# A high menaquinone intake reduces the incidence of coronary heart disease

G.C.M. Gast [a,b,*], N.M. de Roos [a], I. Sluijs [a,b], M.L. Bots [a], J.W.J. Beulens [a], J.M. Geleijnse [b], J.C. Witteman [c], D.E. Grobbee [a], P.H.M. Peeters [a], Y.T. van der Schouw [a]

[a] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands
[b] Department of Human Nutrition, Wageningen University, The Netherlands
[c] Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands

**Abstract** *Background and Aim:* Vitamin K dependent proteins have been demonstrated to inhibit vascular calcification. Data on the effect of vitamin K intake on coronary heart disease (CHD) risk, however, are scarce.
To examine the relationship between dietary vitamins $K_1$ and $K_2$ intake, and its subtypes, and the incidence of CHD.
*Methods and Results:* We used data from the Prospect–EPIC cohort consisting of 16,057 women, enrolled between 1993 and 1997 and aged 49–70 years, who were free of cardiovascular diseases at baseline. Intake of vitamin K and other nutrients was estimated with a food frequency questionnaire. Multivariate Cox proportional hazards models were used to analyse the data.
After a mean ± SD follow-up of 8.1 ± 1.6 years, we identified 480 incident cases of CHD. Mean vitamin $K_1$ intake was 211.7 ± 100.3 μg/d and vitamin $K_2$ intake was 29.1 ± 12.8 μg/d. After adjustment for traditional risk factors and dietary factors, we observed an inverse association between vitamin $K_2$ and risk of CHD with a Hazard Ratio (HR) of 0.91 [95% CI 0.85–1.00] per 10 μg/d vitamin $K_2$ intake. This association was mainly due to vitamin $K_2$ subtypes MK-7, MK-8 and MK-9. Vitamin $K_1$ intake was not significantly related to CHD.
*Conclusions:* A high intake of menoquinones, especially MK-7, MK-8 and MK-9, could protect against CHD. However, more research is necessary to define optimal intake levels of vitamin K intake for the prevention of CHD.
© 2008 Elsevier B.V. All rights reserved.

* Corresponding author. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Room STR 6.131, PO Box 85500, 3508 GA Utrecht, The Netherlands. Tel.: +31 88 755 9360/9384 (secr); fax: +31 88 755 5485.
E-mail address: g.c.m.gast@umcutrecht.nl (G.C.M. Gast).

## Introduction

Vitamin K is a fat-soluble vitamin that occurs in two biologically active forms; phylloquinone (vitamin $K_1$) and menaquinone (MK-4 through MK-10, vitamin $K_2$). Vitamin K is the co-substrate for the enzyme gamma-glutamyl carboxylase. This enzyme catalyzes the carboxylation of specific glutamic residues into gamma-carboxyglutamyl acid residues (Gla) in a limited number of proteins [1]. Gla-proteins have an increased affinity for calcium [2]. One of the Gla-proteins, Matrix-Gla Protein (MGP), is a powerful inhibitor of vascular calcification [3]. Vascular vitamin K deficiency might increase the amount of under-carboxylated, non-functional forms of MGP and thereby lead to increased calcium deposition, and, eventually, cardiovascular disease (CVD) [1,4]. Indeed, MGP-knock out mice were shown to develop severe vascular calcification [5]. MGP has also been found in close association with areas of calcification [4]. Moreover, the drug warfarin, which inhibits Gla residue formation, was shown to increase vascular calcification in in vitro and in vivo data from humans and rats [3,6].

In healthy subjects it has been shown that a substantial fraction of MGP occurs in undercarboxylated form with no biological activity [7], suggesting that most apparently healthy adults may be subclinically vitamin K deficient. Thus, although the intake of vitamin K in Western countries is sufficient for haemostasis, it might be suboptimal to maintain maximal gamma-carboxylation of the extra-hepatic vitamin K dependent proteins and thus to prevent vascular calcification [5]. These data are supported in a population-based survey, in which an inverse association between dietary intake of vitamin K and the presence of aortic calcification in elderly subjects was found [8]. Vitamins $K_1$ and $K_2$ might have different effects in prevention of vascular calcification; a high dose (30 mg/day) of vitamin $K_2$ (subtype MK-4), but not vitamin $K_1$, prevented medial calcification in rats [9]. Thus far, only a few prospective studies have been performed on dietary vitamin K intake and risk of CVD. The Rotterdam study reported a strong inverse association between vitamin $K_2$ intake and coronary heart disease (CHD) [10]. For vitamin $K_1$ the association was less obvious, which was comparable for two studies in US populations [11,12]. Data on vitamin $K_2$ were not available in these studies. The aim of the present study was to examine the relationship between dietary vitamins $K_1$ and $K_2$ intake and the incidence of CHD. In addition, we have analysed which subtypes of dietary vitamin $K_2$ intake are related to the incidence of CHD. For this purpose we used data of a cohort of 16,057 women who were followed for 8.1 years.

