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

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1 **Review article**

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23 **Abstract**

24 Cardiovascular disease is the leading cause of death and disability worldwide. Recent work

25 suggests a link between vitamin K insufficiency and deficiency with vascular calcification, a

26 marker of advanced atherosclerosis. Vitamin K refers to a group of fat-soluble vitamins

27 important for blood coagulation, reducing inflammation, regulating blood calcium metabolism,

28 as well as bone metabolism, all of which may play a role in promoting cardiovascular health.

29 Presently, there is a lack of a comprehensive vitamin K database on individual foods, which

30 are required to accurately calculate vitamin K1 and K2 intake for examination in

31 epidemiological studies. This has likely contributed to ambiguity regarding the recommended

32 daily intake of vitamin K, including whether vitamin K1 and K2 may have separate, partly

33 overlapping functions. This review will discuss the presence of: (i) vitamin K1 and K2 in the

34 diet; (ii) the methods of quantitating vitamin K compounds in foods; and (iii) provide an

35 overview of the evidence for the cardiovascular health benefits of vitamin K in observational

36 and clinical trials.

40

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

66   functional component in photosynthesis.[4] Vitamin K2 (MK) is composed of a group of

67   isoprenologs where the side chain is a polymer of isoprenoid units ranging between four to

68   thirteen repeats. MKs are named according to the number of repeating units,[4] with derivatives

69   including MK-4 to MK-13. For example, MK-4 has four unsaturated isoprenoid units (*Figure*

70   *1*). Despite having analogous structures the origins of MKs differ. MK-4 is synthesised from

71   PK and menadione by animals and humans.[2] All other MKs are synthesised by anaerobic

72   bacteria, which includes species present in the human microbiome.[4] Bacterial MKs are

73   important in metabolic respiration acting as components of the electron transport chain.[5] The

74   synthetic compound, menadione, is also referred to as vitamin K3 and is often added to animal

75   feed.[6]

76   *Dietary vitamin K*

77   Phylloquinone is present in the majority of foods including vegetables, fruits, nuts, bread,

78   cereals, oil, meat and animal products; however, quantities vary greatly.[7, 8] The highest

79   concentrations of PK found in green leafy vegetables, due to its role in photosynthesis.

80   Concentrations in these vegetables range between 400-700 µg/100 g.[4] Vegetable oils, such as

81   olive, canola and soybean, also contain considerable amounts of PK. These oils are widely used

82   in margarine, salad dressings, and the preparation of foods, such as bread. The contribution of

83   oils as part of commercially consumed foods towards total dietary vitamin K intake may be

84   greater than initially thought. For example, in the US, commercially prepared foods and mixed

85   dishes were the second highest contributors to PK intake, after vegetables.[9] The widespread

86   use of plant products in food production and their consumption by animals explains the

87   presence of PK in disparate food groups. The PK content in the Western diet makes up the

88   majority of vitamin K intake. It constitutes up to 90% of dietary vitamin K intake, of which 60-

89   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.

114   For example, the MK content of full fat cheddar cheese is five times higher than the total MK

115   content of reduced-fat cheddar (281 µg/100 g vs. 49 µg/100 g, respectively).[17]

116   Few studies have analysed the MK content in the Western diet. From this limited work, MKs

117   have been reported to constitute approximately 10% of dietary vitamin K intake.[4, 10, 11] Of note,

118   the major MK form is MK-4, which contributes 25-40% of the total MK in the diet.[10, 11] After

119   MK-4, the most nutritionally abundant MKs are MK-9, MK-8 and MK-7.[10, 18] However, as the

120   MK content in individual foods has not been systematically determined to the degree of PK,[2]

121   this may lead to errors in these estimates. Studies have highlighted meat and dairy products as

122   the best sources of MKs.[3] For example, a German study reported that 60% of all MKs came

123   from dairy products, with cheese consumption contributing 43% of total MK intake.[10]

124   Considering, the European diet is typically rich in cheese, it is thought to be a major contributor

125   of MK intake in such populations.[2] Presently, the most comprehensive resources for the

126   different MK content from a variety of animal and plant-based foods is from the Netherlands.[3]

127   Besides the aforementioned Dutch work, there is relatively little data for the MK content in the

128   diets of other countries. This is likely due to the absence of comprehensive databases of MK

129   content in individual foods, which are required to quantify MK intake at individual and

130   population levels.[2]

