

MenaQ7™
(menaquinone-7)
Natural Vitamin K2

Vitamin K2 as MenaQ7™
Monograph

Improve Bone Health and Inhibit Arterial Calcification
May 2008

Leon J. Schurgers, PhD
Senior Researcher
University Maastricht

Vice President VitaK BV



For More Information – North America
Contact PL Thomas
PLT@PLThomas.com
973-984-0900

EU & International
NattoPharma
Info@NattoPharma.com
[47] 22 55 36 30

Introduction

Since its discovery in 1930 it has been thought that blood coagulation was the only function in which vitamin K is involved. During the last decades it has been found, however, that vitamin K also plays an important role in bone and vascular health. Surprisingly, the present recommendations for adequate vitamin K intake are still solely based on the requirement for blood clotting factor synthesis in the liver. The liver is capable of extracting vitamin K from the blood stream with high efficacy, and accumulating evidence suggests that other tissues, such as bone and vasculature may need higher vitamin K intakes to fully activate the vitamin K dependent proteins produced locally. Evidence obtained from population-based studies and from research in experimental animal models shows that postmenopausal bone loss and age-related vascular stiffening can be decreased substantially by extra vitamin K2 intake. Current recommendations are 45 micrograms of natural vitamin K2 as menaquinone-7 (MenaQ7™) per day to promote adequate vitamin K status and to help maintain bone and cardiovascular health.

Function of Vitamin K

The function of vitamin K is unique among other vitamins. It has an unequivocal role as a cofactor for the enzyme gamma-glutamyl carboxylase (figure 1) ^{1,2}. This enzyme activates specific glutamate amino acids within certain proteins which are designated as 'Gla-proteins'. In the absence of vitamin K uncarboxylated species of Gla-proteins are formed, which are biologically inactive³.

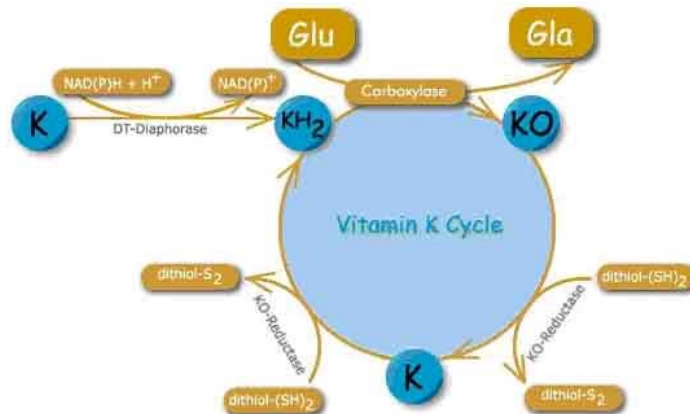


Figure 1

Four types of tissue produce large quantities of Gla-protein; the Gla-containing blood coagulation factors are synthesized in the liver. Osteocalcin (OC) is the most abundant Gla-protein in human bone where it is uniquely synthesized. Matrix Gla-protein (MGP) is expressed in cartilage and in the arterial vessel wall. Finally, Gas6 is also a vitamin K-dependent protein, synthesized by fibroblast and other extravascular cells. Different activities have been attributed to the Gas6 molecule such as anti-apoptotic functions and growth stimulatory activities⁴.

Vitamin K requirement

Like other fat-soluble vitamins, vitamin K is taken up from the food in the intestine by the bile-salt mediated pathway. After solubilisation into lipid droplets, vitamin K is incorporated in lipoprotein particles, and released in the blood stream. All tissues are capable of taking up vitamin K from the blood stream, but it has been demonstrated that the efficacy of vitamin K uptake is much larger for the liver than for the other tissues⁵. Therefore, the dietary vitamin K requirement for complete osteocalcin (in bone), and MGP (in vasculature and cartilage) activation is much higher than that for complete clotting factor activation (in liver). Surprisingly, the present recommendations for vitamin K intake are solely based on the hepatic requirement which is set to 1µg/Kg body weight per day.

The role of vitamin K2 has for the past decade been linked to two of the most important health issues, osteoporosis and cardiovascular disease. This link specifically centers on calcium utilization – implying that there is concurrent arterial calcification and osteoporosis when metabolism of calcium is inadequate. This is called the Calcium Paradox.^{6, 7,8,9 46}

Molecular forms of Vitamin K

Vitamin K is a group name for a family of related compounds, generally subdivided into phyloquinone (K1) and the menaquinones (K2). Menaquinones can be further subdivided into 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 interesting examples.