## Methods

### Population

PROSPECT is one of the two Dutch contributions to the European Prospective Investigation Into Cancer and Nutrition (EPIC) [13]. Between 1993 and 1997, 17,357 women aged 49—70 years, living in Utrecht and surroundings were enrolled in the PROSPECT—EPIC study. Participants were recruited through the regional breast cancer screening program. Women were excluded if they had a dietary intake below 500 kcal or higher than 6000 kcal per day ($n = 92$), did not fill in a general questionnaire ($n = 117$), did not consent to linkage with vital status registries or could not be traced in these registries ($n = 362$), reported a history of CHD (ICD-9; 410—414, 427.5) or CVA (ICD-9; 430—438) prior to the baseline measurements ($n = 654$), or reported use of vitamin K antagonists. The final study population consisted of 16,057 women.

### Baseline measurements

At baseline a general questionnaire containing questions on demographic characteristics, presence of chronic diseases, and risk factors for chronic diseases, such as hypertension, smoking habits and drinking of alcohol were administered. Systolic and diastolic blood pressure were measured twice at the right arm with an automated and calibrated oscil-lomat (Bosch & Son, Jungingen, Germany) with the subject in supine position, and the mean was calculated. Height and weight were measured and body mass index (BMI) was calculated as weight divided by height squared ($kg/m^2$). Smokers were categorized as current ($<10$, $11-20$, $>20$), past or never smokers. Hypertension was defined present based on several aspects: a physician-diagnosed self-report, measured hypertension ($>140$ systolic or $>90$ diastolic) or by the use of hypertensive medication. Hypercholesterolemia and diabetes mellitus were defined present when women reported that these disorders had been diagnosed by a physician [13].

### Dietary intake data

Energy and nutrient intake were estimated from a validated food frequency questionnaire (FFQ) [14]. The FFQ contained questions on the usual frequency of consumption of 77 main food items, preparation methods, and additions during the year preceding enrolment. Colour photographs were used to estimate portion size for 28-food items. Overall, the questionnaire allows the estimation of the average daily consumption of 178 foods, by asking for sub-items for several food items, like fruit and vegetables, in additional questions. Nutrient intake was calculated using the 1996 version of the Dutch national food composition table, except for total folate, for which the 2001 version was used [15,16]. This table does not contain information on vitamin K contents of foods and therefore the concentrations of vitamins $K_1$ and vitamin $K_2$ (MK-4 through MK-9) in a large series of Dutch foods were assessed at the Biochemistry Laboratory, Maastricht University [17]. For some foods, published data by others were used to update the dietary database for vitamin K [18—22]. In total, vitamin K contents of 260 foods were found and added to the NEVO (1996) food database. We used data from our previous validation study to estimate reliability of the FFQ to estimate vitamin K intake against 12—24-h recalls in 58 women, as described in more detail previously [14,23]. We observed a low relative validity of phylloquinone with Spearman correlations of 0.24. Relative validity for intake of menaquinone and MK-4 to MK-9 was reasonable with

Spearman correlation coefficients ranging from 0.51 for MK-7 to 0.72 for MK-5. In our study population of 16,057 women, vegetables contributed 82% of vitamin $K_1$ intake, while cheese contributed 54%, milk products 22% and meat 15% of vitamin $K_2$ intake.

