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Evolving role of vitamin K2-7(Menaquinone) in Osteoporosis & cardiovascular health

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Abstract
Osteoporosis & cardiovascular disorders are one of the commonest global problems. These two disorders not only affect the quality of life but also put a huge financial burden on the family and the nation as a whole. Since ages we have been using calcium supplements for the management of osteoporosis and the recent reports have shown that it can lead to increased cardiovascular complications. Vitamin K2, an age old vitamin has been shown to take care of osteoporosis and cardiovascular complications, since it plays an important role in carboxylation of certain proteins in bone and blood vessel. This review article summarizes the role of vitamin K2 in osteoporosis 7 cardiovascular disorders and also throws light on the clinical evidences available for the same.
Keywords: *Osteoporosis*, Vitamin K2, Menaquinone-7, Carboxylation.

1. Introduction
Vitamin K comprises a group of substances, which are widespread in nature and are an essential co-factor in humans in the synthesis of several proteins that play a role in haemostasis and others that may be important in calcium homeostasis.The K in Vitamin K comes from the Germanic word koagulation, for its ability to clot blood and prevent hemorrhage. Although discovered in the early 1930s, Vitamin K remains one of the least understood Vitamins. The Danish and American biochemists Henrik Dam and Edward Doisy shared the 1943 Nobel Prize: Dam for appreciating the existence of a factor essential for the coagulation of blood and Doisy for elucidating Vitamin K's structure[1].

2. Vitamin K
2.1 Structure and Activity
Vitamin K is fat-soluble and that it has several forms. Vitamin K1 (phylloquinone) is made by plants (phytonadione is identical to K1 but is synthesized commercially). Vitamin K and is found in leafy green vegetables (e.g., lettuce, broccoli, spinach, cabbage) and vegetable oils (e.g., soybean and canola oils).

Vitamin K2 or MK-n (menaquinone-n, a variously sized molecule depending on the number (n) of repeating 5-carbon units) is made by intestinal bacteria. K2 production by bacteria provides only a minor fraction of our daily needs since it is made mostly in the large intestine and colon where it is poorly absorbed.

Vitamin K3 (menadione) is a potent synthetic (manmade) form of Vitamin K. Vitamin K3 cannot exert all the functions of natural Vitamin K because of limited transformation into the fat-soluble Vitamin K forms[1].

Vitamin K takes part in what is called the Vitamin K cycle by assisting enzymes (Vitamin K-dependent carboxylases) that control the activation of several key coagulation factors including prothrombin (II), proconvertin (VII), and Christmas factor (IX). The result is the addition of carbon dioxide (carboxylation) to glutamic acid residues (Glu) in these proteins, converting them to their calcium binding pro-coagulation forms (Gla). Vitamin K then gets recycled back to its usable form through the actions of two reductase enzymes.

2.2 Widespread Role of Vitamin K
Vitamin K-dependent proteins are known to participate in three physiological processes:
a) In blood coagulation (coagulation factors II, VII, IX and X)
b) In bone metabolism (Osteocalcin / Bone Gla-protein)
c) In vascular smooth muscle –Carboxylation of Matrix GLA Protein which inhibits calcification of blood vessels.

The Vitamin K requirement for carboxylation of bone and arterial wall VKD proteins is higher than that for the carboxylation of coagulation factors in the liver. Daily Vitamin K requirements for maximal γ-carboxylation of the extra

hepatic VKD proteins may be significantly higher than recommended by current dietary guidelines. Vitamin K deficiency, resulting in the undercarboxylation of specific VKD proteins, may be an independent risk factor for osteoporosis and arterial calcification[2].

2.3 Vitamin K2

Vitamin K2 is a collective term for a group of Vitamin K compounds called menaquinone. The individual components of Vitamin K2 are also referred to by the number of isoprenyl units in the side chain; generally they are designated as MK-n, where n specifies the number of isoprenoids residues.

The menaquinones most commonly found in food are MK-4, which is a short-chain menaquinone, and the long-chain menaquinones MK-7, MK-8, and MK-9.

K2 (MK-7) is a product of bacterial food fermentation found in foods such as cheeses, cabbage, fermented soy or natto, but it is most economically derived from natto (a traditional soy and rice fermented mixture).

Vitamin K2 was approved in Japan from 1995 in the treatment of osteoporosis.Compared to the other Vitamin K analogues, Vitamin K2 has the most potent gamma-carboxylation activity[3].

