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Review

Vitamin K and osteoporosis: Myth or reality?

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ABSTRACT

Vitamin K is a liposoluble vitamin. The predominant dietary form, phylloquinone or vitamin K1, is found in plants and green vegetables; whereas menaquinone, or vitamin K2, is endogenously synthesized by intestinal bacteria and includes several subtypes that differ in side chain length. Aside from its established role in blood clotting, several studies now support a critical function of vitamin K in improving bone health. Vitamin K is in fact required for osteocalcin carboxylation that in turn regulates bone mineral accretion; it seems to promote the transition of osteoblasts to osteocytes and also limits the process of osteoclastogenesis. Several observational and interventional studies have examined the relationship between vitamin K and bone metabolism, but findings are conflicting and unclear. This systematic review aims to investigate the impact of vitamin K (plasma levels, dietary intake, and oral supplementation) on bone health with a particular interest in bone remodeling, mineral density and fragility fractures.

Contents

Abbreviations: VK, Vitamin K; OC, Osteocalcin; RCTs, Randomized Control Trials; BMD, Bone Mineral Density; BTM, Bone Turnover Markers; VD, Vitamin D; Gla, γ-carboxyglutamic acid; PT, Prothrombin Time; MGP, Matrix Gla Protein; Gas-6, Growth arrest-specific 6 protein; ucOC, undercarboxylated OC; cOC, carboxylated OC; RANKL, Receptor Activator of Nuclear factor Kappa B Ligand; ODF, Osteoclast Differentiation Factor; SXR, Steroid and Xenobiotic Receptor; PXR, Pregnane X Receptor; FFQ, Food Frequency Questionnaire; BALP, Bone Alkaline Phosphatase; CTX, C-terminal Telopeptide of type 1 collagen; P1NP, Procollagen I Intact N-Terminal; NTX, N-terminal Telopeptide of type 1 collagen; QUS, Quantitative Ultrasound; BMC, Bone Mineral Content; CI, Confidence Interval; RR, Relative Risk; HR, Hazard Ratio; OR, Odds Ratio; DXA, Dual-energy X-ray absorptiometry; VKAs, VK antagonists; PTH, Parathormone.

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1. Introduction

The fundamental role of vitamin K (VK) in blood clotting is well established [1–5]. However, the past two decades have seen increasing evidence supporting that VK also plays an important part in bone health. Indeed, VK is needed to carry out the process of osteocalcin (OC) carboxylation [6], it seems to promote the osteoblast-to-osteocyte transition and also limit osteoclastogenesis [7].

Some cross-sectional and randomized control trials (RCTs) have reported that VK plasma levels show a positive correlation with bone mass and a negative one with fracture risk. It has been clearly demonstrated that optimal vitamin D (VD) repletion is necessary to maximize the response to anti-resorbers regarding both bone mineral density (BMD) changes and anti-fracture efficacy [8], but we do not know exactly whether optimal VK status affects the response to anti-osteoporotic drugs. Moreover, most of the studies published on this topic are characterized by several important limitations and provide contrasting evidence. VK supplementation is therefore still not globally recommended to contrast post-menopausal bone loss, although an exception is Japan where it has already been approved for the prevention and treatment of osteoporosis [9].

This systematic review aims to investigate the impact of VK (plasma levels, dietary intake, and oral supplementation) on bone health. In particular, the focus is on the relation between VK and bone remodeling, bone mineral density and bone fragility fractures.

2. Materials and Methods

PubMed, MEDLINE and Cochrane databases were searched according to PRISMA guidelines [10] to identify publications on VK and bone health. Articles investigating the different forms of VK and their effect on bone metabolism were included. Names of different forms of VK such as Phylloquinone (K1), Menaquinone (K2), Menaquinone-4 (MK-4) and Menaquinone-7 (MK-7) were matched with bone turnover markers (BTM), in particular OC, BMD, and bone fragility fractures. Publications in English only were included.

3. Vitamin K

VK is a liposoluble vitamin discovered in 1929 by Henrik Dam, and its name derives from the German word *koagulation*. VK plays a central role in the liver where it is required for the synthesis of functionally active forms of several coagulation factors [11]. Its emerging role in modulating bone metabolism will be discussed in this review.

3.1. *Structure and Sources*

VK exists in two forms that share a methylated naphthoquinone nucleus (menadione) and have a variable aliphatic side chain at the 3′ position.

Phylloquinone (K1) is the predominant form found in human diet and is synthesized by plants and green vegetables, like kale, spinach, broccoli and some fruits and herbs. Certain types of oil such as soybean and canola also contain large amounts of K1 (Table 1) [12].

Menaquinones (K2) consist of several subtypes that differ in the length of the side chain which may range from 1 to 13 residues of unsaturated isoprene: the *n* in the acronym MK-n reflects the number of isoprene residues in the aliphatic side chain. K2 is endogenously synthesized by intestinal bacteria, so it has a more confined distribution in the diet than K1 and it is found in some cheese, eggs, meat and *natto* (fermented soybeans commonly consumed in Japan that are the richest source with a content of 775 mcg/100 g) [13–15].

K1 can be converted into menaquinone-4 (MK4) and accumulated in extrahepatic tissues. It has also been demonstrated that this conversion occurs following oral or enteral administration, but not parenteral or intracerebroventricular administration [16].

3.2. *Vitamin K Status in Humans*

VK status is still not a straightforward estimation. It can be measured in plasma, but an abnormal lipid profile may affect the results. Moreover, VK is a soluble vitamin with many isoforms and VK plasma values alone may not be sufficient to assess the real VK status. VK content of an adult human liver is about 200–300 nmol [17]. It is not clear whether liver storage may be an indicator of VK status, in fact, liver may or

Table 1 – Vitamin K content in common food.

