

openheart The health benefits of vitamin K

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ABSTRACT

Vitamin K has important functions within the body, some of which are still being discovered. Research has shown that vitamin K is an anticalcification, anticancer, bone-forming and insulin-sensitising molecule. Recent data indicate that subclinical vitamin K deficiency is not uncommon. Additionally, vitamin K antagonists such as warfarin may cause detrimental side effects, which may partly be blunted through vitamin K supplementation.

INTRODUCTION

Vitamin K is a fat-soluble vitamin, important for the function of numerous proteins within the body, such as the coagulation factors (II, VII, IX, X and protein C and protein S), osteocalcin (a bone-forming protein) and matrix-Gla protein (MGP) (an anticalcification protein), to name a few.^{1–3} Vitamin K exists naturally as vitamin K₁ (phylloquinone) and vitamin K₂ (menaquinone, MK-4 through MK-10).^{2–5} Vitamin K₁ is mainly found in green leafy vegetables as well as olive oil and soyabean oil, whereas vitamin K₂ (menaquinone) is found in small amounts in chicken, butter, egg yolks, cheese and fermented soyabeans (better known as natto).^{2 6–9}

Vitamin K₁ and vitamin K₂ are required for the γ -glutamyl carboxylation of all vitamin K-dependent proteins.² Despite the fact that mammalian bacterial intestinal flora are able to produce vitamin K₂, the amount produced is thought to be negligible.² The adequate intake (AI) for vitamin K has been proposed to be 90 μ g/day for women and 120 μ g/day for men.^{2 10} However, it has been speculated that the AI for vitamin K (90–120 μ g/day) is not sufficient to induce complete carboxylation of all vitamin K-dependent proteins.^{2 11 12}

VITAMIN K DEFICIENCY

The measurement and treatment of vitamin K deficiency based on blood tests is not perfect. Plasma phylloquinone concentrations fluctuate based on recent dietary intakes.^{2 13} Despite the fact that a high

percentage of undercarboxylated osteocalcin indicates poor vitamin K status, this value can also vary based on recent vitamin K intake and supplementation^{2 14} and may not indicate chronic vitamin K status. Moreover, a normal carboxylated MGP protein in the serum may not necessarily indicate a normal vitamin K status, as carboxylated MGP in the serum could be normal, but suboptimal in the arteries (where vitamin K₂ is needed to prevent vascular calcification.)

VITAMIN K AND BONE HEALTH

Osteoporosis is a leading contributor of fractures worldwide, causing more than 8.9 million fractures annually.¹⁵ Moreover, Osteoporosis affects an estimated 200 million women worldwide (approximately 1/10th of women aged 60, 1/5th of women aged 70, 2/5ths of women aged 80, and 2/3rds of women aged 90).¹⁵ One in 3 women and 1 in 5 men over 50 will experience an osteoporotic fracture.¹⁵ Additionally, 61% of all osteoporotic fractures occur in women.¹⁵ It has been predicted that the incidence of hip fracture is expected to increase by 310% in men and 240% in women by 2050; thus, the economic toll of osteoporosis is expected to significantly increase.¹⁵ Indeed, it has been estimated that there is a 40% lifetime risk for fractures affecting the hip, forearm and vertebrae (similar to the risk for cardiovascular disease),¹⁵ with nearly 75% of these types of fractures occurring in patients aged 65 years age and above.^{15 16} Osteoporosis has been shown to account for more days spent in the hospital than diabetes, heart attacks or breast cancer.¹⁵ It is also a major cause of disability, which has been shown to be greater than that caused by cancer (except lung cancer) and comparable to or greater than disability from rheumatoid arthritis, asthma and high blood pressure related heart disease.¹⁵ The overall mortality within the first 12 months after a hip fracture is approximately 20%, being higher in men than women.¹⁵ Moreover, men make up 20–25% of all hip fractures,¹⁵ and have an estimated 30% lifetime risk of experiencing an osteoporotic



