INTRODUCTION AND SCIENTIFIC ABSTRACT

Vitamin K2

Benefits for heart and bone health

VITAMIN K2 IS THE MOST BIOLOGICALLY ACTIVE FORM OF VITAMIN K.
IT IS ALSO THE MOST BENEFICIAL FOR BONE HEALTH MAXIMIZATION.

CONTENT

Vitamin K2 directs calcium to the bones

Vitamin K2 the forgotten Vitamin

Vitamin K2 is an essential fat-soluble vitamin vital for optimal bone and cardiovascular health. It regulates the transport and distribution of calcium in the body. K2 variant MK-7 surpasses other K2 forms due to its superior bioavailability, longer half-life, and lower dosage requirement. Vitamin K2 has a long history that spans centuries and continents, but it remained 'the forgotten vitamin' for a long time. Thanks to several scientific studies on vitamin K2 spanning almost two decades, K2 was reborn with a new vigor. These studies demonstrate the effects of K2 supplementation on bone and cardiovascular health. The efficacy of vitamin K2 was studied in multiple population segments including women and children. Apart from these scientific milestones, perfecting of organic synthesis of all-*trans* vitamin K2 MK-7 (similar in biological activity and function to fermentation-derived K2) and microencapsulation of K2 MK-7 (which made MK-7 stable in formulation) enabled K2 to enter the mainstream.

Vitamin K2 puts calcium into balance

Calcium is the key link connecting vitamin K2 to bone and cardiovascular health. Vitamin K2 directs calcium to the bones instead of the arteries. K2's mechanism of action involves activation (by carboxylation) of two key proteins – osteocalcin and matrix Gla-protein (MGP). Osteocalcin plays a role in the integration of calcium into the bone matrix whereas K2-activated MGP binds free-floating calcium and prevents calcium deposition in arteries. Fermented food products like the soy-based Japanese dish natto, are the major sources of dietary K2.

Studies have established a link between a K2-rich diet and improved bone-mineral density. Western diets, on the other hand, are deficient in vitamin K2. Hence, nutritional supplementation is the only way to overcome dietary K2 deficiencies. **K2 MK-7 together with calcium and vitamin D3 is an optimal combination for bone health.** Similar formulations for heart health containing K2 and other heart-protecting ingredients can provide a superior health benefit.

Vitamin K2 for bone and heart health

Vitamin K1

Vitamin K1 or phylloquinone accounts for around 90% of the total dietary vitamin K intake. Green leafy vegetables such as green salads, broccoli, kale, and spinach are the major sources of K1. Although the intake of vitamin K in the general population is between 70-250 µg/day, less than 20% of vitamin K1 from dietary sources is absorbed by the body (poor bioavailability). The major function of K1 is to aid blood coagulation by activating blood-clotting proteins.

Vitamin K2

Vitamin K2 or menaquinones comprise a group of structurally similar molecules which differ in their chain length and biological activity. Their side chains can vary from 4-14 isoprenoid units (designated as MK-n, n being the number of side chains.) MK-4 and MK-7 are the most important menaquinones. MK-4 can be obtained from meat, dairy, and eggs whereas MK-7 is mainly found in fermented foods such as cheese, yogurt, and natto (a fermented Japanese soybean product.) Vitamin K2 is essential for optimal bone and heart health.

Vitamin K2 MK-7 is superior to MK-4 regarding biological activity and half-life in the body. MK-7 has a half-life of about 72 hours whereas it is only about 1.5 hours for MK-4. Therefore, MK-7 remains biologically active in the body longer and allows for a better bodily distribution and activation of extra-hepatic K2-dependent proteins such as osteocalcin and matrix gla-protein (MGP). These differences arise from the differences in the distribution and breakdown of the two MK forms. MK-4 is mainly transported in the blood via triglyceride-rich lipoproteins whereas low-density lipoproteins transport MK-7. MK-7 reaches bones and vessel walls easier than MK-4. The daily requirement of MK-7 is much lower compared to MK-4 (around 90-120 µg/day for MK-7 and 45,000 µg three times a day for MK-4.)

Vitamin K2 MK-7 exists in two isomeric forms – *cis* and *trans*. The *trans* isomer is the naturally- occurring and the most bioactive form of MK-7. All-*trans* MK-7 is linear in structure due to an extended system of isoprenoid units whereas the *cis* isomers are non-linear. Hence, the *cis* MK-7 does not fit well into subcellular structures, leaving K2-dependent enzymes and proteins inactivated. Fermentation-derived vitamin K2 MK-7 is usually a mixture of *trans* and *cis* isomers along with other compounds. *Trans* MK-7 must be purified from this mixture to obtain the bioactive substance. Synthetic K2 MK-7, on the other hand, bypasses these issues as the final product obtained is all-*trans* K2 MK-7 with no contamination from *cis* isomers or other compounds.

K2 Deficiency

Western diets are currently deficient in vitamin K2 as the daily dietary intake of K2 has reduced over the past hundred years. In the previous century, it was common to eat vitamin K2-rich foods like cured fish and aged cheese. The exact knowledge of K2 MK-7 intake levels in different countries is lacking. Inactive proteins – osteocalcin and matrix Gla-protein (MGP) – are the biomarkers of vitamin K2 deficiency. Osteocalcin incorporates calcium into bone whereas MGP binds excess calcium in the blood to prevent its deposition in arteries. Both proteins are dependent on vitamin K2 as both need to be activated by K2 in a carboxylation process to function properly. The ratios of carboxylated and uncarboxylated osteocalcin and MGP in the blood are, therefore, important biomarkers of vitamin K2 status in the human body. Multiple scientific studies over the past two decades have shown that in western populations, a high percentage of osteocalcin and MGP remain

in the inactive (uncarboxylated) form as demonstrated by blood samples from healthy subjects.

Supplementation can overcome the K2 deficiency. K2 supplementation increases the amount of activated osteocalcin and MGP. For example, a study with healthy volunteers demonstrated that before supplementation with vitamin K2, levels of inactive osteocalcin were high, whereas afterward they were low and most of the osteocalcin was activated. To achieve sufficient osteocalcin activation, research indicates that 90-120 µg of vitamin K2 as a daily dose is sufficient. Populations that reach the recommended daily dose, such as Japanese people consuming natto, were shown to have lower rates of bone and heart disease. Other epidemiological studies in Japan also strengthen the notion that MK-7 promotes bone health. Studies have also recognized the beneficial effects of vitamin K2 supplementation in reducing levels of inactive osteocalcin and MGP, and thereby improving bone and cardiovascular health.

› Bone ‹

Calcium is the building block of bones. Vitamins D3 and K2 regulate calcium utilization in the body. Vitamin D3 promotes intestinal uptake and transport of calcium ensuring sufficient blood calcium levels for normal mineralization of collagen matrix in the skeleton. Vitamin K2 activates osteocalcin, a protein which helps incorporate calcium into the bone matrix. Hence, optimal levels of calcium and vitamins D3 and K2 must be maintained throughout our lives, particularly during childhood and puberty, when most of the bone mass is made, and during the later years when the risk of fracture is more.

Several studies demonstrate the link between vitamin K2 and bone health in adults and children. Children need high levels of vitamin K2 to support their rapid growth. However, studies show that vitamin K2 levels in children and adolescents are low and that the inactive form of osteocalcin (ucOC, undercarboxylated osteocalcin) is more abundant than the active form (cOC, carboxylated osteocalcin) when compared to other age groups. Vitamin K2 MK-7 supplementation elevates activated osteocalcin levels in children.

Osteoporosis, a condition characterized by brittle bones with low mineral density, is rampant among the aging population. Clinical studies involving postmenopausal women revealed that they benefited from a daily low dose of MK-7 for three years with a resulting reduced age-related decline in bone mineral density. Also, women diagnosed with osteopenia improved their bone microstructure with MK-7 supplementation.

Dietary intake of vitamin K is sufficient to cover our body's needs to activate blood clotting factors. Contrary to blood clotting factors, studies show that most people do not consume the required amount for sufficient activation of proteins involved in calcium transport (recommended daily intake levels of up to 120 µg vitamin K depending on age and gender.) Studies also demonstrate that this deficiency can be compensated by vitamin K2 supplementation to make up dietary shortcomings and thereby increase the activation of K2-dependent proteins required for optimal bone health.

›Heart‹

Excess calcium in the body can have detrimental effects on health. It can accumulate in blood vessels and other soft tissues. Hence, proper calcium utilization by the body is not only relevant for bone health but also for cardiovascular health. Vitamin K2 mediates calcium distribution and utilization in the body. K2 activates matrix gla-protein (MGP) by carboxylation to become cMGP which binds free-floating calcium, preventing it from being deposited in arteries. Low blood K2 levels result in higher levels of inactive MGP (dp-ucMGP, a de-phosphorylated and undercarboxylated form), which is incapable of binding to calcium. This may lead to arterial calcification, a condition resulting from calcium build-up within the vascular smooth muscle cells in tunica media (the middle layer of an artery), reducing the elasticity of the vessel and increasing the risk of cardiovascular events.

Scientific and clinical studies investigating the effect of vitamin K2 MK-7 on cardiovascular health demonstrate a significant reduction in arterial stiffness and slower progression of calcification. High blood levels of non-activated MGP (dp-ucMGP) correlates with low vitamin K status and vascular calcification. Higher levels of non-activated MGP have been observed in people aged 65 and older, people at specific risk for vascular calcification, including those with rheumatoid arthritis, aortic valve disease, aortic stenosis, heart failure, chronic kidney disease (CKD), and patients taking vitamin K antagonists. Furthermore, there is an inverse relationship between non-activated MGP and cardiovascular patient survival. In a study of 577 older individuals, non-activated MGP was associated with increased risk of cardiovascular disease, independent of other risk factors and vitamin D status. This effect was attributed to low vitamin K status.

Vitamin K2 supplementation can mitigate these effects by reducing non-activated MGP levels in healthy adults. In a double-blinded, randomized, placebo-controlled clinical trial, arterial stiffness was investigated in 244 healthy postmenopausal women over a period of 3 years, supplemented with either 180 µg of MK-7 or a placebo. Among the participants with an elevated arterial stiffness at baseline, the stiffness index was significantly improved compared to the placebo group. A beneficial effect of MGP activation was also seen among the participants taking MK-7 which experienced a 50% decrease in circulating dp-ucMGP compared to the placebo group. Vitamin K2 doses from 90 µg and higher improve the carboxylation (thereby activation) of MGP in healthy adults.

Pregnancy

Pregnancy is a time when the body's demand for calcium is very high. Pregnant women not only undergo substantial skeletal remodeling in preparation for giving birth but also need to account for the fetus's high demand for calcium for skeletal formation. In extreme cases, this can result in pregnancy-associated osteoporosis, a condition characterized by severe pain in expectant mothers caused by vertebral fractures. As vitamins K2 and D3 are the vital ingredients that regulate calcium distribution and utilization in the body, K2 supplementation can improve bone health during pregnancy and reduce the risk of bone-related pain. Apart from mothers' health, K2 supplementation may also ensure bone health of infants, even after birth. Skeletal development before birth may set the tone for future bone health.