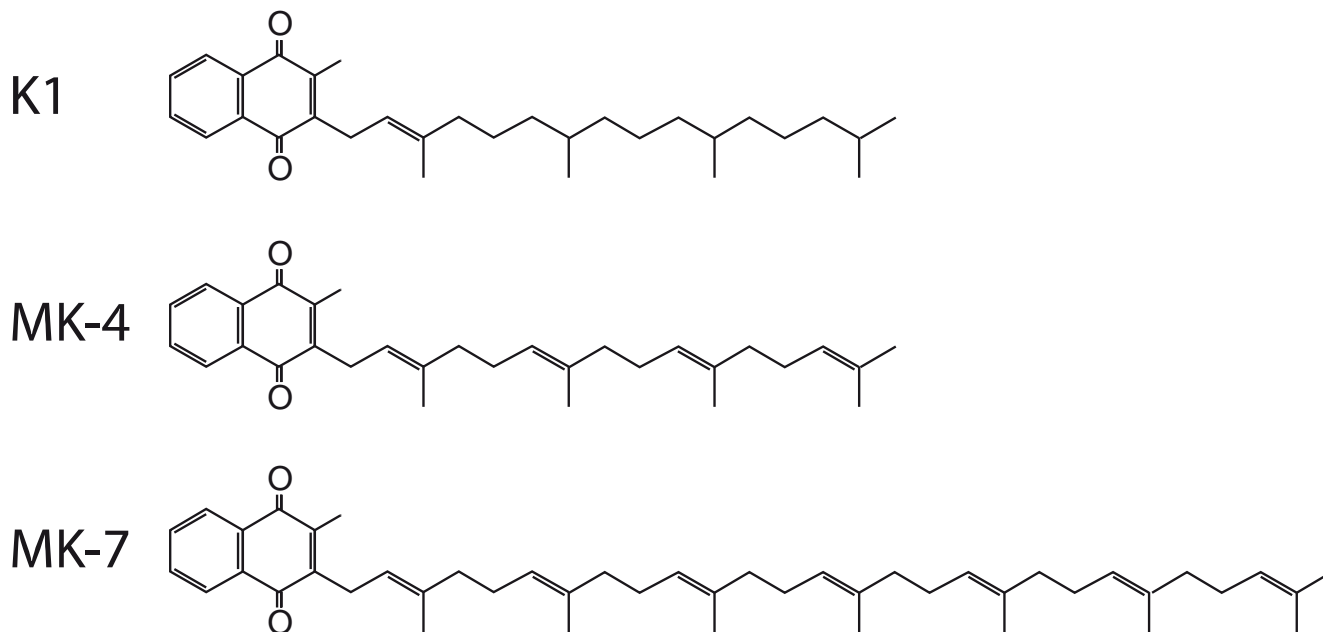


Figure 2

All K-vitamins have a similar function, but since their pharmacokinetic behavior and tissue distribution following absorption vary greatly, it is obvious that adequate supply to different tissues not only depends on the amount of vitamin K taken, but also on **which type of vitamin K** ingested. It has been reported that

the uptake of K1 from green vegetables (which form the main dietary source of K1) varies between 5 and 15%. Although K2 vitamins comprise only some 10% of our total dietary vitamin K intake, they may form **half of the total vitamin K absorbed**; this because of the **much better, nearly complete absorption** and also **significantly longer biological half-life** of the long-chain menaquinones. It seems, therefore, that these long-chain menaquinones (e.g. MK-7) are the main contributors to the vitamin K status in humans!

Since the present recommendations for vitamin K intake are solely based on the hepatic requirement the vitamin K intake is **insufficient** for extra-hepatic tissues. The occurrence of a substantial fraction of under-carboxylated extra-hepatic proteins such as osteocalcin (ucOC) and MGP (ucMGP) in the majority of the healthy adult population suggests sub-clinical vitamin K deficiency in large parts of the population. Therefore, the supplementation with vitamin K (pills or functional foods) is under investigation. Due to legislation and availability, for supplementation only synthetic K1 and MK-4 and natural MK-7 are available.

Poor vitamin K status and fracture risk: scientific evidence

Bones grow and develop most intensively during childhood and adolescence, binding minerals to healthy bone matrix with one goal – to maximize bone mineral density to maintain bone mass and strength later in life. In both men and women, bone mass accumulates until about the age of 30, which is commonly called “peak bone mass.” Once peak bone mass is met, we slowly lose bone throughout the course of life, and women also have accelerated bone starting in perimenopause and continuing throughout menopause due to hormonal changes. Thus, the probability of developing osteoporosis in the elderly is inversely correlated with peak bone mass achieved as young adults. Osteoporosis is a disease of bone reducing the bone mineral density (BMD), disrupting bone microarchitecture, and altering non-collagenous proteins, thus increasing the risk of fracture.

As described above, the recommended dietary allowance (**RDA**) for vitamin K is based solely on hepatic requirements but is too low for extra-hepatic requirement, such as bone. The first evidence that poor vitamin K status is associated with an increased risk of femoral neck fracture was postulated by Hart et al., who found that circulating vitamin K concentrations in patients with hip fractures were extremely low ¹⁰. There is a significant correlation between hip fracture incidence and vitamin K intake. Moreover, regional variations in food patterns, suggest that increasing intake of vegetables and legumes might lead to a decrease in hip fracture incidence in the future¹¹.



Figure 3

This observation was confirmed by others who correlated fracture risk with serum osteocalcin carboxylation¹²⁻¹⁶ or dietary vitamin K1.^{17,18} Most authors also found an association between vitamin K status and bone mineral density (BMD). Only a limited number of intervention studies have been published testing the effect of K1 on bone. A general trend is that K1 (in ranges between 250 µg up to 10 mg/day) alone improves osteocalcin carboxylation, but has little effect on BMD.¹⁹⁻²¹

In combination with calcium and vitamin D, a moderate effect of vitamin K1 on BMD has been reported (35% less bone loss than with calcium and vitamin D alone)¹⁸ In a large number of Japanese studies, K2 has been tested in high doses (45 mg/day)²²⁻²⁴. With these doses vitamin K is not used as a nutritional supplement, but as a pharmaceutical drug. Obviously, the extremely high K2 intake resulted in maximal osteocalcin carboxylation. In a randomized placebo-controlled vitamin K2 intervention trial among 340 Caucasian postmenopausal women it was demonstrated that K2 (45 mg/day during 3 years) had little effect on the BMD, but induced an increase of the BMC and showed that **bone strength** at the site of the femoral neck did not vary during the entire duration of K2 treatment, whereas in the placebo group there was a significant and consistent decline of bone strength²⁵. Re-examination of the DEXA scans of previous vitamin K1 trials did not show such an effect for vitamin K1!

