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Claire R. Palmer
Edith Cowan University

Lauren C. Blekkenhorst
Edith Cowan University

Joshua R. Lewis
Edith Cowan University

Natalie C. Ward

Carl J. Schultz

See next page for additional authors

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Review article

Quantifying dietary vitamin K and its link to cardiovascular health: a narrative review.

Claire R Palmer[1,2], Lauren C Blekkenhorst[2,3], Joshua R Lewis[2,3,4], Natalie C Ward[3,5], Carl J Schultz[3, 6], Jonathan M Hodgson[2,3], Kevin D Croft[1], Marc Sim[2,3]

[1] School of Biomedical Sciences, The University of Western Australia, Perth, Western Australia, Australia

[2] School of Health and Medical Sciences, Edith Cowan University, Perth, Western Australia, Australia

[3] School of Medicine, Faculty of Health and Medical Sciences, The University of Western Australia, Perth, Western Australia, Australia

[4] Centre for Kidney Research, Children's Hospital at Westmead, School of Public Health, Sydney Medical School, The University of Sydney, Sydney, New South Wales, Australia

[5] School of Public Health & Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia

[6] Department of Cardiology, Royal Perth Hospital, Perth, Western Australia, Australia

Corresponding author

Marc Sim

School of Medical and Health Sciences, Edith Cowan University

Joondalup, 6027, Western Australia, Australia

Email: marc.sim@ecu.edu.au

Abstract

Cardiovascular disease is the leading cause of death and disability worldwide. Recent work suggests a link between vitamin K insufficiency and deficiency with vascular calcification, a marker of advanced atherosclerosis. Vitamin K refers to a group of fat-soluble vitamins important for blood coagulation, reducing inflammation, regulating blood calcium metabolism, as well as bone metabolism, all of which may play a role in promoting cardiovascular health. Presently, there is a lack of a comprehensive vitamin K database on individual foods, which are required to accurately calculate vitamin K1 and K2 intake for examination in epidemiological studies. This has likely contributed to ambiguity regarding the recommended daily intake of vitamin K, including whether vitamin K1 and K2 may have separate, partly overlapping functions. This review will discuss the presence of: (i) vitamin K1 and K2 in the diet; (ii) the methods of quantitating vitamin K compounds in foods; and (iii) provide an overview of the evidence for the cardiovascular health benefits of vitamin K in observational and clinical trials.

Key words

Vitamin K, Database, Phylloquinone, Menaquinone, Vascular calcification, Cardiovascular health

Introduction

Vitamin K refers to a group of fat-soluble vitamins important for blood coagulation and has been linked to other biological processes including blood calcium metabolism, as well as bone and vascular health.[1] The two main vitamin K forms are vitamin K1 (phylloquinone; PK) and vitamin K2 (menaquinones; MK). The few studies that have explored the link between vitamin K1 and/or K2 with cardiovascular outcomes have reported conflicting results.[1] Despite the potential importance of vitamin K1 and/or K2 for human health, the adequate intake (AI) recommendations proposed by numerous public health organisations typically only consider vitamin K1. This is likely a result of limited information relating to vitamin K2.[2] Specifically, there is currently a lack of international comprehensive databases on the vitamin K content of individual foods, which are required to accurately calculate vitamin K1 and/or K2 intake.[2] This review discusses the: (i) presence of vitamin K1 and K2 in the diet; (ii) the methods of quantitating vitamin K compounds in foods; and (iii) provides an overview of the evidence for the cardiovascular health benefits of vitamin K in observational and clinical trials. We also propose that the development of a comprehensive national vitamin K databases of individual food items is critical to advance our understanding of vitamin K nutrition for a range of health outcomes including cardiovascular health.

Vitamin K: phylloquinone and menaquinones

Vitamin K is a group of structurally and functionally analogous fat-soluble vitamins, which act as cofactors in the post-translational γ-carboxylation of vitamin K-dependant proteins (VKDP). Vitamin K compounds are characterised by a 2-methyl-1,4-naphthoquinone backbone also called menadione and a lipophilic isoprenoid side chain attached at carbon three *(Figure 1*). Two types of vitamin K exist naturally: vitamin K1 (PK) and vitamin K2 (MK).[3] Phylloquinone has a side chain analogous to the phytyl side chain of chlorophyll; four isoprenoid residues of which three are saturated. It is synthesised by all photosynthetic plants and algae, as it is a

functional component in photosynthesis.[4] Vitamin K2 (MK) is composed of a group of isoprenologs where the side chain is a polymer of isoprenoid units ranging between four to thirteen repeats. MKs are named according to the number of repeating units,[4] with derivatives including MK-4 to MK-13. For example, MK-4 has four unsaturated isoprenoid units (*Figure 1*). Despite having analogous structures the origins of MKs differ. MK-4 is synthesised from PK and menadione by animals and humans.[2] All other MKs are synthesised by anaerobic bacteria, which includes species present in the human microbiome.[4] Bacterial MKs are important in metabolic respiration acting as components of the electron transport chain.[5] The synthetic compound, menadione, is also referred to as vitamin K3 and is often added to animal feed.[6]