131   *Bioavailability of vitamin K*

132   Bioavailability is the rate and extent by which a nutrient is absorbed and becomes available to

133   the target tissue.[19] Alternatively, bioaccessibility relates to the amount of compound that is

134   released from its matrix in the gastrointestinal tract and is available for absorption.[19] Precise

135   quantitative assessments of vitamin K bioavailability in humans is challenging due to

136   unquantified tissue conversion of PK to MK-4 and the contributions of the gut microbiota.[20]

137   Both PK and MKs are absorbed from the intestine using the same mechanisms as other fat

138   soluble vitamins and lipids.[4] Apart from this shared absorption mechanism there are differences

139 in the bioavailability and bioaccessibility of vitamin K compounds.[3] For example, previous

140 work examining the absorption rate of PK and MK in a food matrix reported that MK

141 absorption from natto (Japanese fermeneted soybean) is approximately 10 times greater

142 compared to PK absorption from spinach. Of note, peak serum values for both PK and MK

143 appear to occur at the ~6 h post-prandial mark. It was also reported that bioavailability of PK

144 from foods is significantly reduced by its poor bioaccessibility from the food matrix.[3]

145 Specifically, only 5-10% of PK is absorbed from cooked vegetables; however, this can be

146 increased slightly by the presence of dietary lipids.[3] In contrast, PK absorption from oils and

147 supplements have much higher absorption rates (~200-400%) compared to vegetables.[3, 21, 22]

148 This indicates that PK as a nutrient can be well absorbed, but the vegetable food matrix may

149 present a barrier to absorption. Compared to PK, the limited amount of studies conducted on

150 long-chain MKs suggest they may have higher bioaccessibility from dietary sources[3].

151 Specifically, the absorption of long-chain MKs (MK-7, MK-8 and MK-9) from natto, cheese

152 and egg yolk is close to 100%.[3] Furthermore, these long-chain MKs have a longer half-life. For

153 example, in a study where 6 male volunteers were given 2 μmol of PK, MK-4 and MK-9, MK-

154 9 was still detectable in the serum at 48 h, while PK and MK-4 returned to baseline after 24

155 h.[23] Notably, the long half-life of MK-9 may not necessarily indicate increased bioavailability,

156 but instead non-preferential utilisation by tissues compared to PK and MK-4. A long half-life

157 may also indicate that long-chain MKs may be of particular importance for extrahepatic

158 tissues.[24] Despite the aforementioned work, the bioavailability of vitamin K compounds from

159 different food matrixes remain largely unknown. Nevertheless, current evidence suggest that

160 long-chain MKs may be more important than previously thought for vitamin K status, due to

161 the almost complete absorption of long-chain MKs and poor absorption of PKs from its main

162 dietary sources.[25]

163

164   *Non-dietary sources of menaquinones*

165   Despite, the large proportion of animal-based foods known to contain MKs, not all of the MKs

166   come from dietary sources. There are two known alternative sources, including PK conversion

167   into MK-4, and MK synthesis by the gut microbiota.[4, 26] The full extent of the conversion of

168   PK to MK-4 is unknown, but in the last decade progress has been made in understanding the

169   mechanism. Specifically, intestinal PK is cleaved in enterocytes to produce menadione (often

170   referred to as vitamin K3), which is prenylated by UbiA prenyltransferase-containing domain

171   1 (UBIAD1) to produce MK-4.[4, 26] A recent study in rats demonstrated that only 0.1% of oral

172   PK was converted into MK-4, and 2% converted into menadione.[27] The extent of this

173   conversion in humans remains unclear, but it is estimated to be 5-25% of PK intake.[28]

174   Therefore, MK-4 biosynthesis from PK may be a large source of MKs, but detailed human

175   studies are needed to examine this further. In addition, the contribution of microbial-

176   synthesised MKs to human nutrition is not fully understood. The majority of MKs in the gut

177   are synthesised by *Bacteroides*-one of the largest intestinal genera.[29, 30] *Bacteroides* primarily

178   produce MK-10 and MK-11, with small amounts of MK-7 to MK-9.[29, 31] There is a large pool

179   of MKs in the gut, with previous work suggesting an average of 1.8 mg of vitamin K2 in the

180   intestinal tract of humans.[32] Despite such reserves of vitamin K2 being over 20 times the AI

181   for adult males, the bioavailability of these MKs is thought to be limited.[33] Specifically, vitamin

182   K compounds are absorbed in the small intestine in a process requiring bile salts and pancreatic

183   enzymes, which are absent where the majority of bacteria colonise the colon.[34] Additionally,

184   the majority of microbial-synthesized MKs are bound by bacterial membranes and thus are not

185   available for absorption.[2] Therefore, while a large amount of MKs are produced by the gut

186   microbiota, their poor bioavailability at the point of synthesis limits their contribution to human

187   vitamin K nutrition.[31, 33] Presently, the exact bioavailability of microbial-synthesised MKs is

188   unknown, and more bioavailability studies are required to discern their role in human nutrition.

