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

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1 **Review article**

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

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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.

**37 Key words**

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

40

## 41 **Introduction**

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

## 58 **Vitamin K: phylloquinone and menaquinones**

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

66 functional component in photosynthesis.<sup>4</sup> Vitamin K<sub>2</sub> (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,<sup>4</sup> 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.<sup>2</sup> All other MKs are synthesised by anaerobic  
72 bacteria, which includes species present in the human microbiome.<sup>4</sup> Bacterial MKs are  
73 important in metabolic respiration acting as components of the electron transport chain.<sup>5</sup> The  
74 synthetic compound, menadione, is also referred to as vitamin K<sub>3</sub> and is often added to animal  
75 feed.<sup>6</sup>

#### 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.<sup>7, 8</sup> 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.<sup>4</sup> 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.<sup>9</sup> 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.<sup>4, 10, 11</sup>

90 Menaquinones are found in meat, dairy and fermented foods.<sup>2</sup> MK-4 is synthesised by the  
91 conversion of consumed PK or menadione in human or animal tissues. Therefore, dietary MK-  
92 4 is typically found in animal tissue and products, such as eggs or dairy.<sup>2</sup> The tissue or organ  
93 of an animal, the type of animal and country of origin (possibly related to feed) all affects the  
94 MK-4 content in food products.<sup>2</sup> For example, goose liver is a major storage tissue of vitamin  
95 K compounds and has the highest MK-4 content of any tissue (370 µg/100 g).<sup>3</sup> In comparison,  
96 goose leg has only 60 µg/100 g of MK-4.<sup>3</sup> Alternatively, chicken breast has more MK-4 than  
97 duck breast; 8.9 vs 3.6 µg/100 g, respectively.<sup>3</sup> Poultry and pork, have especially high MK-4  
98 content due to menadione in their feed, which is converted into MK-4.<sup>2, 12</sup> The MK-4 content  
99 of various poultry products vary greatly.<sup>2</sup> Presently, the most comprehensive tool to calculate  
100 the MK-4 content of food is provided by the United States Department of Agriculture (USDA)  
101 Food Composition Databases.<sup>13</sup> However, there is limited information on the MK-4 content in  
102 some commonly consumed meats, such as lamb, veal, and goat.<sup>14</sup> Other MKs (MK5-12) are  
103 synthesised by anaerobic bacteria<sup>4</sup>; however, information on their approximate content in food  
104 products are scarce.

105 Foods fermented with the MK-synthesising bacteria are abundant in long-chain MKs. For  
106 example, natto (fermented soybeans) has been reported to have the highest MK-7 content of  
107 any food, containing 936 µg/100 g.<sup>15</sup> The type of MKs produced is dependent on the type of  
108 bacteria and temperature conditions.<sup>5</sup> Notably, bacteria that produce MK-9 also produce MK-  
109 8, but in four-fold lower amounts. Thus, MK-9 and MK-8 co-occur in foods that are fermented.  
110 Other MKs are produced independent of each other.<sup>16</sup> Not all fermented foods contain MKs.  
111 For example, yeast has lost the enzymes required for MK synthesis,<sup>4, 5</sup> thus bread does not  
112 contain MKs.<sup>2</sup> Fermented dairy products, such as cheese, milk and yogurt, are significant  
113 sources of long-chain MKs.<sup>2, 17</sup> 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  $\mu\text{g}/100\text{ g}$  vs. 49  $\mu\text{g}/100\text{ g}$ , respectively).<sup>17</sup>

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.<sup>4, 10, 11</sup> Of note,  
118 the major MK form is MK-4, which contributes 25-40% of the total MK in the diet.<sup>10, 11</sup> After  
119 MK-4, the most nutritionally abundant MKs are MK-9, MK-8 and MK-7.<sup>10, 18</sup> However, as the  
120 MK content in individual foods has not been systematically determined to the degree of PK,<sup>2</sup>  
121 this may lead to errors in these estimates. Studies have highlighted meat and dairy products as  
122 the best sources of MKs.<sup>3</sup> 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.<sup>10</sup>  
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.<sup>2</sup> Presently, the most comprehensive resources for the  
126 different MK content from a variety of animal and plant-based foods is from the Netherlands.<sup>3</sup>  
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.<sup>2</sup>

