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## **Vitamin K<sub>2</sub> (menaquinone) supplementation and its benefits in cardiovascular disease, osteoporosis, and cancer**

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## Abstract

Vitamin K is known to play an essential role in the coagulation cascade; however, a growing body of research has found that a subtype of this vitamin, vitamin K<sub>2</sub> (menaquinone), may have a beneficial effect in osteoporosis, cardiovascular disease, and cancer. The purpose of this article is to provide a comprehensive review of recent literature regarding menaquinone and its role in human health. This review discusses the physiology of menaquinone, its clinical benefits in cardiovascular disease, osteoporosis, and cancer, and how it may interact with certain medications. The authors conclude that menaquinone supplementation has been shown to improve carboxylation of osteocalcin and matrix-Gla protein to their active forms, two proteins that possess important roles in calcium distribution. In the setting of cardiovascular disease, menaquinone intake has been shown to lower the risk of coronary calcification and coronary heart disease, and a randomized controlled trial has demonstrated that it can reduce arterial stiffness. In osteoporosis, menaquinone has been shown in numerous randomized controlled trials to decrease the rate of bone loss at the lumbar spine and forearm and reduce the risk of fracture. In cancer, menaquinone intake has been shown to reduce overall incidence and mortality; clinical trials have suggested that it may have a role in reducing recurrence and death from hepatocellular carcinoma. However, in all clinical settings, more large randomized controlled trials are needed to definitively determine the clinical benefits of menaquinone supplementation, as many studies have failed to show any significant benefit. Lastly, more research is needed to determine how menaquinone supplementation interacts with medications such as warfarin, bile-acid sequestrants, orlistat, mineral oil and CYP3A4 substrates.

## Keywords

vitamin K<sub>2</sub>, menaquinone, MK-7, MK-4, cardiovascular, coronary heart disease, bone, osteoporosis, cancer

## Introduction

Vitamin K is known to play an essential role in the coagulation cascade; however, a growing body of research has found that it may also have an effect on osteoporosis, cardiovascular disease, and cancer. Many may be unaware that there are actually different forms of vitamin K, and that these forms possess distinct functions. The form primarily involved with the production of clotting factors is K<sub>1</sub> (phylloquinone). The effects of K<sub>2</sub> (menaquinone) have recently become the subject of discussion, as literature has suggested that menaquinone may play a role in the treatment of cardiovascular disease, osteoporosis, and cancer.<sup>1-3</sup> Menaquinone activates proteins such as osteocalcin (OC) and matrix-Gla protein (MGP), which are important regulators of calcium distribution. Through these interactions, menaquinone can assist in the inhibition of vascular calcification and the deposition of calcium into bone.<sup>4</sup> This review discusses the physiology of menaquinone, its clinical benefits in cardiovascular disease, osteoporosis, and cancer, and how it may interact with certain medications.

## Methods

NCBI's PubMed database was utilized to search for literature pertaining to the clinical benefits of menaquinone supplementation. Combinations of the following search terms were used:

vitamin K<sup>2</sup>, K<sup>2</sup>, menaquinone, menatetrenone, MK-4, MK-7, osteoporosis, fracture, bone mineral density, cardiovascular, calcification, coronary artery disease, cancer, hepatocellular carcinoma, warfarin, anticoagulation, pregnane X receptor. Primary and secondary sources were screened for relevance by title and the contents of the abstract. Potential sources were then downloaded and their contents were scrutinized for relevance. Priority was given to level 1 and 2 randomized controlled trials (RCT) and meta-analyses; however, observational studies were also included in the discussion.