189   *Dietary recommendations for vitamin K*

190   Current dietary vitamin K recommendations are typically based on the intake of PK sufficient

191   to maintain coagulation.[20] This is problematic as the tissue-requirements of extrahepatic

192   vitamin K-dependant proteins for PK differs from the requirement of hepatic vitamin K-

193   dependant proteins.[2] Therefore, dietary intakes which are sufficient to maintain coagulation

194   may be inadequate in optimising other aspects of health that might be impacted by vitamin K

195   nutrition.[2, 28] However, any attempts to update the AI values for vitamin K1 or include vitamin

196   K2 would be hampered by a lack of dose-response studies and evidence on the occurrence,

197   absorption, function and content of MKs in the body or organs.[28, 35, 36] Difficulties in

198   quantitating the contribution of MKs synthesised by the gut microflora and tissue conversion

199   of PK are additional challenges when defining dietary requirement.[20] Furthermore, a recent

200   report by the European Commission highlighted the uncertainties in country-wide estimates of

201   vitamin intake data, which are caused by 'borrowing' vitamin K values from other countries

202   and substituting vitamin K values for individual food items for similar foods or food groups.[35]

203   For example, the USDA database[13] which was used to determine the vitamin K Nutrient

204   Reference Values for Australia and New Zealand.[37] Presently, the AI for vitamin K intake is

205   set at 60 and 70 µg/d for adult Australian females and males respectively.[37] Alternatively, it

206   has been proposed that even intakes of 90-120 µg/day (set by the USDA)[13] of vitamin K may

207   not be sufficient to induce complete carboxylation of all VKDPs.[38] Consequently, AI

208   recommendations in Australia for vitamin K intake may not accurately reflect actual vitamin

209   K intake (or demands), and may lead to ambiguity when determining optimal vitamin K

210   nutrition. There is a need for both the development of regional databases and the expansion of

211   existing databases to include both vitamin K1 and K2 content (including a range of MKs) in

212   commonly consumed foods. Such work will allow for improvements in the determination of

213   dietary vitamin K recommendations. Finally, due to differences in the bioavailability and/or

10

214   bioaccessibility of vitamin K2, future guidelines may need to consider distinguishing between

215   dietary vitamin K1 and K2.

216   *Vitamin K databases*

217   Databases of nutrients allow for the calculation of individual and population level intake of

218   nutrients, permitting for epidemiological investigation. Although there appears to be ~70

219   sources containing information relating to the vitamin K content of food, closer analysis of

220   these reveal that current databases are largely incomplete.[25] Specifically, only 12 databases list

221   the vitamin K content of individual food items, which is required to more accurately determine

222   vitamin K intake.[25] The Dutch database is the most comprehensive and includes PK and several

223   types of MKs, ranging from MK-4 to MK-10.[39] Although the largest database (in English) has

224   been developed by the USDA, this database does have limitations. Specifically, food items

225   have only been assessed for PK and MK-4 in the 25th release of the USDA database.[13]

226   Considering no other MKs have been included in the database, this limits its utility for

227   accurately estimating total vitamin K2 intake. The USDA database also lacks some commonly

228   consumed animal products, including lamb, veal and goat, which are potentially rich sources

229   of MKs.[13] The limited availability of comprehensive, national databases that lists both PK and

230   MK content in food hampers investigation of the relationship between dietary vitamin K1

231   and/or K2 intake and a range of health outcomes. Nevertheless, available databases provide

232   researchers with an indication of the types of food that are rich in vitamin K1 and K2 (including

233   the specific MKs). By reviewing pre-existing databases,[3, 13, 15, 17, 39, 40] we have compiled a list

234   of commonly consumed foods and their approximate vitamin K content in *Table 1*. However,

235   estimates of the respective vitamin K1 and K2 content of the foods listed in *Table 1* should be