2.4 Role of Vitamin K2 in Gamma carboxylation

Vitamin K2 is essential cofactor for γ-carboxylase .A large number of vitamin K-dependent proteins (VKD) throughout the body, by carboxylating certain glutamate residues and changing to gamma-carboxyglutamate residues, abbreviated Gla, Gla has the property of binding calcium, and so as soon as VKD proteins get their glutamate residues carboxylated by vitamin K, they are able to bind calcium as well. VKD proteins are osteocalcin, MGP, blood coagulation proteins [2].

2.5 Importance of Gamma carboxylation

Vitamin K2 is an essential cofactor for γ-carboxylase. Incomplete γ- carboxylation of osteocalcin (OC) resulting from Vitamin $K2_7$ deficiency is associated with osteoporosis and increased risk of fracture. Osteocalcin is synthesized only in osteoblasts. Because osteocalcin that is not carboxylated cannot bind to hydroxyapatite, serum levels of under carboxylated osteocalcin is a good biochemical marker of the metabolic turnover of bone.

Vitamin K-dependent (VKD) protein carboxylation uses Vitamin K epoxidation to convert Glus to carboxylated Glus (Glas), rendering VKD proteins active in physiologies that include hemostasis, apoptosis, bone mineralization, calcium homeostasis, growth control, and signal transduction.

Matrix Gla protein (MGP): MGP (matrix Gla Proteins), found in bone, cartilage, and vascular smooth muscle cells. MGP plays a key role in the inhibition of tissue calcification. MGP needs to be carboxylated to function properly, this carboxylation or activation is done by Vitamin $K2_7$. Hence Vitamin $K2_7$ deficiency is a risk factor for vascular calcification[2].

2.6 Role of Vitamin $K2_7$ in Osteoporosis[2, 4]

1. Vitamin K2 is an essential cofactor for γ-carboxylase. Incomplete γ- carboxylation of osteocalcin (OC) and matrix Gla protein (MGP) resulting from Vitamin $K2_7$ deficiency is associated with osteoporosis and increased risk of fracture. Vitamin K-dependent (VKD) protein carboxylation uses Vitamin K epoxidation to convert Glus to carboxylated Glus (Glas), rendering VKD proteins active in physiologies that include hemostasis, apoptosis, bone mineralization, calcium homeostasis, growth control, and signal transduction.
2. Vitamin $K2_7$ was also shown to enhance the induction of OC (Osteocalcin) mRNA levels mediated by co-administered 1α, $25(OH)_2$ Vitamin D3.
3. Vitamin $K2_7$ has a dual role in mediating bone homeostasis. It acts in an anabolic manner to stimulate the synthesis of osteoblastic markers and deposition of bone.
4. Vitamin $K2_7$ decreases bone resorption by inhibiting the formation of osteoclasts as well as their bone resorptive activity.
5. Vitamin $K2_7$ treatment also induces apoptosis of osteoclasts while inhibiting apoptosis of osteoblasts thereby shifting the balance towards bone formation.
6. Vitamin $K2_7$ causes activation of SXR (Steroid Xenobiotic Receptor), which modulates the expression of osteoblastic bone markers: bone alkaline phosphatase, osteoprotegerin and osteopontin.

Vitamin $K2_7$ helps in carboxylation of osteocalcin. Strong correlation was found between Undercarboxylated serum osteocalcin levels and the subsequent risk of hip fracture. Women with abnormally high undercarboxylated osteocalcin concentrations (>1.65 ng/mL) had a RR between 3.1 and 5.9 times higher than those with normal undercarboxylated osteocalcin levels (<1.65 ng/mL)[2].

In the skeleton, bone always undergoes remodeling where old is removed by osteoclasts and new bone tissue replaced by osteoblasts. Vitamin k is essential to this process, helping the remodeling by activating protein necessary to utilize calcium.

It was found that osteocalcin was undercarboxylated by 40% in postmenopausal women when compared with premenopausal women[5].The postmenopausal women responded to Vit. K 2_7 supplementation with an increase in total and carboxylated osteocalcin and a decrease in urinary calcium and hydroxyproline[2].

2.7 Role of Vitamin $K2_7$ in Cardiovascular Health

Arterial calcification occurs at two sites in the vessel wall: the media and the intima. Arterial calcification decreases vessel elasticity and integrity leading to cardiovascular disease (CVD).