### 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.<sup>19</sup> Alternatively, bioaccessibility relates to the amount of compound that is  
134 released from its matrix in the gastrointestinal tract and is available for absorption.<sup>19</sup> 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.<sup>20</sup>  
137 Both PK and MKs are absorbed from the intestine using the same mechanisms as other fat  
138 soluble vitamins and lipids.<sup>4</sup> Apart from this shared absorption mechanism there are differences



139 in the bioavailability and bioaccessibility of vitamin K compounds.<sup>3</sup> 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 fermented 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.<sup>3</sup>  
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.<sup>3</sup> In contrast, PK absorption from oils and  
147 supplements have much higher absorption rates (~200-400%) compared to vegetables.<sup>3, 21, 22</sup>  
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<sup>3</sup>.  
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%.<sup>3</sup> Furthermore, these long-chain MKs have a longer half-life. For  
153 example, in a study where 6 male volunteers were given 2  $\mu\text{mol}$  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.<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup>

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.<sup>4, 26</sup> 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.<sup>4, 26</sup> A recent study in rats demonstrated that only 0.1% of oral  
172 PK was converted into MK-4, and 2% converted into menadione.<sup>27</sup> The extent of this  
173 conversion in humans remains unclear, but it is estimated to be 5-25% of PK intake.<sup>28</sup>  
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.<sup>29, 30</sup> *Bacteroides* primarily  
178 produce MK-10 and MK-11, with small amounts of MK-7 to MK-9.<sup>29, 31</sup> 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.<sup>32</sup> 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.<sup>33</sup> 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.<sup>34</sup> Additionally,  
184 the majority of microbial-synthesized MKs are bound by bacterial membranes and thus are not  
185 available for absorption.<sup>2</sup> 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.<sup>31, 33</sup> 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.<sup>20</sup> 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.<sup>2</sup> 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.<sup>2, 28</sup> 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.<sup>28, 35, 36</sup> 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.<sup>20</sup> 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.<sup>35</sup>  
203 For example, the USDA database<sup>13</sup> which was used to determine the vitamin K Nutrient  
204 Reference Values for Australia and New Zealand.<sup>37</sup> Presently, the AI for vitamin K intake is  
205 set at 60 and 70  $\mu\text{g}/\text{d}$  for adult Australian females and males respectively.<sup>37</sup> Alternatively, it  
206 has been proposed that even intakes of 90-120  $\mu\text{g}/\text{day}$  (set by the USDA)<sup>13</sup> of vitamin K may  
207 not be sufficient to induce complete carboxylation of all VKDPs.<sup>38</sup> 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

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.<sup>25</sup> 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.<sup>25</sup> The Dutch database is the most comprehensive and includes PK and several  
223 types of MKs, ranging from MK-4 to MK-10.<sup>39</sup> 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 25<sup>th</sup> release of the USDA database.<sup>13</sup>  
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.<sup>13</sup> 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,<sup>3, 13, 15, 17, 39, 40</sup> 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.<sup>2</sup>

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.<sup>2</sup> For example,  
241 beef cuts in Japan contain more MK-4 than the United States ( $15.0 \pm 7.0$  vs. 1.1 to 9.3  $\mu\text{g}/100$   
242 g, respectfully).<sup>14, 15</sup> Similarly, the MK-4 content in egg yolk is four times higher in Japan  
243 compared to the United States (64 vs 15.5  $\mu\text{g}/100\text{g}$ ).<sup>2</sup> The variability in MK-4 has been  
244 attributed to the use of menadione in animal feeds and further differences in food production.<sup>2</sup>  
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).<sup>16</sup>  
247 Furthermore, intra-country MK variability can be high (up to 80%), as reported between six  
248 English cheddar cheeses.<sup>16</sup> 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.<sup>41</sup>  
263 A recent review concisely summarised quantification methods for determination of vitamin K