Another explanation for this discrepancy between K1 and K2 (besides bioavailability, half life and bioactivity) is that K2 vitamins have a second function not related to vitamin K activity. We and others have shown that the side chain of K2 vitamins may be regarded as a geranylgeranyl derivative **which inhibits osteoclast activation**, most likely via the inhibition of the mevalonate pathway^{26, 27}. Although menaquinone-4 (MK-4) seems to be the obvious form of K2 to be used at pharmacological doses, arguments are provided that menaquinone-7 (MK-7) may be more effective at doses that do not exceed the present recommendations for daily vitamin K intake²⁶ The fermented soybean product Natto is a natural source of MK-7. Natto is a Japanese breakfast food that has been traditionally eaten for over 1000 years. Studies in Japan have linked vitamin K2 to a reduction of cardiovascular diseases and could explain the lower levels of heart disease in Japan. Additionally, several Japanese studies have been published showing the effect of nutritional MK-7 intake and bone health. At regular intake of nutritional doses of MK-7 from Natto, the long half life of MK-7 lead to accumulation in extra-tissues that may only be reached with much higher and more frequent doses of synthetic MK-4^{28,29}. Moreover, studies performed by Ikeda et al. showed that habitual natto intake was associated with reduced bone loss in postmenopausal women. Natto intake may help to prevent postmenopausal bone loss

Children's bone health: scientific evidence

Bone turnover is highest during childhood and adolescence. Therefore children and elderly have the greatest requirement for vitamin K. It is generally accepted that the higher peak bone mass you achieve, the more protected in later life to develop osteoporosis.

Scientific evidence, both population-based (i.e., epidemiological) studies and clinical trials have linked better vitamin K status in children to stronger and healthier bones. In a recent study published in the British Journal of Nutrition February 2008.⁵⁴ Summeren, et al showed that improved vitamin K status in children over a two-year period resulted in higher bone mineral density. The association between better vitamin K status and better bone health was confirmed by measuring the ratio of active to inactive osteocalcin.

Findings from previous studies also indicated that additional vitamin K intake might improve bone geometry and positively influence bone mass. The contributing effect has also been reported by O'Connor, et al,⁵⁵ who found better vitamin K status related to higher bone mineral density in 223 healthy girls (11-12 years), Coumarin (e.g. warfarin), a widely prescribed blood-thinning medication, inhibits vitamin K activity.

A study in children receiving coumarin, showed a vitamin K deficiency resulting in significantly reduced bone mass.⁵⁶ This illustrates the potential consequences of vitamin K deficiency in young growing bone.

Children have a much higher bone metabolism⁵⁷ than adults – therefore their need for vitamin K is also higher.

Several studies have shown a pronounced vitamin K deficiency in children. In the majority of children a marked elevation of inactive (undercarboxylated) osteocalcin was found, indicative of poor vitamin K status.⁵⁸ Kalkwarf⁵⁹ et al. underlined that the true requirement for K Vitamins may be much higher than the current recommendation.

through the effect of menaquinone 7, which is more abundant in natto than in any other soybean product.³⁰ The study was evaluated in a representative population of over 1200 healthy Japanese women, ages 20-79.

Calcium Supplementation, cause for concern?

Calcium supplementation is frequently given to healthy postmenopausal women for supporting bone health. In a recent study investigating the effects of daily calcium supplementation (1000 mg) for 5 years in healthy postmenopausal women on bone metabolism and bone density questioned the veracity of supplementation. This study found calcium supplementation may be associated with increased cardiovascular events, particularly myocardial infarction. Thus, although the effect of calcium supplementation was beneficial for bone health it was detrimental for CVD⁶. Unfortunately, vitamin K consumption was not measured in this study, however, experts have raised the issue of a need for vitamin K supplementation concomitantly to help support calcium utilization.⁶⁹

Bisphosphonates are by far the most common medications prescribed for osteoporosis treatment. Bisphosphonates are antiresorptive medicines, which mean they slow or stop the natural process that dissolves bone tissue, resulting in maintained bone density and strength. This may prevent the development of osteoporosis. If osteoporosis already has developed, slowing the rate of bone thinning reduces the risk of fractures³¹

Bisphosphonates have also some unwanted side effects such as heartburn, headache and pain in muscles and joints. As it is recommended to take bisphosphonates together with calcium and vitamin D the absorption could be low as it is known that calcium supplements may interfere with the ability to absorb bisphosphonates.

Poor vitamin K status and cardiovascular disease: scientific evidence

Recent human research has unequivocally linked the role of vitamin K to vascular calcification. Vitamin K was considered for over a half century only to be important for normal blood clotting. Oral anticoagulant therapy is a widely used treatment for patients with increased thrombosis risk. Coumarins such as warfarin, inhibit the recycling of vitamin K, thereby reducing the vitamin K-activity status. The effect of anticoagulants is the inhibition of carboxylation of coagulation factors, resulting in the formation of inactive, noncarboxylated proteins induced by vitamin K absence (PIVKA). For more than half a century it was believed that the only effect of coumarins was blocking the activation of clotting factors, synthesized in the liver. Osteocalcin and Matrix Gla protein (MGP) are the two best characterized extra-hepatic vitamin K-dependent calcium binding proteins. In case of vitamin K insufficiency these proteins are sub-optimally carboxylated, and thus not be able to bind calcium as effectively as required for optimal function. The impairment of MGP due to incomplete carboxylation may be regarded as a risk factor for arterial calcification⁴, and impairment of the function of osteocalcin results in an increased risk of osteoporosis³².