MGP (matrix Gla Proteins), found in bone, cartilage, and vascular smooth muscle cells. MGP plays a key role in the inhibition of tissue calcification. MGP needs to be carboxylated to function properly, this carboxylation or activation is done by Vitamin $K2_7$. Hence Vitamin $K2_7$ deficiency is a risk factor for vascular calcification.

Vitamin $K2_7$ deficiency cause inadequate calcium metabolism and utilization. This is called the Calcium Paradox. This paradox suggests that necessary calcium is not being effectively utilized by the body for building bones and other healthy functions, thus increasing to unhealthy levels in the vascular system and eventually leading to heart disease. Vitamin $K2_7$ is necessary to prevent complications of the calcium paradox[3].

3. Impact of Vitamin K2 deficiency

Studies have shown that sub clinical Vitamin K deficiency is present in most healthy adults and children in Western Populations. This is demonstrated by a striking difference in incidence and prevalence of poor bone health and cardiovascular diseases which exists between the populations in westernized and more traditionally Far East countries i.e. Oslo has the highest incidence of fractures, despite the fact that Norwegians drink a lot of milk which is one of the best dietary sources of calcium, compared to Japan and Singapore which have the lowest incidence of fractures. Recently it was shown that foods have less vitamin K than previously thought. Most multi-vitamins don't contain any vitamin K at all. The ones that do don't contain enough for optimal health[3].

The amount of Vitamin $K2_7$ needed for optimal carboxylation of osteocalcin is significantly higher than what is provided by diet alone[2]. Deficiency of Vitamin K2 leads to hemorrhage, undercarboxylation of osteocalcin and MGP cause underutilization of calcium leads to osteoporosis and arterial calcification.

4. Evidence of safety and efficacy of Vitamin K2 in osteoporosis

4.1. Effect of Vitamin K2 supplementation on Bone Mineral Density

Efficacy of Vitamin K2 was evaluated in 241 osteoporotic patients for period of 24 month. Study group divided into control and Vitamin K2 (45 mg/day).Incidence of new vertebral fracture was found 30.3% in control group compared to 10.9% with Vitamin K2 group. The percentages of change from the initial value of Lumbar Bone Mineral density (LBMD) at 6, 12, and 24 months after the initiation of the study were -1.8 ± 0.6%, -2.4 ± 0.7%, and -3.3 ± 0.8% for the control group, and 1.4 ± 0.7%, -0.1 ± 0.6%, and -0.5 ± 1.0% for the Vitamin K2-treated group, respectively.

The changes in LBMD at each time point were significantly different between the control and the treated group ($p = 0.0010$ for 6 months, $p = 0.0153$ for 12 months, and $p = 0.0339$ for 24 months)[6].

Longitudinal study of 17 postmenopausal women given vitamin K2 (45 mg/day) for one year found that K2 was able to suppress the decrease in spinal BMD, with a slight increase (0.23 [+ or -] 0.47%) compared to the control group of 19 postmenopausal women who experienced a decrease (-2.87 [+ or -] 0.51%) in BMD[7].

4.2. Vitamin K2 combination with Vitamin D3 in osteoporosis

Efficacy of combination of Vitamin K2 with D3 was evaluated in primary osteoporosis. Combined administration of Vitamin D3 and Vitamin K2 seems to have the greatest effect on lumbar bone mineral density[8].

In another study with 92 osteoporotic women were randomly divided into four administration groups: Vitamin D3 (1 alpha hydroxyl Vitamin D3, 0.75 microg/day) (D group; n = 29), Vitamin K2 (menatetrenone, 45 mg/day) (K group; n = 22), Vitamin D3 plus Vitamin K2 (DK group, n = 21), and calcium (calcium lactate, 2 g/day) (C group; n = 20). Results indicated that combined administration of Vitamin D3 and Vitamin K2, compared with calcium administration, appears to be useful in increasing the BMD of the lumbar spine in postmenopausal women with osteoporosis[9].

