INTRODUCTION

Bone diseases present large challenges for society

Bone health is critically important to overall health and quality of life. Healthy bones provide the body with the framework that allows for mobility and for protection against injury. Bones stores minerals such as calcium which are vital to the functioning of for instance muscle contraction, nerve signaling and many enzymatic reactions.

A major health challenge is the increasing number of people with weak bones caused by osteopenia (lower than normal bone mineral density [BMD]) and osteoporosis (a disease state with low BMD). An estimated 75 million people in Europe, USA and Japan have osteoporosis and three times as many women are affected [1, 2] compared to men. There is a clear, worldwide correlation between osteoporosis and fractures, with lifetime risk of osteoporotic fractures in women and men 30-50% and 15-30%, respectively [3]. It has been estimated that the fracture incidence is more than 2.3 million cases annually in Europe and the US alone [1]. Brittle bones and osteoporosis result in a huge economic burden worldwide with large implications for the individual as well as for society. The economic burden in the EU is estimated to be € 37 billion, and this is projected to increase, on average, by 25% in 2025 [4]. As such, just a small improvement in bone health may have large positive implications on public health budgets and increase the longevity and quality of life of individuals. The importance of vitamin K for bone health, particularly vitamin K1 and vitamin K2 (MK-4), has been summarized in several review papers [5-7]. K2 MK-4 is approved as a drug in Japan for treatment of osteoporosis (dose; 45 mg/day, 3 X 15 mg/ day). This paper focuses specifically on the documented role of vitamin K2 MK-7, in supplement doses, in relation to bone health and the bone building process.

BONES ARE LIVING TISSUE, CONSTANTLY RENEWED ALL THROUGH LIFE

The skeleton consists of bone (hydroxyapatite) supplemented by ligaments, tendons, muscles and...
cartilage. Calcium is an important building block in the bone matrix. Bone building processes constantly ongoing - in fact the entire skeleton is regenerated every seven years. Bone build-up cells (osteoblasts) and break-down cells (osteoclasts) regulate the strength of bones, and when break-down cell activity dominates, the skeleton becomes increasingly fragile. This may result in development of osteoporosis.

In the cycle of bone remodeling, the osteoclasts remove old or damaged bone (this removal is called bone resorption). The removed bone constituents, including calcium, are released into circulating blood. At the same time the osteoblasts produce new bone (bone formation). In this process, osteoblasts produce osteocalcin (OC), a protein that, in its activated form, can bind calcium and incorporate calcium into bones (8).

![Figure 2. Bone mass as a function of age. During puberty: a large increase in bone mass. From 50 years onwards: a strong decrease in bone mass, particularly in females (© Kappa Bioscience).](image)

It is important throughout life to secure sufficient calcium to maintain a healthy skeleton. Bone mass increases from childhood until the early twenties, before beginning to decline (Figure 2). From 30-40 years onwards, bone mass gradually decreases in both men and women. This implies that sufficient calcium intake is particularly important in childhood/puberty and from age 35 onwards. Further, the risk of broken bones increases with age and the ability to recover decreases. Post-menopausal women are particularly vulnerable for development of brittle bones, leading to fractures.

**HOW CAN WE IMPROVE BONE HEALTH – LOOK TO JAPAN!**

Osteoporosis is to a large extent a preventable disease. Several environmental factors, such as physical exercise and diet (considered mainly to be attributed to sufficient intake of calcium and vitamin D3), can strongly influence our bone quality. Worldwide, the risk of osteoporotic fracture can vary up to 10-fold, depending on country of origin, with Caucasians having the greatest reported risk (9). The fact that major risk factors for osteoporosis are well known, but incidences are increasing worldwide, suggests that important factor) for a good bone health are being overlooked.

Japan is one of the countries with aw frequencies of bone- and cardiovascular diseases, especially in certain regions in the North (10). In these Northern regions the Japanese eat a dish called natto - fermented soybeans which contain high amounts of vitamin K2, menaquinones-7 (MK-7). This food has been used for hundreds of years and is approved as a Health Food in Japan. Many Japanese epidemiological studies have pointed to beneficial effects of vitamin K2, MK-7 for bone health (11, 12). In a large three-year study in Japanese women it was demonstrated that intake of natto resulted in increased BMD at the femoral neck (13). These effects were attributed to intake of MK-7 in the natto food. When the incidence of hip fractures in twelve regions in Japan were an analysed and correlated to intake of the key nutrients calcium, magnesium, vitamin D and vitamin K, the strongest inverse correlation to fractures was found to be intake of vitamin K, in particularly vitamin K2, MK-7, both in men and women (10). Intake of natto food was also inversely correlated with hip fractures in women (14). Furthermore, natto intake was associated with improved BMD in elderly men (15). Thus, several Japanese studies demonstrate the beneficial effects of MK-7 on bone strength and for prevention of fractures. Furthermore, these studies show that MK-7 is safe without any side effects.