It has been shown in experimental animals, creating vitamin K deficiency in the arterial vessels using the vitamin K-antagonist warfarin, that vascular calcifications were present within two weeks³⁴. Also in humans vitamin K-antagonists like warfarin cause a doubling of the arterial calcifications as compared to patients not receiving vitamin K-antagonist^{35, 36}. Calcification results in an increase of aortic stiffness and hence contributes to systolic hypertension and left ventricular hypertrophy, coronary insufficiency, ischemia and congestive heart failure³⁷⁻⁴⁰. Calcification of the arterial vessel wall is common⁴¹. This is why it is a necessity to prevent the deposition of calcifications in the arteries, or even to block or lower already present arterial calcifications.



Figure 4A



Figure 4B

A **key-player** in this is the **vitamin K-dependent matrix Gla-protein (MGP)**. Therefore activation of MGP is extremely important. In a rat model for arterial calcification it was demonstrated that **vitamin K2 completely prevented calcification** (figure 4B), whereas vitamin K1 had no effect at all. (Figure 4A)⁴². MGP, a potent inhibitor of calcification is present in cartilage and the vessel wall. Deficiency of vitamin K, either to nutritional deficiency or due to the use of coumarin-derivatives, results in undercarboxylation of MGP (ucMGP) and impairment of its biological function. There is scientific evidence⁴³ that in atherosclerotic lesions and areas of calcification massive accumulation of ucMGP is present. Therefore, serum ucMGP may become a biomarker to identify people at risk for developing vascular calcification⁴. Recently, it was shown in rats that pre-formed arterial calcifications could be regressed by high vitamin K2 intake, and that elasticity of the arterial vessel wall was restored⁴⁴. In this study, evidence was provided that the effect of vitamin K was caused via activation of MGP. Additionally, in hypercholesterolemic rabbits it has been shown that high doses of vitamin K2 decrease the circulating cholesterol concentrations, and suppress the progression of atherosclerotic plaques, intima thickening and pulmonary atherosclerosis.⁴⁵

In a cross sectional analysis among 5,000 participants of the Rotterdam study, Geleijnse *et al.* reported a strong correlation between long-term nutritional vitamin K2 (mainly long-chain menaquinones) intake and aortic calcification and cardiovascular death.⁴⁶ After sub-division of the cohort into tertiles according to dietary vitamin K2 intake and using the lowest tertile of intake as a reference, the cardiovascular mortality risk was 50% lower in the highest tertile for vitamin K2 intake, whereas the all-cause mortality risk was 25% lower! 45mcg of total menaquinones was also shown to be the highest consumption intakes in the study done on Heidelberg population during cancer study (97731 people during 8.6y)⁴⁷ For vitamin K1 the observed associations were not significant, consistent with data from our group, showing preferential uptake of K2 by the vessel wall and no effect of K1 on arterial calcification in the rat model (see figure 4) ^{5, 42}

There is not enough vitamin K in Western diet

Current daily recommendations for Vitamin K are based exclusively on K1 and the requirement for proper blood clotting which was set to 1µg K1 per kg of body weight (daily). This amount is not sufficient for the optimal function in other tissues such as bone and vasculature which need vitamin K as well.

Hence, the majority of both children and adults are vitamin K deficient. This may have a negative impact on bone and vascular health. It may lead to a lower bone mass, resulting in more fractures; additionally low vitamin K status in the vasculature will lead to impairment of MGP, thereby not able to inhibit unwanted calcification.

Moreover, vitamin K1 is not stored in the body for a long time and people are therefore dependent on the regular intake from the food. However, the regular consumption of foods containing vitamin K is not sufficient for maintaining optimal health. Regular western food contains K1 in green vegetables (poor absorption) and some K2 in fermented products (e.g. especially cheeses). It is therefore important to supplement people with additional vitamin K2. **The only safe and effective Natural vitamin K2 is now commercially available as MenaQ7™.**

MenaQ7™ is natural

Figure 5



Natto food has been consumed in Japan since history. It's consumption in Japan has been linked to lower prevalence of osteoporotic diseases compared to Japanese people not consuming Natto ²⁸. Analyzing Natto for its content of various forms of Vitamin K, it contains predominantly MK-7.

Due to legislation and availability, only three forms of vitamin K are on the market. Synthetic K1 (Konakion), synthetic MK-4 (menatetrenone), and natural MK-7 carefully purified from Natto. **MenaQ7™** is already on the market since 1998. In Asian countries no synthetic products are allowed to give to newborns. Therefore, natural derived MK-7 from natto is used to prevent hemorrhagic disease of the newborn and thus it is successfully given in Asian countries to prevent newborns from bleedings. From this and other studies using natto food with exact content of MK-7 it can be concluded that MenaQ7™ is absolutely safe.

MenaQ7™ has optimal bioavailability, half-life and bioactivity

Many studies have been conducted to demonstrate bioavailability of Vitamin K ⁴⁸⁻⁵¹. In the journal *Blood*, the official journal of the American Society of Hematology, a possible explanation was given for the greater benefits of MK-7 over K1 in promoting bone and cardiovascular health. In this first human study using natural vitamin K2 as a dietary supplement it was demonstrated that natural vitamin K2 as menaquinone-7 (MK-7) was significantly better as compared to synthetic vitamin K1 in several important areas, including better absorption, much longer bioavailability and higher efficacy levels in the body.

