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Action of vitamin K on bone health

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Summary

Objetives: Nowadays it is recognised that vitamin K plays an important role in bone health. It is necessary for the gamma-carboxylation of osteocalcin (the most important non-collagen protein in the bone), making the osteocalcin function. There are two important forms of vitamin K (vitamin K1 and vitamin K2), which come from different sources and have different biological activity.

Epidemiological studies suggest that a diet with high levels of vitamin K is associated with a lower risk of hip fractures in older men and in women. However, controlled randomised clinical trials, carried out with supplements of vitamin K1 or K2 in the white population do not show an increase in bone mineral density (BMD) in most of the different areas of the skeleton. Supplementation with vitamin K1 and K2 may reduce the risk of fracture, but the clinical trials which include fractures as a final result have methodological limitations, so clinical trials with greater numbers of patients, and which are better designed, would be needed in order to prove the efficacy of vitamin K1 and K2 in relation to fractures.

In conclusion, we may say that there is currently insufficient evidence to recommend the routine use of vitamin K for the prevention of osteoporosis and fractures in postmenopausal women.

Key words: *bone mineral density, fractures, menaquinones, osteoporosis, phylloquinones, vitamin K.*

Introduction

In the last two decades the use of nutritional supplements for the prevention of diseases has increased significantly in the developed nations. Calcium and vitamin D are the two main supplements used to achieve better bone health. There is also much interest in vitamin K. Vitamin K is better known for its functions in blood coagulation, but it is also important in bone metabolism.

We have carried out a review of the different forms and sources of vitamin K and its effects on bone mineral density (BMD) and on fractures.

Forms and dietary sources of vitamin K

The term vitamin K represents a group of liposoluble compounds, chemically similar, which differ in their origins and/or their functions. There are two natural forms of vitamin K: vitamin K1 and vitamin K2. Vitamin K1, also called phyloquinone or phytonadione, is synthesised by the plants and is the predominant form of vitamin K in the human diet¹. Its main sources are green-leafed vegetables (e.g. watercress, parsley, cabbage, spinach, lettuce), vegetables of the genus *brassica* (e.g. Brussels sprouts, broccoli), some fruits (e.g. avocado, kiwi fruit and white grapes), some herbs (e.g. parsley and coriander) and green or herbal teas. Other dietary sources are vegetable oils such as soya or olive, these being the supplements which are the most bioavailable¹. It is also found in liver, butter and minced beef.

Vitamin K2 includes a range of forms of vitamin K known as menaquinones-n (MK-n), where the n refers to the number of repeated units of 5-carbon. The principal menaquinones in the diet range from MK-4 to MK-10, and are mainly consumed in foods which contain fats, which favours its absorption and bioavailability over phyloquinone 2. The menaquinones are produced especially by bacteria, except MK-4 (or menatetrenone). In spite of its low bioavailability in foods, MK-4 is the predominant form of vitamin K in the body. For this reason, some scientists suggest that MK-4 is produced through the endogenous conversion of the endogenous phyloquinones as well as MK-7, -8 and -9². MK-4 may also be found in fish, eggs, liver, kidney, milk, butter, fermented cheeses and some vegetables. The quantity and types menaquinones in fermented products depend on the type of bacteria present in those products. Natto, a Japanese condiment of fermented soya is the richest dietary source of menaquinones (especially MK-7) currently known. The menaquinones with the longest chains (MK-10 to MK-13) are produced by the anaerobic bacteria of the colon, but have very low bioavailability and little activity, like vitamin K^{1,2}. MK-7 is the form which has the highest bioavailability and longest half-life compared with the phyloquinones and MK-4³.