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fracture when over 50, similar to the lifetime risk of developing prostate cancer.¹⁵ Fragility fractures are the primary cause of hospitalisation and/or death for US adults \geq age 65 and above.¹⁵ Furthermore, 44% of nursing home admissions are due to fractures.¹⁵ It is obvious that osteoporosis is extremely common and this condition leads to disability, costs and even death. Thus, preventing and treating this disease is of utmost importance. However, the recently updated USA Preventive Services Task Force (USPSTF) has recently stated that there is insufficient evidence that calcium and vitamin D prevent a fracture in premenopausal women or in men who have not experienced a fracture and now recommends against daily supplementation with 400 IU or less of vitamin D3 and 1000 mg or less of calcium for the primary prevention of fractures in non-institutionalised postmenopausal women. Thus, unless you are an institutionalised postmenopausal woman or you have already experienced a fracture, the USPSTF does not recommend calcium and vitamin D for preventing a first-time

fracture, as there is a lack of evidence. The USPSTF also states that “Daily supplementation with \leq 400 IU of vitamin D3 and \leq 1000 mg of calcium has no net benefit for the primary prevention of fractures” and that “Evidence is lacking regarding the benefit of daily supplementation with $>$ 400 IU of vitamin D3 and $>$ 1000 mg of calcium for the primary prevention of fractures in postmenopausal women, and the balance of benefits and harms cannot be determined.”¹⁷ Thus, what else can a clinician prescribe to help to prevent osteoporosis and its consequences? A broad amount of data seems to indicate substantial potential for supplementary vitamin K. However, currently few guidelines recommend vitamin K therapy for prevention or treatment of osteoporosis.

Vitamin K₁ (5 mg daily) given to 440 postmenopausal women with osteopenia for 2 years in a randomised, placebo-controlled, double-blind trial caused a greater than 50% reduction in clinical fractures (9 vs 20, $p=0.04$) versus placebo, despite the fact that there was no improvement in bone mineral density.¹⁸ Moreover, there was a 75% reduction in cancer incidence with vitamin K₁ (3 vs 12, $p=0.02$). The benefit of vitamin K on bone is thought to be unrelated to increasing BMD but rather increasing bone strength.¹⁹ A recent meta-analysis has shown that vitamin K₂ (45 mg/day) significantly reduces hip (77% reduction), vertebral (60% reduction) and all non-vertebral fractures (81% reduction).²⁰ Whether the results of vitamin K₂ at a dose of 45 mg can be translated to over the counter doses of vitamin K₁ (such as 1–5 mg) is still a matter of debate, but vitamin K₁ on its own has already been shown to reduce fractures and cancer in a clinical trial, although more data are needed to confirm these benefits.

Box 1 The health benefits of vitamin K (box 1)

Bone health

May help to prevent fractures due to osteopenia and osteoporosis^{18–20}

Trial evidence

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A recent meta-analysis has shown that vitamin K₂ (45 mg/day) significantly reduces hip (77% reduction), vertebral (60% reduction) and all non-vertebral fractures (81% reduction).²⁰

Cancer (especially liver cancer)

May help to prevent liver cancer and death in patients with liver cirrhosis and hepatocellular carcinoma (HCC)^{54–61}

Trial evidence

Five randomised controlled trials tested vitamin K₂ (45 or 90 mg/day) in patients with HCC. Vitamin K₂ significantly improved 1-year overall survival, (RR=1.03, 95% CI 1.00 to 1.05, $p=0.03$)⁷⁴

Vascular calcifications

May help to prevent vascular calcifications (especially in patients on warfarin)^{5 26 75}

Trial evidence

Significantly delayed the development of coronary artery calcium in a 3-year, double-blind, randomised controlled trial of 452 patients.

In a 3-year, double-blind, placebo-controlled trial, vitamin K₁ (along with vitamin D) significantly delayed the deterioration of arterial elasticity³⁷ in 181 postmenopausal women. This was not found with vitamin D alone.

Coronary heart disease (CHD)

May reduce the risk of CHD, CHD mortality and all-cause mortality^{5 26 76}

Insulin sensitivity

May help improve insulin sensitivity⁴⁰

Warfarin international normalised ratio (INR)

May help to stabilise INR in patients on warfarin⁴¹

VITAMIN K AND VASCULAR CALCIFICATIONS

Coronary artery calcium (CAC) has been shown to have increasing prevalence as kidney function declines.³ Indeed, CAC prevalence has been reported in 13% of ‘healthy’ patients without renal disease,²¹ 40% of patients with chronic kidney disease patients not on dialysis,²¹ 57% of patients starting dialysis²² and 83% of patients on long-term dialysis.²³ Diets lacking vitamin K can precipitate the development of vitamin K deficiency in as little as 7 days.²⁴ Additionally, subclinical vitamin K deficiency is not uncommon, especially in patients receiving warfarin.²⁵ Cross-sectional and cohort data have shown a lower risk of coronary heart disease (CHD), CHD mortality, all-cause mortality and severe aortic calcifications with higher vitamin K₂ (menaquinone) intake^{5 26} (box 1). This was not shown with vitamin K₁ intake (phylloquinone, the major dietary source of vitamin K.^{26 27} Thus, dietary vitamin K₁ intake, without vitamin K₂, may not be sufficient to suppress arterial calcifications and/or reduce risk for subsequent cardiovascular events and death. The menaquinone form of vitamin K (ie, vitamin K₂) has been presumed to be

more effective than vitamin K₁ at preventing and reversing arterial calcifications. It has been proposed that a substantial amount of apparently healthy patients are subclinically vitamin K deficient based on undercarboxylated osteocalcin and MGP,²⁵ presumably increasing the risk of vascular calcifications, cancer and osteoporosis.