## Menaquinone Supplementation

Vitamin K is a family of seven structurally similar compounds, but only phylloquinone and menaquinone have established roles in human physiology. Phylloquinone serves as an essential cofactor for liver enzymes that carboxylate glutamic acid residues on clotting factors II, VII, IX, X, protein C, and protein S.<sup>5</sup> Menaquinone also serves as a cofactor for carboxylase enzymes; however, the targets of these enzymes differ from phylloquinone (Figure 1). One of these targets is the bone matrix protein, OC, which is activated by the carboxylation of three glutamic acid residues and plays a critical role in the binding and deposition of hydroxyapatite mineral in bone.<sup>6</sup> Another target is MGP found in vascular smooth muscle cells; when MGP is carboxylated, it inhibits vascular calcification.<sup>7</sup>

For adults, the recommended dietary intake of phylloquinone is between 50 and 120 µg/day.<sup>8</sup> This can easily be obtained through consumption of leafy green vegetables like kale, collards, and spinach; thus, phylloquinone deficiency is rare.<sup>9</sup> However, sources of phylloquinone do not contain significant quantities of menaquinone. Dietary menaquinone is found primarily in whole milk, other dairy products, egg yolk, chicken, pork, beef, and natto (a Japanese food made from fermented soybeans).<sup>7</sup> The menaquinone in dairy products and natto is synthesized by fermenting bacteria such as *Bacillus subtilis*; in meats, K<sub>1</sub> and K<sub>3</sub> are converted to K<sub>2</sub> by living tissue.<sup>10</sup>

A number of menaquinone subtypes exist; they differ by the number of prenyl units contained on the isoprenoid side chain.<sup>7</sup> Bacteria produce menaquinone of varying side chain lengths, the most notable being the MK-7 subtype.<sup>11</sup> Very high levels of MK-7, as much as 939 µg/100 g are found in natto.<sup>7,12</sup> MK-4 is another important subtype that is produced in animal tissue and can be acquired by consuming meat and dairy products; however, the levels are much lower than those of MK-7 in natto.<sup>7</sup> In the United States, the amount of MK-4 found in meat ranges from 0.2-31.6 µg/100 g, highest being found in chicken; in dairy products it ranges from 0.2-10.2 µg/100 g and is highest in hard cheese.<sup>13</sup> Both MK-7 and MK-4 are available for supplementation; however, the half-life and bioavailability of MK-7 has been found to be superior to that of MK-4.<sup>14,15</sup>

Several studies have examined the effect of MK-7 supplementation on the carboxylation of OC and MGP; this research has found a dose-dependent relationship between the quantity of MK-7 and the carboxylation of OC and MGP.<sup>15-20</sup> The daily amount of MK-7 required to influence carboxylation of OC and MGP has been found to vary from 45 µg to 360 µg.<sup>15-20</sup> This effect is believed to have clinical significance due to the role that these proteins play in bone and the cardiovascular system. High levels of dephospho-uncarboxylated MGP (du-MGP) and

uncarboxylated OC (uOC) have been associated with arterial stiffness and an increased risk of fractures.<sup>21-23</sup>

## Clinical Impact on Cardiovascular Disease

The benefits of menaquinone to the cardiovascular system have been evaluated by several studies (Table 1). Level 2 cross sectional studies from the Netherlands have found that increased menaquinone intake is associated with decreased coronary calcification and mortality from coronary heart disease.<sup>24-27</sup> Beulens et al. administered a food frequency questionnaire to 564 Dutch postmenopausal women and found a decreased relative risk of coronary calcification (RR = 0.80, 95% CI: 0.65-0.98) with increased menaquinone intake but not phylloquinone.<sup>24</sup> Geleijnse et al. and Gast et al. also studied the results of a food questionnaire taken by 4807 and 16,057 postmenopausal Dutch women respectively; these studies found that high menaquinone intake was associated with a lower relative risk for coronary heart disease mortality (RR = 0.43, 95% CI: 0.24-0.77) and a lower hazard ratio for having coronary heart disease (HR = 0.91, 95% CI: 0.85-1.00).<sup>25, 26</sup>