Food	Vitamin K content (mcg/100 g)	Food	Vitamin K content (mcg/100 g)
Vegetables		Proteins\Legumes\ Cheese	
Chard	830	*Natto*	775
Kale	817	Soy	47
Spinach	482.9	Tuna in oil	44
Chicory	297.6	Beans	19
Onion	193.4	Peas	14.5
Lettuce	102.3	Chickpeas	9
Carrot	13.2	Roast veal	7
Tomato	7.9	Lentils	5
Potato	1.9	Hamburger	4.6
		Chicken	4.2
		Cheddar cheese	2.8
		Mozzarella	2.3
		Eggs	0.3
		Fresh meat	<1
		Fresh fish	<1
		Whole milk	<1
Fruits		Fats and oils	
Kiwi	40.3	Soybean oil	183.9
Grape	14.6	Margarine	93
Kaki	2.6	Olive oil	62.3
Peach	2.6	Butter	7
Apple	0.6		
Banana	0.6		
Breads\Grains		Herbs and spices	
White bread	7.7	Sage	1714.5
Pizza	6.6	Thyme	1714.5
Spelt	3.6	Parsley	1640
Flour	0.3	Origan	621.7
Pasta	0.1	Basil	414.8
		Black pepper	163.7
		Chili	108.2

may not supply the required level of VK when dietary intake is insufficient.

VK2 intake in humans can also be assessed through specific Food Frequency Questionnaires (FFQ), but assessing VK2 activity rather than intake seems clinically more appropriate, and it can be done by quantifying undercarboxylated VK2-dependent proteins, like OCN [18]. The degree of γ-carboxylation of OCN seems to be sensitive to VK intake, and therefore it may represent a relative measure of the state of the VK [19], although sensitivity and specificity of OCN assessment are still a concern for the presence of physiological fluctuations in circulating levels and the absence of standardized tests across laboratories [18]. Since the great part of the presented results takes into consideration VK plasma levels and/or data from FFQs, caution is required in their interpretation.

Prothrombin time (PT) is a coagulation test that may reflect VK deficiency [20]. VK deficiency is rare and mostly due to the use of certain medications (VK antagonist anticoagulants, some antibiotics, anticonvulsants) [21] or liver and pancreas diseases [11].

Elderly subjects are at greater risk of developing VK deficiency likely due to reduced endogenous production of VK2. This condition could be determined by a reduced peripheral conversion of VK1 to K2 [22] or a reduced activity of intestinal bacteria as also observed in obese and diabetic subjects [23,24].

3.3. *Dietary Intake*

VK deposits in the body are quite meager, probably because they are easily depleted without regular dietary intake: it is estimated that the majority of VK ingested is cleared within 24 h. The endogenous production of K2 is variable depending on types and distribution of bacteria in the bowel, so its actual contribution to the daily VK requirement is not clear [25]. Conversely, a reduced dietary intake of K1 seems to be responsible for an inadequate VK status [1,26].

According to the National Academy of Sciences, the recommended dietary intake ranges from 2 mcg/day in newborns to 75 mcg/day in adolescents and is the same for both genders up to this stage. Adults' recommended intake is 120 mcg/day for males and 90 mcg/day for females [27]. However, these intakes are not usually sufficient to maintain optimal levels of VK, that are variable according to the ethnic origin and age of the subjects [28].

3.4. *Bioavailability*

VK shows variable bioavailability depending on the form. K1 obtained from green vegetables, for example, is firmly adherent to the cell membranes and is hence less bioavailable compared to that coming from plant oils or K1 supplements [29,30]. Menaquinones, instead, are primarily derived from animal based sources and are consumed in fatty food matrices that may potentially improve absorption leading to higher bioavailability compared to phylloquinone [31]. The bioavailability of K2 is also related to the side chain length. Indeed, there is a positive correlation between lipophilicity, bioavailability, and side chain length [31], the latter being controlled by specific gut bacteria [32]. The absorption of all forms of VK takes place in the small intestine via a process requiring bile salts [33]; however, these are absent in the colon where the majority of menaquinones are produced, suggesting a low rate of absorption of such VK forms [34]. Nonetheless, further studies are required to quantify differences in absorption, bioavailability and body distribution between individual menaquinone forms and phylloquinone.

4. Vitamin K and Osteoporosis

4.1. *Physiological Action of Vitamin K in Bone*

As previously stated, VK plays a critical role in maintaining bone strength by acting as cofactor of the enzyme gamma carboxylase, which in turn activates VK-dependent proteins in bone: OC, matrix Gla protein (MGP protein), Gla-rich protein, protein S, and growth arrest specific 6 protein (Gas6) [1,35] (Fig. 1). Gla proteins are located in body fluids and the extracellular matrix and have calcium binding abilities [36]. OC, an osteoblast derived protein, is located in bone and cartilage, essential for the synthesis and regulation of bone matrix [7]. Some studies carried out on animal models showed that OC, with its high affinity to calcium and hydroxyapatite, is implicated in the osteoclast–osteoblast

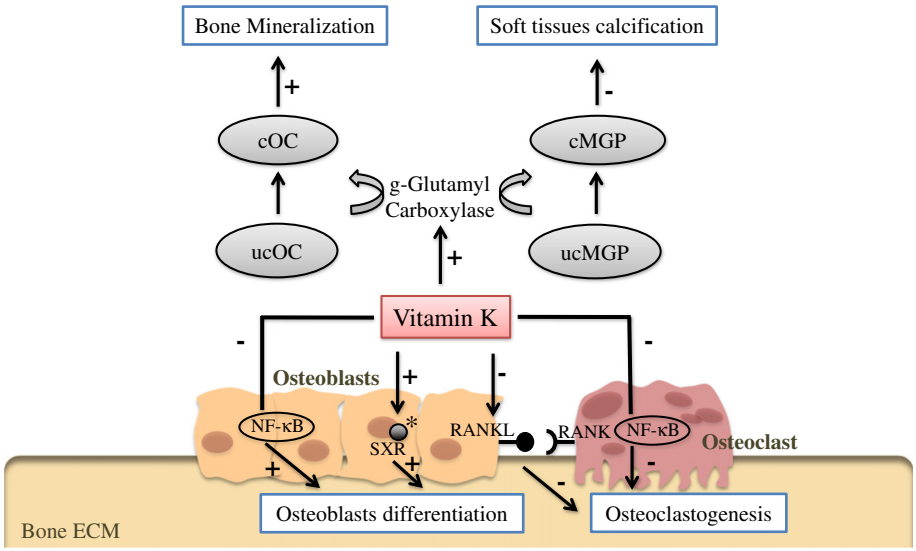

Fig. 1 – Mechanisms of action in bone. *: Evidence in animal models; ucOC: undercarboxylated Osteocalcin; cOC: carboxylated Osteocalcin; ucMGP: undercarboxylated Matrix Gla Protein; cMGP: carboxylated Matrix Gla Protein; NF-κB: nuclear factor κB; SXR: Steroid and Xenobiotic Receptor; RANKL: Receptor Activator of Nuclear factor Kappa B Ligand; RANK: Receptor Activator of Nuclear factor Kappa B; ECM: Extracellular matrix.