Low vitamin K status (indicated by undercarboxylated MGP) is associated with increased vascular calcifications, and these levels can be improved by effective vitamin K supplementation^{28–32}. It was long believed that vitamin K was only involved in forming coagulation factors (ie, maintaining haemostasis). However, other vitamin-K dependent proteins (containing γ -carboxyglutamate or Gla) are dependent on vitamin-K carboxylation for functionality.³³ Vitamin K acts as a cofactor in the conversion of glutamate into Gla. Gla-containing proteins (MGP and osteocalcin) regulate many anticalcification and bone-forming processes in the body, which are dependent on vitamin K in order to be produced. Low levels of vitamin K impair activation of osteocalcin and decrease the activity of osteoblasts (cells important for building bone).^{33–34} Thus, vitamin K is vital to the functionality of proteins such as osteocalcin (important for building bone), (MGP, the most potent arterial calcification inhibitor known) and the growth-arrest sequence-6 protein (Gas6, involved in cell growth regulation³⁵).

Vitamin K has been shown to significantly delay the development of CAC in a 3-year, double-blind, randomised controlled trial of 452 patients (229 patients on vitamin K₁ and 223 patients in the control group).³⁶ All patients were assigned to a multivitamin (containing 1.6 mg thiamine, 1.8 mg riboflavin, 2.1 mg vitamin B-6, 3 μ g vitamin B12, 75 mg vitamin C, 12 mg vitamin E, 6 mg pantothenic acid, 20 mg niacin, 160 μ g folate and 30 μ g of biotin) as well as calcium (600 mg calcium carbonate) and vitamin D (cholecalciferol 400 IU). In the intention-to-treat (ITT) analysis, CAC progression at baseline and at year 3 was measured in 388 participants, which indicated no difference in the progression of CAC. However, a secondary analysis of 295 participants who were compliant with their supplements (predefined as $\geq 85\%$ adherence over 3 years) showed a significantly decreased progression of CAC in the vitamin K₁ group (500 μ g) compared to the control group ($p=0.03$). Moreover, in adherent participants with a CAC >10 at baseline (ie, patients with pre-existing arterial calcification), patients assigned to vitamin K₁ had a 6% less progression in CAC than those in the control group ($p=0.04$), whereas there was no benefit of vitamin K₁ in patients without baseline CAC. Despite the fact that serum MGP increased in the vitamin K₁ group, whereas MGP was decreased in the control group (treatment effect: $p\leq 0.03$ in ITT and secondary analyses), neither baseline nor change in MGP predicted the change in CAC, suggesting that the benefit of vitamin K on CAC progression is not related to increases in serum MGP. However, since the assay for serum MGP did not differentiate between carboxylated versus undercarboxylated

forms of MGP (and it is assumed that only the carboxylated form of MGP is functional as a calcification inhibitor), interpretation of serum MGP is severely problematic. Baseline osteoprotegerin (OPG) concentrations were positively predictive of change in CAC ($p=0.004$ in ITT adjusted for treatment), corroborating previous evidence suggesting that patients with higher calcification scores have higher baseline serum OPG concentrations. This study also indicated that there was no influence of vitamin K₁ on circulating OPG, interleukin 6 and C reactive protein and controlling for the 3-year change in cytokines did not alter the significance of treatment effect on change in CAC. Thus, the effect of vitamin K on CAC might be independent of changes in serum cytokine levels (but not necessarily ruling out vitamin K's benefit on the blunting of the effects of these cytokines). Despite the fact that vitamin K₁ has a beneficial effect on CAC in older men and women, larger studies powered for clinical end points (stroke, myocardial infarction and death) are needed to assess the risks and benefits of vitamin K therapy.

