IMPORTANCE OF VITAMIN K FOR BONE HEALTH

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Abstract

Vitamin K, which serves as a cofactor for the endoplasmic enzyme gammaglutamate-carboxylase, plays an important role in many biological processes, such as bone and vascular protection. At the bone level, poor vitamin K status leads to the undercarboxylation of osteocalcin. In the elderly, this is an independent risk factor for increased postmenopausal bone loss and osteoporotic fractures. High vitamin K intake has been shown to have a bisphosphonate-like activity, thus improving both bone strength and geometry.
Key words: Vitamin K, osteocalcin, bone strength, osteoporosis

Rezumat

Importanţa vitaminei K pentru sănătatea osului

Vitamina K este cofactorul enzimei endoplasmice gamaglutamat-carboxilaza şi astfel joacă un rol important în numeroase procese biologice, cum ar fi protecţia osului şi a vaselor. La nivelul osului, nivelul inadecvat de vitamină K produce carboxilarea insuficientă a osteocalcinei. La persoane vârstnice acesta este un factor de risc independent pentru pierderea de masă osoasă după menopauză şi apariţia fracturilor osteoporotice. Aportul sporit de vitaminaă K are un efect similar cu al bisfosfonaţilor, putând ameliora deopotrivă rezistenţa şi geometria oaselor.
Cuvinte cheie: Vitamina K, osteocalcină, rezistenţa osului, osteoporoză

BACKGROUND

The function of vitamin K is to serve as a cofactor for the endoplasmic enzyme gammaglutamate carboxylase (GGCX). The vitamin K-dependent step is the conversion of protein-bound glutamate residues into gammacarboxy glutamate (Gla). The energy required for this carboxylation reaction is provided by the oxidation of vitamin K hydroquinone (KH_2) into vitamin K 2,3 epoxide (KO). KO can be recycled in two steps to its quinone and subsequently its hydroquinone form by the action of the enzyme vitamin KO reductase (VKORC1) (1). Only a limited number of Gla-containing proteins are known at this time including prothrombin and related clotting factors, as well as osteocalcin and matrix Gla-protein (MGP). In all Gla-proteins presently known the Gla-residues are absolutely required for function. Poor vitamin K status inevitably leads to the production of undercarboxylated or non-carboxylated Glaproteins, which are biologically inactive. Osteocalcin is a small protein exclusively synthesized in bone. At a molecular level its function is not precisely known.

VITAMIN K AND OSTEOCALCIN CARBOXYLATION

Since the liver is capable of extracting vitamin K from the blood stream with high efficacy, sub-optimal vitamin K status is reflected by poor osteocalcin carboxylation rather than by poor prothrombin carboxylation. We have demonstrated that incomplete osteocalcin carboxylation resulting from vitamin K deficiency of bone is common, especially during episodes of high bone metabolism observed in children and in postmenopausal women. It has been shown that poor osteocalcin carboxylation in the elderly is an independent risk factor for increased postmenopausal bone loss and osteoporotic fractures (2).

The function of osteocalcin at a molecular level has remained obscure thus far. Studies in transgenic osteocalcin deficient mice suggest that it is a negative regulator of bone growth, and that it is required for regular deposition of hydroxyapatite crystals in bone. On the other hand it should be kept in mind that bone contains also other Gla-proteins, so that osteocalcin may only serve as a convenient serum marker of bone vitamin K status, whereas other Gla-proteins are equally important (or even more important) for bone strength.

VITAMIN K MAY DECREASE POSTMENOPAUSAL BONE LOSS

We have found that vitamin K may retard bone loss in postmenopausal women (3). The effect, which

is probably attributed by both K_1 and K_2, was only found if vitamin K was combined with minerals (calcium, magnesium, zinc) and vitamin D. An example of such study is given in figure 1, and these data were recently confirmed in a British study. Bone loss is defined here as decrease in bone mineral density (BMD) as measured by dual energy X-ray absorptiometry (DXA). BMD may be regarded as a measure for the quality of bone. In this study (known as the Osteo-1 study), the rate of bone loss decreased by about 40% in the group treated with minerals, and the vitamins D and K.