Dietary vitamin K

Phylloquinone is present in the majority of foods including vegetables, fruits, nuts, bread, cereals, oil, meat and animal products; however, quantities vary greatly.[7, 8] The highest concentrations of PK found in green leafy vegetables, due to its role in photosynthesis. Concentrations in these vegetables range between 400-700 µg/100 g.[4] Vegetable oils, such as olive, canola and soybean, also contain considerable amounts of PK. These oils are widely used in margarine, salad dressings, and the preparation of foods, such as bread. The contribution of oils as part of commercially consumed foods towards total dietary vitamin K intake may be greater than initially thought. For example, in the US, commercially prepared foods and mixed dishes were the second highest contributors to PK intake, after vegetables.[9] The widespread use of plant products in food production and their consumption by animals explains the presence of PK in disparate food groups. The PK content in the Western diet makes up the majority of vitamin K intake. It constitutes up to 90% of dietary vitamin K intake, of which 60-80% is derived from vegetables.[4, 10, 11]

Menaquinones are found in meat, dairy and fermented foods.[2] MK-4 is synthesised by the conversion of consumed PK or menadione in human or animal tissues. Therefore, dietary MK-4 is typically found in animal tissue and products, such as eggs or dairy.[2] The tissue or organ of an animal, the type of animal and country of origin (possibly related to feed) all affects the MK-4 content in food products.[2] For example, goose liver is a major storage tissue of vitamin K compounds and has the highest MK-4 content of any tissue (370 µg/100 g).[3] In comparison, goose leg has only 60 µg/100 g of MK-4.[3] Alternatively, chicken breast has more MK-4 than duck breast; 8.9 vs 3.6 µg/100 g, respectively.[3] Poultry and pork, have especially high MK-4 content due to menadione in their feed, which is converted into MK-4.[2, 12] The MK-4 content of various poultry products vary greatly.[2] Presently, the most comprehensive tool to calculate the MK-4 content of food is provided by the United States Department of Agriculture (USDA) Food Composition Databases.[13] However, there is limited information on the MK-4 content in some commonly consumed meats, such as lamb, veal, and goat.[14] Other MKs (MK5-12) are synthesised by anaerobic bacteria[4]; however, information on their approximate content in food products are scarce.

Foods fermented with the MK-synthesising bacteria are abundant in long-chain MKs. For example, natto (fermented soybeans) has been reported to have the highest MK-7 content of any food, containing 936 µg/100 g.[15] The type of MKs produced is dependent on the type of bacteria and temperature conditions.[5] Notably, bacteria that produce MK-9 also produce MK-8, but in four-fold lower amounts. Thus, MK-9 and MK-8 co-occur in foods that are fermented. Other MKs are produced independent of each other.[16] Not all fermented foods contain MKs. For example, yeast has lost the enzymes required for MK synthesis,[4, 5] thus bread does not contain MKs.[2] Fermented dairy products, such as cheese, milk and yogurt, are significant sources of long-chain MKs.[2, 17] The MK content varies by the percentage of fat present in dairy.

For example, the MK content of full fat cheddar cheese is five times higher than the total MK content of reduced-fat cheddar (281 µg/100 g vs. 49 µg/100 g, respectively).[17]

Few studies have analysed the MK content in the Western diet. From this limited work, MKs have been reported to constitute approximately 10% of dietary vitamin K intake.[4, 10, 11] Of note, the major MK form is MK-4, which contributes 25-40% of the total MK in the diet.[10, 11] After MK-4, the most nutritionally abundant MKs are MK-9, MK-8 and MK-7.[10, 18] However, as the MK content in individual foods has not been systematically determined to the degree of PK,[2] this may lead to errors in these estimates. Studies have highlighted meat and dairy products as the best sources of MKs.[3] For example, a German study reported that 60% of all MKs came from dairy products, with cheese consumption contributing 43% of total MK intake.[10] Considering, the European diet is typically rich in cheese, it is thought to be a major contributor of MK intake in such populations.[2] Presently, the most comprehensive resources for the different MK content from a variety of animal and plant-based foods is from the Netherlands.[3] Besides the aforementioned Dutch work, there is relatively little data for the MK content in the diets of other countries. This is likely due to the absence of comprehensive databases of MK content in individual foods, which are required to quantify MK intake at individual and population levels.[2]