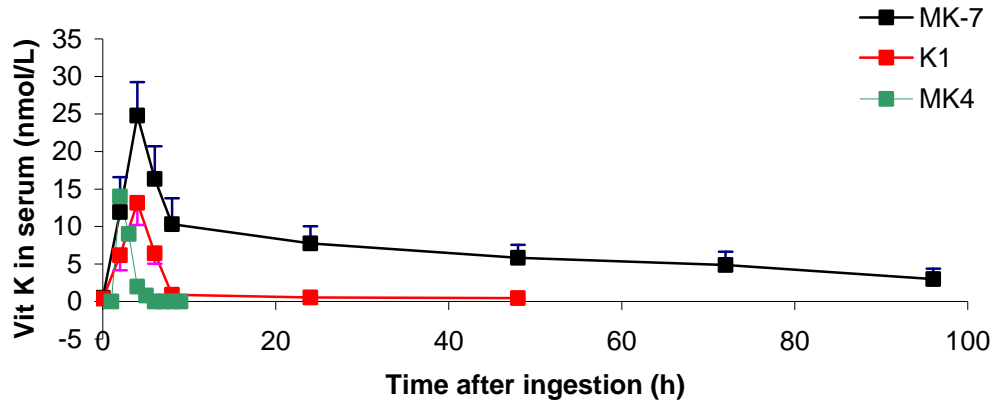


Figure 6

The study showed that MK-7 was absorbed into human blood as quickly as K1, but with a 1.5 fold better absorption. It also remained at significant high levels for a much longer period of time as compared to K1 (36 times!). Moreover, MK-7 also promoted and activated markers of bone building.

MenaQ7™ and serum half-life

The primary reason for MK-7's superiority appears to be its very long half-life in the blood, compared to the half-life of K1, which results in more stable blood levels and **significantly greater accumulation** of vitamin K (MK7) in the blood. This means that if taken in single daily doses, only MK-7 is effectively present in the circulation and available for absorption by various tissues during the 24 hours following intake. If taken on a regular basis, MK-7 accumulates during the first 2 weeks, after which a steady-state level is reached, whereas there is no accumulation of K1.

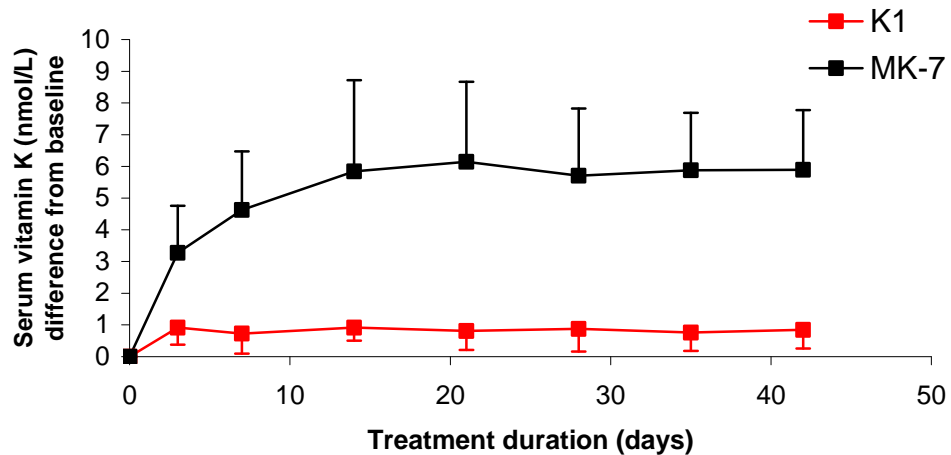


Figure 7

Calculating the half life in plasma from these absorption curves, MK-4 has a half life of 1 hour, K1 has a half life of 1.5 hours, whereas MenaQ7™ **has a half life of 3 days!** Research data show that long chain menaquinones like MenaQ7™ are redistributed by the liver, transported by low and high density lipoproteins in the circulation and made available for extra-hepatic tissues as well⁵ This is not the case for K1, which

remains in the liver⁵². In this way MenaQ7™ reaches besides the liver also extra-hepatic tissues like bone and vasculature.

MenaQ7™ – micrograms – and not milligrams

Most of the articles presenting clinical studies use either K1 or menatetrenone (MK-4). This reason for this is that both synthetic vitamins have been available for many years on the market. The awareness of the beneficial properties of long-chain menaquinones like MenaQ7™ only arose in the last decade. Since both K1 and MK4 have both a short half life in the circulation, the dose needed to build up a store for extra hepatic tissues such as bone and vasculature is high and frequent daily intake is necessary (e.g. three – six times a day). **MenaQ7™ is highly available**, and has a half life of three days which leads to accumulating menaquinone-7 levels in the circulation⁵³. That MenaQ7™ is also the **most bioactive form** was demonstrated by the activation of osteocalcin, the vitamin K-dependent protein reflecting vitamin K status of bone. MenaQ7™ was compared to synthetic K1 and it turned out that MenaQ7™ was significantly better in bioavailability and bioactivity. Therefore the dose of MenaQ7™ needed to have a significant effect is in the microgram and not in the milligram range! MenaQ7™ at dose up to 50 mcg was recognized by GRAS Association in the US as supplement safe to use in dairy food enrichment. This evaluation was based on an independent literature searching led by Panel of scientific Experts. The only safe and effective Natural Vitamin K2 is now commercially available as MenaQ7™.