VITAMIN K_2 (AND NOT K_1) ALSO IMPROVES BMC AND BONE GEOMETRY

Vitamin K is a group name for a series of related compounds differing in their aliphatic side chain. Hence wide differences are found between various K vitamins with respect to their hydrophobicity, plasma transport, biological half-life time and efficacy. Here we will distinguish between K_1 (phylloquinone) and menaquinone-4 (MK-4), which is one of the K_2 vitamins. In a second trial, known as the Osteo-2 study, we have monitored the effect of only MK-4 on BMD, bone mineral content (BMC) at the site of the femoral neck, as well as effects on relevant dimensions of the femoral neck, i.e. the hip axis length (HAL) and the femoral neck width (FNW) (4). Whereas BMD may be regarded as a measure for bone quality, BMC rather stands for the amount of bone present. In important difference with the Osteo-1 study is that in the Osteo-2 study no other vitamins or minerals were administered to the participants.

It was found that BMD was not affected by MK-4 alone, but that BMC increased in the vitamin K-treated group during the trial (see figure 2). So although the quality of bone declined in both the placebo group and the MK-4 group at a similar rate, the amount of bone at the site of the femoral neck was larger in the subjects in the vitamin K_2 group. This was even more obvious when we measured also the FNW: in the vitamin K_2 group we actually found an increase at the narrowest side of the femoral neck (see figure 3). This demonstrates that vitamin K_2 mediates in accrual of more bone at critical sites such as the femoral neck.

MAINTENANCE OF BONE STRENGTH DURING HIGH K_2 INTAKE

From the equations published by Karlamangla et al. (5), we were able to calculate various indices for bone strength at the site of the femoral neck: the compression strength index (CSI), the impact strength index (ISI) and the bending strength index (BSI), the latter being the most important one. The BSI is calculated from the BMD, HAL, FNW and body weight in the following formula:

$$BSI = (BMD * FNW2) / (HAL * weight)$$

As is shown in figure 3, the bone strength at the site of the femoral neck decreased in the placebo group, but remained constant in the vitamin K_2-treated one. A similar effect was observed for both other indices for bone strength (data not shown). This effect was independent of age. After obtaining this result, we have checked the DXA data from previous trials with vitamin K_1. Remarkably, no effect on FNW was ever observed with vitamin K1, however.

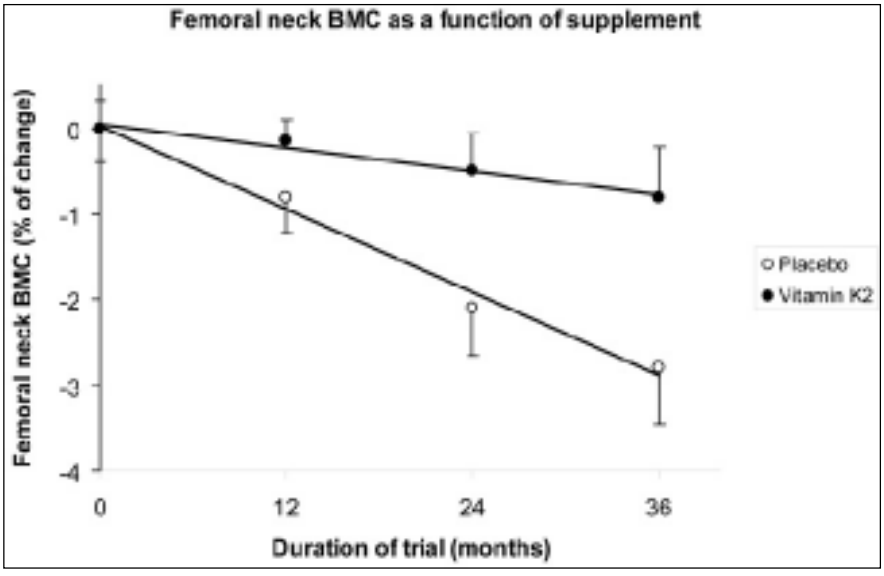

Figure 1
Femoral neck BMD as a function of supplement. 180 postmenopausal women between 55 and 65 years of age participated in this study and were subdivided into three arms of 60 subjects each. The following supplements given were given daily during three years with yearly measurement of the BMD: group I: placebo [□]; group II: calcium (500 mg), magnesium (150 mg), zinc (10 mg) and vitamin D3 (8 µg) [o]*; group III: the minerals as above, vitamin D_3 (8 µg) and vitamin K1 (1 mg)* [●]*. Bars represent SEM.*