A level 2 RCT studied the effect of menaquinone supplementation (MK-7, 180 µg/day) on arterial stiffness in 244 healthy postmenopausal Dutch women over a three year period.<sup>28</sup> The investigators monitored carotid and aortic stiffness, du-MGP levels, inflammatory markers (interleukin-6, C-reactive protein, tumor necrosis factor- $\alpha$ ), and markers of endothelial dysfunction (vascular cell adhesion molecule, E-selectin, advanced glycation endproducts). Menaquinone supplementation was found to significantly decrease the amount of du-MGP by approximately 50%; there was no significant effect on inflammatory or endothelial markers.<sup>28</sup>

Additionally, after three years, the menaquinone treatment group demonstrated a significant overall decrease in Stiffness Index  $\beta$ , a measure of the elastic properties of the arterial wall.<sup>28</sup> Treatment group patients were further divided into two groups based on whether they had a high or low baseline Stiffness Index  $\beta$  (a high Stiffness Index  $\beta$  indicates poorer vascular characteristics). When data was adjusted for confounding variables such as age, BMI, cholesterol lowering medication, and anti-hypertensive medication, menaquinone supplementation was found to significantly decrease the Stiffness Index  $\beta$  of patients with high baseline Stiffness Index  $\beta$  but not those with low baseline Stiffness Index  $\beta$ . This suggests that menaquinone was more beneficial to patients with poorer vascular status. Furthermore, variables such as vessel distention, compliance, distensibility, Young's modulus, and carotid pulse wave velocity demonstrated significant improvement from menaquinone supplementation only in patients with high baseline Stiffness Index  $\beta$ . There was a significant overall improvement in aortic pulse wave velocity, a measure of regional arterial stiffness; however, this effect was not significantly demonstrated in either the high or low Stiffness Index  $\beta$  groups independently.<sup>28</sup>

This study was limited by a small sample size.<sup>28</sup> The original power analysis was performed to measure bone strength, which was the primary outcome measurement of the clinical trial (this work is published separately); this resulted in a power of only 65% for detecting a significant change in aortic pulse wave velocity.<sup>28, 29</sup> Additionally, it should be noted that this clinical trial was funded by NattoPharma<sup>®</sup> (Høvik, Norway), a major producer of commercially available MK-7 supplements, and is therefore subject to funding bias. Despite these limitations, the study

provides evidence that menaquinone supplementation can reduce arterial stiffness, and that this effect seems to be more pronounced in patients with poorer baseline vascular characteristics. More RCTs are needed to both confirm the effect of menaquinone on arterial stiffness, and determine whether this effect significantly reduces the incidence and mortality of cardiovascular disease.

### **Clinical Impact on Osteoporosis**

A number of level 1 RCTs have been performed to study the effects of menaquinone on osteoporosis (Table 2).<sup>16, 29-34</sup> A trial performed by Knapen et al., involving the same 244 patients from their study of menaquinone and arterial stiffness, found that menaquinone supplementation (MK-7, 180 µg/day) decreased the rate of bone loss at the lumbar spine and femoral neck but not at the total hip.<sup>28, 29</sup> Although both the treatment and control groups experienced bone loss over the three year trial, the treatment group exhibited significantly less bone loss in lumbar spine after one year of supplementation. However, the decrease in the rate of bone loss in the femoral neck only became significant in the third year of daily supplementation. Additionally, they found that menaquinone supplementation significantly reduced the level of uOC and increased carboxylated OC (cOC).<sup>29</sup>

Another level 1 RCT by Emaus et al. involved 334 healthy postmenopausal women who were supplemented with MK-7 (360 µg/day) for one year.<sup>16</sup> The investigators measured bone mineral density (BMD) at the total hip, femoral neck, lumbar spine, and total body; they also measured serum levels of cOC and uOC. At 12 months, there was no significant difference in the rate of bone loss between the treatment and control group at any site; however, the treatment group displayed significantly higher serum levels of cOC and reduced levels of uOC.<sup>16</sup>