**THE IMPORTANCE OF COMBINING CALCIUM, VITAMIN D3 AND VITAMIN K2 – “THE TRI-ESSENTIALS”**

The most widely used supplements to support bone health are calcium and vitamin D3. Vitamin D3 increases the intestinal uptake of calcium into the blood stream. To secure an optimal bone building process, however, vitamin K is needed to activate osteocalcin, allowing osteocalcin to bind calcium and transport calcium from blood into the bone. Vitamin K, vitamin D3 and calcium work in concert for building strong bones: the “tri-essentials” for bone health (Figure 3).

The importance of vitamin K2 for bone health in healthy subjects has been recently documented. In a large three year placebo-controlled study of 244 postmenopausal women with daily intake of 180 µg of MK-7, a statistically significant difference in bone quality parameters was found between the MK-7 group and the control (16). It was concluded that MK-7 preserves bone at Lumbar spine L1-L4 (both BMC and BMD).
Furthermore, measured bone strength was preserved in the treatment group. This study demonstrates that beneficial effects on bone health can be obtained with supplement doses of vitamin K2, MK-7 (180µg), Vitamin K1 and MK-4 are also used, but this is documented to be sub-optimal, due to the very high doses of vitamin K1 (1-5 mg) or MK-4 (45 mg) required. (Reviews 5, 6, 7). Such dosages exceed the nutritional vitamin guidelines, and the amount typically available in dietary supplement tablets of 50 - 100µg - far below what is needed for vitamin K1 or MK-4 to have beneficial effects on bone parameters. Vitamins in high (mg) doses are often classified as drugs; for example, MK-4 is registered as a drug for treatment of osteoporosis in Japan and administered at 45mg/daily dose.

OSTEOCALCIN, A VITAMIN K-DEPENDENT PROTEIN IMPORTANT FOR BONE BUILDING

Osteocalcin (OC) is a non-collagenous protein primarily expressed in osteoblasts involved in bone metabolism and calcium binding. OC is dependent on vitamin K in order to carry out its function of building calcium into bone and is important for bone mineralization and calcium homeostasis (7). Vitamin K is a co-factor for the enzyme that activates OC by carboxylation of the protein at certain sites. In the activated (carboxylated) form, OC can bind calcium and transport calcium into the bone matrix (Figure 4). OC is a so-called Gla-protein which is dependent on vitamin K for its function (all Gla-proteins use vitamin K as cofactor for the carboxylation step). From bone, OC partly goes into blood circulation and the fraction of undercarboxylated-osteocalcin (ucOC, inactive) and carboxylated osteocalcin (cOC) can be measured as surrogate markers for vitamin K status (17).

Individuals with low vitamin K2 intake have a larger fraction of non-activated osteocalcin, resulting in reduced capacity for calcium binding. High serum concentration of ucOC is thus correlated with low BMD and is a predictor of hip fracture risk (18-20). Since high ucOC concentration also is a marker of vitamin K deficiency, studies demonstrate the pivotal role of sufficient intake of vitamin K for optimal bone health. As may be expected, low serum concentration of vitamin K, in particular vitamin K2, menaquinone-7 (MK-7), is correlated with increased fracture incidence (21-22, 10). In conclusion, ucOC is an important biomarker for bone health; a high concentration of ucOC is a strong indicator of vitamin K deficiency and relates to low BMD and risk of hip fractures.

VITAMIN K IN FOOD – IS THERE A DEFICIENCY IN THE GENERAL POPULATION?

Vitamin K is a fat-soluble vitamin. Vitamin K comprises a number of structurally related compounds including phylloquinone (vitamin K1) and menaquinones (vitamin K2s). Menaquinones are classified according to the length of their side chains; menaquinone-4 (MK-4) and menaquinone-7 (MK-7) being the most important. Phylloquinone is found in green/leafy vegetables such as green salads, broccoli and spinach. Vitamin K1 is considered as the major dietary source of vitamin K, accounting for approximately 90% of total vitamin K intake (23). Based on different studies of content in food worldwide, Suttie has estimated the intake of vitamin K in the general population to be between 70 - 250 µg/day (24). However, the bioavailability of vitamin K1 from food is low; less than 20% is absorbed (25).