60 patients with chronic glomerulonephritis were randomized to four groups: control, 1-alpha-hydroxyvitamin D3 (0.5 mcg/day), vitamin K2 (45 mg/day), or vitamins K2 with D3. Patients concomitantly received prednisolone at a daily dose of 0.7 mg/kg up to a maximum of 40 mg for four weeks, then tapered to 25 mg daily for another four weeks prior to assessment. The control group experienced a significant decrease from baseline in BMD over the eight-week study: -3.19 [+ or -] 1.11 percent, compared to the vitamins D, K, and D+K groups that maintained baseline levels (0.28 [+ or -] 1.30, 0.50 [+ or -] 1.17, and 0.44 [+ or -] 1.36 percent, respectively)[10].

4.3. Vitamin K2 & Vitamin D3 combination compared with Vitamin K2 alone

172 women with osteoporosis enrolled in study. Combined therapy with Vitamin K2 and D3 for 24 months markedly increased bone mineral density (4.92 ± 7.89%), while Vitamin K 2 alone increased it only 0.135 ± 5.44%. Combination of Vitamin K2 & Vitamin D3 was found to be much superior to Vitamin K2 alone[11].

4.4. Better efficacy of Vitamin K2 in reducing incidence of fracture compared to bisphosphonates

Meta analysis studies found that Vitamin K2 reduces incidence of vertebral fracture by 53 % as compared to alendronate (48%), risedronate (36%), etidornate (37%), and raloxifene (40%) in patients with postmenopausal or age-related osteoporosis[12].

In another study the incidence of vertebral fractures was 8.0 % in patient treated with menatetrenone & 20.8 % in

patients treated with calcium in postmenopausal women with osteoporosis[13].

With respect to the therapeutic effect of menatetrenone treatment on corticosteroid-induced osteoporosis over 2 years, the incidence of a new vertebral fracture was 13.3% in the menatetrenone treatment group versus 41% in the control group, indicating that Vitamin K2 treatment could prevent fractures[14].

4.5. Systematic review and meta-analysis of randomized controlled trials of Vitamin K2

Pooling the 7 trials with fracture data in a meta-analysis, found an odds ratio (OR) favoring menaquinone of 0.40 (95% confidence interval [CI], 0.25-0.65) for vertebral fractures, an OR of 0.23 (95% CI, 0.12-0.47) for hip fractures, and an OR of 0.19 (95% CI, 0.11-0.35) for all non vertebral fractures[15].

4.6. Effect of Vitamin K2 on serum undercarboxylated osteocalcin

20 osteoporotic women with vertebral fractures were randomly divided into two groups: the menatetrenone plus calcium treatment group (n=10) and the calcium alone (control) group (n=10). The duration of the treatment was 14 days. A significant reduction in the serum ucOC level from the baseline was observed 7 and 14 days after the start of treatment in the menatetrenone-treated group, and a significant reduction in the serum ucOC level as compared with the control group was observed 14 days after the start of the treatment in the menatetrenone-treated group[16].

In a double blind randomized placebo-controlled study of 63 postmenopausal women with osteoporosis. The Vitamin K2 group (n = 33) received 45 mg menatetrenone and 1500 mg calcium carbonate per day and the control group (n = 30) received placebo and 1500 mg calcium carbonate per day for 48 weeks. The undercarboxylated OC level decreased by 55.9% in the menatetrenone group and 9.3% in the control group compared with the baseline level[17].

In various clinical trials Vitamin K2 was found to be superior therapeutic agent in glucocorticoids-induced osteoporosis[18].

5. Clinical studies of Vitamin $K2_7$ in Cardiovascular disorders

1. In a population study of 4500 elderly patients and inverse relationship was demonstrated between dietary intake of menaquinone and aortic calcification myocardial infarction and sudden cardiovascular death. Menaquinone cause 50% reduction in arterial calcification, cardiovascular death & 25 % reduction in of all cause of mortality[19].
2. In 16,057 women, aged 49-70 years, who were free of cardiovascular diseases at baseline, mean vitamin K(1) intake was 211.7± 100.3 mug/d and vitamin K(2) intake was 29.1± 12.8 mug/d & Follow-up period of 8.1± 1.6. Study observed an inverse association between vitamin K(2) and risk of CHD with a Hazard Ratio (HR) of 0.91 [95% CI 0.85-1.00] per 10 mug/d vitamin K(2) intake. This association was mainly due to vitamin K(2) subtypes MK-7, MK-8 and MK-9. Vitamin K (1) intake was not significantly related to CHD.A high intake of menaquinones, especially MK-7, MK-8 and MK-9, could protect against CHD[20].