A level 2 meta-analysis by Huang et al. evaluated the clinical impact of menaquinone supplementation on osteoporosis from 19 level 1 and 2 RCTs (6,759 patients).<sup>35</sup> The combined results of 4 trials involving 190 postmenopausal women with osteoporosis revealed a significant decrease in the loss of vertebral BMD after 6 months of MK-4 supplementation (45 mg/day). The results of two studies involving 144 postmenopausal women without osteoporosis demonstrated no significant benefit to vertebral BMD after 6 months of daily MK-4 (45 mg/day). When looking at forearm BMD, the combined results of three studies demonstrated a significant benefit to postmenopausal women with osteoporosis; however, there was no significant benefit in women without osteoporosis. There was also no significant benefit to hip BMD when combining the results of 6 studies; however, these studies only included women without osteoporosis.<sup>35</sup>

Huang et al. also observed a significant overall increase in cOC and decrease in uOC at 6 and 12 months with menaquinone supplementation in women with and without osteoporosis.<sup>35</sup> There was no significant difference in the rate of adverse reactions with menaquinone supplementation, and the most commonly reported adverse reactions were nausea or abdominal pain. Additionally, the investigators pooled the results of 6 trials that evaluated the risk of osteoporotic fractures; they found a significant reduction in the incidence of fractures (RR = 0.50, 95% CI: 0.33-0.74) with menaquinone supplementation.<sup>35</sup>

Many of the trials included in this meta-analysis were limited by small sample sizes (<50 treatment group patients) and suffered from selection and reporting bias. Additionally, 11 of the 19 trials exclusively involved Japanese patients, and 15 trials involved supplementation with MK-4, which has been found to have an inferior half-life and bioavailability compared to MK-7.<sup>14, 15, 35</sup>

Menaquinone supplementation does appear to improve the carboxylation of OC in both postmenopausal women with and without osteoporosis; however the clinical benefit of this effect has not been strongly observed in postmenopausal women without osteoporosis. The current literature indicates that menaquinone supplementation can decrease the rate of bone loss in the lumbar spine and forearm and reduce the risk of fracture in postmenopausal women with osteoporosis. There is considerable heterogeneity in the literature with regards to the subtype (MK-4 vs. MK-7) and amount of menaquinone being administered. More large double-blinded RCTs are needed to definitively understand the clinical impact of menaquinone supplementation, particularly the MK-7 subtype, in postmenopausal women with and without osteoporosis.

### **Clinical Impact on Cancer**

In vitro studies have demonstrated that menaquinone possesses anti-cancer properties by inducing cell cycle arrest and apoptosis in a number of cell types including leukemia, liver, gastric, colorectal, lung, and prostate cancer.<sup>36-44</sup> Level 2 observational studies indicate that menaquinone intake may reduce the incidence and mortality of cancer (Table 3).<sup>45, 46</sup> Nimptsch et al. followed a cohort of 24,340 European adults over a period of ten to fourteen years; participants were asked to fill out food frequency questionnaires to assess their menaquinone intake.<sup>46</sup> The study revealed an inverse relationship between menaquinone intake and overall cancer incidence (HR = 0.86, 95% CI: 0.73-1.01), although this trend was non-significant (p = 0.08). There was a significant inverse relationship between menaquinone intake and overall cancer mortality (HR = 0.72, 95% CI: 0.53-0.98, p = 0.03). When broken down by cancer type, menaquinone intake was associated with a significantly lower incidence and mortality of lung cancer. Menaquinone intake was also associated with a significantly lower incidence of prostate cancer and overall incidence of cancer in men.<sup>46</sup>

The use of menaquinone in the treatment of hepatocellular carcinoma (HCC) has been investigated by a number of level 2 RCTs.<sup>47-55</sup> Two small trials examined the ability of menaquinone (MK-4, 45 mg/day) to suppress the development of HCC in patients with hepatitis C.<sup>54, 55</sup> Both studies observed a lower proportion of patients develop HCC with menaquinone treatment; however, this effect was statistically significant in only one study.<sup>54, 55</sup> Several RCTs have evaluated the effect of menaquinone (MK-4, 45 mg/day) in reducing recurrence and mortality after surgical or ablative treatment of HCC.<sup>47-52</sup> These studies have reported conflicting results; some found a significant decrease in recurrence and mortality while others found no effect.<sup>47-52</sup> The majority of these studies were limited by small sample sizes (<100 patients) and relatively short follow-up time (1-3 years).