Vitamin K in the diet is absorbed in the small intestine through a process which requires the presence of bile salts. After intestinal absorption, the vitamins K1 and K2 are transported in lipoproteins rich in triglycerides (chylomicrons) through the lymphatic system towards the liver and other

tissues. The vitamin K1 is primarily captured by the liver to be metabolized and excreted¹. A small proportion of vitamin K1, which returns to the circulation in particles of lipoproteins with very low density secreted by the liver, is transported to the extra-hepatic tissues¹. The menaquinones are transported by means of low density lipoproteins from the liver to the extra-hepatic tissues, such as the bone. The exception is MK-4, which is transported by both low and high density lipoproteins. Vitamin K1 and the long chain menaquinones are stored, essentially, in the liver, while MK-4 is stored predominantly in the brain, the reproductive organs, the pancreas and the glands¹.

Dietary recommendations

Only a small quantity of vitamin K is accumulated in the body². Although some studies have reported functions of vitamin K beyond its action in coagulation, as well as its role in bone metabolism, the Institute of Medicine has established that the daily recommended intake (DRI) for an adequate intake (AI) for men and women is based on the absence of abnormal haemorrhages⁴. The AI for vitamin K1 is 120 µg/day for men and 90 µg/day for women¹. Due the absence of known toxicity, an upper limit has not been established for vitamin K1⁴. Recent studies have suggested that vitamin K2 may be more biologically active than vitamin K1², but due to the lack of sufficient data to date the Institute of Medicine has not provided DRI values for vitamin K2.

Vitamin K deficiency

Deficiency in vitamin K may occur as a result of diseases of the liver, pancreatic or biliary disease, cystic fibrosis, diseases of malabsorption of fats, ulcerous colitis, regional enteritis or Crohn's disease, short intestine syndrome and intestinal surgery (especially of the terminal ileum where the biliary salts are absorbed), chronic malnutrition, alcoholism, and the taking of medicines such as anticoagulants antagonistic to vitamin K⁵. Deficiency in vitamin K increases the risk of bleeding and may have deleterious effects on bone health.

Vitamin K supplements

Vitamin K1, MK-4 and MK-7 are available in pharmacological forms. Vitamin K1 is the most common form of vitamin K commercially available, referred to as phytonadione. Vitamin K1 supplements are used to treat and prevent vitamin K deficiency, to prevent haemorrhages or problems of coagulation caused by certain medicines or diseases and to counteract the effects of an overdose of anticoagulants. Dietary supplements of MK-4 and MK-7 have been approved in Japan for the prevention and treatment of osteoporosis⁶. Menadione or "vitamin K3" is a synthetic hydrosoluble form of vitamin K which may be converted into vitamin K2 in the body. The US Food and Drugs Administration has not authorised menadione to be sold as a dietary supplement for humans due to its potential deleterious effects⁵.

Vitamin K and bone metabolism

Vitamin K is the essential co-factor for the gamma-carboxylation of proteins with gamma-carboxyglutamic (Gla) residues, facilitating the posttranslational conversion of glutamic acid (Glu) to Gla residues in the proteins dependent on vitamin K and activating them. It is involved in the regulation of the management of calcium in the body. Although vitamin K prevents vascular calcification and that of the soft tissues, it also promotes the integration of calcium into bone.

There are three vitamin K-dependent proteins in the bone: osteocalcin (also called bone Gla protein), Gla matrix protein and protein S. The effect of vitamin K on osteocalcin is perhaps the best understood of these.

Osteocalcin is synthesised by the osteoblasts during the mineralisation phase of bone formation and is essential for the formation of crystals of hydroxyapatite. Glu has three residues, and their capacity to bond to mineral depends on gamma-carboxylation which is dependent on vitamin K. Although the vitamin K-dependent coagulation factors are 100% gamma-carboxylated, as they are found in dietary recommendations for their use, more than 40% of blood osteocalcin may be non-carboxylated. It has been shown that supplements with either MK-4 or MK-7 already produce a level of carboxylation similar to osteocalcin². However, supplementation with MK-7 appears to be more effective in carboxylating osteocalcin than supplementing with phylloquinones².

