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RESEARCH ARTICLE

THE ROLE OF VITAMIN K2 IN REDUCING THE INCIDENCE OF OSTEOPOROSIS AND HEART DISEASE

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ABSTRACT

Natural forms of vitamin K include phylloquinone which is vitamin K_1, and a family of molecules called menaquinones which is MKs or vitamin K_2. Vitamin K2 deficiency may impair the activity of VKDPs and increase the risk of osteoporosis, fractures and heart disease. Phylloquinone is found at high concentrations in green leafy vegetables and certain plant oils, therefore the deficiency of it is very rare. But most menaquinones are usually found in animal livers and fermented foods. In western style diet the consumption of fermented foods is limited and low, also increasing the use of vegetable oils and the reduction of animal fat, organ meat and increasing the use of antibiotics interferes with vitamin k2 absorption. The blood test for vitamin k2 deficiency is not a regular test worldwide, so this important vitamin is deficient in many people. Heart disease is the leading cause of death in the world and Osteoporosis is increasing dramatically. So increasing the use of fermented foods like natto, increasing animal fat and organ meat in the diet, especially older people, may reduce the risk of heart disease and osteoporosis.

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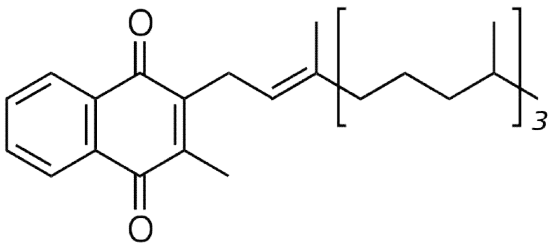
INTRODUCTION

Vitamin K_2 or menaquinonecontains9 related compounds, in general subdivided into the short-chain menaquinones with MK-4 as the most important member, and the long-chain menaquinones, of which MK-7, MK-8 and MK-9 are nutritionally the most known. Vitamin K_2, the main storage form in animals, has several subtypes, which is different in isoprenoid chain length. These vitamin K_2 homologues are called menaquinones, and are characterized by the number of isoprenoid residues in their side chains. Menaquinones are abbreviated *MK-n* as a whole, which *M* stands for menaquinone, the *K* stands for vitamin K. The *n* represents the number of isoprenoid side chain residues. For instance, menaquinone-4 which is the abbreviation of MK-4, has four isoprene residues in its side chain. Menaquinone-4 which is also known as menatetrenone from its four isoprene residues, is the most common type of vitamin K_2 in animal products, since MK-4 is normally synthesized from vitamin K_1 in certain animal tissues, that is arterial walls, pancreas, and testes, by replacement of the phytyl tail with an unsaturated geranylgeranyl tail containing four isoprene units, thus yielding menaquinone-4. This homolog of vitamin K_2 may have enzyme functions distinct from those of vitamin K_1. Menaquinone-7 is different from MK-4 in that it is not produced by human tissue. MK-7 may be converted from phylloquinone which is K_1, in the colon by *E. coli* bacteria. (Vermeer and Braam, 2001) However, bacterially derived menaquinonesor MK-7 appear to contribute minimally to overall vitamin K status. (Suttie, 1995; Weber, 2001) MK-4 and MK-7 are both found in the United States in dietary supplements for bone health.



Picture 1) Vitamin K1 or phylloquinone.both forms of the vitamin contain a functional naphthoquinone ring and an aliphatic side chain. Phylloquinone has a phytyl side chain

MK-4 has been shown to decrease the incidence of fractures up to 87%. (Sato *et al*., 2005) MK-4 with the dosage of 45 mg