Synthetic vs. “Natural” vitamin K2

IN CONCLUSION:

Synthetic vitamin K1 or MK-4

- Quickly appears but also disappears from the blood. Half life 1 - 2 hours!
- Therefore: High pharmacological doses (mg) necessary; multiple daily doses needed
- Large doses interact negatively with persons on blood-thinning medications (warfarin) – very dangerous and medical supervision necessary

Natural Vitamin K2 as Menaquinone-7 (MK-7) from Natto

- Highly bioavailable, highly bioactive and longest serum half life ⁴¹. Half life 3 days!
- Long half life will result in building up a buffer of MK-7, and supplies MK-7 to ALL tissues 24 hours a day.
- More effective at doses that do not exceed the present recommendations for daily vitamin K intake
- At 45mcg/daily, provides levels shown to be the highest consumption in the Rotterdam study
- Not likely to interact negatively with blood-thinning medications (warfarin) at 45 mcg/day!

Vitamin K supplementation and anticoagulation treatment

Patients receiving oral anti-coagulant therapy should not take Vitamin K supplements without consulting their physician first.



Other Indications for K Vitamins

Skin Health Vitamin K plays an important role in skin health by promoting a vitamin-K dependent protein called matrix-GLA protein (MGP). MGP is the most potent inhibitor of soft tissue calcification known, and adequate amounts of vitamin K are necessary for it to function – resulting in protection of elastin in the skin, and protect the vasculature, including capillaries in the skin from calcification^{60, 62, 63}

Varicose veins have an estimated prevalence of between 5% to 30% in the adult population, with a female to male predominance of 3 to 1. The exact mechanisms by which varicose vein develop are still unclear, although several risk factors are known to be involved, including genetic predispositions, age, obesity, physical activity, standing occupations, multiple pregnancies and connective tissue abnormalities. The vitamin K-dependent protein (MGP) is necessary to inhibit increased mineralization of the smooth muscle cells in the vein wall, which may contribute to the development of varicose veins. In a recent study researchers found that in varicose veins the local vascular vitamin K status was insufficient to activate all MGP, and that supplementation could restore the activity of MGP. Active MGP could inhibit both proliferation and mineralization of veins, thereby helping inhibit the development of varicosis⁶⁰.

Cellular Health Antitumor activities of phyloquinone and menaquinones have been observed in various cancer cell lines, including liver, lung, stomach and breast⁶³. The anticancer activity of the natural vitamin K is mediated by antiproliferative effects through inductions of proto-oncogenes, which foster cell cycle arrest and induce apoptosis in several cancer cell lines.

Recent data showed that only long chain menaquinones derived from dairy products (MK5-9) were associated with significantly lower risk of advanced prostate cancer⁴⁷. In addition, several case studies have demonstrated the clinical benefit of vitamin K2 in the treatment of patients with acute myeloid leukemia and myelodysplastic syndrome⁶⁴.

Arthritis Rheumatoid arthritis is a chronic, inflammatory autoimmune disorder that causes the immune system to attack the joints, which can lead to substantial loss of mobility due to pain and joint destruction.

Vitamin K may represent a new agent for the treatment of rheumatoid arthritis, with other disease modifying antirheumatic drugs due to inhibition of proliferation of fibroblast-like synoviocytes through the induction of apoptosis. Research conducted by Okamoto and others showed that vitamin K2 inhibits the development of collagen-induced arthritis in a dose-dependent manner.⁶⁵ Moreover, research conducted by Neogi et al showed the association between low plasma levels of vitamin K and increased prevalence of osteoarthritis manifestations in the hand and knee⁶⁶.

Brain Health Recent research also indicates that vitamin K could contribute to brain health. It is known, that signals from glial cells play essential role for brain development. However, only recently it was discovered that MGP, a potent inhibitor of calcification, is present in neuronal cells, and their level is regulated by glial cells⁶⁷. Vitamin K can penetrate blood brain barrier, and there is a possibility that their deficiency may impairment biological function of neurons.

The hypothesis is now proposed that vitamin K deficiency contributes also to the pathogenesis of Alzheimer's disease (AD) and that vitamin K supplementation may have a beneficial effect in preventing or treating the disease. Vitamin K may also reduce neuronal damage associated with cardiovascular disease⁶⁸

Research involving these conditions, and in many other fields, continues. Check periodically with www.vitamink2.org for the latest information.