In addition to the gamma-carboxylation of osteocalcin vitamin K may affect the genetic transcription required for the expression of the osteoblast markers and thus affect the synthesis of collagen⁷. Furthermore, vitamin K may also suppress bone resorption and osteoclastogenesis^{8,9}. *In vitro* and in animal studies, it has been suggested that MK-4 may be associated with the regulation of inflammation, oxidative stress and apoptosis¹⁰, all of which may reduce bone resorption. In a study carried out in osteoblasts, it was observed that MK-7 suppresses the differentiation of osteoblasts and induces the mRNA of osteocalcin, osteoprotegerin and RANKL¹¹.

Also, vitamin K2 may act on bone through a function which regulates transcription, inducing the expression of the gene for the steroid and xenobiotic receptor (SXR), which is expressed primarily in the liver and the intestine, regulating the expression of the cytochrome P450 enzymes (CYP3A4 and CYP2C8) and of the family of ATP transporters such as MDR1 and MRP2. Vitamin K modulates the expression of osteoblast bone markers through SXR, favouring bone formation, which means that SXR is probably also involved in the maintenance of bone homeostasis¹².

Association between vitamin K and BMD and fractures: observational studies

In the majority of observational studies low blood levels of vitamin K1, low intake of vitamin K1, low intake of vitamin K2 (MK-7) and high blood levels of non-carboxylated osteocalcin have been asso-

ciated with an increased risk of hip fractures¹³⁻¹⁵. For example, in the Nurses' Health Study, carried out in women between 30 and 88 years of age (n=72,327), those with an intake of phylloquinones lower than 109 µg/day had an increased risk of fracture at 10 years compared with those who had a higher intake of phylloquinones¹⁶. Similarly in the Framlingham Heart Study, in a group of 888 men and women with an average of age 75 years and an average intake of phylloquinone of 56 µg/day, it was observed that they had a higher risk of fracture of the hip in the following seven years than those who ingested an average of 254 µg/day. In this study there was no association between the intake of vitamin K and BMD¹⁷.

Although few studies have shown, overall, an association between a low intake of vitamin K and a reduction in BMD in women, there is less evidence of an association between high levels of vitamin K intake and an increase in BMD in observational studies¹⁴. These studies suggest: that an adequate intake of vitamin K may be necessary to reduce bone resorption; that the requirements for the maintenance of adequate bone health should be higher than the adequate intake values proposed; and that, once the vitamin K requirements for bone health are reached no additional intake is needed. In addition, a greater limitation of these studies is that high intakes of vitamin K1 may be an indicator of the consumption of foods which contain other nutrients protective of bone, such as calcium, magnesium, potassium and phytochemicals. Therefore, on the basis of the findings of the observational studies, we cannot conclude that vitamin K has an independent protective effect on bone health.

Effects of the supplementing of vitamin K on BMD and on fractures: clinical trials and meta-analyses

Various clinical trials in different populations have examined the effect of vitamin K on BMD. Two systematic reviews and meta-analyses have made a summary of these clinical trials^{18,19}. In the most recent review published in 2012, Fang et al.¹⁹ collect the data from 17 trials with vitamin K in the healthy population and in patients over 18 years of age with primary and secondary osteoporosis. These include ten trials with vitamin K2 (eight with MK-4 at a dose of 15-45 mg/day and two with MK-7 at a dose of 0.2-3.6 mg/day) and seven trials with vitamin K1 (0.2-10 mg/day). In the general analysis the authors mix the results of all the trials with vitamin K and examine the changes in BMD. They observe that supplementing with vitamin K had no effect on the BMD in the femoral neck, but increased the BMD in the lumbar spine by 1.3% (95% confidence interval [95% CI]: 0.5-2.1) after 6-36 months of supplementation¹⁹. In an analysis of subgroups according to type of vitamin K, vitamin K2 increased BMD in the lumbar spine by an average of 1.8% (95% CI: 0.9-2.8), while vitamin K1 had no effect. The therapeutic effect on BMD in the lumbar spine was much gre-

ater in Asiatic populations than in Western. However, when the authors excluded those studies with a high risk of methodological errors due to the existence of other factors, they found no significant effects of vitamin K in the lumbar region. Fang et al.¹⁹ warned against these estimated errors in the effects of treatment in the meta-analysis, due largely to the differences in the groups studied, differences in methodological quality in the trials selected and to errors in publications. The effect of the supplements of vitamin K2 on fractures is based on eight clinical trials carried out Japanese patients with primary and secondary osteoporosis.