Bioavailability of vitamin K

Bioavailability is the rate and extent by which a nutrient is absorbed and becomes available to the target tissue.[19] Alternatively, bioaccessibility relates to the amount of compound that is released from its matrix in the gastrointestinal tract and is available for absorption.[19] Precise quantitative assessments of vitamin K bioavailability in humans is challenging due to unquantified tissue conversion of PK to MK-4 and the contributions of the gut microbiota.[20] Both PK and MKs are absorbed from the intestine using the same mechanisms as other fat soluble vitamins and lipids.[4] Apart from this shared absorption mechanism there are differences

in the bioavailability and bioaccessibility of vitamin K compounds.[3] For example, previous work examining the absorption rate of PK and MK in a food matrix reported that MK absorption from natto (Japanese fermeneted soybean) is approximately 10 times greater compared to PK absorption from spinach. Of note, peak serum values for both PK and MK appear to occur at the ~6 h post-prandial mark. It was also reported that bioavailability of PK from foods is significantly reduced by its poor bioaccessibility from the food matrix.[3] Specifically, only 5-10% of PK is absorbed from cooked vegetables; however, this can be increased slightly by the presence of dietary lipids.[3] In contrast, PK absorption from oils and supplements have much higher absorption rates (~200-400%) compared to vegetables.[3, 21, 22] This indicates that PK as a nutrient can be well absorbed, but the vegetable food matrix may present a barrier to absorption. Compared to PK, the limited amount of studies conducted on long-chain MKs suggest they may have higher bioaccessibility from dietary sources[3]. Specifically, the absorption of long-chain MKs (MK-7, MK-8 and MK-9) from natto, cheese and egg yolk is close to 100%.[3] Furthermore, these long-chain MKs have a longer half-life. For example, in a study where 6 male volunteers were given 2 μmol of PK, MK-4 and MK-9, MK-9 was still detectable in the serum at 48 h, while PK and MK-4 returned to baseline after 24 h.[23] Notably, the long half-life of MK-9 may not necessarily indicate increased bioavailability, but instead non-preferential utilisation by tissues compared to PK and MK-4. A long half-life may also indicate that long-chain MKs may be of particular importance for extrahepatic tissues.[24] Despite the aforementioned work, the bioavailability of vitamin K compounds from different food matrixes remain largely unknown. Nevertheless, current evidence suggest that long-chain MKs may be more important than previously thought for vitamin K status, due to the almost complete absorption of long-chain MKs and poor absorption of PKs from its main dietary sources.[25]

Non-dietary sources of menaquinones

Despite, the large proportion of animal-based foods known to contain MKs, not all of the MKs come from dietary sources. There are two known alternative sources, including PK conversion into MK-4, and MK synthesis by the gut microbiota.[4, 26] The full extent of the conversion of PK to MK-4 is unknown, but in the last decade progress has been made in understanding the mechanism. Specifically, intestinal PK is cleaved in enterocytes to produce menadione (often referred to as vitamin K3), which is prenylated by UbiA prenyltransferase-containing domain 1 (UBIAD1) to produce MK-4.[4, 26] A recent study in rats demonstrated that only 0.1% of oral PK was converted into MK-4, and 2% converted into menadione.[27] The extent of this conversion in humans remains unclear, but it is estimated to be 5-25% of PK intake.[28] Therefore, MK-4 biosynthesis from PK may be a large source of MKs, but detailed human studies are needed to examine this further. In addition, the contribution of microbial-synthesised MKs to human nutrition is not fully understood. The majority of MKs in the gut are synthesised by *Bacteroides*-one of the largest intestinal genera.[29, 30] *Bacteroides* primarily produce MK-10 and MK-11, with small amounts of MK-7 to MK-9.[29, 31] There is a large pool of MKs in the gut, with previous work suggesting an average of 1.8 mg of vitamin K2 in the intestinal tract of humans.[32] Despite such reserves of vitamin K2 being over 20 times the AI for adult males, the bioavailability of these MKs is thought to be limited.[33] Specifically, vitamin K compounds are absorbed in the small intestine in a process requiring bile salts and pancreatic enzymes, which are absent where the majority of bacteria colonise the colon.[34] Additionally, the majority of microbial-synthesized MKs are bound by bacterial membranes and thus are not available for absorption.[2] Therefore, while a large amount of MKs are produced by the gut microbiota, their poor bioavailability at the point of synthesis limits their contribution to human vitamin K nutrition.[31, 33] Presently, the exact bioavailability of microbial-synthesised MKs is unknown, and more bioavailability studies are required to discern their role in human nutrition.

