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Vitamin K and metabolic bone disease

Cees Vermeer, Marjo H J Knapen, Leon J Schurgers

Originally, vitamin K was defined as a factor concerned with haemostasis, deficiency resulting in a haemorrhagic disease.[1] After the initial discovery of phylloquinone in 1929, various compounds were described which all had "vitamin K activity," that is, the ability to restore normal haemostasis in vitamin K deficient animals.[2] It turned out that "vitamin K" is a group name for several related compounds that are characterised by a common naphthoquinone ring structure substituted with a methyl group at position 2 and an aliphatic side chain at position 3. Differences between the various K vitamers consist of variations in the length and degree of saturation of the aliphatic side chain. Phylloquinone (vitamin K-1) has an aliphatic side chain of four prenyl residues, the first of which is unsaturated. In natural menaquinones (vitamin K-2) the number of prenyl residues may vary from 4 to 13, and all are unsaturated. Menaquinones are usually denominated as MK-n, where n stands for the number of prenyl residues.

Vitamin K is one of the few vitamins with a precisely known function at the molecular level, and its activity can be tested in vitro using purified enzyme systems,[3] where it serves as a cofactor in the post-translational conversion of protein bound glutamate into γ-carboxyglutamate, better known as Gla (fig 1). The reaction is an enzymatic carboxylation reaction, in which the coenzyme vitamin K is oxidised into an epoxide, thus providing the energy to drive this reaction.[4] Vitamin K deficiency thus leads to the synthesis of undercarboxylated proteins. Since Gla residues are calcium binding groups which are essential for the biological activity of the proteins in which they are found, undercarboxylated proteins have a poor affinity for calcium, and a low biological activity in all cases where their function is known.

Department of Biochemistry and Cardiovascular Research Institute, Maastricht University, Maastricht, The Netherlands
C Vermeer
M H J Knapen
L J Schurgers

Correspondence to:
Dr C Vermeer, Department of Biochemistry, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands.

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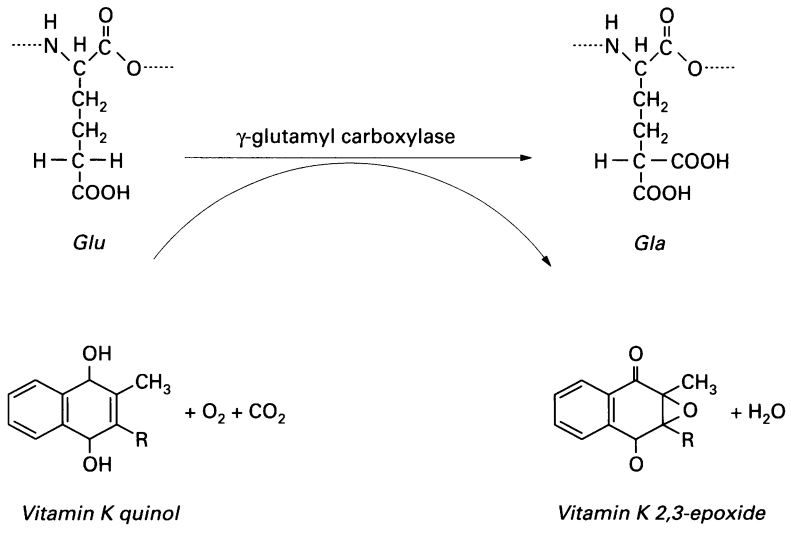

Figure 1 The vitamin K dependent step in the post-translational conversion of glutamate (Glu) into γ-carboxyglutamate (Gla). The energy required for the reaction is obtained from the oxidation of vitamin quinol into an epoxide.

