high proportion of uncarboxylated osteocalcin (ucOC) are independent risk factors for hip fractures.^{27–30}

The production and activation of osteocalcin (OC) is regulated by vitamin K and 1,25-dihydroxyvitamin D [1,25(OH)₂D; calcitriol].^{30,31} 1,25(OH)₂D promotes the transcription of the

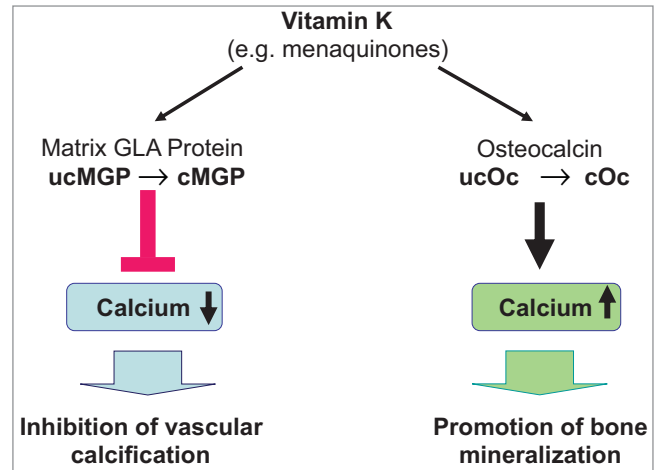


Figure 3. Effect of vitamin K on bone and vascular health.

osteocalcin gene, whereas vitamin K promotes the posttranscriptional carboxylation of Gla residues in the osteocalcin propeptide.^{31,32} Furthermore it was demonstrated that 1,25(OH)₂D enhances the activity of γ -glutamyl carboxylase, suggesting that the carboxylation of osteocalcin is stimulated by vitamin D and that menaquinone-4 stimulates 1,25-dihydroxyvitamin D₃-induced mineralization by human osteoblasts.³³ There is growing evidence about the synergistic effect on bone health of vitamin K and vitamin D. But further data is required in order to have a complete understanding of the complex interaction between vitamin K, vitamin D and bone metabolism.

Whereas carboxylated osteocalcin (cOC) promotes the incorporation of calcium into the bone matrix, thus supporting bone metabolism, the vitamin K-dependent matrix Gla protein (cMGP) counteracts vascular calcification and age-related wear and tear on the arteries and protects the blood vessels from calcium overload (Fig. 3).^{34,35} There are increasing indications that normal dietary intake of the vitamin K allowance recommended by the nutritional associations is insufficient for the γ -carboxylation of osteocalcin and matrix Gla protein.²⁶

Bone health

In the Nurses’ Health Study, which investigated 72,327 women aged from 38–63 years, the effect of daily vitamin K intake on bone fragility was investigated over a 10-year period. It was shown that women with a daily vitamin K intake of ≥ 109 µg had a 30% reduction in the risk of hip fracture compared to women with an intake of < 109 µg (RR: 0.70; 95% CI: 0.53, 0.93).²⁸ In a double-blind, placebo-controlled study with 55 adolescents, the proportion of uncarboxylated osteocalcin (ucOC) was significantly reduced compared to placebo by a daily supplement of 45 µg vitamin K₂ as menaquinone-7 and the proportion of carboxylated osteocalcin (cOC) was increased, indicating improved bone mineralization.³⁶

A meta-analysis of 13 randomized controlled studies investigated the effect of vitamin K supplementation as vitamin K₁ (1–10 mg daily) or vitamin K₂ (15–45 mg MK-4 daily) on the

fracture rate and bone density. It was shown that, compared with placebo, particularly vitamin K₂ as MK-4 reduces the risk of vertebral fractures by 60% (OR: 0.40; 95% CI, 0.25–0.65), of hip fractures by 77% (OR: 0.23; (95% CI, 0.12–0.47) and of non-vertebral fractures by 81% (OR: 0.19; 95% CI, 0.15–0.35).³⁷ In a recent 3-year placebo-controlled study in 244 healthy postmenopausal women, a daily supplement of 180 µg vitamin K₂ as MK-7 led to a significant improvement in bone density, bone health and bone strength. The quotient of ucOC/cOC served as a marker for the vitamin K status and was considerably improved by MK-7.³⁸

Increased levels of ucOC are also found in patients with fractures during treatment with amino-bisphosphonates. In a randomized study in 241 postmenopausal women, a supplement of 45 mg vitamin K₂ (MK-4, menaquinone-4) over a period of 24 months led to a significant rise in carboxylated osteocalcin (cOC) and a significantly reduced fracture rate compared with the control group. The osseous efficacy of the bisphosphonates (e.g., risedronate) used in osteoporosis therapy can be improved by concurrent supplementation with vitamin K₂ (MK-4: 45–60 mg/d), which has been confirmed by the results of clinical studies.^{39–42}