Dietary recommendations for vitamin K

Current dietary vitamin K recommendations are typically based on the intake of PK sufficient to maintain coagulation.[20] This is problematic as the tissue-requirements of extrahepatic vitamin K-dependant proteins for PK differs from the requirement of hepatic vitamin K-dependant proteins.[2] Therefore, dietary intakes which are sufficient to maintain coagulation may be inadequate in optimising other aspects of health that might be impacted by vitamin K nutrition.[2, 28] However, any attempts to update the AI values for vitamin K1 or include vitamin K2 would be hampered by a lack of dose-response studies and evidence on the occurrence, absorption, function and content of MKs in the body or organs.[28, 35, 36] Difficulties in quantitating the contribution of MKs synthesised by the gut microflora and tissue conversion of PK are additional challenges when defining dietary requirement.[20] Furthermore, a recent report by the European Commission highlighted the uncertainties in country-wide estimates of vitamin intake data, which are caused by 'borrowing' vitamin K values from other countries and substituting vitamin K values for individual food items for similar foods or food groups.[35] For example, the USDA database[13] which was used to determine the vitamin K Nutrient Reference Values for Australia and New Zealand.[37] Presently, the AI for vitamin K intake is set at 60 and 70 µg/d for adult Australian females and males respectively.[37] Alternatively, it has been proposed that even intakes of 90-120 µg/day (set by the USDA)[13] of vitamin K may not be sufficient to induce complete carboxylation of all VKDPs.[38] Consequently, AI recommendations in Australia for vitamin K intake may not accurately reflect actual vitamin K intake (or demands), and may lead to ambiguity when determining optimal vitamin K nutrition. There is a need for both the development of regional databases and the expansion of existing databases to include both vitamin K1 and K2 content (including a range of MKs) in commonly consumed foods. Such work will allow for improvements in the determination of dietary vitamin K recommendations. Finally, due to differences in the bioavailability and/or

bioaccessibility of vitamin K2, future guidelines may need to consider distinguishing between dietary vitamin K1 and K2.

Vitamin K databases

Databases of nutrients allow for the calculation of individual and population level intake of nutrients, permitting for epidemiological investigation. Although there appears to be ~70 sources containing information relating to the vitamin K content of food, closer analysis of these reveal that current databases are largely incomplete.[25] Specifically, only 12 databases list the vitamin K content of individual food items, which is required to more accurately determine vitamin K intake.[25] The Dutch database is the most comprehensive and includes PK and several types of MKs, ranging from MK-4 to MK-10.[39] Although the largest database (in English) has been developed by the USDA, this database does have limitations. Specifically, food items have only been assessed for PK and MK-4 in the 25th release of the USDA database.[13] Considering no other MKs have been included in the database, this limits its utility for accurately estimating total vitamin K2 intake. The USDA database also lacks some commonly consumed animal products, including lamb, veal and goat, which are potentially rich sources of MKs.[13] The limited availability of comprehensive, national databases that lists both PK and MK content in food hampers investigation of the relationship between dietary vitamin K1 and/or K2 intake and a range of health outcomes. Nevertheless, available databases provide researchers with an indication of the types of food that are rich in vitamin K1 and K2 (including the specific MKs). By reviewing pre-existing databases,[3, 13, 15, 17, 39, 40] we have compiled a list of commonly consumed foods and their approximate vitamin K content in *Table 1*. However, estimates of the respective vitamin K1 and K2 content of the foods listed in *Table 1* should be interpreted with caution, as there is substantial variability of PK and MK content in food by region.[2]

Regional differences in the vitamin K content of food

The content of both PK and MKs in foods are known to differ between regions.[2] For example, beef cuts in Japan contain more MK-4 than the United States (15.0 ± 7.0 vs. 1.1 to 9.3 µg/100 g, respectfully).[14, 15] Similarly, the MK-4 content in egg yolk is four times higher in Japan compared to the United States (64 vs 15.5 µg/100g).[2] The variability in MK-4 has been attributed to the use of menadione in animal feeds and further differences in food production.[2] Variability of up to 15% has been reported in the MK content (MK-6 to MK-10) of semi-hard cheese varieties in three different European countries (France, Poland, Denmark).[16] Furthermore, intra-country MK variability can be high (up to 80%), as reported between six English cheddar cheeses.[16] Results are also complicated by a range of methodologies (discussed in subsequent section) used to quantify PK and MKs. The regional differences in food production, climate conditions, and dietary consumption patterns highlights the importance of comprehensive and detailed databases that are population specific. Ideally, databases should include information relating to the brand, type, method of creation and region of grown foods alongside their PK and MK content. If regional differences in vitamin K content of food are ignored, this can lead to inaccuracies when estimating vitamin K intake. Although the investment in comprehensive databases is unlikely to occur in most countries, the creation of more region specific databases on the vitamin K contents of foods would ultimately facilitate better estimation of vitamin K1 and K2 intake.