236   interpreted with caution, as there is substantial variability of PK and MK content in food by

237   region.[2]

238

239   *Regional differences in the vitamin K content of food*

240   The content of both PK and MKs in foods are known to differ between regions.[2] For example,

241   beef cuts in Japan contain more MK-4 than the United States (15.0 ± 7.0 vs. 1.1 to 9.3 µg/100

242   g, respectfully).[14, 15] Similarly, the MK-4 content in egg yolk is four times higher in Japan

243   compared to the United States (64 vs 15.5 µg/100g).[2] The variability in MK-4 has been

244   attributed to the use of menadione in animal feeds and further differences in food production.[2]

245   Variability of up to 15% has been reported in the MK content (MK-6 to MK-10) of semi-hard

246   cheese varieties in three different European countries (France, Poland, Denmark).[16]

247   Furthermore, intra-country MK variability can be high (up to 80%), as reported between six

248   English cheddar cheeses.[16] Results are also complicated by a range of methodologies

249   (discussed in subsequent section) used to quantify PK and MKs. The regional differences in

250   food production, climate conditions, and dietary consumption patterns highlights the

251   importance of comprehensive and detailed databases that are population specific. Ideally,

252   databases should include information relating to the brand, type, method of creation and region

253   of grown foods alongside their PK and MK content. If regional differences in vitamin K content

254   of food are ignored, this can lead to inaccuracies when estimating vitamin K intake. Although

255   the investment in comprehensive databases is unlikely to occur in most countries, the creation

256   of more region specific databases on the vitamin K contents of foods would ultimately facilitate

257   better estimation of vitamin K1 and K2 intake.

258   **Measuring dietary vitamin K**

259   *Methods to quantitate phylloquinone and menaquinones in food*

260   To develop databases, a validated method of measuring PK and MKs simultaneously, with

261   accuracy and high throughput, is required. These methods must have high sensitivity and

262   selectivity due to the low vitamin K content in foods, and the complexity of the food matrix.[41]

263   A recent review concisely summarised quantification methods for determination of vitamin K

264 in various biological matrices including blood, urine and tissue.[42] However, the quantification

265 of vitamin K from food were not considered in detail. Existing methods to determine PK and

266 MK levels from food generally use reverse-phase HPLC with fluorescent detection or HPLC–

267 Mass Spectrometry (MS), with K1 or deuterated-K1 as the internal standard, respectively.[16, 43]

268 Gas Chromatography-MS (GC-MS) has also been used with deuterium-labelled internal

269 standards to accurately measure PK in serum.[44, 45] However, there are limitations in using GC

270 for measuring vitamin K, as high temperatures are needed to volatise vitamin K compounds

271 ($>300°C$).[46] Therefore, HPLC is the preferred method to separate vitamin K compounds for

272 analysis.[41] The emerging method to measure vitamin K in foods is a HPLC-MS method

273 developed by Karl et al.[43] This method has high versatility and may be used to measure PK and

274 all MKs in a variety of forms such as food, serum and faeces.[43] Based on current evidence,

275 HPLC-MS appears to be the best method to measure vitamin K compounds from foods.

276 *Methods of extracting vitamin K from the food matrix*

277 Vitamin K compounds are trapped in the matrix of foods and must be liberated in order to be

278 accurately measured.[3] Vitamin K compounds are unstable in alkali conditions and undergo

279 photo-oxidation upon exposure to ultraviolet light, which limits the available extraction

280 methods.[46] Samples must be stored in amber vials and all work done yellow light to prevent

281 this degradation.[43] Due to these limitations, the most common extraction method is liquid-

282 liquid extraction, with the exact process depending on the food matrix.[46, 47] Sample pre-

283 treatment is important to liberate K vitamers from the matrix. Most food groups, with the

284 exception of vegetable oils, are homogenised and sonicated to break up the food matrix.[3, 48]

285 This is especially important for vegetables, as it is essential to liberate PK from the thylakoid

286 membranes.[46] Furthermore, some fatty foods, such as oils or cheeses, undergo lipase digestion

287 or acid treatment to break down fats, thereby increasing purity.[16, 46] To our knowledge, there is

288   currently no standardised method for the extraction of vitamin K compounds from food,

289   however, numerous validated methods exist.[3, 43, 47]