interplay, in bone mineralization and calcium homeostasis [37]. In particular, the undercarboxylated OC (ucOC) is the initial inactive form that shows limited calcium and hydroxyapatite binding activity without VK mediated carboxylation of the Gla domains (17, 21 and 24) [38]. Besides, Gla domains synthesis and activation are VK dependent.

The role of VK as an essential cofactor for carboxylation of bone matrix proteins is well established. However, recent evidence suggests that VK also has a transcriptional regulatory function (Table 2). In particular, VK seems to regulate osteoblastogenesis and osteoclastogenesis through the nuclear factor κB (NF-κB) signal transduction pathway. In fact, while the activation of NF-κB signaling is crucial for osteoclasts development and resorption, it also actively antagonizes osteoblasts function and differentiation. VK2 supports bone formation and suppresses bone resorption by antagonizing basal and cytokine induced activation of NF-κB in a γ-carboxylation-independent manner [18,39].

It has also been found that MK-4 can induce the activation of pregnane X receptor (SXR), a nuclear receptor involved in the transcriptional regulation of enzymes such as cytochrome P450s [40]. Although there are no data available yet about the expression of SXR in normal human bone, its mouse ortholog PXR is found to be abundantly expressed in bone tissue of rodents [41]. VK is able to regulate the transcription of bone markers through activation of PXR in primary osteocytes isolated from wild type mice whereas this effect is abolished in PXR knockout mice (PXRKO) [42]. Furthermore, to support the SXR/PXR effect on skeletal health, a Japanese group has shown that the BMD and the three dimensional bone volume fraction (analysis of femoral trabecular bones performed with Micro computed tomography) of PXRKO mice were significantly decreased compared to the BMD of wild type mice and that the three point bending test revealed that this led to mechanical fragility [43].

On the other hand, MK-4 seems to regulate some genes likely through other intracellular pathways not requiring SXR ligands activation. Therefore, the existence of other still unknown VK receptors has been postulated [44]. Similarly to OC, VK also induces the activation of MGP, a calcification inhibitor expressed in vascular tissue as well as in bone and cartilage. In the skeleton, this protein acts to prevent calcification of blood vessels, soft tissues and cartilages [45]. Finally, protein-S is another VK dependent protein involved in bone turnover. It is secreted by osteoblasts but its role remains unclear [46].

A further consideration in the interpretation of the results from animal studies is that physiological disparities between animals and humans skeleton. Despite, for example, the rat having been proved a good model for osteoporosis research using many experimental protocols including hormonal interventions (ovariectomy, orchiectomy, hypophysectomy, parathyroidectomy), immobilization, and dietary manipulations [47], these models cannot exactly replicate the physiopathology of a human skeleton, as men and rodents differ in some biochemical pathways and weight bearing is different between quadrupeds and bipeds.

4.2. *Vitamin K and Bone Remodeling*

Several studies have investigated the effects of VK on bone remodeling. In particular, a few observational studies, mainly conducted in Asian populations, have shown that VK1 and K2 intake (assessed with a FFQ) and/or plasma levels may be associated with a reduction of ucOC [48–50].

Other studies have investigated the relationship with other bone biomarkers. The largest one was a subsequent analysis of the Framingham Offspring Study, where plasma K1 concentrations and dietary intake resulted to be inversely associated with osteoprotegerin levels, a glycoprotein capable

Table 2 – Major vitamin K effects and mechanisms on bone metabolism.

Major vitamin K effects and mechanisms on bone metabolism		
Cofactor function		
Vitamin K effect	Mechanism	Reference
Cofactor of the enzyme gamma-carboxylase	Activation of vitamin K-dependent proteins in bone: OC, MGP protein, Gla-rich protein, protein S, and Gas6	- Shearer MJ et al. Adv Nutr 2012;3:182–95. - Booth SL. Annu Rev. Nutr 2009;29:89–110.
Transcriptional regulatory function		
Vitamin K effect	Mechanism	Reference
Inhibition of osteoclastogenesis	Suppression of NF-κB in a γ-carboxylation-independent manner.	- Yamaguchi et al. Int J Mol Med. 2011;27:3–14
Stimulation of osteoblastogenesis	Suppression of NF-κB in a γ-carboxylation-independent manner.	- Yamaguchi et al. Int J Mol Med. 2011;27:3–14
	Induction of SXR/PXR, a nuclear receptor involved in the transcriptional regulation of some enzymes such as cytochrome P450s [*].	- Shearer MJ et al. Thromb Haemost 2008;100:530–47. - Tabb MM et al. J Biol Chem 2003;278:43,919–27. - Azuma K et al. Journal of Endocrinology (2010) 207, 257–263.
Prevention of calcification of blood vessels, soft tissues and cartilages.	Activation of MGP, a calcification inhibitor expressed in vascular tissue as well as in bone and cartilages.	- Ichikawa T et al. J Mol Endocrinol 2007;39:239–47.