264 in various biological matrices including blood, urine and tissue.<sup>42</sup> 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.<sup>16, 43</sup>  
268 Gas Chromatography-MS (GC-MS) has also been used with deuterium-labelled internal  
269 standards to accurately measure PK in serum.<sup>44, 45</sup> However, there are limitations in using GC  
270 for measuring vitamin K, as high temperatures are needed to volatilise vitamin K compounds  
271 (>300°C).<sup>46</sup> Therefore, HPLC is the preferred method to separate vitamin K compounds for  
272 analysis.<sup>41</sup> The emerging method to measure vitamin K in foods is a HPLC-MS method  
273 developed by Karl et al.<sup>43</sup> 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.<sup>43</sup> 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.<sup>3</sup> 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.<sup>46</sup> Samples must be stored in amber vials and all work done yellow light to prevent  
281 this degradation.<sup>43</sup> Due to these limitations, the most common extraction method is liquid-  
282 liquid extraction, with the exact process depending on the food matrix.<sup>46, 47</sup> 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.<sup>3, 48</sup>  
285 This is especially important for vegetables, as it is essential to liberate PK from the thylakoid  
286 membranes.<sup>46</sup> Furthermore, some fatty foods, such as oils or cheeses, undergo lipase digestion  
287 or acid treatment to break down fats, thereby increasing purity.<sup>16, 46</sup> 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.<sup>3, 43, 47</sup>

## 290 **Vitamin K and cardiovascular health**

### 291 *Atherosclerotic cardiovascular disease*

292 Cardiovascular disease accounts for approximately 1 in 3 deaths worldwide.<sup>49</sup> 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.<sup>50</sup> 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 stroke. 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.<sup>50</sup> 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.<sup>51</sup> Observational studies have reported that vitamin K intake  
307 is inversely associated with arterial calcification.<sup>52</sup> 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.<sup>52-54</sup> 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.<sup>55</sup> However, their effects are  
312 non-specific and systemic, thus extrahepatic VKDP are inactivated during VKA treatment.<sup>54,</sup>

313 <sup>56</sup> This includes Matrix-Gla Protein (MGP), an inhibitor of arterial calcification,<sup>57</sup> activated  
314 protein-C an inhibitor of inflammation and endothelial cell apoptosis,<sup>58</sup> and osteocalcin that  
315 may regulate metabolic dysfunction and arterial calcification.<sup>59</sup> Studies have shown that the  
316 long-term use of VKA is associated with accelerated arterial calcification.<sup>2, 60</sup> VKDPs have  
317 high affinity for calcium-based matrices through their Gla residues<sup>58</sup> and the potential role of  
318 vitamin K in enhancing/activating anti-calcification processes (especially through MGP) has  
319 gained much interest.<sup>1</sup> 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.<sup>61, 62</sup>

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.<sup>63</sup> Additionally,  
325 patients suffering from Keutel syndrome, where mutations to the MGP gene render the protein  
326 non-functional,<sup>64</sup> also develop extensive soft tissue calcification.<sup>65</sup> Polymorphisms within the  
327 MGP have been associated with both atherosclerosis and vascular calcification.<sup>53, 66</sup> 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.<sup>67</sup> 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.<sup>68</sup> 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.<sup>69</sup> 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.<sup>69</sup> 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.<sup>70</sup> 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.<sup>71</sup> This protective affect was attributed  
355 predominantly to long-chain vitamin K2 subtypes MK-7, MK-8 and MK-9.<sup>71</sup>

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 from the Prospect-EPIC and MORGEN-EPIC cohorts.<sup>72</sup> In contrast, some  
359 studies have reported no association between vitamin K2 intake and CVD mortality or stroke.<sup>73-</sup>  
360 <sup>75</sup> 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.<sup>69</sup> In contrast to MKs, high PK intake has not been