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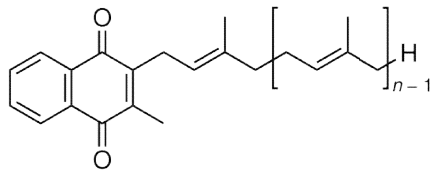
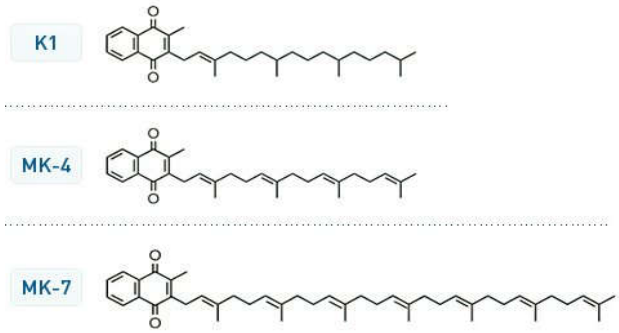
daily, has been approved by the Ministry of Health in Japan since 1995 for the prevention and treatment of osteoporosis. (Iwamoto *et al*., 1999) All K vitamins are very similar in structure, they share a quinone ring, but they are different in the length and degree of saturation of the carbon tail and the number of side chains. (Shearer, 2003) The number of side chains is indicated in the name of the particular menaquinone which is MK-4 means that four molecular units - called isoprene units - are attached to the carbon tail, and this influences the transport to different target tissues. The mechanism of action of vitamin K_2 is similar to vitamin K_1. Traditionally, K vitamins were recognized as the factor required for coagulation, but the functions performed by this vitamin group were revealed to be much more complex. K vitamins play a vital role as cofactor for the enzyme γ-glutamyl carboxylase, which is involved in vitamin K-dependent carboxylation of the gla domain in Gla-proteins,that is in conversion of peptide-bound glutamic acid (Glu) to γ-carboxy glutamic acid (Gla) in these proteins. Carboxylation of these vitamin K-dependent Gla-proteins, besides being essential for the function of the protein, is also an important vitamin recovery mechanism since it serves as a recycling pathway to recover vitamin K from its epoxide metabolitefor reuse in carboxylation.



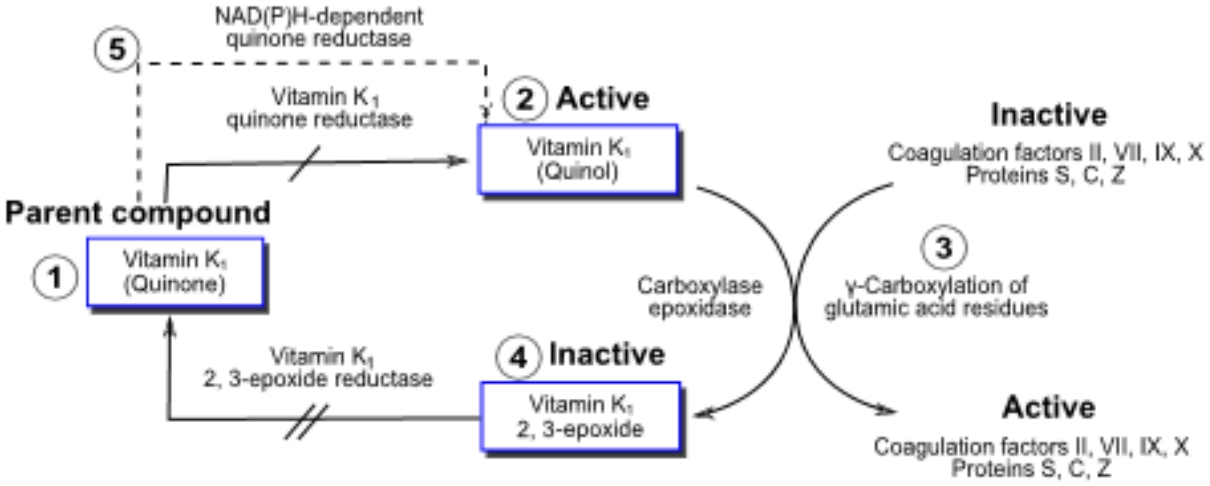
Picture 2) Vitamin K2 ormenaquinone. In menaquinone, the side chain is Composed of a varying number of isoprenoid residues