Vitamin K administration has been shown to significantly delay the progression of CAC and, in addition, it has also been shown to significantly delay the deterioration of arterial elasticity.³⁷ In another 3-year, double-blind, placebo-controlled trial, vitamin D and vitamin K were investigated. The trial included 181 postmenopausal women who were given (1) a placebo, (2) a supplement containing minerals and vitamin D, or (3) the same supplement with the addition of vitamin K₁. The vitamin K₁ group had a significant increase in the distensibility coefficient (8.8%, $p<0.05$), compliance coefficient (8.6%, $p<0.05$), elasticity (13.2%, $p<0.01$) and a decrease in pulse pressure (-6.3% ; $p<0.05$). There was no significant difference between the vitamin D and mineral group without vitamin K and the placebo group. In summary, vitamin K₁ along with vitamin D has beneficial effects on arterial elasticity.

VITAMIN K AND WARFARIN

Since warfarin directly leads to the inhibition of vitamin K, it would be presumed that there could be increased arterial calcifications in patients given warfarin. In fact, many preclinical and prospective studies have shown increased calcifications with patients on warfarin compared to those not receiving warfarin. Furthermore, most individuals taking warfarin are counselled to avoid vitamin K-containing foods, such as green leafy vegetables, which may lead to an even further increase in CAC.³⁶ Vitamin K deficiency can be exacerbated further when warfarin is initiated.³⁸ The negative impact that warfarin can have on the body has long been recognised.³⁹

Warfarin has been shown to cause severe arterial calcifications in the aorta and the carotid arteries of rats. However, when high-dose therapy with vitamins K₁ or K₂ (100 μ g/g of chow) was given, the progression of

calcifications ceased and there was also a 37% reduction in prior calcifications induced by warfarin. Moreover, high-dose vitamin K₁ or vitamin K₂ restored arterial distensibility back to that seen in control rats.³⁸ Thus, animal data indicate that vitamin K (K₁ or K₂) may be able to reverse arterial calcifications and at the same time improve arterial compliance. Warfarin has been shown to prevent the conversion of vitamin K₁ to vitamin K₂.^{40–42} Since warfarin was stopped prior to the introduction of high-dose vitamin K₁ or K₂, it is uncertain if either would have prevented these calcifications during concomitant warfarin administration. However, previous data have shown that vitamin K₂ is more effective than vitamin K₁ at preventing arterial calcifications during concomitant warfarin treatment in rats.³⁴ Thus, if further trials are performed, vitamin K₁ and K₂ should be tested.

Women taking warfarin during the first trimester of their pregnancy may give birth to children with punctate calcifications in the axial skeleton, proximal femurs and calcanei. It has been presumed that prenatal vitamin K deficiency induced by warfarin may be the underlying cause of these calcifications.^{43 44} Indeed, several studies indicate a connection between warfarin and arterial calcifications.

Thirty six patients, 19 on warfarin for more than 10 years and 17 controls from five different thrombosis services in the Netherlands, were studied. Patients on warfarin had over a threefold increase in femoral artery calcifications compared to the control group (77.8% vs 25%, respectively). Also, patients on warfarin had a significantly higher carotid intima-media thickness compared with controls ($p=0.04$). The authors concluded that warfarin was associated with increased arterial calcifications and may increase atherosclerotic development.⁴⁵

Warfarin has been associated with a 1.71-fold increase in mitral valve calcium (MVC), mitral annular calcium (MAC) or aortic valve calcification on two-dimensional echocardiograms compared to controls. In a population of 1155 patients, MVC, MAC and aortic valve calcium was present in 65% of patients with warfarin compared to only 52% of patients who were not on warfarin ($p<0.0001$).⁴⁶

Forty five aortic valves were examined after cardiac replacement surgery to look at calcified deposits. In the warfarin group, during a period of approximately 1–3 years in duration, there was significantly more calcium content contained within the aortic valves of patients with warfarin compared to the control group. Patients receiving preoperative warfarin treatment had a twofold increase in calcifications compared to non-treated patients. The authors concluded that warfarin might induce cardiovascular calcifications.

Finally, multislice spiral CT was used to determine if patients on warfarin have a significantly greater amount of coronary calcification compared to controls. Indeed, patients on warfarin had increased coronary calcium,

determined by a significantly higher Agatston score (1561 ± 1141) compared to controls (738 ± 978) ($p=0.024$ for the difference).⁴⁸ Moreover, patients on warfarin had significantly more valvular calcium compared to patients without anticoagulation treatment (valvular Agatston score 2410 ± 1759 vs 1070 ± 1085 , $p=0.002$). Thus, warfarin seems to be associated with increased valvular and coronary calcium in patients with aortic valve disease, which may be related to warfarin's inhibition of vitamin-K's ability to carboxylate anticalcification proteins, particularly MGP.