The largest RCT involved 548 former HCC patients who were randomized to receive placebo, 45 mg/day MK-4, or 90 mg/day MK-4; menaquinone treatment was not found to reduce disease occurrence or death (HR = 1.150, 95% CI: 0.843-1.570, p = 0.811), and the study was

discontinued after three years (Table 3).<sup>47</sup> A level 2 meta-analysis of 5 RCTs observed significantly reduced HCC recurrence at two and three years, but not one year; there was no benefit to survival.<sup>56</sup> Another level 2 meta-analysis of 6 RCTs and one cohort study also found no significant reduction in recurrence at one year, but a significant reduction at two and three years; this study found a significant improvement in overall survival.<sup>57</sup> However, due to the small sample sizes and relatively short follow-up times of the available clinical trials, more research is needed to definitively understand the benefit of menaquinone in the prevention and treatment of HCC.

## Menaquinone Drug Interactions

Perhaps the most well-known drug interaction associated with vitamin K is the coumarin derivative anticoagulants, namely warfarin. Although this interaction is classically described with phylloquinone, recent evidence indicates that menaquinone can have a similar, if not more potent reversal of coumarin-induced anticoagulation. Studies by Theuwissen et al. and Schurgers et al. examined the effects MK-7 on the stability of oral anticoagulation with vitamin K antagonists (coumarin derivatives) and found that MK-7 not only reverses anticoagulation, but is even more effective than phylloquinone in reversing undercarboxylation of both hepatic (blood coagulation factors) and extra-hepatic proteins (OC and MGP).<sup>58, 59</sup> Thus, clinicians should be aware that even low dose menaquinone supplementation (50 µg/day) could impact coumarin-induced anticoagulation.<sup>58</sup>

Recently, menaquinone has been demonstrated to be a ligand for the pregnane X receptor (PXR).<sup>60</sup> PXR is a nuclear receptor with both a ligand and DNA binding domain. Once bound, menaquinone forms a heterodimer with PXR, and this complex acts as a transcriptional regulator for certain CYP 450 isozymes. One of the major targets of the PXR-K<sub>2</sub> complex is the gene for CYP3A4.<sup>60</sup> This enzyme is responsible for metabolizing a wide variety of commonly used medications, and induction can lower plasma concentrations of CYP3A4 substrates.<sup>61</sup> Important CYP3A4 substrates include members of the HMG-CoA reductase family, certain anti-epileptic drugs and a wide variety of antimicrobials and antineoplastics.<sup>62</sup> Although more research is required to further elucidate the exact effect of menaquinone on the CYP3A4 enzyme, the results of these studies at least support that caution should be exercised when menaquinone is taken concurrently with known substrates.

Additionally, drugs such as bile-acid-sequestrants (cholestyramine, colestipol, colesevelam), orlistat, and mineral oil, that interfere with the absorption of fat soluble vitamins, may interfere with menaquinone supplementation by decreasing absorption of the vitamins.<sup>63-65</sup> Patients taking these medications should be instructed to take menaquinone at least an hour before or several hours after their other medications.