Vascular health

In the Rotterdam Study, a large-scale, population-based study with 4,807 Dutch women and men (age: 55+), the effect of dietary vitamin K₁ and vitamin K₂ over a 10-year period (1990 to 2000) was investigated with regard to the risk of coronary heart disease, arterial calcification and overall mortality. This study found that vitamin K₁ (intake: ~250 µg/day) had no protective effect on the cardiovascular system or overall mortality. Vitamin K₂ (intake: ~25 µg/day) reduced the relative risk of dying of heart disease by 57%. Vitamin K₂ also markedly reduced the occurrence of coronary heart disease (by 41%) and overall mortality (by 36%). Vitamin K₂ even reduced the risk of severe arterial calcification by 52% (OR: 0.48).⁴³

Carboxylated MGP is an important inhibitor of vascular calcification. Accordingly, uncarboxylated MGP (ucMGP) is an independent risk factor for arteriosclerosis. In a recent placebo-controlled study, a daily supplement of 180 µg or 360 µg MK-7 led to a significant reduction in uncarboxylated MGP (ucMGP) of 31% and 46%, respectively, compared with placebo.⁴⁴ Due to premature vascular calcification, dialysis patients have a higher cardiovascular risk. In those affected, elevated levels of uncarboxylated MGP are frequently present, indicating insufficient dietary intake of vitamin K. In a recent study with dialysis patients, supplementation with 360 µg, 720 µg or 1080 µg MK-7 3 times weekly over an 8-week period significantly reduced the proportion of inactive MGP by 17%, 33% and 46%, respectively.^{45,46} It must therefore be assumed that vitamin K₂ supplementation can improve the individual cardiovascular risk in cardiovascular and dialysis patients.

In addition, the protective effect of vitamin K₂ in vascular disease makes it of interest for patients with diabetes mellitus. In patients with diabetes, elevated levels of uncarboxylated MGP are also associated with an increased risk of vascular calcification. Furthermore, a recent placebo-controlled study in 42 healthy men showed that supplementation with 30 mg vitamin K₂ as MK-4 (3× daily) improved the insulin production and insulin sensitivity of the cells through activation of osteocalcin (ucOC → cOC), compared with placebo. Carboxylated osteocalcin (cOC) appears to be an endogenous hormone, which also improves insulin metabolism.^{45,46} Also of interest are the results of animal studies, which showed that accumulation of vitamin K₂ (MK-4) in the arterial wall was 3 times higher than that of vitamin K₁. In this context, arterial calcification triggered by warfarin was also completely prevented by vitamin K₂ but not by vitamin K₁. As a result of its isoprenoid-rich structure, vitamin K₂ also appears to have a favorable effect on cholesterol values.⁴⁷

Further therapeutic indications

Due to its anti-inflammatory, anti-oxidative and anticarcinogenic properties, vitamin K, particularly MK-7, may be of interest in a number of other diseases (e.g., cancer, diabetes, age-related macular degeneration [AMD]); over the next few years, studies will show whether this is the case. Furthermore, because of its structural similarity to coenzyme Q10, it is likely that MK-7 is a Q10 mimetic with respect to the mitochondria and supports mitochondrial adenosine triphosphate (ATP) production in the respiratory chain.

K Vitamers: Differences in Efficacy

Of the various K vitamers, i.e., phylloquinone and vitamin K₂ as menaquinone-4 (MK-4) or menaquinone-7 (MK-7), vitamin K₁ and menaquinone-7 are of most interest as food supplements. MK-7, which is obtained from natto, shows some physico-chemical

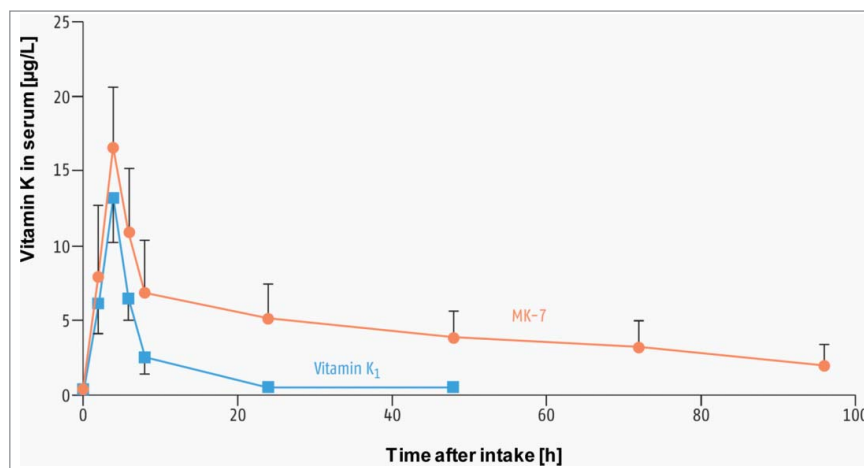


Figure 4. Comparison of oral bioavailability of vitamin K₁ and MK-7: vitamin K serum levels following a single dose of 1 mg vitamin K₁ or 1 mg MK-7.⁴⁹