363 associated with CVD mortality,<sup>73</sup> CHD incidence and mortality<sup>69, 71, 73, 76, 77</sup> and PAD<sup>72</sup>.  
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.<sup>76,</sup>  
366 <sup>77</sup> 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.<sup>76,77</sup> 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.<sup>78</sup> 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).<sup>79</sup> 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  $\beta$  scores  $\geq 10.8$ ) at baseline, improvements  
393 in local carotid pulse wave velocity were recorded, suggesting better elasticity.<sup>80</sup> 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.<sup>81</sup>

398 To date, few trials have used PK supplements and tracked cardiovascular outcomes.<sup>72</sup> 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%.<sup>82</sup> 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.<sup>83</sup> In  
405 participants with high adherence to the supplements ( $\geq 85\%$ , 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.<sup>84</sup> 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.<sup>69</sup> 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.<sup>52, 84</sup> 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,<sup>43</sup> 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.<sup>2</sup> 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.

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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 <sup>1</sup>
Milk	Full cream milk	0.5	0.8	0.1	UD	UD	UD	UD	UD	-	-	-	NL <sup>3</sup>
	Reduced fat milk	0.2	UD	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
	Soya milk	3.0	-	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
Fats & Oils	Margarine	93.2	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>3</sup>
	Butter	14.9	15	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>3</sup>
	Olive	53.7	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>3</sup>
	Butter & margarine blend	86.5	1.7	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
	Canola	71.3	UD	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
Cheese	Cheddar	2.4	9.5	UD	0.9	0.8	5.6	175	42.9	42.2	1.3	UD	USA <sup>17</sup>
	Low fat cheddar	0.5	1.8	UD	UD	0.7	4.0	22.6	1.5	16.3	UD	UD	USA <sup>17</sup>
	Cottage cheese	0.3	0.3	0.5	0.5	0.6	2.5	8	0.4	39.1	UD	UD	USA <sup>17</sup>
	Brie	4.9	12.5	UD	UD	UD	UD	UD	UD	-	-	-	FRN <sup>40</sup>
	Camembert	2.5	8.0	1.3	0.1	3.2	1.5	4	UD	-	-	-	FRN <sup>40</sup>
	Parmesan	20.6	UD	UD	0.01	0.1	0.2	UD	UD	-	-	-	ITL <sup>40</sup>
	Cream cheese	2.4	UD	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
Creams	Yogurt (regular full-fat)	0.4	0.7	UD	UD	UD	UD	13.2	1.6	8.4	UD	UD	USA <sup>17</sup>
	Standard full cream	2.4	9.3	UD	UD	UD	UD	442	85.2	44.3	2.6	UD	USA <sup>17</sup>
	Ice cream	0.3	UD	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
Breads	White bread	3.4	-	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
	Multigrain bread	1.5	-	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
	Rye bread	0.7	UD	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>3</sup>
Meats	Beef	0.02	1.4	UD	UD	1.3	3.7	UD	UD	-	-	-	NL <sup>40</sup>
	Minced Meat	1.1	7.6	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>40</sup>
	Deer	2.4	0.9	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>40</sup>
	Chicken	UD	10.1	UD	UD	UD	UD	UD	UD	-	-	-	NL <sup>40</sup>

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

	Cashews	34.1	-	-	-	-	-	-	-	-	-	-	USA <sup>13</sup>
	Tofu (firm)	12.0	0.01	-	-	UD	-	-	-	-	-	-	JPN <sup>15</sup>
<b>Fermented vegetables</b>	Natto	32.1	UD	7.2	12.4	996.5	82.4	UD	UD	-	-	-	NL <sup>40</sup>
	Sauerkraut	22.4	0.4	0.9	1.6	0.2	0.9	1.5	UD	-	-	-	NL <sup>40</sup>

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

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