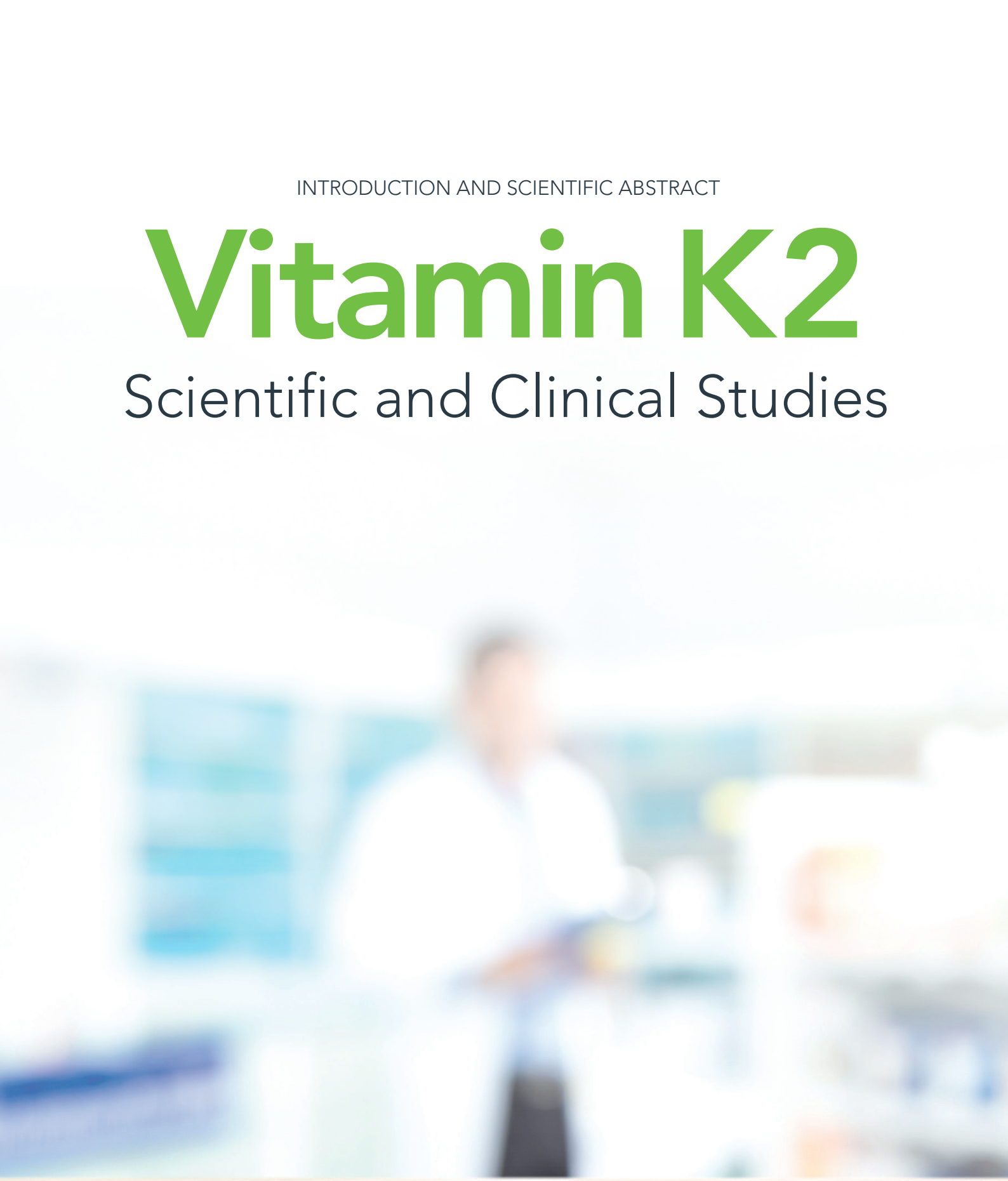
Studies involving expectant mothers demonstrate the benefits of vitamin K2 supplementation during pregnancy. Vitamin K2 is poorly transported from a mother to unborn child. This can be remedied through K2 supplementation. In a Japanese study, women given 20 mg of vitamin K2 about a week before they gave birth had more K2 in their blood compared to the control group (women who were not given K2). Notably, K2 levels were also elevated in umbilical cord blood, indicating the increased transfer of K2 to the child. As a result, none of the children born to K2-treated mothers showed signs of vitamin K deficiency at birth, compared to 90% of the children whose mothers were not given vitamin K. Interestingly, levels of vitamin K2 in breast milk on the fifth day after birth were significantly higher in women who were given K2 before they gave birth.

The levels of vitamin K in breast milk are typically very low at less than 1 ng/ml. A study showed that infants fed only breast milk during the first six months of life had inadequate vitamin K intake (less than 1 µg/kg/day). The mothers of the infants were getting enough vitamin K (more than 1 µg/kg/day), so the problem seems to be an inadequate transfer of vitamin K from the mother's body to her milk. Infants fed a typical supplemented formula were getting approximately 100 times more vitamin K than the breastfed infants, and their circulating vitamin K levels were approximately 20 times higher. Similarly, lactating mothers can also increase the levels of vitamin K2 in their breast milk by taking K2. A study showed increased levels of vitamin K in the breast milk of new mothers who took 5 mg/day of vitamin K1. Additionally, vitamin K levels were also elevated in the blood of their breastfed children. However, it remains unclear whether K2 delivery via breast milk is adequate, so additional supplementation for infants might be required.

INTRODUCTION AND SCIENTIFIC ABSTRACT

Vitamin K2

Scientific and Clinical Studies



SEVERAL STUDIES DEMONSTRATE THE **LINK BETWEEN VITAMIN K2 AND BONE HEALTH** IN ADULTS AND CHILDREN.
STUDIES WITH **VITAMIN K2 MK-7 ON CARDIOVASCULAR HEALTH.**

REFERENCE A

Schwalfenberg, G.K., *Vitamins K1 and K2: The Emerging Group of Vitamins Required for Human Health.*

Objective:

The objective of this literature review was to evaluate the evidence for the use of vitamin K supplementation in osteoporosis, vascular calcification, arthritis, cancer, renal caluci (kidney stones), diabetes, and warfarin therapy..

Main Evaluation:

Vitamin Ks exist in several types where the most common versions are Vitamin K1 and Vitamin K2 as MK-4 and MK-7. An overview of function and sources are shown in figure 1. A more detailed overview of food sources is found in figure 2.
The authors findings: "Vitamin K2 may be a useful adjunct for the treatment of osteoporosis, along with vitamin D and calcium, rivaling bisphosphonate therapy without toxicity. It may also significantly reduce morbidity and mortality in cardiovascular health by reducing vascular calcification. Vitamin K2 appears promising in the areas of diabetes, cancer, and osteoarthritis. Vitamin K use in warfarin therapy is safe and may improve INR control, although a dosage adjustment is required."

Results:

Vitamin K supplementation may be useful for several chronic conditions with a growing prevalence in an ageing population.

Food source of vitamins K1 and K2

Vitamin K1	Vitamin K2
	
1. Boiled spinach	1. Natto (fermented soy)
2. Cooked broccoli	2. Hard cheese (Gouda)
3. Coleslaw with homemade dressing	3. Soft cheese (blue cheese)
4. Cooked asparagus	4. Egg yolk
5. Soybean oil	5. Butter
6. Red or green grapes	6. Chicken liver
7. Plums	7. Salami
8. Kidney beans	8. Chicken breast
9. Yogurt	9. Ground beef
10. Mayonnaise	10. Sauerkraut
11. Margarine	11. Fermented milk (kefir)
	

Vitamin K types, functions, and sources

Type of vitamin K	Function in the human body	Sources of vitamin
Vitamin K1	(i) Participates in blood clotting. Serves as a cofactor for carboxylation of protein bound glutamate residues by converting them to carboxy glutamate (GLa). GLa containing proteins are found in Factors II, VII, IX, and X	(i) Green leafy vegetables and some plant oils
Vitamin K2, menaquinone-4 (MK-4)	(i) Osteocalcin (synthesized in bone) (ii) Matrix GLa protein (synthesized in cartilage and in blood vessel walls) It is involved in calcium transport, preventing calcium deposition in the lining of blood vessel walls, and helps improve bone density [1] (iii) Short chain form with shorter half-life	(i) Butter, eggs yolks, lard, and animal based foods (ii) Synthesis by bacteria in the intestinal tract (however, synthesized MK-4 is bound to the membranes of bacteria in the gut and very little is absorbed in humans) (iii) Over-the-counter (OTC) supplements
Vitamin K2, menaquinone-7 (MK-7)	(i) As for MK-4 (ii) Long chain form with longer half-life	(i) Fermented foods, some cheese (ii) Extracted from Nattō (fermented soy) as an OTC supplement

REFERENCE B

Fujita, Y., et al., *Association between vitamin K intake from fermented soybeans, natto, and bone mineral density in elderly Japanese men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study*

Objective:

The primary objective was to investigate a possible association between intake of MK-7 as natto and bone mineral density (BMD). The secondary objective was to examine any association between uncarboxylated osteocalcin levels and BMD and to evaluate the role of vitamin K in that association.

Serum concentration of ucOC	BMD[a], g/cm²
	Mean ± SD
Lumbar spine	
1st quartile (≤ 2.04 ng/ml)	1.058±0.014
2nd quartile (2.04 ng/ml < and ≤ 2.94 ng/ml)	1.046±0.015
3nd quartile (2.94 ng/ml < and ≤ 4.36 ng/ml)	1.035±0.015
4th quartile (4.36 ng/ml <)	0.999±0.016* *** ******
p value for trend	0.045
Total hip	
1st quartile (≤ 2.04 ng/ml)	0.911±0.008
2nd quartile (2.04 ng/ml < and ≤ 2.94 ng/ml)	0.903±0.009
3nd quartile (2.94 ng/ml < and ≤ 4.36 ng/ml)	0.884±0.009* ****
4th quartile (4.36 ng/ml <)	0.844±0.009* *** *****
p value for trend	0.048
Femoral neck	
1st quartile (≤ 2.04 ng/ml or less)	0.768±0.008
2nd quartile (2.04 ng/ml < and ≤ 2.94 ng/ml)	0.760±0.008
3nd quartile (2.94 ng/ml < and ≤ 4.36 ng/ml)	0.752±0.008**
4th quartile (4.36 ng/ml <)	0.717±0.009* *** *****
p value for trend	0.069

Method:

A cross-sectional clinical trial of 1,662 senior men (≥65 years) in Japan. The trial included 2,174 volunteers; 2,012 of them were included in all study items. 350 volunteers were excluded due to missing information on food intake and/or their medical history. BMD was measured by dual-energy X-ray absorptiometry (DXA scan) at the lumbar spine (L2–4) and the right hip. Blood samples were taken for detection of undercarboxylated osteocalcin levels (ucOC) as a biomarker of vitamin K intake and total osteocalcin (OC) as a marker of bone formation by immunoassays. Natto consumption was measured by counting the number of natto packs consumed over a 1-week period (1 pack contains about 20 µg of vitamin K1 and about 380 µg of vitamin K2).