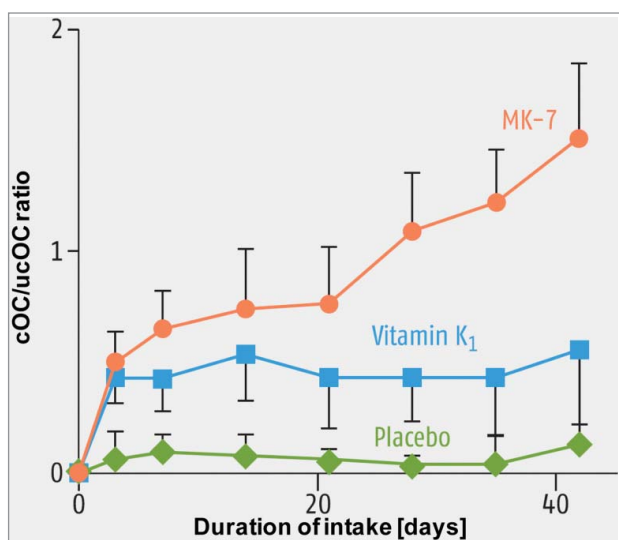


Figure 5. Carboxylation of osteocalcin by vitamin K₁ and MK-7: Vitamin K₁ or MK-7 were supplemented daily at a dose of 0.22 μmol in the form of tablets or capsules. Initially, the ratio between carboxylated (cOC) and uncarboxylated osteocalcin (ucOC) was 1.74 in the MK-7 group, 1.8 in the vitamin K₁ group and 1.7 in the placebo group. In the placebo group, only vitamin K₁ was determined. After approximately 3 days, vitamin K₁ and MK-7 increased the carboxylation of osteocalcin, but only the intake of MK-7 led to a further increase in the degree of carboxylation.⁴⁹

advantages compared with vitamin K₁. Due to its molecular structure, menaquinone-7 (MK-7) is more lipophilic and has a much longer half-life (3 days) than vitamin K₁. Regular MK-7 intake therefore results in blood levels that are not only more stable but also approximately 7–8 times higher. Compared with vitamin K₁, distribution of MK-7 in the various tissues is significantly better. MK-7 is thus more efficient in the carboxylation of extrahepatic (e.g., osteocalcin) and hepatic (e.g., prothrombin) proteins (Figs. 4, 5).^{48,49} Compared with MK-7, no oral bioavailability has been determined for MK-4 at nutritional doses (e.g., 420 μg MK-4). Therefore, the small quantities of MK-4 contained in the diet do not contribute to the buildup of vitamin K status or to the degree of carboxylation of vitamin K-dependent proteins.⁵⁰

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Interaction with Vitamin K Antagonists

As a result of the considerably better bioavailability of MK-7, the risk of a pharmacodynamic interaction with vitamin K antagonists is also markedly higher than with vitamin K₁. Whereas studies showed a reduction in the International Normalized Ratio (INR) value (from 2 to 1.5) at an intake of >300 μg vitamin K₁ daily, this occurred at >100 μg with MK-7. Many nutritional supplements are currently marketed at a daily dosage of 45 μg and over. Recent dose-finding studies at the University of Maastricht, which investigated the effect of 10 μg , 20 μg and 45 μg MK-7 daily on the anticoagulant properties of vitamin K antagonists, showed that even with a daily supplement of < 10 μg MK-7, a significant disturbance of blood coagulation control can occur. Dr Theuwissen's working group therefore advises against MK-7 supplements in patients undergoing treatment with vitamin K antagonists.^{48,51}

Summary for Clinical Practice

Vitamin K—particularly MK-7—is currently enjoying a genuine boom in the health products branch, comparable with the vitamin D boom of around 3 years ago. For preventive purposes, a recommended daily supplement of 0.5–1.0 μg MK-7 per kg body weight is an acceptable guideline. In the treatment of diseases such as osteoporosis, the equivalent daily dose would be 2–4 μg per kg body weight. Medical and pharmaceutical practitioners should be conversant with the basic aspects and particular features of the K vitamers in order to offer patients competent advice. Whereas recent studies show that vitamin K₂ is gaining importance in the prevention and therapy of bone and vascular disease, its high interaction potential with anticoagulants remains a problem!

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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