Vitamin K and cardiovascular health

290

291   *Atherosclerotic cardiovascular disease*

292   Cardiovascular disease accounts for approximately 1 in 3 deaths worldwide.[49] Atherosclerotic

293   cardiovascular disease includes coronary heart disease (CHD), peripheral arterial disease and

294   cerebrovascular disease. Atherosclerotic diseases are caused by endothelial dysfunction,

295   inflammation and the slow build-up of cholesterol, fats and calcium deposits forming plaques

296   in arterial walls.[50] The plaques decrease lumen area of the artery, increasing resistance to blood

297   flow. Vulnerable plaques can rupture or erode leading to blood clots, which can obstruct blood

298   flow to the heart causing a heart attack, or to the brain causing a stoke. These plaques frequently

299   develop calcium mineral deposits, which further adds to plaque volume and leads to reduced

300   arterial compliance leading to an increased risk of organ damage.[50] As such, strategies capable

301   of preventing or delaying the progression of atherosclerosis are fundamental to improving

302   cardiovascular health.

303   *Vitamin K and vascular calcification*

304   The arterial (vascular) calcification process was once thought to be passive and a natural

305   consequence of ageing. However, it is now understood to be a complex biomineralisation

306   process which is actively regulated.[51] Observational studies have reported that vitamin K intake

307   is inversely associated with arterial calcification.[52] The best evidence for a causal role for

308   vitamin K in arterial calcification comes from vitamin K antagonists (VKA), genetic studies

309   and short-term randomised controlled trials.[52-54] VKA such as warfarin are anticoagulants used

310   to prevent thrombosis and pulmonary emboli. VKAs reduce the bioactivation of the hepatic

311   VKDP, such as prothrombin, which contributes to coagulation.[55] However, their effects are

312   non-specific and systemic, thus extrahepatic VKDP are inactivated during VKA treatment.[54,

313 [56] This includes Matrix-Gla Protein (MGP), an inhibitor of arterial calcification,[57] activated

314 protein-C an inhibitor of inflammation and endothelial cell apoptosis,[58] and osteocalcin that

315 may regulate metabolic dysfunction and arterial calcification.[59] Studies have shown that the

316 long-term use of VKA is associated with accelerated arterial calcification.[2, 60] VKDPs have

317 high affinity for calcium-based matrices through their Gla residues[58] and the potential role of

318 vitamin K in enhancing/activating anti-calcification processes (especially through MGP) has

319 gained much interest.[1] However, a fine balance may exist as anti-thrombotic blocking agents

320 of vitamin K on blood coagulation are an important treatment for preventing heart attacks and

321 strokes. Although controversial, this has led to suggestions that individuals on anti-coagulants

322 should consider avoiding vitamin K rich foods.[61, 62]

323 MGPs ability to reduce arterial calcification has been demonstrated in MGP knockout mice,

324 which rapidly develop severe calcification and die from blood-vessel rupture.[63] Additionally,

325 patients suffering from Keutel syndrome, where mutations to the MGP gene render the protein

326 non-functional,[64] also develop extensive soft tissue calcification.[65] Polymorphisms within the

327 MGP have been associated with both atherosclerosis and vascular calcification.[53, 66] As

328 summarised in *Figure 2*, these results suggest that VKDP, and in particular MGP, may play a

329 pivotal role in preventing vascular calcification in humans. As a result of the aforementioned

330 work, the importance of vitamin K for cardiovascular health has recently been investigated in

331 several observational studies and clinical trials.

332 *Observational studies of vitamin K intake and clinical cardiovascular disease*

333 To date, there are few prospective studies investigating the role of dietary vitamin K intake on

334 the risk of CVD. These studies are particularly challenging as most databases have very limited

335 data on the MK content in food, presenting a significant limitation. Likewise, PK intake is

336 susceptible to social bias, because vegetable intake is generally over-reported, and is likely to

337 be influenced by variation in vegetable intake across populations.[67] Validating the FFQs used

338 to estimate vitamin K2 intake is also challenging, as MKs are not typically detected in the

339 circulation unless large quantities of MK-rich foods are consumed.[68] Furthermore, dietary PK

340 is likely to be influenced by variation in vegetable intake across populations. The Rotterdam

341 Study (n= 4807), one of the largest investigations to date, found that the relative risk (RR) of

342 CHD mortality was reduced for individuals within the middle (21.6–32.7 µg/d) and upper

343 (>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)

344 compared to the lowest tertile (<21.6 µg/d) of vitamin K2 intake. Similarly, individuals in the