Results:

Volunteers who consumed higher amounts of natto/MK-7 had significantly lower levels of inactive/uncarboxylated osteocalcin. Also, volunteers who took natto/MK-7 several times per week had a higher BMD (total hip and femoral neck) compared to those who consumed natto less frequently. The study also found a significant association between low levels of uncarboxylated osteocalcin and increased BMD (lumbar spine and total hip).

	TOTAL		Natto intake frequency								*p* value[a]
			Less than 1 pack/week		1 pack/week		Several packs/week		1 pack/day or more		
	N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD)	
Biochemical marker of bone turnover											
OC (ng/ml)	1,626	4.9 (3.2, 6.4)	931	4.9 (3.1, 6.6)	285	4.8 (3.0, 6.1)	260	4.8 (3.2, 6.6)	150	5.0 (3.9, 6.6)	0.184
TRACP-5b (mU/dl)	1,656	209.3 (203.8, 214.9)	948	211.3 (121.1, 368.5)	290	199.3 (114.7, 346.5)	264	211.2 (125.0, 356.9)	154	213.0 (124.5, 364.4)	0.426
ucOC (ng/ml)	1,626	2.9 (2.9, 3.0)	931	3.4 (1.9, 6.0)	285	2.7 (1.6, 4.7)	260	2.4 (1.4, 4.2)	150	2.1 (1.2, 3.7)	<0.001
BMD, g/cm²											
Lumbar spine	1,580	1.029±0.188	893	1.020±0.192	284	1.036±0.182	255	1.045±0.182	148	1.044±0.190	0.169
Total hip	1,658	0.882±0.123	950	0.873±0.124	289	0.886±0.123	265	0.905±0.122	154	0.897±0.117	<0.001
Femoral neck	1,658	0.743±0.114	950	0.732±0.112	289	0.746±0.113	265	0.770±0.117	154	0.759±0.190	<0.001

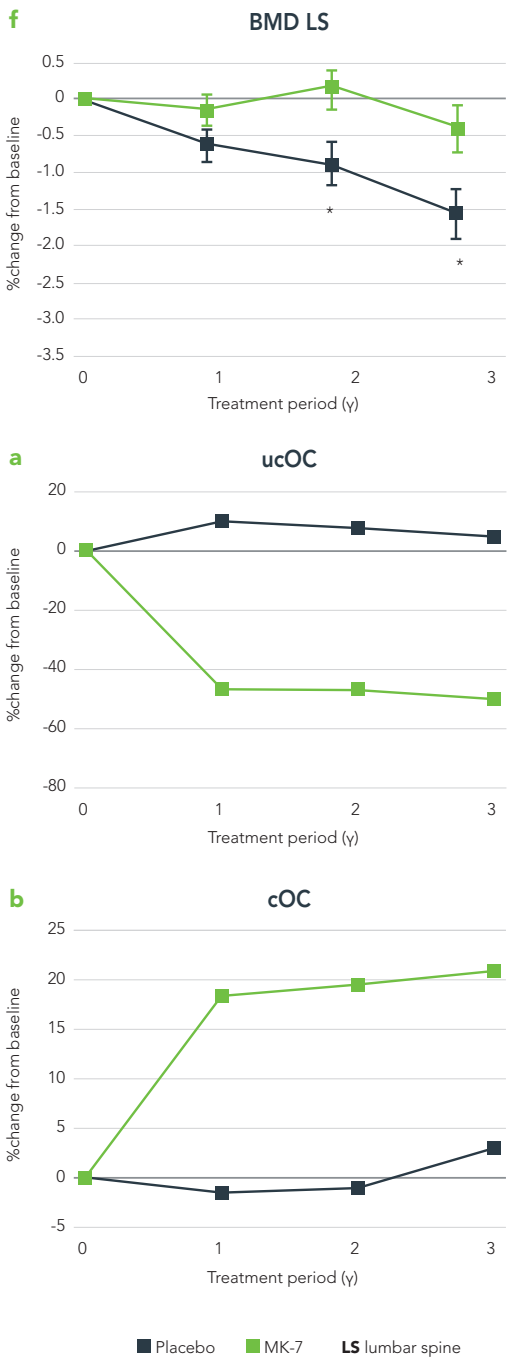
REFERENCE C

Knapen, M.H., et al., *Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women*

Objective:
Vitamin K is recognized for its role in maintaining bone health. The objective of this study was to investigate potential beneficial effects of long-term, low-dose vitamin K2 MK-7 supplementation on bone health.

Method:
A double-blind, randomized, placebo-controlled trial in healthy postmenopausal women who received either MK-7 (180 µg per day) or placebo over a period of three years. A total of 244 participants were included, and 223 completed the 3-year supplementation period. Bone mineral content (BMC) and bone mineral density (BMD) of the femoral neck, total hip, and lumbar spine (L1–L4) were measured at baseline and followed up on a yearly basis for three years by dual-energy X-ray absorptiometry (DXA scan). Circulating biomarkers – serum levels of carboxylated and uncarboxylated osteocalcin (cOC and ucOC) – were detected by immunoassays.

Results:
Long-term supplementation of MK-7 significantly decreased age-related BMD and BMC decline at the lumbar spine and femoral neck. It also reduced the loss of vertebral height of the lower thoracic region at the mid-site of the vertebrae. MK-7 intake also significantly increased serum levels of active/carboxylated osteocalcin and lowered the levels of inactive/uncarboxylated osteocalcin. These findings suggest that long-term vitamin K2 MK-7 supplementation at the right dose may help prevent age-related bone loss in postmenopausal women.

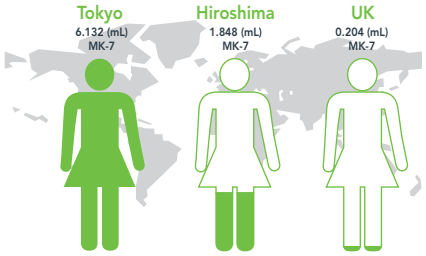


REFERENCE D

Kaneki, M., 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*

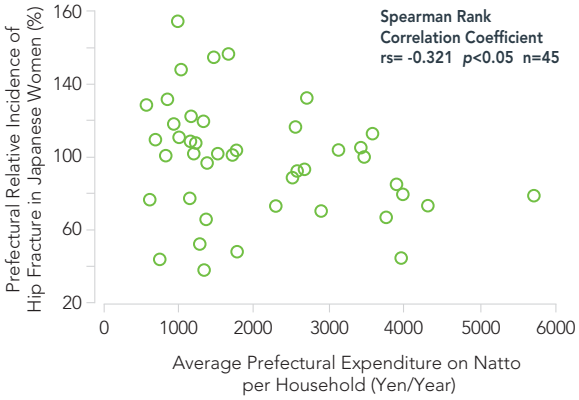
Objective:

The objective was to investigate the determinants of differences in the circulating levels of vitamin K2 among subjects living in different geographic regions and to explore potential implications for the risk of hip fracture.



Serum Concentration of Vitamin K in Postmenopausal Women in the United Kingdom, Hiroshima and Tokyo

Location	*n*	PK (ng/mL)	MK-7 (ng/mL)	Age (y)
United Kingdom	31	0.497 ± 0.537 (2)	**0.371 ± 0.204 (24)**	58.5 ± 9.4
Hiroshima	25	0.741 ± 0.581 (1)	**1.221 ± 1.848 (9)**	57.2 ± 3.3
Tokyo	49	0.727 ± 0.461 (0)	**5.268 ± 6.132 (8)**	67.4 ± 8.3
AGE ≤ 65 y AGE > 65 y	19 30	0.714 ± 0.567 (0) 0.734 ± 0.391 (0)	3.865 ± 3.846 (3) 6.157 ± 7.138 (5)	59.1 ± 4.8 72.7 ± 4.8



Method:

The trial consisted of three groups of postmenopausal women living in Tokyo (n=49), Hiroshima (n=25) and London (n=31). Serum levels of MK-7 were determined by blood samples and analyzed by chromatography. The relative incidence of fracture was determined by the ratio between the reported and expected number of hip fractures among the 45 prefectures throughout Japan. For the correlation, the annual household expenditure for MK-7/natto (Japanese government figures) was used. Natto-loading test was performed in 8 postmenopausal women in Tokyo (who abstained from natto consumption 2-weeks before and after the test). Blood samples were taken to analyze MK-7 levels before and after natto consumption and were continued at regular intervals for up to 2-weeks.

Results:

There were large variations in the levels of circulating MK-7 levels based on geography, with the highest circulating MK-7 levels in those areas of Japan (in Tokyo) where consumption of natto was highest. British women had the lowest levels among those tested. Natto intake is one of the major contributors to the higher serum MK-7 concentration as demonstrated by the natto-loading test. MK-7 levels were elevated after natto ingestion and were decreased after a 2-week deprivation. Areas that spend less on MK-7/natto had the highest incidence of fractures (significant correlation, $p<0.05$).

REFERENCE E

Geleijnse, J.M., et al., *Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study*

Objective:
Vitamin K-activated matrix Gla-protein (MGP) prevents soft tissue and vascular calcification. The objective of this study was to investigate if intake of vitamin K1 or K2 was related to aortic calcification and coronary heart disease.

Method:
A population-based study of 4,807 participants with no history of myocardial infarction followed for 7.2 ± 1.9 years (mean ± SD). Participants were monitored for incidents of coronary heart disease (CHD), all-cause mortality, and aortic atherosclerosis and their correlation to vitamin K intake. Aortic calcification was diagnosed by detecting calcified deposits in the abdominal aorta parallel and anterior to the lumbar spine (L1–L4) and was graded as "absent or mild" (≤1 cm calcification), "moderate" (>1 and <5 cm), or "severe" (≥5 cm) calcification. Vitamin K intake was calculated using participant-provided dietary intake data for the period of the study. The risk of coronary events and all-cause mortality was determined by statistical analysis (using a Cox regression model).