Vitamin K2, menaquinones, are found in animal products such as meat, dairy, eggs (mainly MK-4) and fermented food, e.g. cheese, yoghurt, and fermented soybean products/natto (mainly MK-7). In the Netherlands the average intake of MK-4 and long-chain menaquinones from eggs and cheese has been reported to be in the range of 7µg/day and 22µg/day, respectively (28). Limited knowledge exists regarding intake of longer menaquinones from food in other European countries or the US, though fermented cheeses may be the most important source (23, 29). However, the most efficacious vitamin K, specifically MK-7, is found to a very low extent in these fermented products (23). These factors likely lead to a deficiency of vitamin K2 intake in most countries.

VITAMIN K DEFICIENCY RESULTS IN LOW BMD AND HIGH RISK OF FRACTURES IN SOME DISEASES

Several medical conditions and drug treatments may result in vitamin K deficiency. For instance, treatment with anti-coagulants (warfarin/coumarins) that block the re-use of vitamin K in the so-called vitamin K cycle result in low serum concentration of vitamin K. This has been shown to affect bone health in both adults and children.

In young people aged 8.6 to 18.8 years that received warfarin for more than 12 months, significant reduction in bone mineral density was observed. In another study, more than 50% of pediatric patients on warfarin/coumarin were reported to have osteopenia (30). Long-term treatment with anti-inflammatory drugs (corticosteroids) is another example of drugs that have detrimental effects on bone quality (31).

People with various diseases affecting intestinal absorption of vitamin K (and other vitamins and minerals) such as...
celiac disease and cystic fibrosis are reported to have poor bone health (32, 33). The correlation of poor bone health associated with cystic fibrosis and vitamin K deficiency (increased ucOC) is well established (32) and supplementation with vitamin K is known to have beneficial effect on bone health in young people with cystic fibrosis (34).

**VITAMIN K2, MK-7 - THE VITAMIN K OF CHOICE**

The major difference between vitamin K1 and vitamin K2 as MK-7 are their respective half-lives. All fat-soluble vitamins are absorbed well in the presence of fat. Vitamin K1 however, quickly disappears from circulation (half-life approx. 1-2 hours). MK-7 in contrast, has a very long half-life (approx. 2-3 days) (35). This considerable difference in half-lives is due to the MK-7 molecular structure, resulting in a difference in uptake, transport in blood, distribution and breakdown in the body. This leads to the large difference in the steady state concentration. Serum concentration of MK-7 is substantially higher than for vitamin K1 when subjects are given equimolar amounts (same number of molecules) (Figure 5). As a result, MK-7 remains in serum and is far more accessible for extra-hepatic organs such as bone and vessel walls, resulting in more efficacious carboxylation of osteocalcin and MGP (36). In the EU the recommended daily allowance (RDA)

![Figure 5. Concentration of vitamin K in serum after intake of vitamin K1 and vitamin K2 (MK-7), including natto food in various amounts. Only intake of MK-7 in dietary doses gives a serum concentration above 1ng/ml. DS = dietary supplement (modified from ref 42, 29, 46, own data, unpublished), (© Kappa Bioscience).](image-url)
of vitamin K is 75 µg/day and is based on requirements for activation of clotting factors in the blood (37). In healthy individuals the intake of vitamin K from food is sufficient to fully activate clotting factors in the liver (meaning the clotting factors are always 100% activated) (35, 38). In contrast to the coagulation factors, several studies have demonstrated that both OC and matrix Gla-protein in serum are far from fully activated in the general population (10-40%) and that supplementation with vitamin K increases the degree of activation (38-43).

In a recent study of healthy volunteers it was documented that the concentration of undercarboxylated OC (ucOC) was high, pointing to an extra-hepatic vitamin K deficiency (44). Interestingly, both children and adults above age 40 year had high concentrations of circulating ucOC. The authors concluded that by increasing the extra-hepatic carboxylation of Gla-proteins (i.e. supplementation with vitamin K2) one can contribute to better bone health (44). Recent published data supports that doses of vitamin K2 in the range of 90-120 µg/day (US RDA) can to a great extent reduce circulating ucOC (40, 43).

CONCLUSIONS

Vitamin K2 deficiencies seem to be quite common in the general population, except for people eating natto. Dietary supplement tablets typically contain 50 -100 µg vitamin K. Based on data from the literature, only vitamin K2, as MK-7, seems to be efficacious with respect to beneficial effects on bone health in supplement doses. Vitamin K2 is also documented to be safe with no side effects even in very high doses. Increased intake of vitamin K2 in the general population to facilitate healthy bones may have substantial beneficial effects for the individual and huge positive implications on the public health and health budgets worldwide.

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