[*] Vitamin K induction of the activation of PXR, the mouse ortholog of the human SXR, has been shown in animal studies only.

of inhibiting the differentiation of the osteoclast precursor into a mature osteoclast, thus reducing bone resorption [51]. A study conducted on a large cohort of perimenopausal women showed that K1 intake was associated with reduced pyridinoline crosslink levels suggesting a decrease in bone resorption [52], whereas in elderly northern Europeans, plasma K1 levels negatively correlated with bone alkaline phosphatase (BALP), a marker of bone formation [53].

Poor evidence is available regarding the causal association between VK intake and supplementation and bone biomarkers but most of the interventional studies seem to confirm the inverse correlation between VK supplementation and ucOC [19,54–63]. Conversely, K1 and K2 do not seem to affect other bone biomarkers in healthy pre- and postmenopausal women [55,58,63].

Interestingly, postmenopausal women subjected to a 4 week depletion of K1 showed no effect in terms of bone biomarkers, but K1 repletion to recommended intake reduced serum NTX significantly, indicating decreased bone turnover [64].

A recent metanalysis on 19 RCTs, five of these mentioned above, conducted among a total of 6759 participants, has investigated the effect of K2 on ucOC and OC. A significant decrease of ucOC and an increase of OC have been reported in the VK2 groups compared to control [65].

A few observational studies, mainly conducted in Asian populations, have shown that VK1 and K2 intake and/or plasma levels may be associated with a reduction of ucOC. Several RCTs confirmed the inverse correlation between VK supplementation and ucOC. There is not enough evidence to draw definitive conclusions regarding the association between VK plasma levels and/or intake and other bone biomarkers in healthy (mainly pre- and postmenopausal women) and osteoporotic subjects, although the data available seem to suggest no correlation (Table 3).

4.3. *Vitamin K and BMD*

Several studies, both cross-sectional and RCTs, have investigated the effect of VK on BMD change.

In cross-sectional studies, plasma VK1 levels seem to strictly correlate with BMD. In particular, in the Framingham Heart Study, among 741 men and 863 women, low plasma VK1 was associated with low femoral neck BMD in men and low spine BMD in postmenopausal women [66].

Several studies evaluated the relation between VK intake, assessed by FFQ, and BMD. Most of the studies supported the hypothesis that adequate VK intake improves BMD. Indeed, women in the lowest quartile of K1 intake had a significantly lower mean BMD after adjustment for confounding factors, like age and menopausal status [52,67]. Furthermore, a cross sectional analysis carried out on elderly subjects showed that consistent VK intake was significantly associated with higher BMD and better quantitative ultrasound parameters [68]. In the Fujiwara-kyo Osteoporosis Risk in Men study, greater intake of *natto* was associated with higher total hip and femoral neck BMD [50]. Similarly, women enrolled in the Japanese Population based Osteoporosis Study showed a significant positive correlation between habitual *natto* intake and total hip and distal third of the radius BMD after 3 years even after adjusting for age, weight, lifestyle factors and calcium intake [69].

In few contrasting observational studies, no significant association between VK intake and BMD was found. These data were observed in adult men [67,70], in elderly men and women participating in the Framingham Heart Study [71], and in perimenopausal women participating in the Danish Osteoporosis Prevention Study [72].

Many authors have designed prospective studies aiming to evaluate the effect of VK supplementation on BMD change, and most have confirmed a positive correlation. In an RCT, healthy postmenopausal women received 200 mcg/day K1, 400 IU VD + 1000 mg calcium/day, combined K1 + D + calcium or placebo. After a 2 year follow-up, women who took K1 + D1 + calcium showed a significant and sustained increase in both

Table 3 – Cross sectional studies and randomized controlled trials on the relationship between vitamin K and bone turnover markers (BTM).

Cross sectional studies

Author, year	Country	n	Subjects	Results
Tsugawa N et al. 2006 [48]	Japan	396	Healthy women aged 30–88	Inverse relationship between K1/MK-7 and ucOC
Yamauchi M et al. 2010 [49]	Japan	221	Healthy pre and post-menopausal women	Inverse relationship between vitamin K intake and ucOC
Fujita Y et al. 2012 [50]	Japan	1662	Healthy men aged ≥65 years	Inverse relationship between intake of *natto* and ucOC
Shea MK et al. 2008 [51]	US	1381	Men (48%) and women (52%); mean age 59 years	Inverse relationship between K1 levels/intake and circulating inflammatory markers
Macdonald HM et al. 2008 [52]	UK	3000	Early postmenopausal women	Inverse relationship between K1 intake and pyridinoline crosslinks
Torbergsen AC et al. 2015 [53]	Norway	189	Hip fractured patients vs. controls	Inverse relationship between K1 levels and BALP

Randomized controlled trials

Author, year	Country	n	Subjects	Trial duration	Intervention	Co-interventions*	Results
Kruger MC et al. 2006 [54]	New Zealand	82	Premenopausal women aged 20–35	16 weeks	K1 (80 mcg/day) vs. placebo	Fortified skim milk (Ca 1000 mg/day)	Decreased ucOC, CTX, P1NP
Bügel S et al. 2007 [55]	Denmark	48	Postmenopausal women	6 weeks	K1 (200, 500 mcg/day) vs. placebo	Vitamin D3 10 mcg/day	Decreased ucOC, increased cOC, increased total OC with maximum dose supplementation. No differences in other BTM.
Bolton-Smith C et al. 2007 [56]	UK	209	Healthy postmenopausal women	2 years	K1 (200 mcg/day) and/or vitamin D (400 IU) plus calcium (1000 mg/day) vs. placebo		Decreased %ucOC and PTH
Kanellakis S et al. 2012 [19]	Greece	219	Postmenopausal women	1 year	K1 or K2 (100 mcg/day) vs. placebo	Fortified dairy products (vitamin D 10 mcg and calcium 800 mg)	Decreased %ucOC and urine deoxypyridinoline levels vs. placebo and vs. group without vitamin K addiction
Cheung AM et al. 2008 [63]	Canada	440	Postmenopausal women with osteopenia and normal levels of vitamin D	4 years	K1 (500 mcg/day) vs. placebo		Decreased ucOC and total OC levels, no differences in CTX
Koitaya N et al. 2013 [57]	Japan	50	Healthy postmenopausal women	1 year	MK-4 (1.5 mg/day) vs. placebo		Decreased ucOC
Binkley N et al. 2009 [58]	US	381	Postmenopausal women	1 year	MK-4 (45 mg/day), K1 (1 mg/day) vs. placebo	Calcium and Vitamin D	Decreased ucOC, no differences in BALP and NTX
Emaus N et al. 2010 [59]	Norway	334	Healthy early post-menopausal women	1 year	MK-7, in the form of *natto* capsules.		Decreased ucOC, increased cOC
Knapen MHJ et al. 2013 [60]	Netherlands	244	Healthy postmenopausal women	3 years	MK-7 (180 mcg/day) vs. placebo		Decreased ucOC, increased cOC
Martini LA et al. 2006 [64]	US	21	Postmenopausal women	84 days	K1 depletion and repletion up to 450 mcg/day		No effects of acute K1 depletion in terms of bone biomarkers, repletion reduced serum NTX
Yasui T et al. 2006 [61]	Japan	34	Postmenopausal women with osteopenia or osteoporosis	1 year	K2 (45 mg/day) or K2 and vitamin D3 (0.75 mcg/day)		Decreased ucOC in both groups, decreased OC and BALP in K2 plus vitamin D group
Miki T et al. 2003 [62]	japan	20	Elderly osteoporotic women with vertebral fractures	2 weeks	MK-4 (45 mg/day)	Calcium (600 mg/day)	Decreased ucOC, no change in OC