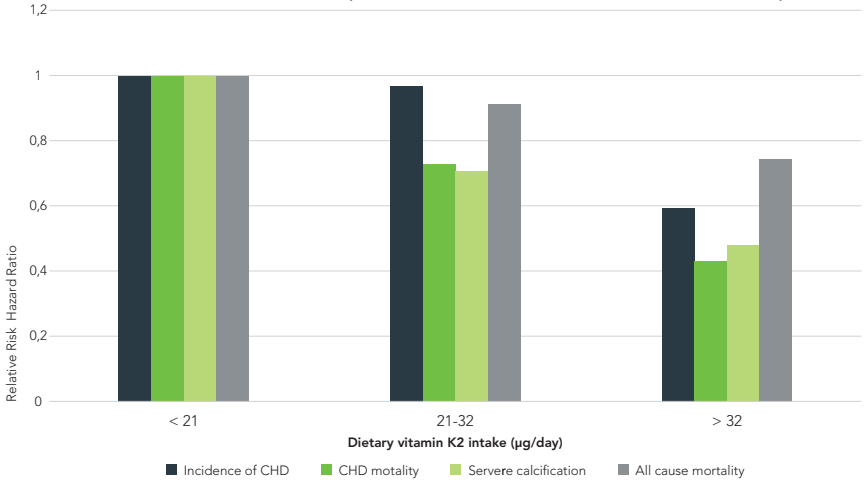
Results:
A strong inverse relationship was observed between vitamin K2 intake and severe arterial calcification. Further, there were significant inverse correlations between K2 intake and incidents of CHD, CHD mortality, and all-cause mortality. On the other hand, there were no consistent correlations between vitamin K1 intake and CHD, mortality, or aortic calcification.

Association of Aortic Calcification with Intake of Menaquinone in 4473 Dutch Men and Women Aged 55yrs and Over

Energy-adjusted menaquinone intake (µg/d)				
	<21.6	*21.6-32.7*	*>32.7*	*P for trend*
n	1468	1493	1512	
Median intake, µg/d	15.1	26.9	40.9	
Moderate calcification				
Controls, *n*	916	958	1000	
Cases, *n*	454	452	453	
OR, model 1[3]	1	0.93 (0.79, 1.10)	0.94 (0.80, 1.11)	0.49
OR, model 2[4]	1	0.91 (0.77, 1.09)	0.93 (0.76, 1.12)	0.45
Severe calcification				
Controls, *n*	916	958	1000	
Cases, *n*	98	83	59	
OR, model 1	1	0.75 (0.54, 1.03)	0.56 (0.39, 0.80)	0.001
OR, model 2	1	0.71 (0.50, 1.00)	0.48 (0.32, 0.71)	<0.001

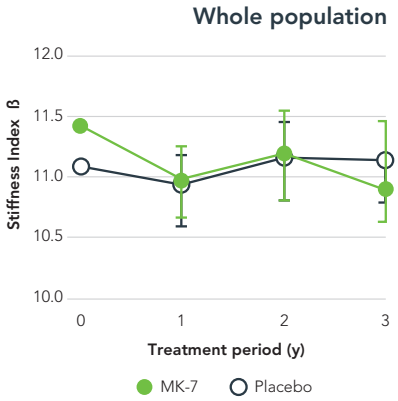
The Rotterdam study showing the benefits of vitamin K2 on cardiovascular health

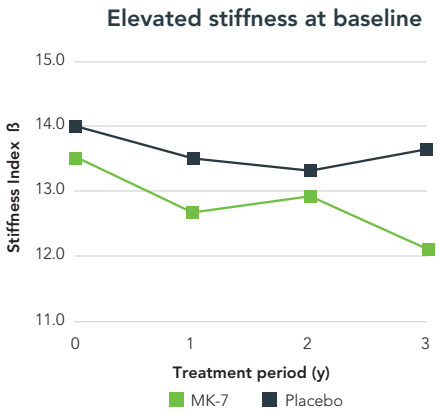
The Rotterdam study: Effect of vitamin K2 intake on heart and cardiovascular health (modified from Geleijnse et al. 2004).

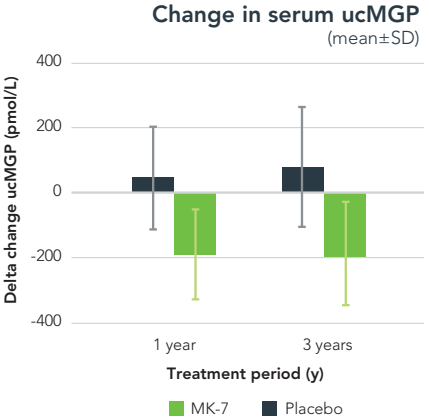


REFERENCE F

Knapen, M.H., et al., *Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women. A double-blind randomised clinical trial*







Objective:
Vitamin K2 intake is linked to cardiovascular health. The objective was to investigate the long-term effects of MK-7 supplementation on activation of matrix Gla-protein and its effects on arterial stiffness in postmenopausal women.

Method:
A double-blind, placebo-controlled clinical trial consisting of 244 healthy postmenopausal women. Participants received either MK-7 (180 µg per day) or placebo for 3 years; 223 participants completed the trial. Circulating markers – desphospho-uncarboxylated matrix Gla-protein (dp-ucMGP) and acute phase markers like Interleukin-6 (IL-6), high-sensitive C-reactive protein (hsCRP), tumor necrosis factor-α (TNF-α) and markers for endothelial dysfunction Vascular Cell Adhesion Molecule (VCAM), E-selectin, and Advanced Glycation Endproducts (AGEs) – were measured by immunoassays. Local arterial stiffness was assessed by echotracking to determine vascular characteristics of the carotid artery. Pulse wave velocity assessed regional arterial stiffness.

Results:
Three-year MK-7 supplementation improved arterial stiffness in healthy postmenopausal women. Supplementation of MK-7 decreased the levels of uncarboxylated MGP at the 1-year measurement and persisted throughout the 3-year study period, but did not influence the markers for acute phase and endothelial dysfunction. For participants with elevated arterial stiffness at baseline, there was a significant reduction in arterial stiffness.

REFERENCE G

Kurnatowska, I., et al., *Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages*

Objective:
Atherosclerosis and vascular calcification are common in patients with chronic kidney disease (CKD). This study assessed the effect of vitamin K2 MK-7 on the progression of atherosclerosis and calcification in non-dialyzed patients with stage 3–5 CKD.

Method:
A double-blind, placebo-controlled trial of 42 non-dialyzed patients suffering from CKD. Patients received either a combination of MK-7 (90 µg) and vitamin D (10 µg), or vitamin D (10 µg) alone, for 9 months. Immunoassays were performed to measure total levels of calcification modulators – matrix Gla protein (MGP), desphosphorylated-uncarboxylated MGP (dp-ucMGP), osteoprotegerin (OPG), fetuin A, osteocalcin (OC), and fibroblast growth factor 23 – before and after study intervention. Ultrasonography determined the progression of atherosclerosis as reflected in common carotid intima-media thickness (CCA-IMT) and a multiscan CT assessed coronary artery calcification score (CACS) level.

Results:
The study reported a significant increase of total MGP and a decrease of dp-ucMGP for the combined (vitamins K + D) treatment group. The increase of CCA-IMT (ΔCCA-IMT) was significantly lower in the vitamins K+D treatment group compared to the vitamin D group. The increase in CACS (ΔCACS) was only slightly lower in the K+D group than in the D group. Hence, vitamin K2 MK-7 may reduce the progression of atherosclerosis (assessed by CCA-IMT) in non-dialysis subjects with CKD stages 3–5, but does not seem to affect the progression of calcification (assessed by CACS) significantly.

Parameter	Vit. K + D (n= 28)	Vit. D (n = 12)	*p* Value
Δ CACS, AU	58.1 ± 106.5 11.0 (0-55.5)	74.4 ± 127.1 20.5 (8.0-119.0)	0.7 0.2
	ANCOVA		0.3
	16.7% ± 23.3% 13.9 (0.0-26.7)	15.9% ± 12.9% 11.1 (4.8-19.7)	0.9 0.8
Δ CCA-IMT, mm	0.06 ± 0.08 0.000 (0.000-0.1)	0.136 ± 0.05 0.100 (0.1-0.2)	0.005 0.004
	ANCOVA		0.007
	6.0% ± 7.1% 0.0 (0.0-12.5)	13.8% ± 4.9% 13.3 (10.0-18.2)	0.003 0.009

Parameter	Vitamin K + D (n = 28)			Vitamin D (n = 12)			*p* value[a]
	before treatment	*after treatment*	*p value*	*before treatment*	*after treatment*	*p value*	
MGP, pg/ml	639.6 ± 187 595.1 (533.6-831.2)	742.8 ± 249.1 684 (555.6-888.4)	0.06	640.7 ± 195.4 560.3 (516.3-718.2)	615 ± 165.9 594.9 (489.1-680.0)	0.6	0.1
ucMGP, pmol/l	1077.1 ± 507.7 1004 (590-1670)	961.5 ± 506.7 812 (510-1580)	0.02	793.9 ± 400.3 715 (467-1190)	820.7 ± 565.2 710 (490-1119)	0.7	0.5

REFERENCE H

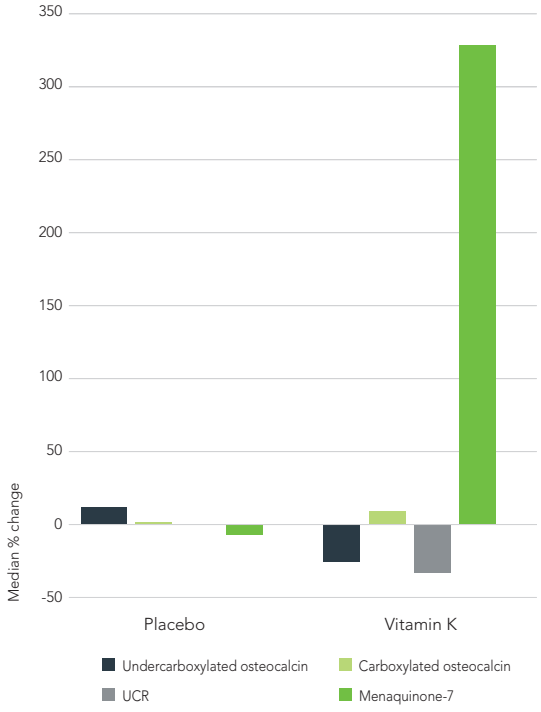
van Summeren, M.J., et al., *The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children*

Objective:
Vitamin K2 beneficially affects bone health probably via its role in osteocalcin activation. This study investigated the effect of K2 MK-7 supplementation on circulating levels of uncarboxylated osteocalcin (ucOC) and carboxylated osteocalcin (cOC) in healthy prepubertal children.

Method:
A double-blind, placebo-controlled clinical trial of 55 children (both genders) who received either MK-7 (45 µg/day) or placebo for eight weeks. Circulating markers – ucOC and cOC – were measured at baseline and after eight weeks by immunoassays, together with MK-7 levels (measured by HPLC.) The ucOC:cOC ratio (UCR) was used as an indicator of vitamin K status. Two major outcomes were calculated: primary outcome was the percentages of change in UCR from baseline (0 weeks) to endpoint (8 weeks) in both treatment groups and secondary outcomes were the percentage of change in the absolute concentrations of circulating ucOC, cOC, and MK-7. Additionally, bone markers and coagulation parameters were measured to confirm the stability of bone metabolism and to rule out any procoagulant effect of MK-7, respectively.