Evidence that somewhat challenges the above data is The Warfarin and Coronary Calcification Study. This study of 70 patients found no significant relationship between duration of warfarin treatment and CAC score.⁴⁹ Therefore, larger randomised controlled trials are required to determine if warfarin increases the risk of vascular calcifications. When vitamin K₁ is given to patients with warfarin, a more stable international normalised ratio (INR) has been noted. This has been shown in one retrospective and two prospective studies.⁴¹

VITAMIN K AND INSULIN SENSITIVITY

In a 3-year randomised, double-blind, controlled trial of 355 patients, vitamin K significantly improved insulin sensitivity in men with diabetes. Vitamin K is involved in pancreatic β -cell proliferation, insulin sensitivity, production of adiponectin and increased glucose tolerance, all of which may have contributed to these results. As a vitamin K inhibitor, warfarin may potentially negate these effects. In summary, vitamin K may improve insulin sensitivity in men with diabetes.⁴⁰

VITAMIN K AND CANCER

Vitamin K₂ has been shown to inhibit the growth of human cancer cell lines, including hepatoma lines, as well as to treat myelodysplastic syndrome.^{50–53} Two trials seem to indicate that vitamin K₂ 45 mg/day reduces the development of hepatocellular carcinoma (HCC) in patients with liver cirrhosis and that vitamin K₂ significantly reduces the recurrence of HCC in patients following the curative treatment of HCC with an associated reduction in all-cause mortality in these patients.^{54–61} The possible reduction in mortality with vitamin K₂ may be explained by multiple mechanisms. The mechanisms responsible include (1) activation of growth-inhibiting proteins requiring vitamin K₂, such as prothrombin,⁶² (2) arylation pathways,⁶³ (3) activation of growth arrest genes such as gas 6,⁶⁴ and (4) and increased c-Jun and c-Myc mRNA expression in hepatoma cells.⁵⁷

Preventing the recurrence of HCC is an important strategy, especially considering the fact that even after patients undergo curative therapy recurrence rates remain high.⁶⁵ While triple combination therapy with boceprevir or telaprevir, pegylated interferon (IFN) and ribavirin is effective for the treatment of hepatitis C virus

(HCV), information on preventing HCC development and/or recurrence as well as all-cause mortality is lacking.^{66–67} Moreover, this combination therapy is expensive, requires injections (IFN) and is often not well tolerated due to adverse events (ie, fever and pancytopenia). Conversely, vitamin K₂ 45 mg once daily is less expensive, orally administered and safe, as it is currently used for the treatment of osteoporosis.^{55–68} In these trials, there were few if any adverse events associated with vitamin K₂ 45 mg once daily, even out to approximately 8 years of duration. However, not all of the trials were blinded and most trials included a relatively small number of patients (n=40–61); however, follow-up was quite long (median=36 months to mean 65 months). Finally, all trials were performed in Japanese patients, and thus these data may not be generalisable to those of other ethnicities.

More information is needed to further evaluate the use of vitamin K₂ 45 mg once daily on top of current optimal medical therapy in patients with HCV, liver cirrhosis or HCC, especially in those who are resistant to current therapies. Combining vitamin K₂ with an ACE inhibitor (perindopril) has shown synergistic effects on HCC recurrence and survival, and thus this combination should also be further explored.⁶⁰ Moreover, additional studies should be explored combining vitamin K₂ with acyclic retinoids, as this combination has also shown positive results on human HCC cell lines.⁶⁹ The Health Benefits of vitamin K have been summarised in [box 1](#).

VITAMIN K AND HAEMODIALYSIS

Pilkey *et al.*⁷⁰ demonstrated that 29% of patients with haemodialysis have coexisting subclinical vitamin K deficiency. Later, Cranenburg *et al.*⁷¹ while evaluating vitamin K status and intake in such patients, reported subnormal levels in 45% of study participants. Finally, in the recent Vitamin K Italian (VIKI) dialysis study, 23.5% of patients were found to be vitamin K deficient, and this deficiency was found to be the strongest predictor of vertebral fractures (OR: 2.94; 95% CI 1.38 to 6.26).⁷² Other data have shown that vitamin K₂ may improve bone remodelling in patients with haemodialysis with low serum parathyroid hormone levels.⁷³ Supplementing with at least 200 µg menaquinone-7 (a form of vitamin K₂) daily may help to achieve near maximal protection from vascular calcification, osteoporosis and cancer (measured by maximal gamma carboxylation of vitamin K dependent proteins).³⁵

CONCLUSION

Vitamin K has a plethora of potential implications, including prevention and treatment of arterial calcifications, coronary heart disease and cancer, improvements in bone strength and reduced risks of fractures as well as improvements in insulin sensitivity. Additionally, vitamin K may even play a vital role in the stabilisation of INR control for patients on warfarin. On the basis of

previously presented data, warfarin may increase arterial calcifications and osteoporosis through the inhibition of vitamin K. Larger trials should be performed to further elucidate the negative long-term health consequences of warfarin and if these can perhaps be prevented through the institution of supplemental vitamin K.