Picture 3) Vitamin K structures. MK-4 and MK-7 are both subtypes of K2

Vitamin K is absorbed along with dietary fat from the small intestine and transported by chylomicrons in the circulation. Most of vitamin K_1 is carried by triacylglycerol-rich lipoproteins and rapidly cleared by the liver. Only a small amount is released into the circulation and carried by LDL and HDL. MK-4 is carried by the same lipoproteins (TRL, LDL, and HDL) and also cleared fast. The long-chain menaquinones are absorbed in the same way as vitamin K_1 and MK-4, but are efficiently redistributed by the liver in predominantly LDL and VLDL. Since LDL has a long half-life in the circulation, these menaquinones can circulate for the extended times yielding in higher bioavailability for extra-hepatic tissues as compared to vitamin K_1 and MK-4. Accumulation of vitamin K in extra-hepatic tissues has direct relevance to vitamin K functions not related to hemostasis. (Martin J. Shearer and Paul Newman, 2008) Vitamin K_2 is preferred by the extra-hepatic tissues which mean bone, cartilage and vasculature, and this may be produced as MK-4 by the animal from K_1, or may be of bacterial origin (MK-7, MK-9, and other MK numbers). The latter may be consumed already prepared by bacteria. Discussion is ongoing as to what extent $K_2$is produced by intestinal bacteria contributes to daily vitamin K_2 needs. If, however, intestinal bacterial supply was enough to supplement all tissues needing K_2, we would not find high fractions of under-carboxylated Gla-proteins in human studies.

Natural K2 is also found in bacterial fermented foods, like mature cheeses. The MK-4 form of K2 is often found in relatively small quantities in meat and eggs. The richest source of natural K2 is the traditional Japanese natto (Kaneki *et al*., 2001) which is made from fermented soybeans and Bacillus subtilis, providing an unusually rich source of K_2 as long-chain MK-7. Its consumption in Northern Japan has been linked to significant improvement in K vitamin level and bone health. The intense smell and strong taste, make this soy-food a less attractive source of K_2 for Westernstyle tastes which is one of the main reasons western people do not like fermented foods. Supplement food companies sell natto extract, standardized for K_2 content in capsules. It is not known if *B. subtilis* will produce K2 with legumes chickpeas, beans, lentils or not. Food sources of vitamin K_2 include fermented or aged cheeses, eggs, meats such as chicken, beef and their fat, livers and organs, and in fermented vegetables, especially natto, as well as sauerkraut and kefir. (Food Sources For Vitamin K_2 2013) Vitamin K_2 is synthesized by animal tissues and is found in meat, eggs, and dairy products. (Elder *et al*., 2006) Menaquinone-7 is synthesized by bacteria during fermentation and is found in fermented soybeans, and in most fermented cheeses. (Tsukamoto *et al*., 2000) Innatto, none of the vitamin K is from menaquinone-4, and in cheese only 2 to 7% is. (On the Trail of the Elusive X-Factor: Vitamin K2 Revealed) Recent studies found a clear association between long-term anticoagulant treatment (OAC) and reduced bone quality due to reduction of active osteocalcin. OAC might lead to an increased incidence of fractures, reduced bone mineral density/bone mineral content, osteopenia, and increased serum levels of undercarboxylated osteocalcin. (Caraballo *et al*., 1999) Bone mineral density was significantly lower in stroke patients with long-term warfarin treatment compared to untreated patients and osteopenia was probably an effect of warfarin-interference with vitamin K recycling. (Sato *et al*., 1997) Furthermore, OAC is often linked to an undesired soft-tissue calcification in both children and adults. (Barnes *et al*., 2005; Hawkins and Evans, 2005) This process has been shown to be dependent upon the action of K vitamins. Vitamin K deficiency results in undercarboxylation of MGP. Vascular calcification was shown to appear in warfarin-treated experimental animals within two weeks. (Price *et al*., 1998) Also in humans on OAC treatment, two-fold more arterial calcification was found as compared to patients not receiving vitamin K antagonists. (Schurgers *et al*., 2004; Koos *et al*., 2005) Among consequences of anticoagulant treatment: increased aortic wall stiffness, coronary insufficiency, ischemia, and even heart failure. Arterial calcification might also contribute to systolic hypertension and ventricular hypertrophy. (Zieman *et al*., 2005; Raggi *et al*., 2004) Coumarins, by interfering with vitamin K metabolism, might also lead to an excessive calcification of cartilage and tracheobronchial arteries.



Picture 4) Biochemistry and mechanism of action of vitamin K

Anticoagulant therapy is usually instituted to avoid life-threatening diseases and a high vitamin K intake interferes with the anticoagulant effect. Patients on warfarin or Coumadin treatment, or treatment with other vitamin K antagonist drugs, are therefore advised not to consume diets rich in K vitamins. But, the latest research proposed to combine vitamins K with OAC to stabilize the INR (International normalized ratio, a laboratory test measure of blood coagulation).