ucOC: undercarboxylated Osteocalcin; NTX: n-telopeptide of type 1 collagen; CTX: C-terminal telopeptide; BALP: Bone Alkaline Phosphatase; cOC: carboxylated Osteocalcin; P1NP: total Procollagen I Intact N-Terminal; PTH: Parathormone; MK-4: Menaquinone-4; MK-7: Menaquinone-7.

* Administered to all groups.

BMD and bone mineral content (BMC) at the ultradistal radius, with no other significant difference among groups [56]. Kanellakis et al. compared three groups of postmenopausal women taking fortified dairy products with calcium 800 mg and VD3 10 mcg alone (CaD) or in combination with K1 or MK7 100 mcg daily (CaDK1 or CaDMK-7). A significant increase in total body BMD was observed in all intervention groups compared to control, while a significant increase in lumbar spine BMD was observed only in CaDK1 and CaDMK-7 compared to the control group after adjusting for changes in serum VD levels and calcium intake [19].

In another similar RCT, postmenopausal women received fortified dairy products plus K1 (100 mcg), K2 (100 mcg) or placebo with one control group on a normal diet. After 12 months, the intervention groups showed a significant increase in total BMD compared to control with no significant differences in QUS parameters [73].

Knapen et al. studied healthy postmenopausal women who received either placebo or MK-7 (180 mcg/day) capsules for 3 years. During the first year, the rate of bone loss was similar in both groups, but after 3 years, MK-7 positively affected bone health as compared to placebo even after adjustment for age and BMI. Additional analysis showed no differences in terms of femoral neck width, hip-axis length and bending strength, but slight differences in terms of compression strength and a significant difference in age adjusted impact strength [60].

Another 2 year long RCT was conducted in postmenopausal women affected by osteopenia/osteoporosis comparing the effect of VD, MK-4, D + MK-4 and normal diet alone on BMD [74]. The MK-4 group showed an increased BMD starting at 18 months compared to control, whereas subjects receiving D3 + MK-4 showed an increased BMD at 6 months already up to 24 months [74].

A further RCT study conducted on healthy postmenopausal women supplemented with a daily formulation of placebo or calcium, magnesium, zinc, and VD (8 mcg/day), or the same formulation with additional K1 (1 mg/day) showed reduced bone loss of the femoral neck in subjects receiving the formulation with K1 at 3 years, with a difference compared to placebo of 1.7% (95% Cl 0.35–3.44) and compared to VD of 1.3% (95% Cl 0.10–3.41). No significant differences were observed regarding spine BMD [75].

If most of the available literature supports a positive effect of VK on BMD, few studies have shown no significant correlation. However, these studies tested significantly different supplementation dosages and cointerventions and/or had shorter follow-ups, aspects that could potentially explain such contrasting data.

Supplementation of K1 for 3 years was investigated in an RCT conducted among men and postmenopausal women who received calcium (600 mg) and VD (400 UI) daily. At three years, no differences regarding BMD at any site were found among subjects who received K1, calcium and VD compared to control [76], showing analogous results to the ECKO trial, where postmenopausal women with osteopenia and normal levels of VD taking K1 5 mg or placebo daily had no significant BMD difference after 4 years [63]. Another similar study conducted on postmenopausal women receiving calcium and VD3 plus K1 (1 mg daily), MK-4 (45 mg daily), or placebo for 12 months showed no significant difference in lumbar or hip BMD/geometric parameters [58].

A recent randomized, double blind, placebo controlled trial conducted on postmenopausal women investigating the effects of MK-4 1.5 mg daily on various BTMs and BMD showed no significant decrease in BMD in the MK-4 group [57] after one year essentially confirming the results of Emaus et al. who showed no significant effect of *natto* on BMD in healthy postmenopausal Norwegian women (50–60 years) [59].

A systematic review and a metaanalysis of 16 RCTs (of which five mentioned above) examined the overall treatment effect of K1 and K2 on BMD using the weighted mean difference. In particular, Fang et al. examined the absolute changes from baseline lumbar spine and femoral neck BMD for each VK group and control group. Lumbar spine seemed to be more sensitive to the positive effect of VK supplementation (absolute change was 21.60 mg/cm^2 [95% CI 3.63, 39.56]). However, at the subgroup analyses, this feature was confirmed only when the authors took into consideration Asian participants, women participants and K1 supplementation. Indeed, in western participants, in subjects supplemented with K2, in participants without secondary osteoporosis and in postmenopausal women, no BMD modification was recorded. Instead, femoral neck BMD was never found to be affected by treatment with VK [77].