Results:
In the MK-7 group, there was a significant increase in the blood levels of MK-7. Additionally, there was a significant reduction in the serum levels of ucOC and UCR ($p<0.001$) compared to the placebo group. In both groups, there were no changes in the bone markers and coagulation parameters. Finally, cOC levels increased in children who took MK-7 ($p=0.067$). These findings suggest that supplementation with MK-7 (around 45 µg/day) increases circulating concentrations of MK-7 and increases osteocalcin carboxylation in healthy prepubertal children.

Changes in Vitamin K Parameters and Other Parameters in Placebo and Vitamin K Groups



Group	Placebo			Vitamin K			*p* value[a]
	n	*Median % change*	*Range*	*n*	*Median % change*	*Range*	
Undercarboxylated osteocalcin (%)	26	+ 12.2	- 48.1 - 344.1	26	- 25.6	- 73.3 - 28.9	<0.001
Carboxylated osteocalcin (%)	26	+ 1.2	- 33.3 - 386.1	26	+ 12.2	- 78.6 - 87.0	0.067
UCR (%)	26	+ 0.0	- 45.5 - 80.0	26	- 33.3	- 59.1 - 16.7	<0.001
Menaquinone-7 (%)	22	- 7.1	- 100.0 - 290.0	22	+ 328.5	16.7 - 1907.1	0.001

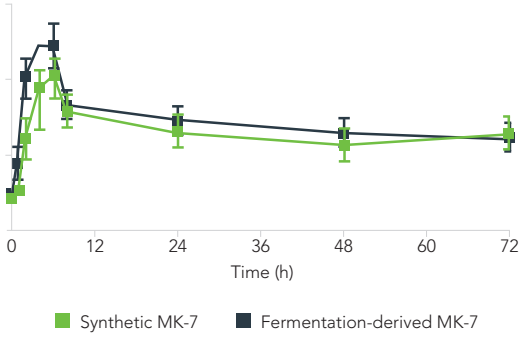
REFERENCE I

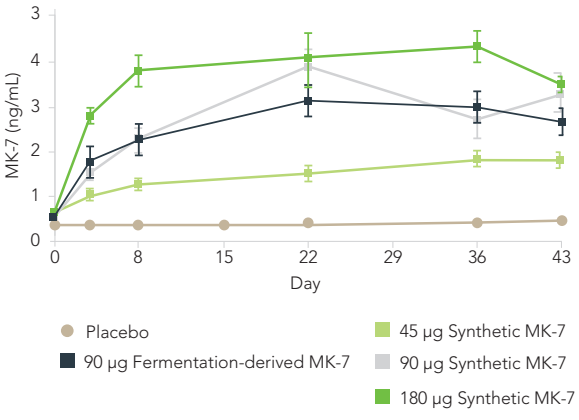
Moller, M., et al., *Bioavailability and Chemical/Functional Aspects of Synthetic MK-7 vs Fermentation-Derived MK-7 in Randomised Controlled Trials*

Objective:
This study investigated the uptake and biological activity of both synthetic and fermentation-derived MK-7 in healthy volunteers, by single dose and over time via multiple dosing.

Method:
This paper describes two clinical trials – a bioavailability study and a functional study. The bioavailability study was a single-blind, two-way cross-over trial with 17 subjects who received a single dose of either synthetic or fermentation-derived MK-7 (180 µg). MK-7 in blood samples were measured 72 hours post-intake. The functional study was a double-blind, placebo-controlled trial where 43 volunteers received synthetic MK-7 (45, 90, or 180 µg), or fermentation derived MK-7 (90 µg), or placebo for six weeks. Blood samples were taken regularly for measurement of MK-7 and uncarboxylated osteocalcin (ucOC) levels.

Results:
Both synthetic and fermentation-derived MK-7 displayed identical pharmacokinetics for single dose intake. Multiple dose intake of synthetic MK-7 showed a dose-dependent increase of MK-7 as well as a dose-dependent reduction of ucOC in the blood. Equivalent doses of 90 µg of the synthetic and the fermentation-derived MK-7 demonstrated equal MK-7 levels in the blood and equal biological activity (as measured by ucOC levels).





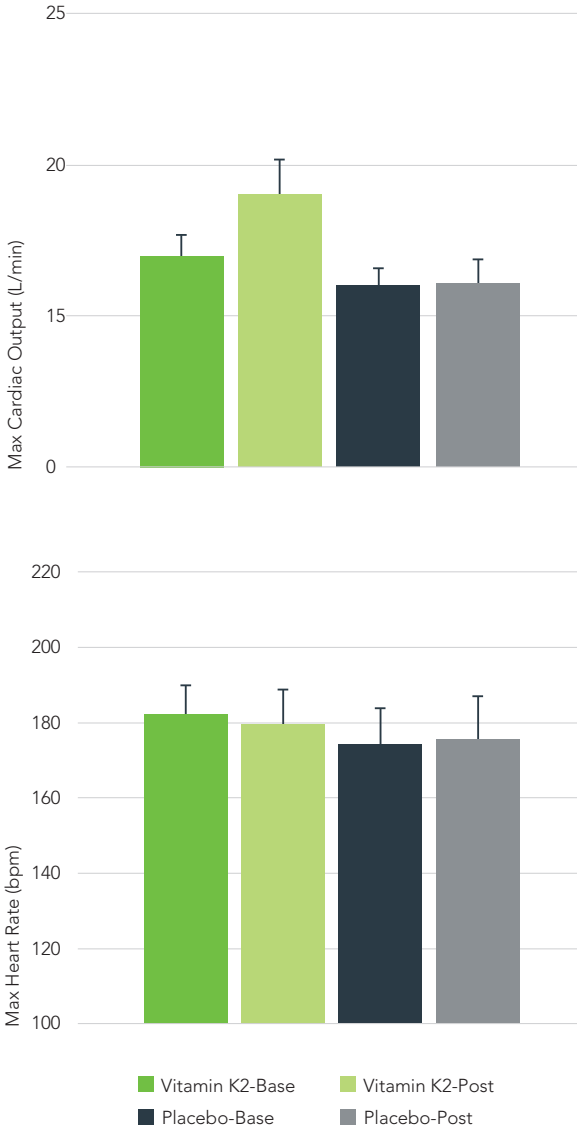
REFERENCE J

McFarlin, B.K., A.L. Henning, and A.S. Venable, *Oral Consumption of Vitamin K2 for 8 Weeks Associated With Increased Maximal Cardiac Output During Exercise*

Objective:
This study was conducted to determine if an 8-week vitamin K2 MK-7 intake could influence cardiovascular response to a cycle ergometer test among athletes.

Method:
An 8-week double-blind, placebo-controlled trial with 26 trained male and female athletes. Subjects received 320 µg/day MK-7 for weeks 1 to 4, followed by 160 µg/day MK-7 for weeks 5 to 8, or placebo for eight weeks. Oxygen consumption, carbon dioxide production, respiratory rate, and respiratory exchange ratio were measured using a graded exercise test on an electronically braked cycle ergometer (both pre-and post-intervention). Also, noninvasive cardiac output, stroke volume, and heart rate were measured using skin-mounted electrodes.

Results:
Supplementation of MK-7 gave a 12% significant increase in maximal cardiac output (p=0.031), and a trend towards an increase in heart rate (p=0.07).



REFERENCE K

Riphagen, I.J., et al., *Riphagen, I.J., et al., Prevalence and Effects of Functional Vitamin K Insufficiency: The PREVEND Study.*

Objective:
Soft tissue calcification is common in several highly prevalent diseases like hypertension, chronic kidney disease (CKD) and cardiovascular disease (CVD). A strong inhibitor for soft tissue calcification is matrix Gla Protein (MGP). Intake of vitamin K2 is shown to active/carboxylate MGP cMGP), and the inactive/uncarboxylated form of MGP (ucMGP) is a good marker for vitamin K insufficiency. The objective of this study was to study the prevalence and effects on vitamin K insufficiency with a particular focus on hypertension, type 2 diabetes, CKD, CVD, and mortality.

Method:
A total of 4275 participants (aged 53±12 yrs, 46.0% male) were included in this general population-based study. The participants were followed up for 10 yrs.

Figure 1

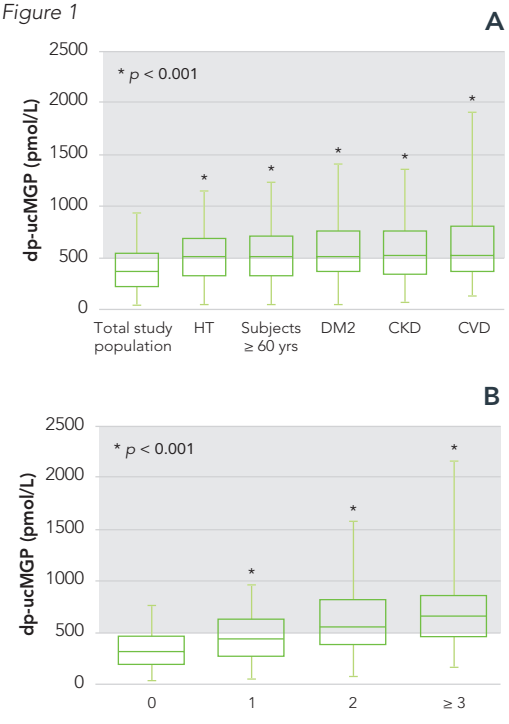
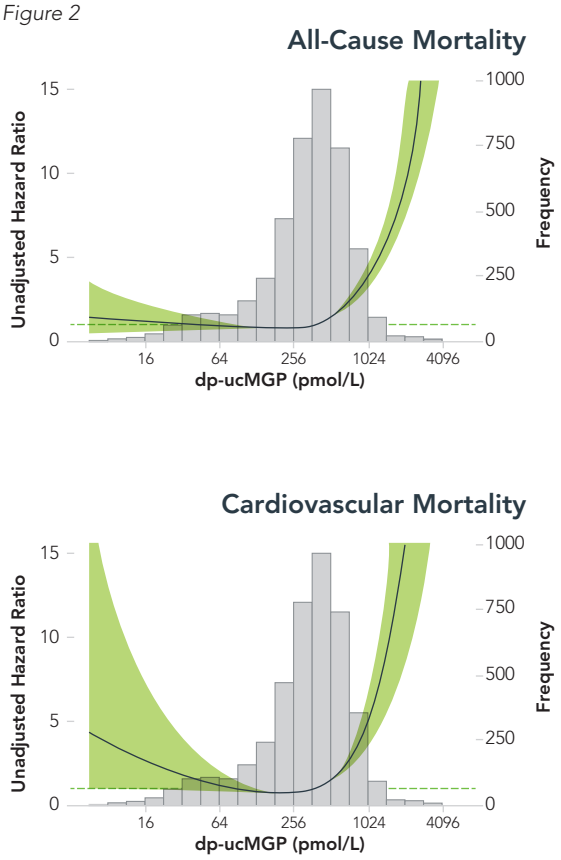


Figure 2



Results:
The participants that were either hypertensive (HT), were ≥60 years of age, had type 2 diabetes (DM2), CKD or CVD had a significantly higher level of the inactive ucMGP (Figure 1A). There was also a significant association between the number of comorbidities (HT, DM2, CKD, CVD) and ucMGP (Figure 1B). After 10 years of follow-up, 279 participants had died, and 74 of these deaths were due to cardiovascular disease. There was a significant association of ucMGP with both all-cause mortality (p<0.001) and cardiovascular mortality (p<0.001) (Figure 2).