345 highest tertile of vitamin K2 intake had lower odds (OR 0.48 95%CI 0.32-0.71) for severe

346 aortic calcification compared to individuals in the lowest tertile of vitamin K2 intake.[69] Of

347 interest, in the Rotterdam study vitamin K1 intake was not related to CHD. However, this was

348 based on 233 events and there was a statistically non-significant 11% lower relative hazard

349 (HR 0.89 95%CI 0.63-1.25) for individuals with the highest intake of PK.[69] Noteworthy, this

350 data should be interpreted with caution as the reproducibility and validity of the FFQ used in

351 the Rotterdam study for estimating vitamin K intake has been reported to be problematic.[70] The

352 finding that higher dietary K2 is inversely related (per 10 µg/d, HR 0.91 95%CI 0.85-1.00) to

353 incident CHD has been replicated in the Prospect-EPIC cohort of 16,057 women (aged 49–70

354 years) free of cardiovascular diseases at baseline.[71] This protective affect was attributed

355 predominantly to long-chain vitamin K2 subtypes MK-7, MK-8 and MK-9.[71]

356 Higher dietary MK intake (per 10 µg/d) has also been associated with an 8% decrease in the

357 relative hazard (HR 0.92 95%CI 0.85-0.99) for peripheral arterial disease (PAD) in 36,629

358 Dutch individuals form the Prospect-EPIC and MORGEN-EPIC cohorts.[72] In contrast, some

359 studies have reported no association between vitamin K2 intake and CVD mortality or stroke.[73-

360 75] It should be noted that the majority of these observational studies have been performed in

361 the Netherlands; likely due to the Dutch having one of the most comprehensive databases for

362 vitamin K (especially K2) content in food.[69] In contrast to MKs, high PK intake has not been

363 associated with CVD mortality,[73] CHD incidence and mortality[69, 71, 73, 76, 77] and PAD[72].

364 However, in two studies where inverse associations between PK intake and CHD incidence

365 were identified, this relationship was attenuated once other dietary factors were considered.[76,

366 77] This suggests that other components of the diet may also be contributing to observed

367 findings, and that the healthy dietary patterns associated with high PK intake may at least

368 partially explain any relationship with reduced CHD risk, rather than PK itself.[76, 77] Similarly,

369 studies examining the link between MK and CHD may be influenced by other components in

370 MK-rich foods (e.g. saturated fats and sodium from processed meats) associated with CHD.

371 Additionally, the lack of comprehensive databases for vitamin K1 and K2, and more

372 specifically the long-chain MK (e.g. MK-5 to MK-13) content of commonly consumed foods

373 limits our understanding of the role of vitamin K on vascular ageing and clinical cardiovascular

374 events. As such, caution needs to be exercised when interpreting epidemiological studies of

375 vitamin K with CHD outcomes.

376 *Vitamin K supplementation trials with surrogate vascular outcomes*

377 To our knowledge, no RCT have examined the effects of vitamin K (e.g. non-pharmaceutical)

378 on clinical CVD outcomes. Here we discuss the evidence from RCTs investigating surrogate

379 vascular outcomes such as coronary artery calcification (CAC), arterial stiffness and aortic

380 valve calcification (AVC). A short 8-week open-label single-arm trial in renal transplant

381 patients (n= 60) reported a significant decrease in arterial stiffness and 14.2% reduction in

382 mean carotid femoral pulse wave velocity with higher doses (360 µg/day) of MK-7

383 supplementation. Of interest, the most benefit was gained in patients presenting with a vitamin

384 K deficiency (undercarboxylated-MGP >500 pmol/L) at baseline.[78] Similar findings were

385 reported in a separate single arm study that supplemented individuals (n=26, with at least one

386 coronary risk factor) with 45 µg/day of MK-4 for one year. Specifically, reduced arterial

387 stiffness (but no changes on CAC) were recorded only in the subset of vitamin K deficient

388   patients (n=4).[79] The aforementioned work indicate that the vitamin K status of populations

389   need to be considered when examining potential beneficial of vitamin K for vascular outcomes.