Measuring dietary vitamin K

Methods to quantitate phylloquinone and menaquinones in food

To develop databases, a validated method of measuring PK and MKs simultaneously, with accuracy and high throughput, is required. These methods must have high sensitivity and selectivity due to the low vitamin K content in foods, and the complexity of the food matrix.[41] A recent review concisely summarised quantification methods for determination of vitamin K

in various biological matrices including blood, urine and tissue.[42] However, the quantification of vitamin K from food were not considered in detail. Existing methods to determine PK and MK levels from food generally use reverse-phase HPLC with fluorescent detection or HPLC–Mass Spectrometry (MS), with K1 or deuterated-K1 as the internal standard, respectively.[16, 43] Gas Chromatography-MS (GC-MS) has also been used with deuterium-labelled internal standards to accurately measure PK in serum.[44, 45] However, there are limitations in using GC for measuring vitamin K, as high temperatures are needed to volatise vitamin K compounds (>300°C).[46] Therefore, HPLC is the preferred method to separate vitamin K compounds for analysis.[41] The emerging method to measure vitamin K in foods is a HPLC-MS method developed by Karl et al.[43] This method has high versatility and may be used to measure PK and all MKs in a variety of forms such as food, serum and faeces.[43] Based on current evidence, HPLC-MS appears to be the best method to measure vitamin K compounds from foods.

Methods of extracting vitamin K from the food matrix

Vitamin K compounds are trapped in the matrix of foods and must be liberated in order to be accurately measured.[3] Vitamin K compounds are unstable in alkali conditions and undergo photo-oxidation upon exposure to ultraviolet light, which limits the available extraction methods.[46] Samples must be stored in amber vials and all work done yellow light to prevent this degradation.[43] Due to these limitations, the most common extraction method is liquid-liquid extraction, with the exact process depending on the food matrix.[46, 47] Sample pre-treatment is important to liberate K vitamers from the matrix. Most food groups, with the exception of vegetable oils, are homogenised and sonicated to break up the food matrix.[3, 48] This is especially important for vegetables, as it is essential to liberate PK from the thylakoid membranes.[46] Furthermore, some fatty foods, such as oils or cheeses, undergo lipase digestion or acid treatment to break down fats, thereby increasing purity.[16, 46] To our knowledge, there is

currently no standardised method for the extraction of vitamin K compounds from food, however, numerous validated methods exist.[3, 43, 47]

Vitamin K and cardiovascular health

Atherosclerotic cardiovascular disease

Cardiovascular disease accounts for approximately 1 in 3 deaths worldwide.[49] Atherosclerotic cardiovascular disease includes coronary heart disease (CHD), peripheral arterial disease and cerebrovascular disease. Atherosclerotic diseases are caused by endothelial dysfunction, inflammation and the slow build-up of cholesterol, fats and calcium deposits forming plaques in arterial walls.[50] The plaques decrease lumen area of the artery, increasing resistance to blood flow. Vulnerable plaques can rupture or erode leading to blood clots, which can obstruct blood flow to the heart causing a heart attack, or to the brain causing a stoke. These plaques frequently develop calcium mineral deposits, which further adds to plaque volume and leads to reduced arterial compliance leading to an increased risk of organ damage.[50] As such, strategies capable of preventing or delaying the progression of atherosclerosis are fundamental to improving cardiovascular health.

Vitamin K and vascular calcification

The arterial (vascular) calcification process was once thought to be passive and a natural consequence of ageing. However, it is now understood to be a complex biomineralisation process which is actively regulated.[51] Observational studies have reported that vitamin K intake is inversely associated with arterial calcification.[52] The best evidence for a causal role for vitamin K in arterial calcification comes from vitamin K antagonists (VKA), genetic studies and short-term randomised controlled trials.[52-54] VKA such as warfarin are anticoagulants used to prevent thrombosis and pulmonary emboli. VKAs reduce the bioactivation of the hepatic VKDP, such as prothrombin, which contributes to coagulation.[55] However, their effects are non-specific and systemic, thus extrahepatic VKDP are inactivated during VKA treatment.[54,

[56] This includes Matrix-Gla Protein (MGP), an inhibitor of arterial calcification,[57] activated protein-C an inhibitor of inflammation and endothelial cell apoptosis,[58] and osteocalcin that may regulate metabolic dysfunction and arterial calcification.[59] Studies have shown that the long-term use of VKA is associated with accelerated arterial calcification.[2, 60] VKDPs have high affinity for calcium-based matrices through their Gla residues[58] and the potential role of vitamin K in enhancing/activating anti-calcification processes (especially through MGP) has gained much interest.[1] However, a fine balance may exist as anti-thrombotic blocking agents of vitamin K on blood coagulation are an important treatment for preventing heart attacks and strokes. Although controversial, this has led to suggestions that individuals on anti-coagulants should consider avoiding vitamin K rich foods.[61, 62]

MGPs ability to reduce arterial calcification has been demonstrated in MGP knockout mice, which rapidly develop severe calcification and die from blood-vessel rupture.[63] Additionally, patients suffering from Keutel syndrome, where mutations to the MGP gene render the protein non-functional,[64] also develop extensive soft tissue calcification.[65] Polymorphisms within the MGP have been associated with both atherosclerosis and vascular calcification.[53, 66] As summarised in *Figure 2*, these results suggest that VKDP, and in particular MGP, may play a pivotal role in preventing vascular calcification in humans. As a result of the aforementioned work, the importance of vitamin K for cardiovascular health has recently been investigated in several observational studies and clinical trials.