Conversely, a recent metanalysis conducted on 19 trials with six of those already been mentioned above has suggested a positive role of K2 supplementation on BMD. Some studies included in the metanalysis have reported a significant lumbar spine BMD increase (mean difference 2.01, 95% CI 0.21 to 3.81, p 0.03) in subjects affected by osteoporosis; meanwhile, participants without osteoporosis demonstrated no significant lumbar spine BMD difference compared to control group. Only three RCTs have shown no significant hip BMD changes in non-osteoporotic participants [65].

In young and elderly women, low VK intake seems to be associated with a reduction of BMD. Few studies have evaluated this possible correlation in men and the results are contrasting. RCTs have also provided contrasting evidence. If most of the available literature seems to suggest that VK supplementation has a positive effect on BMD, the heterogeneity in terms of population, intervention, follow up and outcomes makes a definitive conclusion hard to be drawn. It appears that co-supplementation with calcium and VD provides better results in terms of BMD and that K1 may be more effective compared to MK- 4 and -7. Total BMD is also more commonly improved whereas there is contrasting evidence regarding lumbar and hip BMD (Table 4).

4.4. *Vitamin K and Bone Quality*

Only a few studies have addressed VK impact on bone quality. In particular, Matsumoto et al. have investigated bone tissue property changes by micro-computed tomography analysis after MK-4 supplementation in growing male Wistar rats, where increased trabecular volume fraction and thickness were found in the MK-4 treated group [78].

Table 4 – Cross sectional studies and randomized controlled trials on the relationship between vitamin K and bone mineral density (BMD).

Cross sectional studies

Author, year	Country	n	Subjects	Results
Booth SL et al. 2004 [66]	US	1604	Men and women aged 32–86	Direct relationship between K1 levels and femoral neck BMD in men and spine BMD in postmenopausal women not on estrogen replacement
Macdonald HM et al. 2008 [52]	UK	3000	Early postmenopausal women	Direct relationship between K1 intake and mean BMD after adjustment for confounding factors
Booth SL et al. 2003 [67]	US	2591	Men and women mean age 59	Direct relationship between K1 intake and mean BMD in women after adjustment for confounding factors, no significant association in men
Bulló M et al. 2011 [68]	Spain	365	Elderly subjects	Direct relationship between K intake and BMD/QUS parameters
Fujita Y et al. 2012 [50]	Japan	1662	Healthy men aged ≥65 years	Direct relationship between intake of *natto* and BMD at the total hip and femoral neck
Ikeda Y et al. 2006 [69]	Japan	944	Women aged 20–79	Direct relationship between intake of *natto* and total hip and distal third of radius BMD
Booth SL et al. 2000 [71]	US	888	Elderly subjects mean age 75	No significant association between K intake and BMD
Rejnmark L et al. 2006 [72]	Denmark	2016	Perimenopausal women	No significant association between K1 intake and BMD

Randomized controlled studies

Author, year	Country	n	Subjects	Trial duration	Intervention	Co-interventions *	Results
Bolton-Smith C et al. 2007 [56]	UK	209	Healthy postmenopausal women	2 years	K1 (200 mcg/day) and/or vitamin D (400 IU/day) plus calcium (1000 mg/day) vs. placebo.		Increased ultradistal radius BMD and BMC in K1 plus vitamin D plus calcium group
Kanellakis S et al. 2012 [19]	Greece	219	Postmenopausal women	1 year	K1 or K2 (100 mcg/day) vs. placebo	Fortified dairy products (vitamin D 10 mcg and calcium 800 mg)	Increased total BMD in all groups vs. placebo, increased lumbar spine BMD in CaDK1 and CaDMK-7 vs. placebo after adjusting for changes in serum vitamin D levels and dietary calcium intake

Moschonis G et al. 2011 [73]	Greece		Postmenopausal women	1 year	K1 (100 mcg/day) or K2 (100 mcg/day) vs. placebo. Fortified milk and yoghurt, one control group on normal diet.	Calcium (800 mg) and vitamin D (10 μg)	Increased total BMD vs. placebo. No difference in QUS parameters
Knapen MHJ et al. 2013 [60]	Netherlands	244	Healthy postmenopausal women	3 years	MK-7 (180 mcg/day) vs. placebo		Decreased bone loss at lumbar spine and femoral neck vs. placebo after adjusting for age and BMI. Increased impact strength vs. placebo after adjusting for age
Ushiroyama T et al. 2002 [74]	Japan	172	Postmenopausal women affected by osteopenia or osteoporosis	2 years	MK-4 (45 mg/day) and/or Vitamin D3 (1 mcg/day) vs. placebo		Increased %BMD change at 18 and 24 months in MK4 group vs. placebo, increased BMD at 6 months up to 24 months in MK-4 plus D3 group
Braam LAJLM et al. 2003 [75]	Netherlands	181	Healthy postmenopausal women aged 50–60	3 years	Calcium (500/day) mg, magnesium (150 mg/day), zinc (10 mg/day), vitamin D (8 mcg/day) with or without K1 (1 mg/day) vs. placebo		Decreased bone loss of the femoral neck vs. placebo [1.7% (95% CI: 0.35–3.44)], and vs. vitamin D group [1.3% (95% CI: 0.10–3.41)]. No difference in change of BMD at lumbar spine
Booth SL et al. 2008 [76]	US	452	Men and postmenopausal women aged 60–80	3 years	K1 (500 mcg/day) vs. placebo	Calcium (600 mg) and vitamin D (400 UI)	No difference in BMD at any site
Cheung AM et al. 2008 [63]	Canada	440	Postmenopausal women with osteopenia and normal levels of vitamin D	4 years	K1 (5 mg/day) vs. placebo		No difference in BMD
Binkley N et al. 2009 [58]	US	381	Postmenopausal women	1 year	MK-4 (45 mg/day), K1 (1 mg/day) vs. placebo	Calcium and Vitamin D	No difference in lumbar spine or proximal femur BMD or proximal femur geometric parameters
Emaus N et al. 2010 [59]	Norway	334	Healthy early post-menopausal women	1 year	MK-7, in the form of *natto* capsules.		No difference in BMD
Koitaya N et al. 2013 [57]	Japan	50	Healthy postmenopausal women	1 year	MK-4 (1.5 mg/day) vs. placebo		No difference in BMD

QUS: Quantitative UltraSound; BMC: bone mineral content, CI: confidence interval; MK-4: Menaquinone-4; MK-7: Menaquinone-7; CaDK1: Group receiving Calcium, Vitamin D and vitamin K1; CaDMK-7: Group receiving Calcium, Vitamin D and Menaquinone 7.