Literature

1. Vermeer C. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochem J.* 1990;266(3):625-636.
2. Shearer MJ. Vitamin K. *Lancet.* 1995;345(8944):229-234.
3. Vermeer C, Hamulyak K. Pathophysiology of vitamin K-deficiency and oral anticoagulants. *Thromb Haemost.* 1991;66(1):153-159.
4. Cranenburg EC, Vermeer C, Koss R, Boumans ML, Hackeng TM, Bouwman FG, Kwaijtaal M, Brandenburg VM, Kettler M, Schurgers LJ. The Circulating Inactive Form of Matrix Gla Protein (ucMGP) as a Biomarker for Cardiovascular Calcification. *J Vasc Res.* 2008; 10;45(5):427-436.
5. Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. *Biochim Biophys Acta.* Feb 15 2002;1570(1):27-32.
6. Wallin, R., Wajih, N., Greenwood, G.T. Et al. Arterial calcification : a review of mechanisms, animal models and the prospects for therapy. *Med. Res. Rev.* 2001; 21:274-301
7. Bagger YZ, Tankó LB, Alexandersen P, Qin G, Christiansen C; Prospective Epidemiological Risk Factors Study Group. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. *J Intern Med.* 2006 ;259(6):598-605.
8. Farhat GN, Newman AB, Sutton-Tyrrell K, Matthews KA, Boudreau R, Schwartz AV, Harris T, Tylavsky F, Visser M, Cauley JA; Health ABC Study. The association of bone mineral density measures with incident cardiovascular disease in older adults. *Osteoporos Int.* 2007 ;18(7):999-1008.
9. Hofbauer LC, Brueck CC, Shanahan CM, Schoppet M, Dobnig H. Vascular calcification and osteoporosis--from clinical observation towards molecular understanding. *Osteoporos Int.* 2007 Mar;18(3):251-9. Epub 2006 Dec 7. Review.
10. Hart JP, Catterall A, Dodds RA, et al. Circulating vitamin K1 levels in fractured neck of femur [letter]. *Lancet.* 1984;2(8397):283.
11. . Yaegashi Y, Onoda T, Tanno K, Kuribayashi T, Sakata K, Orimo H. Association of hip fracture incidence and intake of calcium, magnesium, vitamin D, and vitamin K. *Eur J Epidemiol.* 2008; 23(3):219-25.
12. Booth SL, Tucker KL, Chen H, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr.* 2000;71(5):1201-1208.
13. Szulc P, Chapuy MC, Meunier PJ, et al. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest.* 1993;91(4):1769-1774.
14. Szulc P, Chapuy MC, Meunier PJ, et al. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study [see comments]. *Bone.* 1996;18(5):487-488.
15. Vergnaud P, Garnero P, Meunier PJ, et al. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab.* 1997;82(3):719-724.
16. Väänänen HK, Luukinen H, Käkönen S-M, et al. Strong prediction of fractures among the elderly by the ratio of carboxylated and total serum osteocalcin. *Calcif Tissue Int.* 1999;64:S79.
17. Booth SL, Broe KE, Gagnon DR, et al. Vitamin K intake and bone mineral density in women and men. *Am J Clin Nutr.* Feb 2003;77(2):512-516.
18. Feskanich D, Weber P, Willett WC, et al. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr.* 1999;69(1):74-79.
19. Braam LA, Knapen MH, Geusens P, et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. *Calcif Tissue Int.* Jul 2003;73(1):21-26.
20. Braam LA, Knapen MH, Geusens P, et al. Factors affecting bone loss in female endurance athletes: a two-year follow-up study. *Am J Sports Med.* Nov-Dec 2003;31(6):889-895.
21. Bolton-Smith C, Mole PA, McMurdo MET, et al. Two-Year intervention with phylloquinone (vitamin K1), vitamin D and calcium effect on bone mineral content of older women. *Ann Nutr Metab.* 2001;45 (suppl 1):46.
22. Shiraki M, Shiraki Y, Aoki C, et al. Vitamin K2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J Bone Miner Res.* 2000;15(3):515-521.

23. Iwamoto J, Takeda T, Ichimura S. Effect of combined administration of vitamin D3 and vitamin K2 on bone mineral density of the lumbar spine in postmenopausal women with osteoporosis. *J Orthop Sci.* 2000;5(6):546-551.
24. Iwamoto J, Takeda T, Ichimura S. Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison with the effect of etidronate. *J Orthop Sci.* 2001;6(6):487-492.
25. Knapen MH, Schurgers LJ, Vermeer C. Vitamin K(2) supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int.* Feb 8 2007.
26. Schurgers LJ, Knapen MH, Vermeer C. Vitamin K2 improves bone strength in postmenopausal women. *ISNAO.* 2007.
27. Hara K, Akiyama Y, Nakamura T, et al. The inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. *Bone.* 1995;16(2):179-184 issn: 8756-3282.
28. Kaneki M, Hedges SJ, Hosoi T, et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition.* Apr 2001;17(4):315-321.
29. Yamaguchi M, Taguchi H, Gao YH, et al. Effect of vitamin K2 (menaquinone-7) in fermented soybean (natto) on bone loss in ovariectomized rats. *J Bone Miner Metab.* 1999;17(1):23-29.
30. Ikeda Y, Iki M, Morita A, Kajita E, Kagamimori S, Kagawa Y, Yoneshima H. Intake of fermented soybeans, Natto, is associated with reduced bone loss in postmenopausal women: Japanese Population-Based Osteoporosis (JPOS) Study *J Nutr.* 2006; 136(5):1323-8.
31. Bone JG, et al. Ten years' experience with alendronate for osteoporosis in postmenopausal women. *New England Journal of Medicine,* 2004; 350(12): 1189-1199.
32. Cranenburg EC, et. al., Vitamin K: The coagulation vitamin that became omnipotent. *Thromb Haemost.* 2007; 98(1):120-5.
33. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ.* 2008; 2;336(7638):262-6.
34. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol.* 1998;18(9):1400-1407.
35. Schurgers LJ, Aebert H, Vermeer C, et al. Oral anticoagulant treatment: friend or foe in cardiovascular disease? *Blood.* Nov 15 2004;104(10):3231-3232.
36. Koos R, Mahnken AH, Muhlenbruch G, et al. Relation of oral anticoagulation to cardiac valvular and coronary calcium assessed by multislice spiral computed tomography. *Am J Cardiol.* Sep 15 2005;96(6):747-749.
37. Lehto S, Niskanen L, Suhonen M, et al. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol.* Aug 1996;16(8):978-983.
38. Ziemann SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* May 2005;25(5):932-943.
39. Rosenhek R, Binder T, Porenta G, et al. Predictors of outcome in severe, asymptomatic aortic stenosis. *N Engl J Med.* Aug 31 2000;343(9):611-617.
40. Raggi P, Shaw LJ, Berman DS, et al. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. *J Am Coll Cardiol.* May 5 2004;43(9):1663-1669.
41. Iribarren C, Sidney S, Sternfeld B, et al. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. *Jama.* 2000;283:2810-2815.
42. Spronk HM, Soute BA, Schurgers LJ, et al. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. *J Vasc Res.* Nov-Dec 2003;40(6):531-537.
43. Sweatt A, Sane DC, Hutson SM, Wallin R. Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. *J Thromb Haemost.* 2003 Jan;1(1):178-85
44. Schurgers LJ, Spronk HM, Soute BA, et al. Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood.* Nov 30 2006.
45. Kawashima H, Nakajima Y, Matubara Y, et al. Effects of vitamin K2 (menatetrenone) on atherosclerosis and blood coagulation in hypercholesterolemic rabbits. *Jpn-J-Pharmacol.* 1997;75(2):135-143.