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Competing interests Dr DiNicolantonio works for a company that sells vitamin K but he does not directly profit from their sales. JHO'K has a major ownership interest in CardioTabs, and is also founder and Chief Medical Officer for this nutraceutical company that has products containing vitamin K.

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REFERENCES

- Booth SL. Roles for vitamin K beyond coagulation. *Annu Rev Nutr* 2009;29:89–110.
- Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. *J Nutr* 1998;128:785–8.
- Krueger T, Westenfeld R, Schurgers L, *et al.* Coagulation meets calcification: the vitamin K system. *Int J Artif Organs* 2009;32:67–74.
- Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. *Thromb Haemost* 2008;100:593–603.
- Beulens JW, Booth SL, van den Heuvel EG, *et al.* The role of menaquinones (vitamin K(2)) in human health. *Br J Nutr* 2013;110:1357–68.
- Kaneki M, Hodges SJ, Hosoi T, *et al.* Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition* 2001;17:315–21.
- Thane CW, Bolton-Smith C, Coward WA. Comparative dietary intake and sources of phyloquinone (vitamin K1) among British adults in 1986–7 and 2000–1. *Br J Nutr* 2006;96:1105–15.
- Schurgers LJ, Vermeer C. Determination of phyloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis* 2000;30:298–307.
- Elder SJ, Haytowitz DB, Howe J, *et al.* Vitamin k contents of meat, dairy, and fast food in the u.s. Diet. *J Agric Food Chem* 2006;54:463–7.
- Dietary reference intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington DC: The National Academies Press, 2001:800.
- Binkley NC, Krueger DC, Kawahara TN, *et al.* A high phyloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. *Am J Clin Nutr* 2002;76:1055–60.
- Booth SL, Martini L, Peterson JW, *et al.* Dietary phyloquinone depletion and repletion in older women. *J Nutr* 2003;133:2565–9.
- Booth SL, O'Brien-Morse ME, Dallal GE, *et al.* Response of vitamin K status to different intakes and sources of phyloquinone-rich foods: comparison of younger and older adults. *Am J Clin Nutr* 1999;70:368–77.
- Booth SL, Al Rajabi A. Determinants of vitamin K status in humans. *Vitam Horm* 2008;78:1–22.
- International Osteoporosis Foundation. Facts and Statistics (cited 21 May 2015). <http://www.iofbonehealth.org/facts-statistics>
- Melton LJ III, Crowson CS, O'Fallon WM. Fracture incidence in Olmsted County, Minnesota: comparison of urban with rural rates and changes in urban rates over time. *Osteoporos Int* 1999;9:29–37.
- USPTF. Clinical Summary: Vitamin D and Calcium to Prevent Fractures: Preventive Medication. 2014 (cited 21 May 2015). <http://www.uspreventiveservicestaskforce.org/Page/Document/ClinicalSummaryFinal/vitamin-d-and-calcium-to-prevent-fractures-preventive-medication>