## Conclusion

Menaquinone supplementation has been shown to consistently improve carboxylation of OC and MGP to their active forms; however, the clinical benefit of this effect has not been uniformly demonstrated. In the setting of cardiovascular disease, menaquinone intake has been shown to lower the risk of coronary calcification and coronary heart disease, and an RCT has

demonstrated that it can reduce arterial stiffness. In osteoporosis, menaquinone has been shown by numerous RCTs to decrease the rate of bone loss at the lumbar spine and forearm and reduce the risk of fracture. In cancer, menaquinone intake has been shown to reduce overall incidence and mortality; RCTs have suggested that it may have a role in reducing recurrence and death from hepatocellular carcinoma. However, in all clinical settings, more large RCTs with ethnically diverse patient populations are needed to definitively determine the clinical benefits of menaquinone supplementation, as many studies have failed to show any significant clinical benefit. Lastly, more research is needed to determine how menaquinone supplementation interacts with medications such as warfarin, bile-acid sequestrants, orlistat, mineral oil and CYP3A4 substrates.

### Figures and Tables

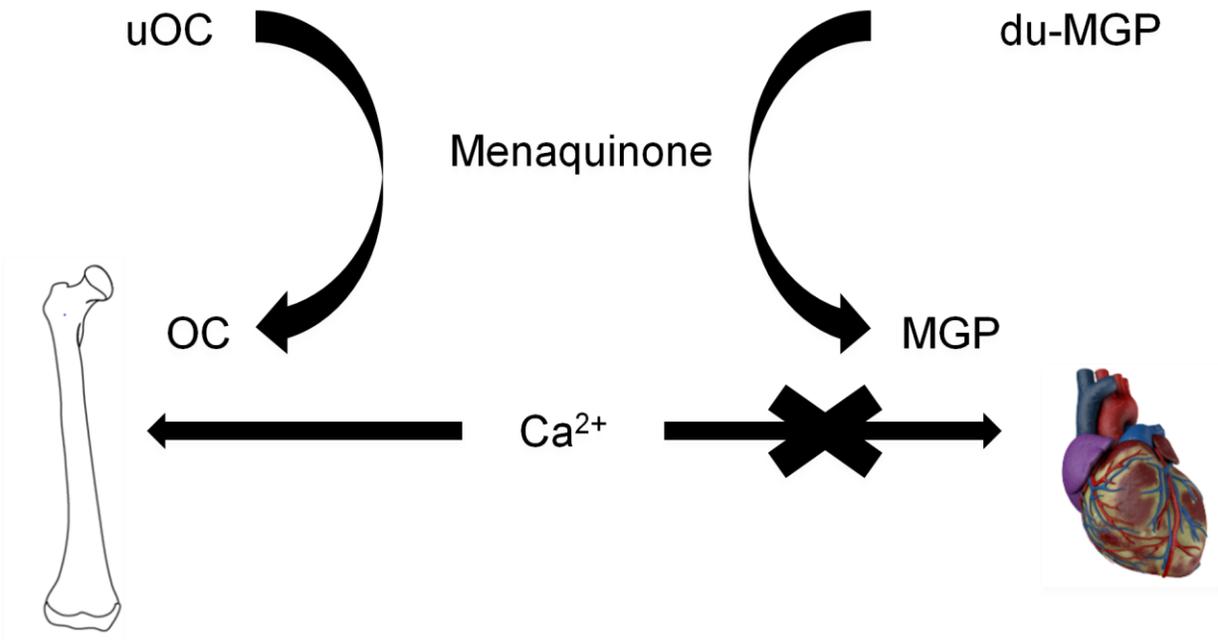


Figure 1. Mechanism of action for menaquinone in calcium regulation.