Table 1 Vitamin K content (ng/g) of various food items

Food item	*Phylloquinone*	*Menaquinone-4*	*Menaquinone-9*
Meat, fish	10–40	10–100	0–20
Pork liver	2–5	3–5	10–20*
Milk, yoghurt	4–10	4–10	0–20
Cheese, curd	20–100	20–100	400–700
Green vegetables	2000–8000	0	0
Fruit	1–30	0	0
Bread	5–30	0	9–20

The various food items were homogenised in a blender and extracted with hexane. Vitamin K analysis was performed after prepurification of the hexane fraction as described earlier.[6] All data are based on fresh food and are expressed per g wet weight.
*No MK-9 present; data are for the sum of MK-7 + MK-8.

Vitamin K in food

Various assay procedures for vitamin K have been developed, and most of them are based on the reduction of the quinone into the quinol form followed by fluorescent or electrochemical detection.[5] It appeared that phylloquinone is present in many different food items, but in general the concentrations are extremely low. High phylloquinone concentrations are only found in green vegetables like spinach, kale, and broccoli, with intermediate values for dairy produce (table 1). Menaquinones (notably MK-7, MK-8, and MK-9) are mainly found in animal liver, curd and cheese, and fermented foods like sauerkraut and natto. Meat may contain substantial amounts of MK-4, which probably originates from the menadione present in the fortified foods that our cattle receive nowadays to ensure optimal performance. From table 1 it can be seen that people who do not regularly eat green vegetables will have a low nutritional vitamin K intake. This may decrease further if cheese and curd do not form part of the daily menu. Using food frequency questionnaires, Jie *et al* observed that between 60 and 70 years of age the mean nutritional vitamin K intake drops by roughly 50%.[7]

Vitamin K and bone

In 1975 it became clear that babies from mothers using vitamin K antagonists were at risk of a bone defect known as chondrodysplasia punctata, or the fetal warfarin syndrome.[8] This discovery initiated the search for Gla proteins in bone, three of which are presently known: osteocalcin,[9 10] matrix Gla protein,[11] and protein S.[12] Although it is generally assumed that at least osteocalcin and matrix Gla protein have a regulatory function in the formation of the bone mineral matrix, we do not know the precise function of any of these proteins. Recently, it was reported that both menaquinone-4 and

its aliphatic side chain alone inhibit osteoclast activity by inducing apoptosis.[13 14] This process does not seem to be mediated through Gla proteins, and it is unique to menaquinone-4.

The liver produces more than 80% of all known Gla proteins (blood coagulation factors), which are secreted into the bloodstream after their synthesis has been completed. About 15% of the protein bound Gla residues are formed in bone tissue, but only a small fraction is released into the blood stream. Most of the bone Gla proteins remain bound to the hydroxyapatite matrix, which results in very long half life times. Therefore the bone Gla proteins form about 80% of our total body store of Gla, whereas osteocalcin is one of the most abundant proteins in the human body. Since the liver is capable of extracting vitamin K from the blood stream with very high efficiency, a nutritional vitamin K deficiency leading to impaired blood coagulation is very rare. To maintain optimal carboxylation of the blood coagulation factors, a daily intake of about 0.5–1 μg/kg body weight is required.[15] Optimal carboxylation of the bone Gla proteins requires a higher vitamin K intake, and in recent years we have shown that undercarboxylation of osteocalcin is quite common, notably in the elderly.[16 17] Thus it is important to realise that vitamin K deficiency is a state which should be defined on the basis of individual tissues, not the whole organism.