* Administered to all groups.

Furthermore, in an RCT conducted on postmenopausal women, Knapen et al. have evaluated the effect of MK-4 45 mg/day on bone strength showing a significant increase in femoral neck width at 3 years with no differences found in terms of DXA-BMD [79].

In addition, the combined effect of VK and antiresorption drugs on mechanical strength and other parameters of bone quality were assessed using the ovariectomized rat osteoporosis model. Contrasting results were provided: Otomo et al. did not observe any effects of K2 supplementation plus Risedronate on bone quality [80], while Matsumoto and coll. have shown that prior MK-4 treatment enhanced the positive effects of risedronate on femur strength [81]. Moreover, the group receiving Alendronate plus K2 supplementation reported better results in biomechanical tests such as the 3-point bending and the compression tests compared to control [82].

A recent study has also suggested that VK supplementation enhanced BMD and bone strength improvements induced by teriparatide treatment [83].

4.5. *Vitamin K and Bone Fractures*

Low plasma VK concentration was associated with increased risk of fracture in different populations. These data were observed in both elderly northern Europeans [53,84] and elderly Asians [85] of both genders, but also in the Japanese general population as observed in a national survey on the incidence of hip fractures [86]. This inverse association was also observed in healthy Asian women aged 30–88 years [87]. Other three large observational studies have confirmed these previous findings showing that subjects in the lowest quintiles or quartiles of VK intake assessed by FFQ had an increased relative risk of hip fractures [71,88,89].

Some contrasting studies support the lack of effect of VK intake on bone fracture risk. In particular, in a cross sectional study conducted on an elderly Asian population (1605 men and 1339 women over 65 years old), VK intake was not associated with fracture risk in both genders after almost 7 years of follow-up, even after adjustment for confounding factors [90].

Furthermore, in the DOPS study conducted on perimenopausal women over 10 years, a logistic regression analysis showed no differences regarding VK intake between patients who sustained a fracture and controls [72].

Moreover, Kawana et al. have measured circulating levels of K1, MK-4 and MK-7 in patients with vertebral and hip fractures and no significant differences were observed, although most of the patients had undetectable levels VK1, MK-4 and MK-7 potentially affecting the results [91].

Finally, no association was found between MK-4 and MK-7 and vertebral fractures in healthy Asian women [87] and between K2 intake and vertebral fractures in the Hordaland Health Study [89].

Few prospective studies have been conducted to evaluate the potential effect of VK supplementation on fracture risk. In the previously described ECKO Trial, fewer women in the VK group suffered clinical fractures compared to control (n 9 vs. 20, p 0.04, HR 0.41, 95% CI 0.15–1.18, p 0.08 at 2 years and HR 0.45, 95% CI 0.20–0.98, p 0.04 at 4 years) [63].

Knapen et al. investigated the ability of MK-7 (180 mcg/day) to prevent vertebral fractures, estimated by vertebral fracture assessment performed by DXA, in healthy postmenopausal women for 3 years compared to placebo. The height loss of the vertebrae was significantly lower in the MK-7 group than in the placebo group after 2 and 3 years [60].

In contrast with the study above, the effect of K2 in addition to Risedronate was investigated in an RCT on 101 elderly women with postmenopausal osteoporosis showing no significant difference in terms of vertebral fracture incidence [92].

Finally, a metanalysis conducted by Cockayne and coll. on different trials than those mentioned above assessed the effect of oral VK supplementation on BMD and fracture risk. The authors included data from 7 trials investigating the relation between fractures and VK. K2 supplementation appeared to reduce hip, vertebral and nonvertebral fracture risk (hip OR 0.23; 95% CI, 0.12–0.47; vertebral OR 0.40; 95% CI, 0.25–0.65; non vertebral sites OR 0.19; 95% CI, 0.11–0.35). However, the finding of a positive effect on fracture risk was prevalently due to a study on a Japanese population treated with menaquinone [93]. Nonetheless, Huang and coll. [65] have confirmed the positive effect of K2 on fracture risk (RR 0.47, 95% CI 0.32–0.70, p 0.0002, I2 0%) even after rejecting that study [94] inducing heterogeneity.

Low concentrations of VK seem to be associated with an increased risk of fracture in different populations, but contrasting findings were obtained from several observational studies. A few RCTs investigated the effects of K1 and K2 on fracture risk showing a potential positive effect. It appears that healthy subjects benefit from VK supplementations whereas the positive effect is somewhat lost or masked in patients at high risk of fracture. However due to the poor evidence available, further studies are indeed required to confirm such observation (Table 5).

5. Vitamin K Antagonists and Bone Health

VK antagonists (VKAs) might negatively affect the risk of fractures through different mechanisms, primarily by inhibiting the carboxylation reaction in OC [95].

Long term VKAs therapy on animal models impaired cortical bone structure leading to reduced hip strength, probably due to a reduction of OC [96].

In subjects with atrial fibrillation and high risk atherosclerosis, warfarin therapy was associated with increased levels of RANKL [97]. In men with atrial fibrillation, but not in women, a positive association between osteoporotic fractures and long term warfarin use was found (OR 1.63; 95% CI 1.26–2.10) [98].

In a population based retrospective cohort study among subjects at their first lifetime venous thromboembolism event, it was shown that oral VKAs treatment was associated with an increased risk of vertebral and rib fractures [99].

However, the real impact of VKAs on bone health is still controversial. Indeed, a large prospective observational study has shown no association between warfarin treatment and fracture risk in elderly postmenopausal women [100].