Table 1. Summary of studies evaluating the effect of menaquinone supplementation on cardiovascular disease. Therapeutic levels of evidence are listed as described by Wright et al.<sup>66</sup>

Study	Level of Evidence	Patient Population	Supplementation	Main Outcome Measurement	Results
Beulens et al. 2009 <sup>24</sup>	Level II - Cross sectional study	564 Post menopausal Dutch women	Dietary menaquinone intake estimated by food frequency questionnaire, average 31.6 ± 12.3 µg/day	Coronary calcification	Menaquinone intake associated with decreased coronary calcification (RR = 0.80, 95% CI: 0.65-0.98, p = 0.03)
Geleijnse et al. 2004 <sup>25</sup>	Level II – Cohort study	4807 men and women over age 55	Dietary menaquinone intake estimated by food frequency questionnaire, average 30.8 ± 18.0 µg/day (men) and 27.0 ± 15.1 µg/day (women)	Coronary heart disease	Relative risk for CHD mortality was lower in the mid and upper tertiles of menaquinone intake (mid: RR = 0.73, 95% CI: 0.45-1.17; upper: RR = 0.43, 95% CI: 0.24-0.77)
Gast et al. 2009 <sup>26</sup>	Level II – Cohort study	16,057 post-menopausal Dutch women	Dietary menaquinone intake estimated by food frequency questionnaire, average 29.1 ± 12.8 µg/day	Coronary heart disease	Menaquinone intake was associated with a decreased risk of CHD (HR = 0.91, 95% CI: 0.85-1.00)
Knapen et al. <sup>28</sup>	Level II – RCT	244 post-menopausal Dutch women	MK-7, 180 µg/day	Carotid and aortic stiffness, du-MGP	Menaquinone supplementation demonstrated a significant decrease in arterial wall stiffness after three years and a 50% decrease in du-MGP

Table 2. Summary of studies evaluating the effect of menaquinone supplementation on osteoporosis.

Study	Level of Evidence	Patient Population	Supplementation	Main Outcome Measurement	Results
Emaus et al. 2010 <sup>16</sup>	Level I – RCT	334 healthy postmenopausal women	MK-7, 360 µg/day	BMD, cOC, and uOC	No significant difference in BMD between treatment and control group at 12 months (p = 0.48). Treatment group had significantly higher cOC and lower uOC (p < 0.001).
Knapen et al. 2013 <sup>29</sup>	Level I – RCT	244 healthy postmenopausal Dutch women	MK-7, 180 µg/day	BMD, cOC, uOC	Daily menaquinone supplementation significantly decreased bone loss at lumbar spine and femoral neck after 3 years (p = 0.014). Treatment group had significantly higher cOC and lower uOC (p < 0.001).
Huang et al. 2015 <sup>35</sup>	Level II – Meta-analysis	19 RCTs with 6759 participants	MK-4, 45 µg/day (13 studies); MK-7, 100-360 µg/day (4 studies)	BMD, cOC, uOC, fractures	Significant decrease in vertebral bone loss in women with osteoporosis (p = 0.0005). Significant overall increase in cOC and decrease in uOC (p = 0.001). Significant reduction in

					fracture incidence (RR = 0.50, 95% CI: 0.33-0.74)
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Table 3. Summary of studies evaluating the effect of menaquinone supplementation on cancer.

Study	Level of Evidence	Patient Population	Supplementation	Main Outcome Measurement	Results
Nimptsch et al. 2008	Level II – Cohort study	24,340 European adults	Dietary menaquinone intake estimated by food frequency questionnaire	Cancer incidence and mortality	Significant inverse relationship between menaquinone intake and cancer mortality (HR = 0.72, 95% CI: 0.53-0.98, p = 0.03)
Yoshida et al. 2011	Level I – RCT	548 former HCC patients	MK-4, 45 or 90 µg/day	Disease recurrence, mortality	Menaquinone treatment was not found to decrease recurrence or mortality, study discontinued after three years
Riaz et al. 2012	Level II – Meta-analysis	Five RCTs with 754 former HCC patients	MK-4, 45-90 µg/day	Disease recurrence, survival	Reduced HCC recurrence at two and three years, but not one; no benefit to survival
Zhong et al. 2013	Level II – Meta-analysis	6 RCTs and 1 cohort study with 930 former HCC patients	MK-4, 45-90 µg/day	Disease recurrence, survival	Reduced HCC recurrence at two and three years, but not one; improved survival

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