## Morbidity and mortality

Data on morbidity were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. Admission files have been filed continuously from all general and university hospitals in The Netherlands from 1990. Data on sex, date of birth, dates of admission and discharge were recorded whenever a patient was discharged from hospital. One mandatory principal diagnosis, and up to nine optional additional diagnoses were reported. The principal diagnosis was used for the morbidity endpoint. All diagnoses were coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9). The database was linked to the cohort on the basis of birth date, gender, postal code, and general practitioner with a validated probabilistic method [24]. Information on vital status was obtained through linkage with the municipal administration registries. Causes of death were obtained from the Dutch Central Bureau of Statistics, coded according to the International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10). Whenever multiple events occurred, the first diagnosis was taken as endpoint. For our analyses, coronary events (ICD-9; 410−414, 427.5 or ICD-10; I20, I23−I25) were the endpoints of interest. For all women who had a CHD event, follow up ended at the date of diagnosis or, when hospital admission had not occurred, at the date of death. Moving out of The Netherlands ($n = 56$) and death due to causes other than cardiovascular disease ($n = 513$) were considered censoring events. All others ($n = 14,879$) were censored on January 1st 2004.

## Data analysis

Characteristics of the study population are described by means $\pm$ SD for normally distributed continuous variables and numbers and frequencies for categorical variables. Correlation between vitamins $K_1$, $K_2$ and its subtypes and other variables were calculated using Pearson correlation analysis. The person-time for each woman was calculated from the month of return of the baseline questionnaire to the month of diagnosis of CHD, the month of death from other causes, or the end of the study period (January 1, 2004). Cox proportional hazards regression models were used to estimate the HR for CHD event with 95% confidence intervals (CIs). Vitamins $K_1$, $K_2$ (per 10 μg increase) and its subtypes (per 1 μg increase) were evaluated as continuous variables. Univariate analysis (model 1) was performed to identify the crude relation between vitamin K and CHD.

Additionally, three multivariate models were used: model 2, including age at baseline, model 3, including classical cardiovascular risk factors and model 4, including dietary factors. Since calcium intake was strongly correlated with vitamin $K_2$ intake ($r > 0,5$), an additional model (5) was used that included calcium intake in the analyses for vitamin $K_2$ and its subtypes. Classical risk factors for CHD that were included as confounders were age, BMI, smoking, diabetes, hypertension, hypercholesterolemia, and alcohol intake. Dietary confounding factors for vitamin $K_1$ intake were dietary fibre, protein from plant origin and folic acid. The dietary confounding factors for vitamin $K_2$ included saturated fat, poly-unsaturated fatty acids and energy (kJ). All nutrients were adjusted for total energy intake by using the residual method [25]. Results were considered statistically significant at 2-sided $P \leq 0.05$. Statistical analyses were performed by using the program SPSS, version 11.5.

## Results

The study had a mean follow-up of $8.1 \pm 1.6$ years (y) and comprised 130,062 person-years. The mean age of the study group was $57.0 \pm 6.0$ y. Mean vitamin $K_1$ intake was $211.7 \pm 100.3$ μg/d and vitamin $K_2$ intake was $29.1 \pm 12.8$ μg/d. The subtypes MK-4, MK-8 and MK-9 were the major contributors to vitamin $K_2$ intake (Table 1). Vitamins $K_1$ and $K_2$ intake were not correlated ($r = -0.02$, $P = 0.01$).

Prevalence of hypertension, hypercholesterolemia and diabetes was more common among women with a high vitamin $K_1$ intake compared to those with a low vitamin $K_1$ intake. Intake of vitamins from fruits and vegetables, such as vitamin C and folic acid, were higher in the women with high vitamin $K_1$ intake (data available on request). Women with a high vitamin $K_2$ intake were more likely to be diabetic, but less likely to have hypercholesterolemia compared to those with a low vitamin $K_2$ intake. Intake of protein, saturated fat, cholesterol and calcium became higher with increasing quartiles of vitamin $K_2$ intake (Table 2).

In total, 480 women experienced a CHD event during follow-up, of which 32 were fatal. Vitamin $K_1$ intake was not associated with risk of CHD, a pattern that did not change after multivariate adjustment (Table 3).

For vitamin $K_2$, we found an inverse association with CHD (model 1, Table 4), with a HR of 0.89 [95% CI 0.82−0.96]. Additional adjustment for all other confounders did not change these results. When finally adding calcium to the model (model 5, Table 4), the estimate was still in the same direction, but the CI's became wider (0.92 [95% CI 0.83−1.01]).