REFERENCE L

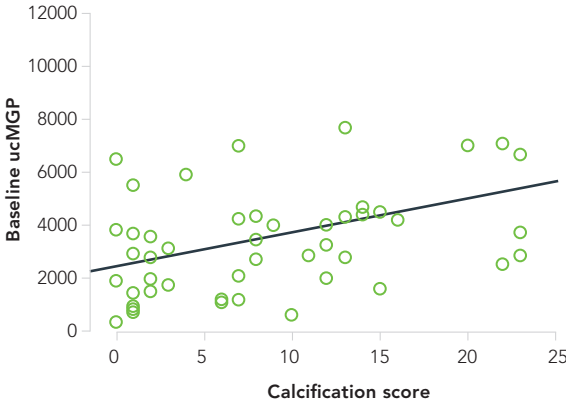
Aoun, M., et al., *High Dephosphorylated-Uncarboxylated MGP in Hemodialysis Patients: Risk Factors and Response to Vitamin K2, a Pre-Post Intervention Clinical Trial*

Objective:
Vascular calcification is very common in hemodialysis patients, a population with high levels of inactive/uncarboxylated MGP (ucMGP). This pre-post intervention clinical trial examined the correlation between the degree of calcification and ucMGP levels and assessed the reduction in ucMGP levels following MK-7 supplementation.

Method:
Fifty hemodialysis patients received a daily dose of 360 µg MK-7 for 4 weeks. At baseline, the calcification score was performed by two independent physicians, one of them was blinded. Blood samples for detection of ucMGP were collected at baseline and at the end of the trial.

Results:
At baseline, the calcification score correlated significantly with ucMGP serum levels (p=0.002). The median level of ucMGP reduced from 3179 µM at baseline to 295 µM at the end of the trial, an 86% reduction.

Correlation between inactive MGP (ucMGP) and calcification score

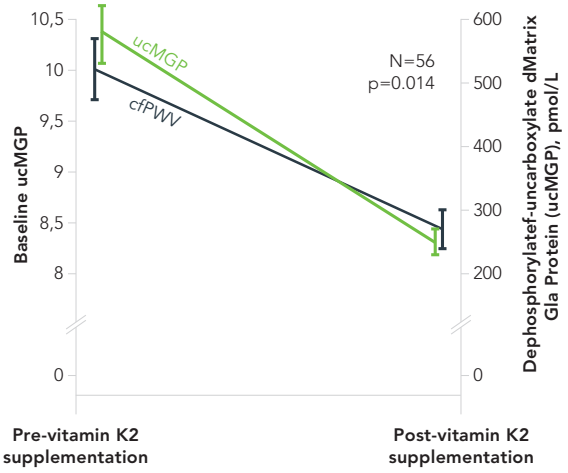


REFERENCE M

Mansour, A. G., et al., *Vitamin K2 Supplementation and Arterial Stiffness Among Renal Transplant Recipients – A Single-Arm, Single-Center Clinical Trial*

Objective:
Renal transplant recipients have an increased risk of arterial stiffness and cardiovascular disease. This population also display a subclinical vitamin K deficiency, detected by high levels of inactive, uncarboxylated matrix gla-protein (ucMGP). This prospective, single-arm clinical study examined the effects of MK-7 supplementation on arterial stiffness and ucMGP levels.

Effects of MK-7 supplementation on arterial stiffness and ucMGP levels



Method:
Sixty renal transplant recipients were supplemented with a daily dose of 360 µg MK-7 for 8 weeks. Arterial stiffness was measured using carotid-femoral pulse wave velocity (cfPWV). Blood samples were collected for assessment of ucMGP.

Results:
The patients experienced a 14.2% improvement in arterial stiffness ($P < 0.001$) and a 55.1% decrease in mean serum levels of ucMGP ($P < 0.0001$), at the end of the study. In conclusion, MK-7 supplementation improved subclinical vitamin K deficiency and arterial stiffness in renal transplant recipients with stable graft function.

REFERENCE N

Marles, R.J., A.L. Roe, and H.A. Oketch-Rabah, *US Pharmacopeial Convention Safety Evaluation of Menaquinone-7, a Form of Vitamin K*

Objective:
The authors reviewed the chemistry, dietary sources, intake levels, and pharmacokinetics of MK-7, together with non-clinical toxicity and adverse events associated with MK-7 intake.

Main Evaluation:

1. **Adverse events from clinical trials:** 14 clinical trials reported adverse events. However, for treatment populations with ≤120 individuals, the right MK-7 dosage (≤180 µg/day for 3 years, ≤360 µg/day for 12 weeks, and ≤1080 µg thrice weekly for 8 weeks) showed no significant adverse effects compared to placebo.
2. **Pharmacovigilance:** A search on MK-7 was performed in all major databases, globally. The findings are summarized in the figure.
3. **Anticoagulants:** MK-7 can have risky interactions with anticoagulant drugs. However, through dose titration and patient counseling, the physician or pharmacist may be able to mitigate that risk to maintain stable anticoagulation control, so long the patient's vitamin K intake is known.
4. **Animal toxicology studies:** Rats given ≤10 mg/kg/day of MK-7 for ≤90 days showed no significant adverse effects, genotoxicity or mutagenicity.

Results:
In conclusion, MK-7 as a dietary supplement does not pose any serious health risks.

Pharmacovigilance – reports for MK-7

Database	Reports
FDA MedWatch program	No reports
Canada Vigilance Adverse Reaction Online Database	No reports on menaquinone-7 or MK-7 One report on "menaquinones" which was a non-serious incidence of diarrhea and vomiting associated with a "suspected" 92 ingredient product containing 6 µg of vitamin K2 (vitamer not specified)
UK Medicines and Healthcare products Regulatory Agency Drug Analysis Prints A-Z	No reports
Australian Therapeutic Goods Administration Database of Adverse Event Notifications – medicines	Two reports associated with menaquinones: ■ Hot flush and blurred vision ■ One case of myocardial infarction and splenic infarction associated with an unspecified menaquinone-containing product taken in combination with multiple prescription drugs that included human prothrombin complex (also a suspected product in the adverse event)
New Zealand MedSafe Suspected Medicine Adverse Reaction Search	No reports

REFERENCE O

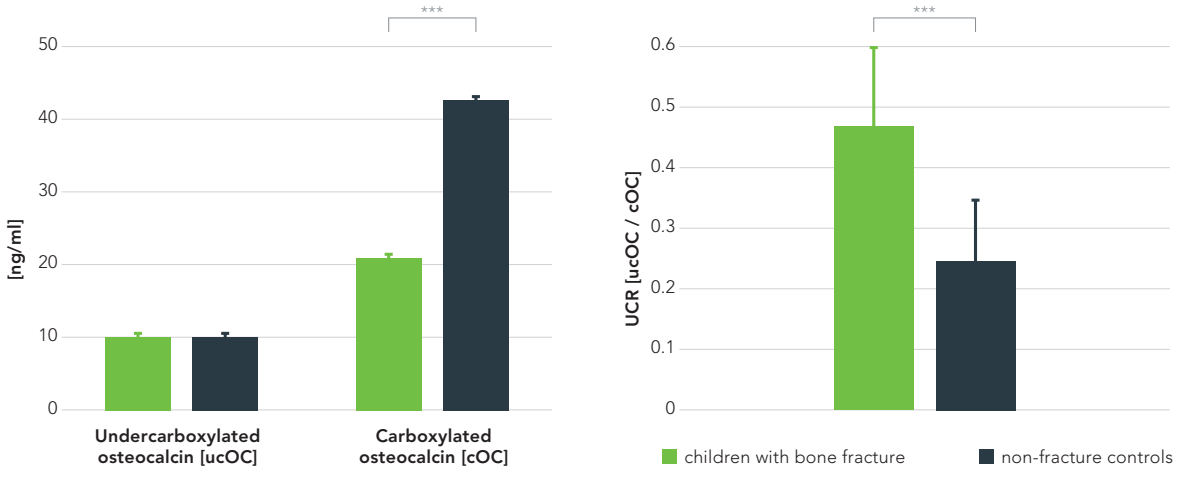
Popko, J., et al., *Decreased Levels of Circulating Carboxylated Osteocalcin in Children with Low-Energy Fractures: A Pilot Study*

Objective:
Vitamins K and D are involved in the regulation of bone health and in the prevention of fractures. The study assessed the status of these vitamins in children with low-energy fractures and in children with no fractures.

Method:
The trial recruited 39 children. The test group, children with low-energy fractures, included 20 children (14 boys and 6 girls) aged 5–15 years, and the control group, children with no fractures, included 19 children (9 boys and 10 girls) aged 7–17 years. Blood samples were collected for the assessment of vitamin K status (by measuring the carboxylated osteocalcin and uncarboxylated osteocalcin levels) and total vitamin D status (by measuring the 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 levels).

Results:
Activated or carboxylated osteocalcin levels were significantly higher in the group with no fractures. The ratio of uncarboxylated to carboxylated osteocalcin (UCR) was significantly higher in the group with low-energy fractures. Vitamin D status were similar in both groups.

Significant correlation between fractures and activation status of osteocalcin



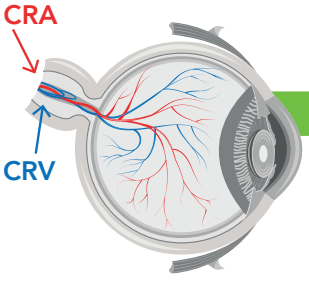
REFERENCE P

Wei, F.F., et al., *Inactive Matrix Gla-Protein is a Novel Circulating Biomarker Predicting Retinal Arteriolar Narrowing in Humans*

Objective:
The diameter of retinal micro-vessels carries important prognostic information; smaller arteriolar diameter is a predictive marker for cardiovascular mortality and coronary heart disease. Matrix gla-protein (MGP) is expressed in the retinal vasculature. The trial investigated the association between inactive or uncarboxylated MGP (ucMGP) and retinal circulation (measured by retinal arteriolar and venular narrowing).

Method:
The trial recruited 935 participants (mean age of 40.9 years and 50.3% women). Retinal vascular diameters (central retinal arteriole and venule) were assessed. Blood samples were collected for ucMGP assessment.