46. Geleijnse JM, Vermeer C, Grobbee DE, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr.* Nov 2004;134(11):3100-3105.
47. Nimptsch K, Rohrmann S, Linseisen J. Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg). *Am J Clin Nutr.* 2008; 87(4):985-92.
48. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. *Br J Nutr.* 1996;76(2):223-229.
49. Schurgers LJ, Vermeer C. Determination of Phylloquinone and Menaquinones in Food. effect of food matrix on circulating vitamin K concentrations. *Haemostasis.* 2000;30(6):298-307.
50. Schurgers LJ, Shearer MJ, Hamulyak K, et al. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. *Blood.* Nov 1 2004;104(9):2682-2689.
51. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. *J Nutr.* 1998;128(5):785-788.
52. Lamou-Fava S, Sadowski JA, Davidson KW, et al. Plasma lipoproteins as carriers of phylloquinone (vitamin K1) in humans. *Am J Clin Nutr.* 1998;67(6):1226-1231.
53. Schurgers LJ, Teunissen KJ, Hamulyak K, et al. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood.* Apr 15 2007;109(8):3279-3283.
54. Van Summeren M, et al. Vitamin K status is associated with childhood bone mineral content, *British Journal of Nutrition* (Published online by Cambridge University Press 18 Feb 2008)
55. O'Connor E., et al. Serum percentage undercarboxylated osteocalcin, a sensitive measure of vitamin K status, and its relationship to bone health indices in Danish girls, *British Journal of Nutrition* (2007), 97, 661-666
56. Barnes C., et al. (2005) Reduced bone density in children on long-term warfarin. *Pediatric Research* 57:578-581
57. Szulc P et al., Biochemical Measurements of Bone Turnover in Children and Adolescents. *Osteoporos Int* (2000) 11:281-294;
58. Van Summeren M, et al. Pronounced Elevation of Undercarboxylated Osteocalcin in Healthy Children. *Pediatric Research*, Vol. 61, No. 3, 2007
59. Kalkwarf HJ, et al. (2004) Vitamin K, bone turnover, and bone mass in girls. *The American Journal of Clinical Nutrition* 80, 1075-1080
60. Cario-Toumaniantz C at al., Identification of differentially expressed genes in human varicose veins: involvement of matrix gla protein in extracellular matrix remodeling. *J Vasc Res.* 2007;44(6):444-59.
61. Li Q, Jiang Q, Uitto J. Pseudoxanthoma elasticum: oxidative stress and antioxidant diet in a mouse model (Abcc6^{-/-}). *J Invest Dermatol.* 2008;128(5):1160-4.
62. Gheduzzi D, Boraldi F, Annovi G, DeVincenzi CP, Schurgers LJ, Vermeer C, Quagliano D, Ronchetti IP. Matrix Gla protein is involved in elastic fiber calcification in the dermis of pseudoxanthoma elasticum patients. *Lab Invest.* 2007 Oct;87(10):998-1008. Epub 2007 Aug 27. Lamson DW at al., The anticancer effects of vitamin K. *Altern. Med. Rev* 2003; 8:303-18.
63. Takami A at al., Successful therapy of myelodysplastic syndrome with menaterenone, a vitamin K2 analog. *Int J Hematol.* 1998; 69, 24-26.
64. Fujita H at al., Vitamin K2 combined with all-trans retinoic acid induced complete remission of relapsing acute promyelocytic leukemia. *Br J Haematol.* 1998; 103, 584-585.
65. Okamoto H at al., Anti-arthritis effects of vitamin K(2) (menaquinone-4)--a new potential therapeutic strategy for rheumatoid arthritis. *FEBS J.* 2007 ; 274(17):4588-94.
66. Neogi T, Booth SL, Zhang YQ, Jacques PF, Terkeltaub R, Aliabadi P, Felson DT. Low vitamin K status is associated with osteoarthritis in the hand and knee. *Arthritis Rheum.* 2006 Apr;54(4):1255-61.
67. Goritz C et al. Glia-induced neuronal differentiation by transcriptional regulation. *Glia.* 2007,15;55(11):1108-22.
68. Allison AC. The possible role of vitamin K deficiency in the pathogenesis of Alzheimer's disease and in augmenting brain damage associated with cardiovascular disease. *Medical Hypotheses* , 2001; 57 (2): 151 - 155.
69. Schurgers, LJ, Vermeer, C, " The need for vitamin K in calcium supplements" *British Medical Journal RAPID RESPONSES*, 1 February 2008