390   A double blind, placebo-controlled trial found MK-7 supplementation (180 µg/d) over three

391   years reduced arterial stiffness in 244 healthy post-menopausal women. Furthermore, in

392   women with higher arterial stiffness (stiffness index β scores ≥10.8) at baseline, improvements

393   in local carotid pulse wave velocity were recorded, suggesting better elasticity.[80] Contrary to

394   the aforementioned positive findings, a recent double blind, placebo-controlled trial

395   supplementing MK-7 (360 µg/d) over 6 months in older individuals (~69 y, 24% female) with

396   type II diabetes and CVD found no significant reduction in femoral arterial calcification

397   measured by sodium fluoride positron emission tomography.[81]

398   To date, few trials have used PK supplements and tracked cardiovascular outcomes.[72] An open-

399   label randomised placebo controlled trial of 72 (82% male, mean age 69 years) individuals

400   reported that 12 months of high dose PK supplementation (2 mg/d) in patients with

401   asymptomatic or mildly symptomatic aortic valve calcification (AVC) reduced AVC

402   progression by ~12%.[82] Another 3-year double-blind placebo controlled trial examined the

403   effect of a multivitamin supplement with 500 µg/d of PK (n=200) vs. an identical multivitamin

404   supplement without PK (n=188) provided in healthy post-menopausal women and men.[83] In

405   participants with high adherence to the supplements (≥ 85%, n=367), there was less CAC

406   progression in the PK group than in the control group. Furthermore, in individuals with pre-

407   existing CAC (Agatston score >10), those supplemented with PK had 6% less CAC

408   progression. A randomised placebo controlled trial in postmenopausal women (n=150)

409   receiving a supplement containing PK (1 mg/day) and vitamin D for 3 years demonstrated

410   better carotid artery compliance and elasticity, measured by ultrasound of the common carotid

411   artery.[84] Specifically, when comparing the vitamin K and D supplemented group to the placebo

412   group, better arterial compliance coefficient (8.6%), distensibility coefficient (8.8%), and pulse

413 pressure (6.3%) were recorded. Collectively, these large population studies and RCTs suggest

414 that vitamin K (PK and/or MKs) supplementation may play a vital role in blood vessel health.

415 An important consideration for future work is to examine if the aforementioned cardiovascular

416 health benefits observed after PK and/or MK supplementation may be limited only to

417 populations presenting with vitamin K insufficiency and/or deficiency.

418 **Conclusion**

419 Until recently, a large proportion of research into vitamin K often only considered PK, with

420 the role of MKs remaining largely ambiguous. It is still unclear as to what the most appropriate

421 dietary recommendations for daily vitamin K should be, including if a differentiation between

422 vitamin K1 and K2 need to be considered in nutritional guidelines promoted by public health

423 organisations. Due to difficulties in the measurement of vitamin K2, the MK derivatives have

424 also not been extensively tested for basic pharmacokinetic data or their concentration in

425 numerous food items. Presently, epidemiological data suggest that high dietary MK intake may

426 be protective against CHD mortality and coronary artery calcification.[69] However, the bulk of

427 evidence for health benefits are limited to the Netherlands; as it currently possesses the most

428 comprehensive vitamin K database, particularly for MKs. Therefore, observational studies are

429 still needed to examine these findings in countries with different dietary patterns. There is also

430 limited evidence from RCTs highlighting the benefit of PK supplements in regards to delaying

431 the progression of CAC, and improving carotid artery compliance.[52, 84] Further investigation

432 using vitamin K supplementation and its effect on clinical outcomes are still needed.

433 Unfortunately, current research relating to vitamin K is hampered by the absence of

434 comprehensive national databases that list both PK and MK content in food. However, with

435 recent advances in the measurement of PK and MKs using HPLC-MS,[43] this could lead to the

436 development of comprehensive region-specific vitamin K databases. This is especially

437 important as regional variability in vitamin K content in food may limit the use of out of region

438   vitamin K databases.[2] Such work will also enable future investigations to explore the potential

439   importance of dietary PK and MKs (separately or collectively) intake for a range of health

440   outcomes. The application of these databases to large cohort studies will advance our

441   understanding of the importance of vitamin K for human health, especially in the

442   cardiovascular field. This will inform well design RCTs to establish causal effects between

443   dietary vitamin K and a range of health outcomes.

444   **Conflicts of interest**

445   There are no conflicts of interest to declare.

446   **Acknowledgements**

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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.

Type	Food	Vitamin K content (µg/100 g or µg/100 ml)											Country[1]
		K1	MK4	MK5	MK6	MK7	MK8	MK9	MK10	MK11	MK12	MK13	
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]
	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]
Vegetables	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]

707   [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

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