**Table 1** Intake of energy adjusted vitamins $K_1$, $K_2$ and vitamin $K_2$ subtypes in 16,057 postmenopausal Dutch women.

| Vitamin K | Mean $\pm$ SD (μg/d) | Range (μg/d) |
|---|---|---|
| Vitamin $K_1$ | $211.7 \pm 100.3$ | 9.1−991.1 |
| Vitamin $K_2$ | $29.1 \pm 12.8$ | 0.9−128.0 |
| MK-4 | $7.1 \pm 2.1$ | 0.5−28.2 |
| MK-5 | $0.3 \pm 0.2$ | 0−2.1 |
| MK-6 | $0.3 \pm 0.2$ | 0−1.5 |
| MK-7 | $0.4 \pm 0.3$ | 0−2.2 |
| MK-8 | $6.0 \pm 3.4$ | 0−32.8 |
| MK-9 | $14.7 \pm 8.1$ | 0−81.9 |

**Table 2** Baseline characteristics of all 16,057 postmenopausal women according to quartiles of energy adjusted vitamin $K_2$ intake.

| | Energy adjusted vitamin $K_2$ intake ($\mu$g/d) | | | |
| --- | --- | --- | --- | --- |
| | <20 (N = 4014) | 20–27 (N = 4014) | 27–36 (N = 4018) | >36 (N = 4011) |
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Follow-up time, months | 96.6 ± 19.8 | 96.7 ± 19.3 | 97.4 ± 18.7 | 97.1 ± 19.2 |
| Age, years | 57.1 ± 6.1 | 57.3 ± 6.1 | 56.9 ± 5.9 | 56.8 ± 5.9 |
| BMI, kg/m$^2$ | 25.9 ± 4.0 | 26.0 ± 4.0 | 26.1 ± 4.0 | 25.9 ± 4.2 |
| Systolic blood pressure, mm Hg | 133.8 ± 20.6 | 133.6 ± 19.9 | 133.0 ± 19.7 | 132.1 ± 19.9 |
| Diastolic blood pressure, mm Hg | 79.5 ± 10.5 | 79.6 ± 10.4 | 79.3 ± 10.5 | 79.0 ± 10.4 |
| *Smoking status* | N (%) | N (%) | N (%) | N (%) |
| Never | 1694 (42.2) | 1809 (45.1) | 1776 (44.2) | 1748 (43.6) |
| Past | 1302 (32.4) | 1367 (34.1) | 1412 (35.1) | 1449 (36.1) |
| Current, <10 | 406 (10.1) | 403 (10.0) | 412 (10.3) | 408 (10.2) |
| Current, 11–20 | 440 (11.0) | 307 (7.6) | 296 (7.4) | 282 (7.0) |
| Current, >20 | 172 (4.3) | 128 (3.2) | 122 (3.0) | 124 (3.1) |
| Hypertension | 1163 (29.0) | 1155 (28.8) | 1125 (28.0) | 1108 (27.6) |
| Diabetes | 88 (2.2) | 94 (2.3) | 111 (2.8) | 131 (3.3) |
| Hypercholesterolemia | 263 (6.6) | 210 (5.2) | 162 (4.0) | 129 (3.2) |
| *Daily dietary intake*[a] | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Energy, kJ/d | 7549 ± 1888.6 | 7633 ± 1821.7 | 7612 ± 1757.6 | 7408 ± 1821.6 |
| Protein, g/d | 64.9 ± 10.0 | 68.8 ± 8.8 | 71.7 ± 8.8 | 75.4 ± 9.7 |
| Total fat, g/d | 66.8 ± 11.0 | 68.3 ± 10.1 | 69.1 ± 9.6 | 71.2 ± 10.4 |
| Mono-unsaturated fat, g/d | 25.1 ± 5.0 | 25.4 ± 4.5 | 25.6 ± 4.3 | 26.0 ± 4.5 |
| Poly-unsaturated fat, g/d | 13.7 ± 3.9 | 13.3 ± 3.5 | 12.9 ± 3.3 | 12.3 ± 3.2 |
| Saturated fat, g/d | 27.1 ± 5.2 | 28.7 ± 4.5 | 29.7 ± 4.6 | 32.1 ± 5.3 |
| Cholesterol, mg/d | 181.9 ± 49.3 | 196.1 ± 50.9 | 205.1 ± 53.4 | 215.4 ± 56.1 |
| Alcohol, g/d | 9.5 ± 15.0 | 8.8 ± 12.6 | 8.7 ± 12.1 | 9.4 ± 12.2 |
| Calcium, mg/d | 867.4 ± 276.5 | 1014.3 ± 256.6 | 1113.7 ± 252.5 | 1306.0 ± 291.3 |
| Folic acid, $\mu$g/d | 185.2 ± 41.4 | 187.6 ± 38.0 | 189.0 ± 37.9 | 187.6 ± 38.4 |

[a] All nutrients were adjusted for energy.