MATERIALS AND METHODS

By reviewing several important and applied studies and researches, there is one mainly reason to understand that vitamin K2 can regulate the incidence of heart disease and osteoporosis. This important reason is the calcification process in human body. Calcium build-up in the arteries around the heart is a huge risk factor for heart disease, which cannot be underestimated. (Kramer *et al*., 2013; Detrano *et al*., 2008; Thompson and Partridge, 2004) In the Rotterdam study, those who had the highest intake of Vitamin K2 were 52% less likely to develop calcification of the arteries, and had a 57% lower risk of dying from heart disease, over a 7-10 year period (27). This result mentions the effectiveness of vitamin K2 in reducing the incidence of heart disease. Another research of 16,057 women found that participants who had the highest intake of vitamin K2 had a much lower risk of heart disease. For every 10 micrograms of K2 they consumed in 24 hours, the risk of heart disease was reduced by nine percent (27). Vitamin K1 had no influence in either of those studies. However, keep in mind that the studies which mentioned above are observational studies, which cannot prove cause and effect. Unfortunately, the few controlled trials that have been done used the K1 form, which seems to be ineffective (The top 10 causes of death, 2014). This means Vitamin K1 may not be effective in reducing the incidence of heart disease or osteoporosis. There is a highly plausible biological mechanism for its effectiveness, and strong correlations found in observational studies. The importance of this cannot be overstated. Cardiovascular diseases is the world most common cause of death. It has killed 14 million people in the year 2012 alone (Kyla Shea and Rachel M. Holden, 2012). There is several evidences from controlled trials that vitamin K2 has major benefits for bone health. A 3-year study in 244 post-menopausal women found that those taking vitamin K2 supplements had much slower decreases in age-related bone mineral density (Knapen *et al*., 2013). Lengthy studies on Japanese women have shown similar benefits, although they did use very high doses. Out of 13 trials, only one failed to show significant improvement. Seven of those trials also reported fractures and found that vitamin K2 reduced spinal fractures by 60%, hip fractures by 77% and all non-spinal fractures by 81% (Cockayne *et al*., 2006). This is a high percentage which shows the effectiveness of vitamin K2 in osteoporosis. In line with these findings, the Japanese officially recommend vitamin K supplementation for the prevention and treatment of osteoporosis (Ishida, 2008). Calcium build-up in the arteries around the heart is a huge risk factor for heart disease (Kramer *et al*., 2013; Detrano *et al*., 2008; Thompson and Partridge, 2004). Therefore, anything that can reduce this accumulation of calcium may help prevent heart disease. This is where vitamin K2 is believed to help, by helping to prevent calcium from being deposited in the arteries (Margueritta *et al*., 2014). Osteoporosis is a common problem in Western countries. It is especially common among elderly women and strongly raises the risk of fractures. Vitamin K2 plays a central role in calcium metabolism, the main mineral found in bones. Vitamin K2 activates the calcium-binding activity of two proteins called Matrix gla protein and osteocalcin, which help to build and maintain bones (Martin *et al*.; Sarah L. Booth *et al*., 2013).

DISCUSSION

In 1997, researchers from the University of Texas and the University of Montreal developed mice that lacked the gene that codes for MGP. These mice appeared normal for the first two weeks of their lives, after which they developed faster heart beats, stopped growing and died within two months with the rupture of their heavily calcified aortas. The disorganization of their cartilage cells not only produced short stature, but also produced osteopenia and spontaneous fractures. (Luo *et al*., 1997) The bones of mice that lack the osteocalcin gene mineralize just as well as those of mice that do not lack the gene, but the mineral deposits are organized differently. This could mean that osteocalcin is important to the functional quality of bone and the ability to regulate its shape. (Koshihara *et al*., 1997) Isolated human osteoblasts, the cells that lay down