Observational studies of vitamin K intake and clinical cardiovascular disease

To date, there are few prospective studies investigating the role of dietary vitamin K intake on the risk of CVD. These studies are particularly challenging as most databases have very limited data on the MK content in food, presenting a significant limitation. Likewise, PK intake is susceptible to social bias, because vegetable intake is generally over-reported, and is likely to be influenced by variation in vegetable intake across populations.[67] Validating the FFQs used

to estimate vitamin K2 intake is also challenging, as MKs are not typically detected in the circulation unless large quantities of MK-rich foods are consumed.[68] Furthermore, dietary PK is likely to be influenced by variation in vegetable intake across populations. The Rotterdam Study (n= 4807), one of the largest investigations to date, found that the relative risk (RR) of CHD mortality was reduced for individuals within the middle (21.6–32.7 µg/d) and upper (>32.7 µg/d) tertiles (RR 0.73 95%CI: 0.45-1.17 and RR 0.43 95%CI 0.24-0.77, respectively) compared to the lowest tertile (<21.6 µg/d) of vitamin K2 intake. Similarly, individuals in the highest tertile of vitamin K2 intake had lower odds (OR 0.48 95%CI 0.32-0.71) for severe aortic calcification compared to individuals in the lowest tertile of vitamin K2 intake.[69] Of interest, in the Rotterdam study vitamin K1 intake was not related to CHD. However, this was based on 233 events and there was a statistically non-significant 11% lower relative hazard (HR 0.89 95%CI 0.63-1.25) for individuals with the highest intake of PK.[69] Noteworthy, this data should be interpreted with caution as the reproducibility and validity of the FFQ used in the Rotterdam study for estimating vitamin K intake has been reported to be problematic.[70] The finding that higher dietary K2 is inversely related (per 10 µg/d, HR 0.91 95%CI 0.85-1.00) to incident CHD has been replicated in the Prospect-EPIC cohort of 16,057 women (aged 49–70 years) free of cardiovascular diseases at baseline.[71] This protective affect was attributed predominantly to long-chain vitamin K2 subtypes MK-7, MK-8 and MK-9.[71]

Higher dietary MK intake (per 10 µg/d) has also been associated with an 8% decrease in the relative hazard (HR 0.92 95%CI 0.85-0.99) for peripheral arterial disease (PAD) in 36,629 Dutch individuals form the Prospect-EPIC and MORGEN-EPIC cohorts.[72] In contrast, some studies have reported no association between vitamin K2 intake and CVD mortality or stroke.[73-75] It should be noted that the majority of these observational studies have been performed in the Netherlands; likely due to the Dutch having one of the most comprehensive databases for vitamin K (especially K2) content in food.[69] In contrast to MKs, high PK intake has not been

associated with CVD mortality,[73] CHD incidence and mortality[69, 71, 73, 76, 77]and PAD[72]. However, in two studies where inverse associations between PK intake and CHD incidence were identified, this relationship was attenuated once other dietary factors were considered.[76, 77] This suggests that other components of the diet may also be contributing to observed findings, and that the healthy dietary patterns associated with high PK intake may at least partially explain any relationship with reduced CHD risk, rather than PK itself.[76, 77] Similarly, studies examining the link between MK and CHD may be influenced by other components in MK-rich foods (e.g. saturated fats and sodium from processed meats) associated with CHD. Additionally, the lack of comprehensive databases for vitamin K1 and K2, and more specifically the long-chain MK (e.g. MK-5 to MK-13) content of commonly consumed foods limits our understanding of the role of vitamin K on vascular ageing and clinical cardiovascular events. As such, caution needs to be exercised when interpreting epidemiological studies of vitamin K with CHD outcomes.

Vitamin K supplementation trials with surrogate vascular outcomes

To our knowledge, no RCT have examined the effects of vitamin K (e.g. non-pharmaceutical) on clinical CVD outcomes. Here we discuss the evidence from RCTs investigating surrogate vascular outcomes such as coronary artery calcification (CAC), arterial stiffness and aortic valve calcification (AVC). A short 8-week open-label single-arm trial in renal transplant patients (n= 60) reported a significant decrease in arterial stiffness and 14.2% reduction in mean carotid femoral pulse wave velocity with higher doses (360 µg/day) of MK-7 supplementation. Of interest, the most benefit was gained in patients presenting with a vitamin K deficiency (undercarboxylated-MGP >500 pmol/L) at baseline.[78] Similar findings were reported in a separate single arm study that supplemented individuals (n=26, with at least one coronary risk factor) with 45 µg/day of MK-4 for one year. Specifically, reduced arterial stiffness (but no changes on CAC) were recorded only in the subset of vitamin K deficient

patients (n=4).[79] The aforementioned work indicate that the vitamin K status of populations need to be considered when examining potential beneficial of vitamin K for vascular outcomes. A double blind, placebo-controlled trial found MK-7 supplementation (180 µg/d) over three years reduced arterial stiffness in 244 healthy post-menopausal women. Furthermore, in women with higher arterial stiffness (stiffness index β scores ≥10.8) at baseline, improvements in local carotid pulse wave velocity were recorded, suggesting better elasticity.[80] Contrary to the aforementioned positive findings, a recent double blind, placebo-controlled trial supplementing MK-7 (360 µg/d) over 6 months in older individuals (~69 y, 24% female) with type II diabetes and CVD found no significant reduction in femoral arterial calcification measured by sodium fluoride positron emission tomography.[81]