18. Cheung AM, Tile L, Lee Y, *et al.* Vitamin K supplementation in postmenopausal women with osteopenia (ECKO trial): a randomized controlled trial. *PLoS Med* 2008;5:e196.
19. Knapen MH, Schurgers LJ, Vermeer C. Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int* 2007;18:963–72.
20. Cockayne S, Adamson J, Lanham-New S, *et al.* Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* 2006;166:1256–61.
21. Russo D, Palmiero G, De Blasio AP, *et al.* Coronary artery calcification in patients with CRF not undergoing dialysis. *Am J Kidney Dis* 2004;44:1024–30.
22. Block GA, Spiegel DM, Ehrlich J, *et al.* Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int* 2005;68:1815–24.
23. Raggi P, Boulay A, Chasan-Taber S, *et al.* Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? *J Am Coll Cardiol* 2002;39:695–701.
24. Israels LG, Israels ED, Saxena SP. The riddle of vitamin K1 deficit in the newborn. *Semin Perinatol* 1997;21:90–6.
25. Cranenburg EC, Schurgers LJ, Vermeer C. Vitamin K: the coagulation vitamin that became omnipotent. *Thromb Haemost* 2007;98:120–5.
26. Geleijnse JM, Vermeer C, Grobbee DE, *et al.* Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr* 2004;134:3100–5.
27. Villines TC, Hatzigeorgiou C, Feuerstein IM, *et al.* Vitamin K1 intake and coronary calcification. *Coron Artery Dis* 2005;16:199–203.
28. Westenfeld R, Krueger T, Schlieper G, *et al.* Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis* 2012;59:186–95.
29. Dalmeijer GW, van der Schouw YT, Magdeleyns E, *et al.* The effect of menaquinone-7 supplementation on circulating species of matrix Gla protein. *Atherosclerosis* 2012;225:397–402.
30. Dalmeijer GW, van der Schouw YT, Vermeer C, *et al.* Circulating matrix Gla protein is associated with coronary artery calcification and vitamin K status in healthy women. *J Nutr Biochem* 2013;24:624–8.
31. Schurgers LJ, Barreto DV, Barreto FC, *et al.* The circulating inactive form of matrix gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. *Clin J Am Soc Nephrol* 2010;5:568–75.
32. Rennenberg RJ, de Leeuw PW, Kessels AG, *et al.* Calcium scores and matrix Gla protein levels: association with vitamin K status. *Eur J Clin Invest* 2010;40:344–9.
33. Berkner KL. The vitamin K-dependent carboxylase. *Annu Rev Nutr* 2005;25:127–49.
34. Spronk HM, Soute BA, Schurgers LJ, *et al.* Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. *J Vasc Res* 2003;40:531–7.
35. Vermeer C. Vitamin K: the effect on health beyond coagulation—an overview. *Food Nutr Res* 2012;56.
36. Shea MK, O'Donnell CJ, Hoffmann U, *et al.* Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr* 2009;89:1799–807.
37. Braam LA, Hoeks AP, Brouns F, *et al.* Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. *Thromb Haemost* 2004;91:373–80.
38. Schurgers LJ, Spronk HM, Soute BA, *et al.* Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood* 2007;109:2823–31.
39. Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy. *Am J Med* 1980;68:122–40.
40. Yoshida M, Jacques PF, Meigs JB, *et al.* Effect of vitamin K supplementation on insulin resistance in older men and women. *Diabetes Care* 2008;31:2092–6.
41. Ford SK, Moll S. Vitamin K supplementation to decrease variability of International Normalized Ratio in patients on vitamin K antagonists: a literature review. *Curr Opin Hematol* 2008;15:504–8.
42. Ryan-Harshman M, Aldoori W. Bone health. New role for vitamin K? *Can Fam Physician* 2004;50:993–7.
43. Demer LL, Tintut Y, Parhami F. Novel mechanisms in accelerated vascular calcification in renal disease patients. *Curr Opin Nephrol Hypertens* 2002;11:437–43.
44. Jaillot J, Robert-Gnansia E, Till M, *et al.* Biliary lithiasis in early pregnancy and abnormal development of facial and distal limb bones (Binder syndrome): a possible role for vitamin K deficiency. *Birth Defects Res A Clin Mol Teratol* 2005;73:188–93.
45. van Varik BJ, Rennenberg RJMW, Kroon AA, *et al.* Vascular calcifications after chronic use of vitamin-k antagonists. *Artery Res* 2009;3:151.
46. Lerner RG, Aronow WS, Sekhri A, *et al.* Warfarin use and the risk of valvular calcification. *J Thromb Haemost* 2009;7:2023–7.
47. Schurgers LJ, Aebert H, Vermeer C, *et al.* Oral anticoagulant treatment: friend or foe in cardiovascular disease? *Blood* 2004;104:3231–2.
48. Koos R, Mahnken AH, Muhlenbruch G, *et al.* Relation of oral anticoagulation to cardiac valvular and coronary calcium assessed by multislice spiral computed tomography. *Am J Cardiol* 2005;96:747–9.