A randomised clinical trial among 325 postmenopausal women who received a placebo or 45 mg/day of vitamin K2 (MK-4 or menatetrenone) over a period of three years²⁰, evaluated the bone mineral content (BMC) and the geometry of the hip using DXA. The indices of bone strength were calculated using DXA (BMD), femoral neck width (FNW) and femoral axis length (FAL). It was observed that vitamin K did not affect the BMD, but the BMC and FNW were increased in comparison to the placebo. In the group treated with vitamin K2 the bone strength in the hip did not vary, while it reduced significantly in the group treated.

A systematic review carried out in 2006 with a meta-analysis of seven clinical trials showed that supplementing with MK-4, 15-45 mg/day over 12-24 months, reduced significantly fractures of the hip (odds ratio [OR]: 0.23, 95% CI: 0.12-0.47) and vertebrae (OR: 0.40, 95% CI: 0.25-0.65), and non-vertebral fractures (OR: 0.19, 95% CI: 0.11-0.35)¹⁸. However, another major trial published in 2009, came to a different conclusion²¹. This was a wide, open trial in phase IV carried out in 4,378 Japanese osteoporotic women with or without prevalent vertebral fractures who received over three years supplements of MK-4 and calcium, with a year of follow up during which no kind of restriction in the use of medication for osteoporosis was imposed. The combined treatment with MK-4, 45 mg/day (divided into three doses of 15 mg each) and calcium, or the treatment with calcium only, was not associated with changes in the incidence of vertebral fractures after 3 year years (5.9% vs 5.7%) or in the incidence of all clinical fractures after 4 years (2.5% vs 2.1%). However, in one of the 11 non-adjusted groups, in a post-hoc analysis the researchers found a statistically significant reduction in the appearance of new vertebral fractures in a subgroup of women with more than five prevalent vertebral fractures (20.3% vs 33.2%, $p=0.03$).

More recently, a double blind randomised trial, carried out in 2013 with MK-7 compared with a placebo over 3 years in 244 postmenopausal Dutch women without osteoporosis found one woman with a new vertebral fracture in the MK-7 group ($n=120$) and six in the placebo group ($n=124$)²², but there were too few fractures for the difference to be statistically significant between the two groups.

Examining all the literature published to date, vitamin K2 supplements may protect against fractures but the data are not very consistent.

The evidence of the effect of supplementation with vitamin K1 on fractures is more limited, and based principally on a single study⁶, since the rest of the trials with vitamin K1 were not designed to analyse fractures. This study is a controlled, randomised double blind clinical trial carried out in 440 postmenopausal Canadian women with osteopenia. It showed a statistically significant effect with the administration of vitamin K1, 5 mg/day, in reducing all fractures after 2-4 years of supplementation (9 women with 11 fractures in the group treated with vitamin K1 vs 20 women with 21 fractures in the placebo group [hazard ratio 0.48, 95% CI 0.20-0.98]), although the fracture was a secondary result of the trial.

Effects of vitamin K supplements associated with other agents for the treatment of osteoporosis

There have been few studies carried out which compare the possible additive effects of vitamin K on the bone of patients being treated for osteoporosis. A Japanese group²³ has studied the effects of risedronate, alone or associated with vitamin K2, on the levels of carboxylated or non-carboxylated osteocalcin (OC). They observed that there was no difference in the levels of OC between the groups, but those patients with vertebral fractures had levels of non-carboxylated OC higher than those patients without fractures in the group treated only with risedronate.