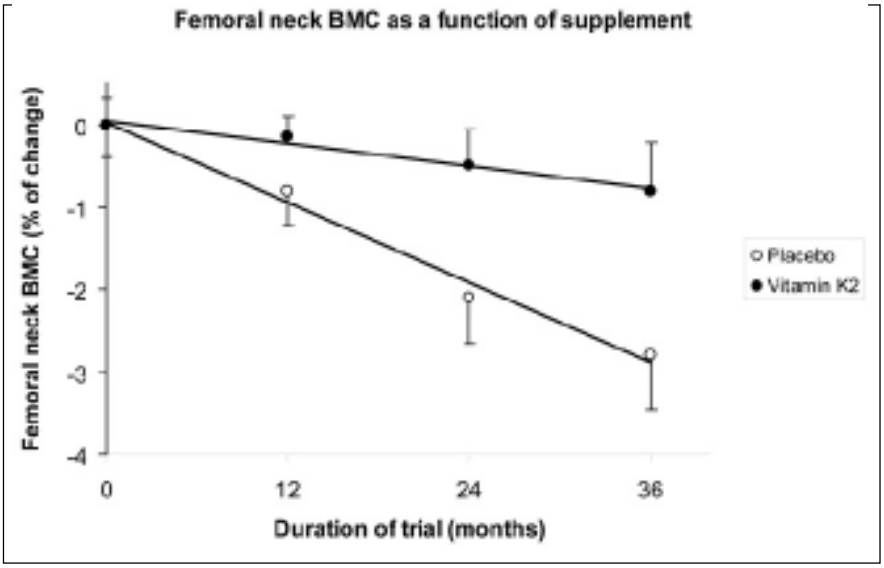

Figure 2
Femoral neck BMC as a function of supplement. 325 postmenopausal women between 55 and 75 years of age participated in this study and were randomized in two groups receiving either placebo or vitamin K_2 (MK-4, 45 mg/day for three years). Symbols: placebo [o]*, vitamin K_2* [●]*. Bars represent SEM.*

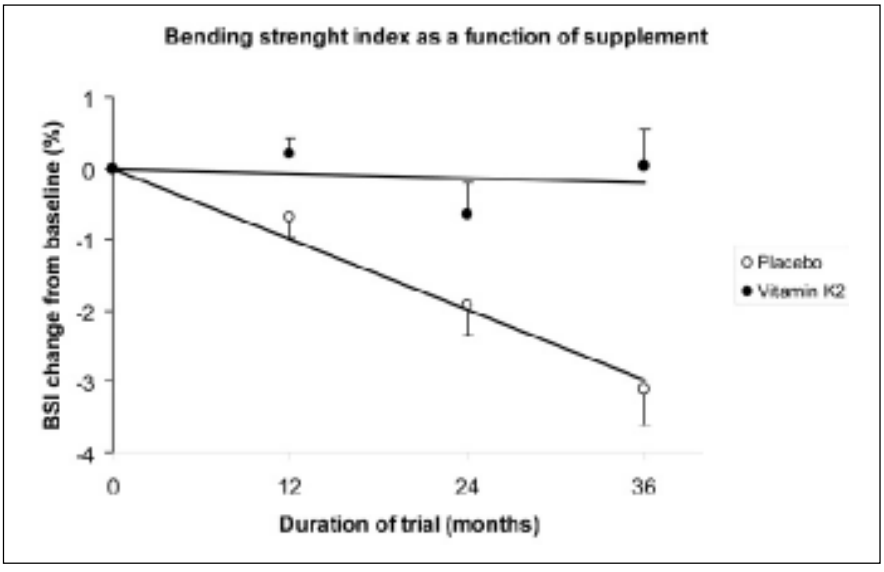

Figure 3
Bending strength index as a function of supplement. Data are from the Osteo-2 study. Further details are as described in the legend to figure 2.