The protective effect of vitamin $K_2$ appeared to be due mainly to its subtypes MK-7 through MK-9 (Table 5), although correction for confounders slightly attenuated all associations.

Menaquinones mostly occur in animal products like meat, milk products and cheese [17]. We therefore also investigated the association between these products and CHD. All results remained similar after adjusting findings of $K_1$ for other dietary variables included in the models for $K_2$ and the other way around.

**Table 3** Hazard ratio's for CHD risk by energy adjusted vitamin $K_1$ intake per 10 $\mu$g increase.

| | HR (95% CI) | P-value |
| --- | --- | --- |
| Model 1[a] | 1.01 (1.00–1.01) | 0.02 |
| Model 2[b] | 1.00 (1.00–1.02) | 0.14 |
| Model 3[c] | 1.00 (0.99–1.01) | 0.51 |
| Model 4[d] | 1.00 (1.00–1.02) | 0.35 |

[a] Model 1 = Univariate (crude) model.
[b] Model 2 = Adjusted for age at baseline (continuous).
[c] Model 3 = Additionally adjusted for classical risk factors for CHD: BMI (<23, 23–27, >27), smoking (never, past, current <10, 11–20, >20), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), and energy adjusted alcohol intake (<1, 1–5, 5–15, 15–30, >30 g/d).
[d] Model 4 = Further adjusted for energy-adjusted dietary factors: dietary fibre, protein from plant origin and folic acid.

**Table 4** Hazard ratio's for CHD risk by energy adjusted vitamin $K_2$ intake per 10 $\mu$g increase.

| | HR (95% CI) | P-value |
| --- | --- | --- |
| Model 1[a] | 0.89 (0.82–0.96) | 0.003 |
| Model 2[b] | 0.90 (0.84–0.98) | 0.009 |
| Model 3[c] | 0.93 (0.86–1.00) | 0.05 |
| Model 4[d] | 0.91 (0.85–1.00) | 0.04 |
| Model 5[e] | 0.92 (0.83–1.01) | 0.08 |

[a] Model 1 = Univariate (crude) model.
[b] Model 2 = Adjusted for age at baseline (continuous).
[c] Model 3 = Additionally adjusted for classical risk factors for CHD: BMI (<23, 23–27, >27), smoking (never, past, current <10, 11–20,>20), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), and energy adjusted alcohol intake (<1, 1–5, 5–15, 15–30, >30 g/d).
[d] Model 4 = Further adjusted for energy-adjusted dietary factors: saturated fat, poly-unsaturated fatty acids and energy (kJ).
[e] Model 5 = Model 4 *with* calcium.

**Table 5** Hazard ratio's for CHD risk by subtypes of energy adjusted vitamin $K_2$ intake per 1 $\mu$g increase.

|  | HR (95% CI) | P-value |
|---|---|---|
| MK-4[a] | | |
| Model 1[a] | 1.04 (0.99–1.10) | 0.11 |
| Model 2[b] | 1.05 (1.00–1.11) | 0.08 |
| MK-5[a] | | |
| Model 1[a] | 0.82 (0.46–1.45) | 0.49 |
| Model 2[b] | 1.03 (0.43–2.51) | 0.94 |
| MK-6[a] | | |
| Model 1[a] | 0.70 (0.40–1.24) | 0.22 |
| Model 2[b] | 0.75 (0.37–1.51) | 0.42 |
| MK-7[a] | | |
| Model 1[a] | 0.68 (0.46–1.00) | 0.05 |
| Model 2[b] | 0.67 (0.42–1.08) | 0.10 |
| MK-8[a] | | |
| Model 1[a] | 0.97 (0.94–1.00) | 0.03 |
| Model 2[b] | 0.96 (0.93–1.00) | 0.05 |
| MK-9 | | |
| Model 1[a] | 0.99 (0.97–1.00) | 0.02 |
| Model 2[b] | 0.98 (0.97–1.00) | 0.03 |