Results:
The retinal arteriolar diameter was significantly smaller among participants with high circulating ucMGP than those with low circulating ucMGP. Therefore, circulating ucMGP is a predictor of smaller retinal arteriolar narrowing. Vitamin K supplementation might promote retinal health.



CRA - central retinal artery
CRV - central retinal vein

The association between inactive or uncarboxylated MGP (ucMGP9 and retinal circulation

Characteristics	Category of ucMGP			*p* Value
Limits (µg/L)	< 2.88	2.88 – 4.58	≥ 4.58	
Central retinal arteriolar calibre (µm)	152.1 (14.3)	151.0 (13.9)	148.8 (13.5)	0.005
Central retinal venular calibre (µm)	218.7 (18.4)	219.2 (18.9)	217.0 (19.9)	0.33
Arteriolar-to-venule ratio	0.70 (0.06)	0.69 (0.06)	0.69 (0.06)	0.080

REFERENCE Q

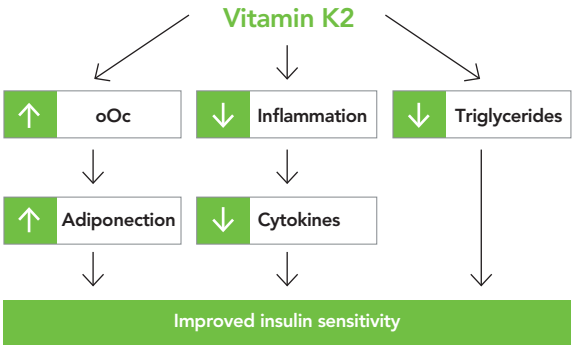
Li, Y., et al., *Effect of Vitamin K2 on Type 2 Diabetes mellitus: A Review*

Objective:
Type 2 diabetes mellitus (T2DM) is a major public health problem, globally. Type 2 diabetes develops when the body becomes resistant to insulin. At first, the pancreas tries to compensate by producing more insulin, eventually, the insulin production diminishes and blood sugar increases. Prospective observational studies and clinical trials show a reduced risk of developing T2DM with vitamin K2 supplementation. The literature review assessed the effects of vitamin K2 on T2DM and its pathogenesis.

Main Evaluation:
Clinical data (one cohort study and two clinical trials) indicate that vitamin K2 intake improves insulin sensitivity and reduces the risk of T2DM.

1. Increased activation of osteocalcin (cOC) can increase adiponectin, lowering plasma glucose levels and increasing insulin sensitivity
2. Reduced inflammation leading to a reduced secretion of cytokines can enhance glucose uptake
3. Reduced serum levels of triglycerides can lead to increased glucose uptake

Results:
The authors propose vitamin K2 supplementation as a potential therapeutic strategy for T2DM in the future.



Effect of vitamin K2 on type 2 diabetes mellitus: A review

Author/year	Country	Design, Participants	Outcome measure	Results and conclusion
Beulens 2010	**Netherlands**	Prospective cohort study (N=38.094) where 918 incidents of diabetes were documented, 10.3 years of follow-up	Documented cases of T2DM were compared to intake levels of vitamin K2	Vitamin K2 may be associated with a reduced risk of T2DM
Choi 2011	**Korea**	Placebo-controlled clinical trial, N=42 healthy males aged 29 years were included, 4 weeks of intervention	Insulin sensitivity index, acute insulin response to glucose, disposition index, adiponectin, interleukin-6	Supplementation with vitamin K2 improves insulin sensitivity
Zatollah 2016	**Iran**	Double-blinded, randomized, placebo-controlled trial, N=66, patients with T2DM and coronary heart disease, aged 40-85 years, 12 weeks of intervention	Serum insulin levels, HOMA index	Significant reduction in serum insulin and HOMA-index

REFERENCE R

Myneni, V.D. and E. Mezey, *Regulation of Bone Remodeling by Vitamin K2*

Objective:
Bone structures are tightly regulated and require amino acids, fatty acids, carbohydrates, minerals, vitamins and water for optimal development and maintenance. Vitamin K2 regulates bone remodeling, a continuous process that helps maintain bone structure, bone volume, and calcium homeostasis. This review summarizes the current data on how K2 regulates bone remodeling.

Main Evaluation:
Old and micro-damaged bone are continuously replaced through the bone remodeling cycle, which consists of five distinct and overlapping phases.

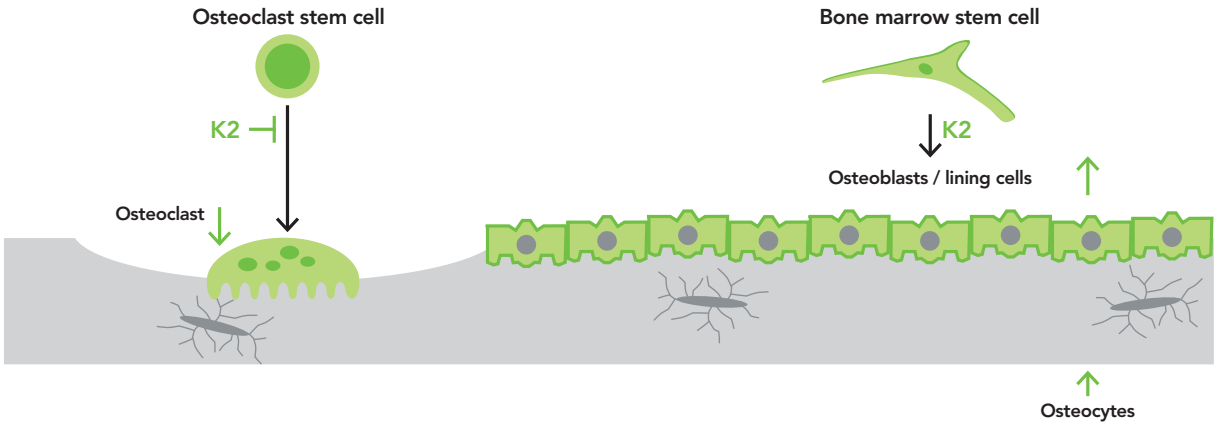
1. **Initiation:** Osteocytes sense deformed bone and secretes signaling molecules to activate osteoclast precursor cells to osteoclasts.
2. **Resorption:** Osteoclasts form bone-resorbing compartments. They secrete proteolytic enzymes and hydrogen ions to lower the pH. This facilitates the dissociation of bone minerals and the degradation of the bone matrix.
3. **Reversal:** Osteoclasts are inactivated, and osteoblasts are recruited.
4. **Formation:** Osteoblasts deposit an organic matrix that is mineralized. They secrete osteocalcin into the bone extracellular matrix where it binds to hydroxyapatite crystals.
5. **Resting:** Most of the osteoblasts that participated in the now bone formation dies, some develop to lining cells and some to osteocytes. Thereafter, the cycle is completed, followed by a resting phase.

The bone remodeling cycle can be imbalanced. If less bone mass is formed than resorbed, it can evolve into osteopenia and eventually osteoporosis.

Results:
Vitamin K2 regulates the bone remodeling cycle at several levels:

1. **Osteoclasts:** Vitamin K2 inhibits osteoclast differentiation
2. **Osteoblasts:** Vitamin K2 stimulates osteoblast development and protects them from cell death leading to increased osteoblast/lining cells and osteocytes
3. **Osteocalcin:** Vitamin K2 activates or carboxylates osteocalcin that allows binding the calcium and hydroxyapatite crystals.

Regulation of bone remodeling by vitamin K2



REFERENCE S

van Ballegooijen, A.J. and J.W. Beulens, *The Role of Vitamin K Status in Cardiovascular Health: Evidence from Observational and Clinical Studies*

Objective:
A growing number of studies support a beneficial effect of vitamin K on cardiovascular health. This literature review summarizes the published studies on vitamin K status and cardiovascular outcomes.

Main Evaluation:
A biomarker for low vitamin K status is inactive, dephosphorylated and uncarboxylated matrix gla-protein (ucMGP). The authors screened all available published literature where dp-ucMGP and cardiovascular endpoints have been studied.

Results:
Observational studies indicate that low vitamin K status measured by high ucMGP concentrations plays a role in cardiovascular disease.

Studies on circulating ucMGP and cardiovascular-related outcomes

Author/year	Country	Participants	Outcome measure	Results and conclusion
Cross-sectional studies				
Ueland, 2010	**Norway**	N=147, calcific valvular aortic stenosis patients, age 74 ± 10, 45% female	Echocardiographic measures	Higher circulating ucMGP was associated with decreased cardiac function
Ueland, 2011	**Norway**	179 heart failure patients and 33 healthy individuals, age 56±12, 22% female	Systolic function	Higher circulating ucMGP was associated with increased heart failure
Dalmeijer, 2013	**Netherlands**	N=195, post-menopausal women, age 67±6	Coronary artery calcification (CAC) score	Higher circulating ucMGP was slightly associated with high CAC (p=0.06)
Liabeuf, 2014	**France**	N=198, type 2 diabetes, men>50 and women >60 years	Peripheral arterial calcification score	Higher circulating ucMGP was associated with peripheral arterial calcification score
Pivin, 2015	**Switzerland**	N=1001, age 47±17, 52% female	Aortic pulse wave velocity	Higher circulating ucMGP was associated with increased arterial stiffness
Mayer, 2016	**Czech Republic**	N=1087, age 55±14, female	Aortic and distal pulse wave velocity	Higher circulating ucMGP was associated with increased aortic pulse wave velocity
Sardana, 2016	**USA**	N=66, type 2 diabetes, age 62±12 years, 9% female	Carotid-femoral pulse wave velocity	Higher circulating ucMGP was associated with increased arterial stiffness
Longitudinal studies among cardiac patients				
Ueland, 2010	**Norway**	N=147 calcific valvular aortic stenosis patients, age 74 ± 10, 45% female, 23 months of follow-up	All-cause mortality	Higher circulating ucMGP was associated with long-term mortality
Ueland, 2011	**Norway**	179 heart failure patients and 33 healthy individuals, age 56±12, 22% female 2.9 years of follow-up	Mortality due to heart failure	Higher circulating ucMGP was associated with decreased long-term heart transplant-free survival
Mayer, 2014 & 2016	**Czech Republic**	N=799, prior CVD patients, age 65 years, 29% female, 5.6 years of follow-up	All-cause mortality, CVD mortality	Higher circulating ucMGP was associated with increased mortality risk

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van den Heuvel, J.M., et al.,