VITAMIN K_2 (AND NOT K_1) HAS BISPHOSPHONATE-LIKE ACTIVITY

To find out differences in the molecular mechanism of vitamin K_1 and K_2 action, we have compared both vitamins in a well known in vitro system for monitoring the efficacy of bisphosphonates: the induction of apoptosis in J774 cells (6). J774 cells are osteoclast-like cells, and induction of apoptosis strongly in vitro suggests decreased bone resorption and retardation of bone loss in vivo. In the experiment described below we have compared the well known bisphosphonates risedronate with vitamins K_1, K_2, ubiquinone-4, and a-tocopherol. Obviously, only K_1 and K_2 have classical vitamin K activity (i.e. they serve as cofactors for gammaglutamate carboxylase via the naphthoquinone group); K_1, K_2, and ubiquinone-4 all three have a 4-isoprenoid side chain, which is phytol (only one unsaturated bond) in the case of vitamin K_1 and geranylgeraniol (four double bonds) in the case of K_2 and ubiquinone-4. a-Tocopherol was taken as a negative control. From table 1 it is clear that only the compounds with a geranylgeraniol side chain had bisphosphonates activity, and that this was independent of the classical vitamin K activity.

POTENTIAL MECHANISM FOR SYNERGISTIC EFFECT OF BISPHOSPHONATES AND VITAMIN K_2

N-containing bisphosphonates such as risedronate inhibit the mevalonate pathway at the step at which pyrophosphate is coupled to geranylgeraniol to form geranylgeraniol pyrophosphate, which is an activator of growth factors required for osteoclast activation. The bisphosphonates act as structural analogs and competitive inhibitors for pyrophosphate on the enzyme involved in the coupling of pyrophosphate and geranylgeraniol. Both vitamin K_2 (menaquinone-4) and ubiquinone-4 contain the geranylgeraniol side chain, and it seems at least plausible

Table 1
Apoptosis induction by various compounds. Mouse osteoblast-like macrophage J774 cells were grown in 12-well plates to a density of 5 x 105 cells/well. At that time the medium was replaced by medium containing one of the test compounds. Apoptotic body formation was quantified by flow cytometry using FITC-labeled annexin-V and propidium iodide followed by off-line calculation of the percentage of events with reduced forward and sideward scatter.

Concentration of additive	100 µM	25 µM
Compound added	% of cells in apoptosis	% of cells in apoptosis
None	19	19
Risedronate	87	n.d.
Vitamin K_1	15	13
Vitamin K_2 (MK-4)	68	76
Ubiquinone-4	79	74
α-Tocopherol	21	n.d.

that these compounds may act as competitive inhibitors in the same reaction. This explains why K_2, via its side chain, may have an extra function, which was not found for K_1 and it also explains why the dosages required for this effect are at least 100-fold higher than required for full osteocalcin carboxylation. If this will turn out to be true, we may expect to see synergistic effects of bisphosphonates and vitamin K_2, since together they may block the entire active site of geranylgeranyl pyrophosphate synthetase. New clinical trials have to be initiated to find out whether the efficacy of bisphosphonates may be increased by combining them with vitamin K_2 therapy.

CONCLUSIONS

The present recommended intake for vitamin K (all forms) is 90-120 µg/day, which is sufficient for optimal carboxylation of all Gla-containing blood clotting factors. Our research demonstrates, however, that at this intake the extra-hepatic Gla-proteins including osteocalcin and MGP are incompletely carboxylated; hence only a fraction of these proteins occurs in a fully active form. Our studies demonstrate that increased vitamin K intake may result in improved bone health, decreased bone loss and increased bone strength after menopause. The data presently available suggest that vitamin K_2 has a higher efficacy and may have an extra function, which is not displayed by vitamin K_1. Therefore, recommendations for dietary vitamin K intake should discriminate between K_1 and K_2. Until the outcomes of ongoing studies will be available, we recommend for healthy adults a daily intake of 100 µg vitamin K_1 and 50 µg K_2, preferably in the form of one of the higher menaquinones: menaquinone-7, -8, or -9.

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