6. Recommended Dose

The recommended daily intake of Vitamin $K2_7$ is 100-120 mcg/day[21].

7. Pharmacokinetic of Vitamin $K2_7$

Among the various forms of menaquinones, the length of the side chain plays an additional role in bioavailability, as menaquinones with medium-length side chains (*e.g.*, MK-7) are better absorbed compared to those with short (MK-4) or long (*e.g.*, MK-8 and MK-9) side chains.Vitamin $K2_7$ appears to be absorbed rapidly and unchanged from the gastrointestinal tract, is carried in the lymph in mixed micelles composed of bile salts, and subsequently released into the circulation. As with other lipid-soluble compounds, optimal absorption is dependent on the presence of bile acids. The liver is the principal site of Vitamin K metabolism, involving oxidative degradation of the side-chain and resulting in subsequent elimination via the bile or urine[22].

8. Safety of Vitamin K2 in hepatic failure patients

Patients with primary biliary cirrhosis experience osteodystrophy and increased fracture rate and fat malabsorption that results in deficiencies of vitamins D and K. Serum levels of vitamin K have been found to be low in this population. In a randomized, controlled trial of 27 patients with primary biliary cirrhosis, the treatment group (n = 14) received vitamin K2 (45 mg/day) for two years. After one year the control group (n = 13) experienced a 3.5 [+ or -] 1.2 percent decrease in BMD, while the vitamin K2 group demonstrated a 0.3 [+ or -] 2.3 percent increase in BMD. After two years, the control group demonstrated a 6.9 [+ or -] 2.1 percent decrease in BMD, compared to only a 0.8 [+ or -] 3.4 percent decrease in the K2 group. BMD was significantly higher in the vitamin K2 group during the two-year period compared to controls[23].Study clearly demonstrated safety of Vitamin K2 in hepatic failure patients.

9. Safety of Vitamin $K2_7$ with Respect to Hypercoagulation in Humans

From a large number of clinical trials using dosages in excess of 40 mg/day, there were no reports of side effects associated with any type of hypercoagulable state[24].

In a clinical study, 29 elderly, osteoporotic patients were given Vitamin K2 (15 mg three times daily, 30 minutes post meals) for 12 weeks and monitored for any change in hemostatic balance. After 12 weeks of administration, all

hemostatic markers remained within normal range[25].

Result from human intervention studies did not report adverse effects of Vitamin $K2_7$ on blood coagulation of at least 6 mcg/kg bw/day[22].

In double blind study daily supplementation with 150 mcg vitamin $K2_7$ along with warfarin therapy can lead to a more stable anticoagulation in patients[26].

A recent small retrospective study in which a 100 mcg daily oral dose of vitamin K was administered on a long-term basis to 8 patients with unstable control of anticoagulation suggested that vitamin K supplementation, by increasing and stabilizing the body's stores of the vitamin, allowed for more steady activation of vitamin K–dependent clotting factors and better control of anticoagulation[26].

10. Long term safety of Vitamin $K2_7$

The Japanese population based osteoporosis study has investigated effect of 200 mcg of Vitamin $K2_7$ on bone mineral density in 944 pre-and postmenopausal women over 3 years, no serious adverse event were reported[22].

10.1 Adverse events with Vitamin $K2_7$

Generally Vitamin $K2_7$ is very well tolerated; rare side effects are noted such as flushing or redness of skin, dizziness, fast and / or weak heartbeat, increased sweating, and low blood pressure (temporally).

11. Conclusion

Numerous studies have demonstrated the importance of Vitamin K2 in bone health. Cell studies have helped delineate the mechanism by which menaquinone promotes bone Mineralization and inhibits resorption. Human and animal studies have clearly demonstrated that Vitamin K2 can improve bone health by increasing bone mass and reducing bone loss.The combination of menaquinone and vitamin D3 has additive beneficial effects on sustaining lumbar BMD and preventing osteoporotic vertebral fractures in postmenopausal women with osteoporosis.

Moderately high doses of vitamin K2 do not produce hypercoagulable or toxic states in humans.Because of very low toxicity and potentially beneficial effects on both bone mineralization and attenuation of arterial calcification, Vitamin $K2_7$ should be strongly considered as therapeutic agent in the treatment of Osteoporosis and Cardiovascular disorder.

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