To date, few trials have used PK supplements and tracked cardiovascular outcomes.[72] An open-label randomised placebo controlled trial of 72 (82% male, mean age 69 years) individuals reported that 12 months of high dose PK supplementation (2 mg/d) in patients with asymptomatic or mildly symptomatic aortic valve calcification (AVC) reduced AVC progression by ~12%.[82] Another 3-year double-blind placebo controlled trial examined the effect of a multivitamin supplement with 500 µg/d of PK (n=200) vs. an identical multivitamin supplement without PK (n=188) provided in healthy post-menopausal women and men.[83] In participants with high adherence to the supplements (≥ 85%, n=367), there was less CAC progression in the PK group than in the control group. Furthermore, in individuals with pre-existing CAC (Agatston score >10), those supplemented with PK had 6% less CAC progression. A randomised placebo controlled trial in postmenopausal women (n=150) receiving a supplement containing PK (1 mg/day) and vitamin D for 3 years demonstrated better carotid artery compliance and elasticity, measured by ultrasound of the common carotid artery.[84] Specifically, when comparing the vitamin K and D supplemented group to the placebo group, better arterial compliance coefficient (8.6%), distensibility coefficient (8.8%), and pulse

pressure (6.3%) were recorded. Collectively, these large population studies and RCTs suggest that vitamin K (PK and/or MKs) supplementation may play a vital role in blood vessel health. An important consideration for future work is to examine if the aforementioned cardiovascular health benefits observed after PK and/or MK supplementation may be limited only to populations presenting with vitamin K insufficiency and/or deficiency.

Conclusion

Until recently, a large proportion of research into vitamin K often only considered PK, with the role of MKs remaining largely ambiguous. It is still unclear as to what the most appropriate dietary recommendations for daily vitamin K should be, including if a differentiation between vitamin K1 and K2 need to be considered in nutritional guidelines promoted by public health organisations. Due to difficulties in the measurement of vitamin K2, the MK derivatives have also not been extensively tested for basic pharmacokinetic data or their concentration in numerous food items. Presently, epidemiological data suggest that high dietary MK intake may be protective against CHD mortality and coronary artery calcification.[69] However, the bulk of evidence for health benefits are limited to the Netherlands; as it currently possesses the most comprehensive vitamin K database, particularly for MKs. Therefore, observational studies are still needed to examine these findings in countries with different dietary patterns. There is also limited evidence from RCTs highlighting the benefit of PK supplements in regards to delaying the progression of CAC, and improving carotid artery compliance.[52, 84] Further investigation using vitamin K supplementation and its effect on clinical outcomes are still needed. Unfortunately, current research relating to vitamin K is hampered by the absence of comprehensive national databases that list both PK and MK content in food. However, with recent advances in the measurement of PK and MKs using HPLC-MS,[43] this could lead to the development of comprehensive region-specific vitamin K databases. This is especially important as regional variability in vitamin K content in food may limit the use of out of region

vitamin K databases.[2] Such work will also enable future investigations to explore the potential importance of dietary PK and MKs (separately or collectively) intake for a range of health outcomes. The application of these databases to large cohort studies will advance our understanding of the importance of vitamin K for human health, especially in the cardiovascular field. This will inform well design RCTs to establish causal effects between dietary vitamin K and a range of health outcomes.

Conflicts of interest

There are no conflicts of interest to declare.

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706 **Table 1:** A compilation of commonly consumed foods that contain vitamin K1 and/or K2 obtained from a range of existing vitamin K resources.