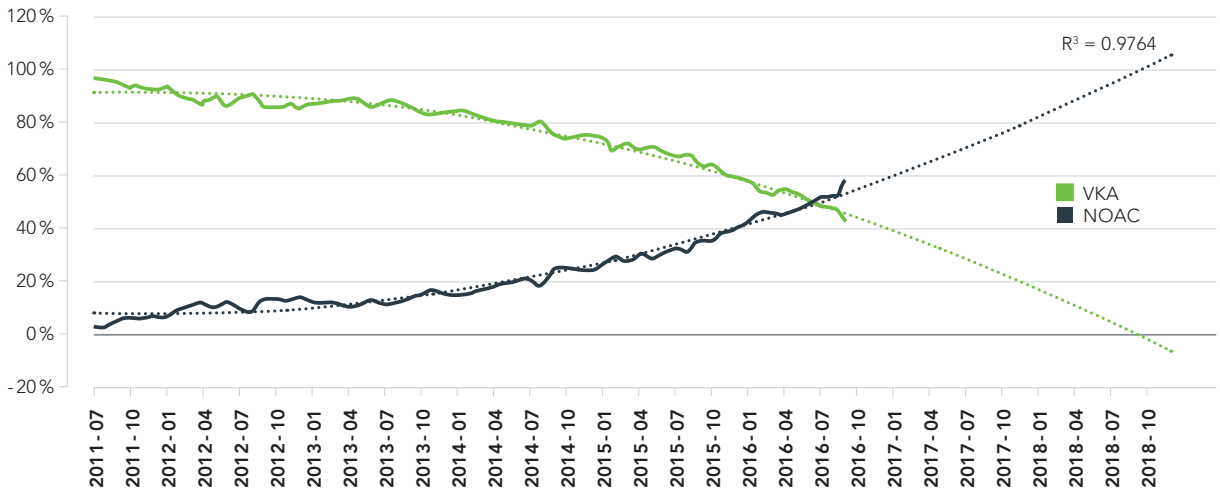
NOACs Replace VKA as Preferred Oral Anticoagulant Among New Patients: A Drug Utilization Study in 560 Pharmacies in The Netherlands

Objective:
Vitamin K antagonists (VKA) and non-VKA oral anticoagulants (NOACs) are the two main categories of oral anticoagulants prescribed for thromboembolic diseases. VKA interferes with the vitamin K cycle and has a small therapeutic window. Treating patients with VKA requires titration of the dose, and the required dose can differ largely among patients. NOACs are a new group of drugs that entered the market in 2011. NOACs do not interfere with the vitamin K cycle and do not require frequent monitoring of the blood, which is a benefit for the patient. This study compared the use and adherence of NOACs to VKA.

Method:
Data from 560 pharmacies and 247,927 patients prescribed from 2011 to 2016 were analyzed. Use in new patients and switch from VKA to NOAC were registered. Adherence to the therapies were calculated.

Results:
Since its launch in 2011, NOACs has grown up to 57% of prescriptions to new patients. Among the NOAC users, 70% were new naïve patients and around 26% switched from VKA. 88% of all NOAC users were adherent to the therapy. In conclusion, NOAC has taken over VKA as the major treatment prescribed to new users. The number of patients starting on VKA is decreasing.

NOACs versus VKA Share in Naive Starter



› K2VITAL® DELTA – verified stability ‹

Vitamin K2 MK-7, like other fat-soluble vitamins, is unstable in certain formulations and environments. It is particularly susceptible to decomposition in combination with minerals such as calcium and magnesium. This is a prominent issue because calcium and magnesium are core components of almost all bone and many cardiac health products. Other factors with accelerate the degradation of vitamin K2 include heat, moisture, light, and acid or alkaline environments. Unprotected

STABILITY

K2 degrades at a very rapid rate which disqualifies manufacturing overage as a solution to the stability issue. Therefore, K2 MK-7 needs to be protected to ensure longer shelf-life and that finished products meet label claim.

Studies on K2 products (around 100 products) on the market revealed the alarming extent of K2's stability issue. 81% of the products tested did not conform to the information printed on the label. 40% of these products did not contain any detectable levels of vitamin K2. These unexpected findings revealed that without a solution to the stability problems of K2 MK-7, the use of the vitamin together with minerals would be risky regarding consumer protection.

K2VITAL® DELTA is a protected, microencapsulated vitamin K2 MK-7, stable with minerals and resistant to harsh environments. It features a unique two-layered water-dispersible coating. During the microencapsulation process, the emulsion of K2 is followed by atomization and a modified spray drying process with a powdering agent. A starch layer on the outside of the 300-micron beadlet protects the vitamin K2 oil droplets. The resulting product is stable with good flow and handling properties compared to natural-fermented K2. **K2VITAL® DELTA** is tolerant to manufacturing processes and with less overage in product manufacturing.

Stability of **K2VITAL® DELTA** has been verified in 12 and 24-month stability tests, in comparison with unprotected, natural-fermented K2. **K2VITAL® DELTA** plus calcium or magnesium demonstrated a minimal loss in assay over a 12-month period at both normal and accelerated temperatures (25°C and 40°C). In comparison, natural-fermented K2 MK-7 showed very significant decreases of MK-7 under the same conditions.

VITAMIN K2 - A PLUS IN COUMARIN THERAPY

Vitamin K2 and anticoagulant therapy

Coumarins are among the classic and commonly used oral anticoagulants. They are widely used for the treatment and prophylaxis of thromboembolic diseases and in particular for the protection of strokes in patients with atrial fibrillation. Warfarin, phenprocoumon and acenocoumarol are the typical representatives of this class of drugs.

As vitamin K antagonists (VKA), these agents inhibit the vitamin K-dependent activation of coagulation factors by competitively displacing vitamin K, thus reducing the coagulation ability of the blood. In addition, they prevent the synthesis of anticoagulant proteins S and C in the liver.

The effectiveness of coumarins is susceptible to interference: interactions with drugs and food are common. Therefore, the blood coagulation ability of VKA-treated patients must be closely monitored. In particular, vitamin K has a major impact on the effects of coumarins. A low-dosage vitamin diet is often recommended during therapy to prevent the sensitive enzyme cascade system of blood clotting to be too strongly influenced, which may lead to spontaneous bleeding or thrombus formation.

An interesting role is suggested particularly for vitamin K2, since in a mammalian trial it is shown that individuals treated with vitamin K2 and Warfarin were protected from calcifying vessels, something not seen for individuals with Warfarin and vitamin K1 treatment [9].

MK-7 and anticoagulants

Healthy individuals titrated with Warfarin to reach a target international normalized ratio (INR) of 2.0 will show changes/normalizations in the INR if they start supplementation with vitamin K1 or vitamin K2 (MK-7) [2]. There are several reports documenting that patients that are taking AOC will benefit from taking a stable dose of vitamin K [3-7].

Sconce et al from 2005 [7] investigated the dietary intake of vitamin K in patients on warfarin. They discovered that patients with an unstable control of their INR blood values had a significantly lower intake of vitamin K when compared to the patients that had a stable control of their INR blood values. Further, Scones el al from 2007 [6] investigated if intake of vitamin K could improve the stability of anticoagulation for patients with high variability in the response to warfarin. In this double-blinded placebo controlled clinical trial the patients taking vitamin K significantly lowered the variability and stabilized the INR values compared to the placebo group. A Canadian research group confirmed their latter findings also in a double-blinded placebo controlled trial [5]. Li et al showed that dietary vitamin K intake may be optimal when initiating warfarin therapy [4]. In a double-blinded randomized placebo controlled study Gebuis and colleagues showed that for patients starting with vitamin K antagonists, supplementation with vitamin K resulted in an improvement of time that anticoagulation was within the therapeutic range. [3]. They also found that there were no differences in thromboembolic and hemorrhagic complications between the placebo group and the test groups.

Recently, the US Pharmacopeial Convention published a safety evaluation of MK-7 [1]. Important sections in this evaluation were interactions between MK-7 and anticoagulants, and pharmacovigilance. The conclusion from the US Pharmacopeial Convention: *"In summary, there is a risk of interaction between MK-7 or other forms of vitamin K and warfarin or other anticoagulant therapy. However, through dose titration and patient counselling, the physician or pharmacist may be able to mitigate that risk to maintain stable anticoagulation control, so long as the patients' vitamin K intake is known."*

The new range of novel non-vitamin K antagonist oral anticoagulants (NOACs) which in contrast to the VKAs suppressing the synthesis on the vitamin K-dependent factors, works by directly inhibiting the key proteases (factors IIa and Xa). These NOACs seem, according to a comparison conducted by Mekaj et al in 2015, to have important advantages in terms of safety, convenience of use, minor drug and food interactions, a wide therapeutic window and no need for laboratory monitoring [8]. It is expected that these NOACs will dominate the market going forward, which ultimately will have impact on the level of concerns associated with use of anticoagulant treatment drugs in relation to vitamin K supplementation.

Pharmacovigilance

In order to look for potential side effects where MK-7 could be involved, Marles et al did, in 2017, a search for published case reports or reviews in the FDA MedWatch program, the Canadian Vigilance Adverse Reaction Online Database, the UK Medicines and Healthcare Products Regulatory Agency Drug Analysis Prints A-Z, the Australian Therapeutic Goods Administration Database of Adverse Event Notifications – medicines, and the New Zealand MedSafe Suspected Medicine Adverse Reaction Search [1].

- **MedWatch-FDA:** No relevant reports
- **Canada Vigilance Database:** No reports on "Menaquinone-7" or "MK-7". One report retrieved with search word "menaquinones". This was a non-serious event of diarrhea and vomiting where the suspected product was a 92-ingredient product containing only 6 µg vitamin K2 (vitamin not specified).
- **UK Medicines and Healthcare Products Regulatory Agency Drug Analysis Prints database:** No relevant reports
- **Australian Therapeutic Goods Administration Database of Adverse Event Notifications – medicines:** Two reported adverse events associated with menaquinones. One event was a report of "hot flush" and "blurred vision" associated with MK-7. The other event was a myocardial infarction and splenic infarction associated with an unspecified menaquinone product (vitamin not specified) taken in combination with human prothrombin complex (also one of the suspected product in the report) and acetylsalicylic acid.
- **New Zealand MedSafe Suspected Medicine Adverse Reaction Search:** No relevant reports.

Conclusion

The conclusion from the US Pharmacopeial Convention: *"The clinical and nonclinical data reviewed here, together with the finding of reviews conducted by reputable bodies such as the EFSA, UK EVM, the IOM and WHO, support the conclusion that MK-7, when ingested as a dietary supplement at levels typically recommended, is not likely to be associated with any serious risk to individual or public health that is not already addressed by current practices regarding the marketing and use of dietary supplements containing vitamin K in its various forms."*

While Warfarin and Coumarin may prevent stroke and pulmonary embolism, it may, over time, contribute to complications associated with low vitamin K activity, such as reduced bone mineral density, osteopenia, osteoporosis, increased bone fractures, and calcification of arteries. Several studies report that a combination of vitamin K and Warfarin may be beneficial circumventing the negative effects. However, since warfarin and coumarin are partially blocking the vitamin K cycle, a sudden intake of vitamin K (both vitamin K1 and vitamin K2) could potentially be dangerous. Patients taking warfarin should consult a physician regarding the optimal balance of vitamin K-family intake, and it will be important to maintain a stable intake of vitamin K when the patient has reached a therapeutic INR range.

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