Another group of Japanese authors²⁴ studied the effect of alendronate associated, or not, with vitamin K2 in postmenopausal women with rheumatoid arthritis. In a study of 62 patients with osteopenia or osteoporosis, those with low levels of non-carboxylated OC were treated with alendronate plus vitamin K2, and those who had normal levels, only with alendronate. After a year of treatment the levels of bone markers (alkaline phosphatase and N-terminal telopeptide of collagen I) decreased equally in both groups. There was no difference in the changes in bone mass in the lumbar spine between the two groups, but there was a statistically significant increase in the BMD in the femoral neck in the group supplemented with vitamin K.

Methodological limitations of the current evidence

The differences in the findings of the various studies on the effect of vitamin K on the BMD and on fractures may be explained by the different forms of vitamin K used, by the underlying intake of vitamin K of each of the groups, by the level of intake of calcium and vitamin D in each of the groups, or by differences in the populations studied. For example, the Japanese studies use MK-4 as vitamin K2, and the European studies use MK-7, while North American studies mainly use vitamin K1. The Japanese clinical trials with MK-4 had

various problems in their methodology, such as a lack of blind studies, high rates of abandonment and lack of randomisation. The participants of these studies were older, with primary or secondary osteoporosis, possibly with low levels of vitamin D and with a poor calcium intake, and with a high underlying risk of fracture. For these reasons, it is not possible to generalise these results with those obtained in trials with MK-4 carried out in healthy postmenopausal women with normal levels of vitamin D and an acceptable intake of calcium. Furthermore, no studies have been carried out with vitamin K supplements in which fractures were considered to be the principal outcome. Therefore, we cannot make a definitive conclusion as to the overall effect of vitamin K supplements on the prevention of fractures.

Safety and adverse effects of vitamin K supplements

Vitamin K supplements are well-tolerated and safe in most cases. Some studies have reported rare effects of supplementation with MK-4 (menatetrenone), such as the incidence of skin lesions and minor gastro-intestinal effects⁵. A few studies have shown that supplements with vitamin K1 may affect the lipid profile, sensitivity to insulin and levels of glycemia⁵.

Vitamin K may reduce the effect of anticoagulants such as warfarin. People who take warfarin should be warned to avoid supplements and foods which may contain vitamin K. There have also been reported interactions with antilipemics and antidiabetics⁵.

Problems of knowledge and future research

It has been proposed that the non-carboxylation of osteocalcin adversely affects its capacity to bond with the bone mineral. However, those studies carried out in which an adequate or maximum level of carboxylated osteocalcin was achieved, did not correspond with an improvement in BMD. It is possible that the effects of vitamin K on BMD may be more pronounced in those populations which have osteoporosis or those with a vitamin D deficit, since there is an interaction between vitamin K and vitamin D.

There is also no effect observed of vitamin K on BMD in subjects with adequate levels of vitamin K. It is probable that the effects of vitamin K on BMD are more positive in those subjects with a vitamin K deficit, such as those with malnutrition or affected by diseases which interfere with its synthesis or absorption.

In spite of the minimal effects on BMD, vitamin K may have a protective effect against fractures. It is possible that vitamin K exerts its effect through the carboxylation of the GLA protein of the matrix, an effect which is not detected in the measurement of BMD. In addition to the role vitamin K plays in gamma-carboxylation there are other mechanisms in the bone which are dependent on vitamin K and which may affect the risk of fracture. For example, the effect of vitamin K on

fractures may be mediated through its effects on bone quality, geometry or strength^{6,20}. Further studies would be needed to clarify these points.

In conclusion, vitamin K is important for bone health. A low intake of vitamin K, low blood levels of the vitamin or high levels of non-carboxylated osteocalcin are associated with an increase in hip fractures in the observational studies¹³. However, the results of the clinical trials are not conclusive, raising doubts as to whether or not general supplementation with vitamin K1 or K2 reduces the risk of vertebral or non-vertebral fractures. It is likely that carrying out new studies in populations with low blood levels, or a low intake, of vitamin K could clarify the role of vitamin K in the prevention of fractures²⁵.

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