49. Villines TC, O'Malley PG, Feuerstein IM, *et al.* Does prolonged warfarin exposure potentiate coronary calcification in humans? Results of the warfarin and coronary calcification study. *Calcif Tissue Int* 2009;85:494–500.
50. Takami A, Nakao S, Ontachi Y, *et al.* Successful therapy of myelodysplastic syndrome with menatetrenone, a vitamin K2 analog. *Int J Hematol* 1999;69:24–6.
51. Miyazawa K, Yaguchi M, Funato K, *et al.* Apoptosis/differentiation-inducing effects of vitamin K2 on HL-60 cells: dichotomous nature of vitamin K2 in leukemia cells. *Leukemia* 2001;15:1111–17.
52. Nishimaki J, Miyazawa K, Yaguchi M, *et al.* Vitamin K2 induces apoptosis of a novel cell line established from a patient with myelodysplastic syndrome in blastic transformation. *Leukemia* 1999;13:1399–405.
53. Sakai I, Hashimoto S, Yoda M, *et al.* Novel role of vitamin K2: a potent inducer of differentiation of various human myeloid leukemia cell lines. *Biochem Biophys Res Commun* 1994;205:1305–10.
54. Hosho K, Okano JI, Koda M, *et al.* Vitamin K2 has no preventive effect on recurrence of hepatocellular carcinoma after effective treatment. *Yonago Acta Med* 2008;51:95–9.
55. Habu D, Shiomi S, Tamori A, *et al.* Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA* 2004;292:358–61.
56. Hotta N, Ayada M, Sato K, *et al.* Effect of vitamin K2 on the recurrence in patients with hepatocellular carcinoma. *Hepatogastroenterology* 2007;54:2073–7.
57. Kakizaki S, Sohara N, Sato K, *et al.* Preventive effects of vitamin K on recurrent disease in patients with hepatocellular carcinoma arising from hepatitis C viral infection. *J Gastroenterol Hepatol* 2007;22:518–22.
58. Kojima K, Tamano M, Akima T, *et al.* Effect of vitamin K2 on the development of hepatocellular carcinoma in type C cirrhosis. *Hepatogastroenterology* 2010;57:1264–7.
59. Yoshida H, Shiratori Y, Kudo M, *et al.* Effect of vitamin K2 on the recurrence of hepatocellular carcinoma. *Hepatology* 2011;54:532–40.
60. Yoshiji H, Noguchi R, Toyohara M, *et al.* Combination of vitamin K2 and angiotensin-converting enzyme inhibitor ameliorates cumulative recurrence of hepatocellular carcinoma. *J Hepatol* 2009;51:315–21.
61. Mizuta T, Ozaki I, Eguchi Y, *et al.* The effect of menatetrenone, a vitamin K2 analog, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment: a pilot study. *Cancer* 2006;106:867–72.
62. Carr B, Wang Z, Kar S, *et al.*, eds. *Prothrombin inhibits hepatocyte DNA synthesis (DNA-S) and expression of the α5 integrin gene*. Proc AACR, 1995.
63. Kar S, Carr BI. Growth inhibition and protein tyrosine phosphorylation in MCF 7 breast cancer cells by a novel K vitamin. *J Cell Physiol* 2000;185:386–93.
64. Varnum BC, Young C, Elliott G, *et al.* Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. *Nature* 1995;373:623–6.
65. Zimmerman MA, Ghobrial RM, Tong MJ, *et al.* Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. *Arch Surg* 2008;143:182–8; discussion 8.
66. Zeuzem S, Andreone P, Pol S, *et al.* Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;364:2417–28.
67. Poordad F, McCone J Jr, Bacon BR, *et al.* Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;364:1195–206.
68. Orimo H, Shiraki M, Tomita A, *et al.* Effects of menatetrenone on the bone and calcium metabolism in osteoporosis: A double-blind placebo-controlled study. *J Bone Miner Metab* 1998;16:106–12.
69. Kanamori T, Shimizu M, Okuno M, *et al.* Synergistic growth inhibition by acyclic retinoid and vitamin K2 in human hepatocellular carcinoma cells. *Cancer Sci* 2007;98:431–7.
70. Pilkey RM, Morton AR, Boffa MB, *et al.* Subclinical vitamin K deficiency in hemodialysis patients. *Am J Kidney Dis* 2007;49:432–9.

71. Cranenburg EC, Schurgers LJ, Uiterwijk HH, *et al.* Vitamin K intake and status are low in hemodialysis patients. *Kidney Int* 2012;82:605–10.
72. Fusaro M, Noale M, Viola V, *et al.* Vitamin K, vertebral fractures, vascular calcifications, and mortality: Vitamin K Italian (VIKI) dialysis study. *J Bone Miner Res* 2012;27:2271–8.
73. Nakashima A, Yorioka N, Doi S, *et al.* Effects of vitamin K2 in hemodialysis patients with low serum parathyroid hormone levels. *Bone* 2004;34:579–83.
74. Zhong JH, Li H, Li LQ, *et al.* Adjuvant therapy options following curative treatment of hepatocellular carcinoma: a systematic review of randomized trials. *Eur J Surg Oncol* 2012;38:286–95.
75. Beulens JW, Bots ML, Atsma F, *et al.* High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis* 2009;203:489–93.
76. Gast GC, de Roos NM, Sluijs I, *et al.* A high menaquinone intake reduces the incidence of coronary heart disease. *Nutr Metab Cardiovasc Dis* 2009;19:504–10.