		Vitamin K content (µg/100 g or µg/100 ml)											
Type	**Food**	**K1**	**MK4**	**MK5**	**MK6**	**MK7**	**MK8**	**MK9**	**MK10**	**MK11**	**MK12**	**MK13**	**Country[1]**
Milk	Full cream milk	0.5	0.8	0.1	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Reduced fat milk	0.2	UD	-	-	-	-	-	-	-	-	-	USA[13]
	Soya milk	3.0	-	-	-	-	-	-	-	-	-	-	USA[13]
Fats & Oils	Margarine	93.2	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Butter	14.9	15	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Olive	53.7	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Butter & margarine blend	86.5	1.7	-	-	-	-	-	-	-	-	-	USA[13]
	Canola	71.3	UD	-	-	-	-	-	-	-	-	-	USA[13]
Cheese	Cheddar	2.4	9.5	UD	0.9	0.8	5.6	175	42.9	42.2	1.3	UD	USA[17]
	Low fat cheddar	0.5	1.8	UD	UD	0.7	4.0	22.6	1.5	16.3	UD	UD	USA[17]
	Cottage cheese	0.3	0.3	0.5	0.5	0.6	2.5	8	0.4	39.1	UD	UD	USA[17]
	Brie	4.9	12.5	UD	UD	UD	UD	UD	UD	-	-	-	FRN[40]
	Camembert	2.5	8.0	1.3	0.1	3.2	1.5	4	UD	-	-	-	FRN[40]
	Parmesan	20.6	UD	UD	0.01	0.1	0.2	UD	UD	-	-	-	ITL[40]
	Cream cheese	2.4	UD	-	-	-	-	-	-	-	-	-	USA[13]
Creams	Yogurt (regular full-fat)	0.4	0.7	UD	UD	UD	UD	13.2	1.6	8.4	UD	UD	USA[17]
	Standard full cream	2.4	9.3	UD	UD	UD	UD	442	85.2	44.3	2.6	UD	USA[17]
	Ice cream	0.3	UD	-	-	-	-	-	-	-	-	-	USA[13]
Breads	White bread	3.4	-	-	-	-	-	-	-	-	-	-	USA[13]
	Multigrain bread	1.5	-	-	-	-	-	-	-	-	-	-	USA[13]
	Rye bread	0.7	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
Meats	Beef	0.02	1.4	UD	UD	1.3	3.7	UD	UD	-	-	-	NL[40]
	Minced Meat	1.1	7.6	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]
	Deer	2.4	0.9	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]
	Chicken	UD	10.1	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]

	Pork	UD	1.4	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]
	Salmon	1.3	5.7	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]
	Salami	2.3	9	UD	UD	UD	UD	UD	UD	-	-	-	NL[40]
	Goose liver	10.9	369	UD	UD	UD	UD	UD	-	-	-	-	NL[40]
	Egg yolk	2.1	31.4	UD	0.7	UD	UD	UD	UD	-	-	-	NL[40]
	Bacon	0.3	3	UD	UD	UD	UD	UD	2.0	29.7	UD	UD	USA[17]
	Lamb	4.5	UD	-	-	-	-	-	-	-	-	-	USA[13]
	Ham	UD	4.3	-	-	-	-	-	-	-	-	-	USA[13]
	Pork sausage	0.3	18.3	-	-	-	-	-	-	-	-	-	USA[13]
	Tinned tuna (in water)	2.5	0	-	-	-	-	-	-	-	-	-	USA[13]
Fruits	Apples	3.0	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Pears	5.2	-	-	-	-	-	-	-	-	-	-	USA[13]
	Avocardos	21	-	-	-	-	-	-	-	-	-	-	USA[13]
Vegetables	Kale	817.0	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Broccoli	156.0	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Spinach	387.0	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Peas	36.0	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL[3]
	Cucumber	16.4	-	-	-	-	-	-	-	-	-	-	USA[13]
	Lettuce (green)	126.3	-	-	-	-	-	-	-	-	-	-	USA[13]
	Lettuce (red)	140.3	-	-	-	-	-	-	-	-	-	-	USA[13]
	Celery	29.3	-	-	-	-	-	-	-	-	-	-	USA[13]
	Carrots	13.2	-	-	-	-	-	-	-	-	-	-	USA[13]
	Cabbage	76.0	-	-	-	-	-	-	-	-	-	-	USA[13]
	Cauliflower	20.2	-	-	-	-	-	-	-	-	-	-	USA[13]
	Potatoes	2.0	-	-	-	-	-	-	-	-	-	-	USA[13]
	Tomatoes	7.9	-	-	-	-	-	-	-	-	-	-	USA[13]
	Capsicum (green)	7.4	-	-	-	-	-	-	-	-	-	-	USA[13]
	Zucchini	4.2	-	-	-	-	-	-	-	-	-	-	USA[13]
	Kidney beans	19.0	-	-	-	-	-	-	-	-	-	-	USA[13]

	Cashews	34.1	-	-	-	-	-	-	-	-	-	-	USA[13]
	Tofu (firm)	12.0	0.01	-	-	UD	-	-	-	-	-	-	JPN[15]
Fermented vegetables	Natto	32.1	UD	7.2	12.4	996.5	82.4	UD	UD	-	-	-	NL[40]
	Saurkraut	22.4	0.4	0.9	1.6	0.2	0.9	1.5	UD	-	-	-	NL[40]

[1] indicates origin of produce and/or country where vitamin K measurement was performed followed by reference. UD: undetected; -: unknown; USA: United States of America; NL: the Netherlands, JPN: Japan; FRN: France; ITY: Italy