Table 5 – Cross sectional studies and randomized controlled trials on the relationship between vitamin K and bone fractures.

Cross sectional studies

Author, year	Country	n	Subjects	Results
Torbergsen AC et al. 2015 [53]	Norway	189	Hip fractured patients vs. controls	Low concentration of vitamin K1 was associated with increased risk of fracture
Nakano T et al. 2011 [85]	Japan	147	Hip fractured patients vs. controls	Low concentration of vitamin K1 was associated with increased risk of fracture
Yaegashi Y et al. 2008 [86]	Japan	118,500	Hip fractured patients	Low concentration of vitamin K1 was associated with increased risk of fracture
Tsugawa N et al. 2008 [87]	Japan	379	Healthy Asian women aged 30–88 years	Low concentration of vitamin K1 was associated with increased risk of fracture
Feskanich D et al. 1999 [88]	US	72,327	Women aged 38–63 years	Quintiles from 2nd to 5th of vitamin K intake had a significantly lower age-adjusted relative risk (RR: 0.70; 95% CI: 0.53, 0.93) of hip fracture compared with women in the lowest quintile
Booth SL et al. 2000 [71]	US	888	Elderly subjects mean age 75	Highest quartile of vitamin K intake had a significantly lower relative risk (0.35; 95% CI: 0. 13, 0.94) of hip fracture than lowest quartile, after adjustment for confounding factors
Apalset EM et al. 2011 [89]	Norway	2807	Men and women (71–75 years)	Patients in the lowest quartile of vitamin K had an increased hip fracture risk (HR = 1.57 [95% CI 1.09, 2.26]) compared to the highest quartile
Chan R et al. 2012 [90]	China	2940	Elderly over 65 years	No association between vitamin K intake and fracture risk in both genders
Rejnmark L et al. 2006 [72]	Denmark	2016	Perimenopausal women	No different vitamin K intake in fractured patients vs. controls after logistic regression analysis
Finnes TE et al. 2015 [84]	Norway	21,774	Men and women aged 65–79	Inverse relationship between vitamin K1 and D serum levels and risk of hip fractures
Kawana K et al. 2001 [91]	Japan	74	Elderly women aged 52–93 with hip or vertebral fractures vs. control	No difference in K1, MK-4 and MK-7 levels vs. controls
Tsugawa N et al. 2008 [87]	Japan	379	Healthy Asian women aged 30–88 years	No association between MK-4 and MK-7 and vertebral fractures
Apalset EM et al. 2011 [89]	Norway	2807	Men and women (71–75 years)	No association between K2 intake and vertebral fractures

Randomized controlled trials

Author, year	Country	n	Subjects	Trial duration	Intervention	Co-interventions*	Results
Cheung AM et al. 2008 [63]	Canada	440	Postmenopausal women with osteopenia and normal levels of vitamin D	4 years	K1 (500 mcg/day) vs. placebo		Fewer women in the vitamin K group had clinical fractures compared to control group
Knapen MHJ et al. 2013 [60]	Netherlands	244	Healthy postmenopausal women	3 years	MK-7 (180 mcg/day) vs. placebo		The height loss of the vertebrae was significantly lower in the MK-7 group vs. placebo
Kasukawa Y et al. 2014 [92]	Japan	101	Women with postmenopausal osteoporosis aged >60 years	1 year	Vitamin K2 (45 mg/day)	Risedronate (17.5 mg/week)	No significant difference in terms of vertebral fracture incidence

RR: relative risk, CI: confidence interval; HR: hazard ratio; MK-4: Menaquinone-4; MK-7: Menaquinone-7.
* Administered to all groups.

6. Conclusion and Major Limitations

In young and elderly women, low VK intake seems to be associated with bone deterioration, suggesting a potential positive effect of VK on bone health. Nonetheless, routine VK supplementation is not globally recommended yet in postmenopausal women affected by osteoporosis, as low quality cross sectional and RCTs have provided contrasting evidence.

In fact, most of the studies that have analyzed the interaction between bone health and VK are characterized by several limitations and the findings should be therefore addressed with caution.

These are some of the major limitations:

- Most of the studies had a small sample size to investigate the impact of VK on bone fractures.
- Most of the studies were undertaken in Asia and only a few were carried out in Caucasian populations.
- Clinical populations were often heterogeneous and sometimes there was no differentiation between pre and postmenopausal women.
- Type and dosage of VK supplements administered were variable.
- VK intake was often self-reported by the patient and the results of the trials sometimes did not take into consideration the lifestyle of the subjects (for instance, the primary dietary sources of phylloquinone such as green vegetables and vegetable oils are characteristic of a healthy lifestyle).
- Bone fractures were often self-reported by the patient.
- Only a few studies evaluated the interplay between VK and bone health in men.
- Only a few trials reported exactly how the randomization process was conducted.
- We did not have access to all studies included in the three meta-analysis cited, making their quality hard to assess and interpretation more challenging.
- Only a few studies reported baseline VD and calcium status.
- The estimation of VK status is unclear, and the great part of the studies here presented is based on VK plasma levels.
- Nutrition and bone health related cross sectional and observational studies do not prove causality between a nutrient deficiency or excess and osteoporosis.
- The interpretation of bone/nutrition literature is challenging due to the numerosity of the potential outcomes such as BMD, BTMs and fracture risk and the confusion about the optimal nutritional marker to measure.

In conclusion, larger and longer pragmatic studies are needed to further investigate the real impact of VK on fracture risk and clarify the contrasting data available. As the emerging consensus in nutritional research suggests, a more comprehensive approach while studying the effect of nutrients on health is required. Analyzing the effect of whole foods or food patterns and thus taking into account the synergy of nutrients will most likely allow to overcome the current challenges and may provide stronger evidence.

Author Contributions

A.P., D.T., L.D., M.W. conceived and designed the review; A.P., D.T., L.D., M.W., D.M., A.M., V.G., R.B., N.N., P.P. and S.M. wrote the paper.

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Conflicts of Interest

None.

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