As long ago as 15 years it was reported that low circulating phylloquinone levels were associated with osteoporotic hip fractures.[18 19] More recently these data were confirmed,[20] and it was found that plasma menaquinone concentrations were also substantially reduced in these patients.[21] Because it was not clear from these data whether the observed low circulating vitamin K levels were associated with a detectable vitamin K deficiency in bone tissue, Knapen *et al* developed an assay to assess the ratio between normal and undercarboxylated osteocalcin, and found that the fraction of undercarboxylated osteocalcin was increased in postmenopausal women.[16 17] Szulc *et al* reported that undercarboxylated osteocalcin is inversely correlated with bone mass,[22] and that undercarboxylated osteocalcin is an independent predictive marker of hip fracture risk.[23 24] We have confirmed these data in a group of women who were between one and 10 years postmenopausal, and we have found that age, body weight, and undercarboxylated osteocalcin are three independent variables from which the bone mineral density may be calculated with an accuracy of over 70% (Knapen M H J, *et al*, unpublished data). Remarkably, pathological effects of oral anticoagulants (vitamin K antagonists) on bone have only been reported for (human) fetuses[8] and for young animals,[25 26] while the data on adult bone are conflicting[27 28]—yet long term oral anticoagulant treatment is often given, for instance to prevent thrombosis or myocardial (re)infarction. The apparent discrepancy between vitamin K deficiency, and the use of vitamin K antagonists is consistent with an additional role for vitamin K in bone, independent of the formation of Gla residues in proteins.

Table 2 Effects of vitamin K supplementation on bone markers in postmenopausal women

Markers tested		*Effect*
Bone formation markers in serum:	bone alkaline phosphatase	↑
	osteocalcin	↑
Bone resorption markers in urine:	hydroxyproline/ creatinine	↓
	deoxypyridinoline/ creatinine	↓
Markers for vitamin K status:	undercarboxylated osteocalcin	↓
	urinary Gla excretion	↑

Data are from intervention studies in which fasting blood and urine samples were taken before and at the end of a period of vitamin K treatment (Konakion, 10 mg/d) ranging from two weeks to three months.

Vitamin K supplementation studies

So it became tempting to investigate whether vitamin K supplementation in postmenopausal women would reduce the rate of bone loss and thus postpone the occurrence of osteoporotic fractures. Several such studies have already been completed in Japan, and all showed a retardation of postmenopausal bone loss with vitamin K supplementation.[29 30] It remains to be seen, however, whether Japanese and Europid women are comparable in this respect, and it is regrettable that similar trials have not yet been reported from western countries. Data on the effect of vitamin K supplementation on bone markers are available, however, and they are summarised in table 2. In two subsequent studies, among 50 and 140 postmenopausal women respectively, it was shown that the daily administration of 1–10 mg of vitamin K is paralleled by a moderate increase in the serum markers for bone formation (osteocalcin and bone alkaline phosphatase), by a slight decrease in urinary hydroxyproline excretion (a marker for bone resorption), and by a reduction of urinary calcium loss.[16 17] The most prominent effects were obtained in fast losers of calcium. In later studies similar observations were made for bone loss due to hypo-oestrogenism and amenorrhoea in female elite athletes, and in microgravity induced bone loss during a six month space flight (Vermeer C, unpublished data).

Conclusions

Without doubt vitamin K is essential for the biosynthesis of three abundant bone proteins. The precise function of these proteins in bone biology is unknown, however. In cell culture systems, vitamin K may have an additional role, namely the regulation of cell growth. The physiological importance of this activity remains to be determined.

Vitamin K status should be considered in relation to individual tissues, not to the whole organism. Whereas nutritional vitamin K deficiency of the liver is very rare in humans, biochemically detectable vitamin K deficiency of bone tissue is quite common, notably in the elderly. This will result in increased serum

levels of undercarboxylated osteocalcin, which is associated with low bone mass and increased fracture risk.

Increased undercarboxylated osteocalcin concentrations may reflect either an isolated vitamin K deficiency state, or a more general nutritional deficiency associated with bone fragility. The former possibility is suggested by a limited number of clinical trials in which supplementation of postmenopausal women with vitamin K concentrates resulted in an improved balance between markers for bone formation and bone resorption. Although in some Japanese studies on osteoporotic patients a beneficial effect of vitamin K on bone mass was also observed (by dual energy x ray absorptiometry), there are insufficient data available to justify the recommendation of increased vitamin K intake by subjects at risk for osteoporosis the western populations.

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