the calcified matrix of bone, secrete osteocalcin in response to vitamins A and D. (Berkner, 2005) The protein-rich matrix surrounding these cells will only accumulate this osteocalcin, however, if it is activated by vitamin K_2. Calcification of the extracellular matrix occurs in parallel with the accumulation of osteocalcin, but it is not clear whether this protein plays a direct role in laying down the calcium salts or if its accumulation simply reflects the higher amount of vitamin K_2 that is available to activate other proteins involved more directly in mineralization such as MGP. (Koshihara *et al*., 1997) When there is an insufficient amount of vitamin K to keep up with the production of vitamin K-dependent proteins, many of these proteins are secreted into the blood in an inactive form. Circulating cells then take up these useless proteins and destroy them. (Berkner, 2005) By drawing a person's blood and testing the percentages of circulating osteocalcin that are active and inactive, we can determine whether that person's bone cells have enough vitamin K to meet their needs. People with the highest percentages of inactive osteocalcin are at a more than five-fold increased risk of hip fracture, (Luukinen *et al*., 2000) confirming the value of the test. By using this test, we can also show that vitamin K_2 is the preferred K vitamin of the bones. It takes one milligram per day of a highly absorbable pharmacological preparation of vitamin K_1 to maximally activate osteocalcin in human subjects; (Binkley *et al*., 2002) it appears, however, that humans are not capable of absorbing much more than one fifth this amount from whole foods. (McKeown *et al*., 2002) By contrast, large amounts of vitamin K_2 are readily absorbed from foods. (Schurgers and Vermeer, 2000) Even when using highly absorbable forms of these vitamins, vitamin K_2 is much more effective. Researchers from the University of Maastricht in the Netherlands showed that over the course of 40 days, vitamin K_2 was three times more effective than vitamin K_1 at raising the percentage of activated osteocalcin. Moreover, the effect of vitamin K_1 reached a plateau after just three days, whereas the effect of vitamin K_2 increased throughout the entire study. Had it lasted longer, the study may have shown an even greater superiority of vitamin K_2. (Schurgers *et al*., 2006)

We can therefore regard the percentage of inactive osteocalcin primarily as a marker for vitamin K_2 status. In the healthy adult population, one hundred percent of the vitamin K-dependent blood coagulants produced by the liver are in their active form. By contrast, in this same population between ten and thirty percent of circulating osteocalcin is in its inactive form. Researchers rarely encounter individuals whose osteocalcin is fully activated. (Vermeer *et al*., 2004) This suggests that vitamin K_2 deficiency is universal, and that variation in K_2 status within the population simply reflects varying degrees of deficiency. Vitamin K_1 supplements produce modest decreases in bone loss in the elderly. A number of Japanese trials, on the other hand, have shown that vitamin K_2 completely reverses bone loss and in some cases even increases bone mass in populations with osteoporosis. (Vermeer *et al*., 2004) The pooled results of seven Japanese trials show that vitamin K_2 supplementation produces a 60 percent reduction in vertebral fractures and an 80 percent reduction in hip and other non-vertebral fractures. (Cockayne *et al*., 2006) These studies used extremely high amounts of vitamin K_2 and did not observe any adverse effects over the course of several years. Since they used such high doses of K_2, however, and no studies have tested lower doses, they do not constitute definitive proof that the vitamin activity rather than some drug-like action unique to the high dose produced such dramatic results. The balance of the evidence, however, suggests that vitamin K_2 is essential to skeletal health and that it is a key substance that modern diets do not adequately provide.

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Conclusion

Humans can partly convert vitamin K1 to K2 in the body. This is useful because the amount of vitamin K1 in a typical diet is ten times that of vitamin K2, which make vitamin K2 intake more important. However, current evidence which mentioned in the research, indicates that the conversion process is inefficient, because we benefit much more from eating vitamin K2 directly. Vitamin K2 is also produced by gut bacteria in the large intestine, and there is some evidence that broad-spectrum antibiotics can contribute to K2 deficiency. (Conly and Stein, 1992; Conly and Stein, 1994) Unfortunately, the average intake of this important nutrient is incredibly low in the modern diet. This important fat soluble vitamin is mainly found in certain animal foods and fermented foods, which most people don't eat much of. Rich animal sources include high-fat dairy products from *grass-fed* cows, liver and other organs, as well as egg yolks. (Hofman *et al*.) Vitamin K is fat-soluble, which means low-fat and lean animal products don't contain much of it. Animal foods contain the MK-4 subtype, while fermented foods like sauerkraut, natto and miso contain more of the longer subtypes, MK-5 to MK-14. (Haemostasis, 2000) If those foods are inaccessible to you, then supplementation is a valid alternative. The benefits of supplementing K2 may be enhanced even further when combined with a vitamin D supplement, because the two vitamins have synergistic effects. (Kidd, 2010) Although this needs to be studied a lot further, the current research on Vitamin K2 and health is extremely promising. It could have life-saving implications for a lot of people and may reduce the epidemic of heart disease and osteoporosis in the world population.

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