[a] Model 1 = Adjusted for age at baseline, classical risk factors for CHD: BMI (<23, 23–27, >27), smoking (never, past, current <10, 11–20, >20), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), energy adjusted alcohol intake (<1, 1–5, 5–15, 15–30, >30 g/d), energy-adjusted dietary factors: saturated fat, poly-unsaturated fatty acids, energy (kJ).
[b] Model 2 = Model 1 *with* calcium.

## Discussion

This study shows that a higher dietary intake of vitamin $K_2$ was significantly associated with a lower incidence of CHD. The association was mainly driven by vitamin $K_2$ subtypes MK-7, MK-8 and MK-9. No association between vitamin $K_1$ intake and CHD was observed after correction for confounders.

The main strengths of our study are its size, the small percentage of loss to follow-up, and the long follow-up, the latter enabling us to study clinically manifest CHD endpoints, which are more informative than intermediate endpoints. The main limitation of this study is the relative validity of our FFQ to estimate intake of vitamin K. For vitamin $K_1$ intake the relative validity was low, suggesting that our results regarding vitamin $K_1$ should be interpreted with caution. For vitamin $K_2$ intake, however the relative validity was reasonable, with correlation coefficients very similar compared to other nutrients estimated using FFQ's [23].

Our study population was limited to postmenopausal women. Although this limits generalisibility of our results to men, this population may be of particular relevance for this research question. During the menopausal phase, an acceleration of bone loss is seen with a rate of 3–10%. Recent studies have shown that reduced bone mineral density may be a risk factor for CVD [26].

In our analyses we did not take lipid lowering drugs into account, because only 12 women reported the use of these medications. Instead, we have corrected for self reported hypercholesterolemia. This is due to the fact that only hypercholesterolemia is not an indication for treatment in The Netherlands, but treatment is indicated for women with a high enough 10-year risk, such is a manifest myocardial infarction or stroke. However, we excluded women with prevalent disease.

In our analyses for vitamin $K_2$ we additionally corrected for calcium intake. Obviously, it is a point of discussion whether or not it is necessary to adjust for calcium intake, as calcium and vitamin $K_2$ intake were strongly correlated with each other. However, it seems that adding calcium to the model has led to an imprecise estimate, as the estimate remained similar, but the CI's became wider. This was also observed for the subtypes of vitamin $K_2$. More research into this is necessary.

In our study, we did not have access to patient charts and were therefore not able to validate the diagnosis of CHD. However, validation studies for non-fatal coronary outcomes have already been conducted in other centres of the EPIC study using similar registries to identify potential cases. Overall, 89% of suspected non-fatal coronary outcomes were confirmed against internationally agreed criteria [27]. These findings suggest that outcome misclassification is probably not a major issue in our study.

Our findings that usual intakes of vitamin $K_2$ were not strongly, but statistically significantly associated with lower incidence of CHD, support the data from the Rotterdam study [10]. Moreover, recent results from our group show similar inverse associations for vitamin $K_2$ with coronary calcification [28]. Mechanistically, there are indications that vitamin $K_2$ is involved in vascular calcification. Arteries with atherosclerotic plaques were found to have a 20–50 fold lower vitamin $K_2$ concentration than arteries without plaque in the same human body [2], suggesting that vitamin $K_2$ protects against calcification. In addition, high doses of vitamin $K_2$ (subtype MK-4) were shown to prevent medial calcification in rats [9].

Until now, only the Nurses' Health Study did find a borderline significant protective effect of vitamin $K_1$ and CHD. However, as this weak association was attenuated by dietary factors associated with risk of CVD, the authors concluded that dietary and lifestyle patterns associated with vitamin $K_1$ intake, rather than the nutrient itself might account for this association [11]. Indeed, the Rotterdam study [10] and the Health Professionals' Follow-up Study [12] could not confirm a protective effect for vitamin $K_1$. Similarly, recent findings from our group [28] in addition to our results did not show an association between vitamin $K_1$ and coronary calcification and CHD respectively. Residual confounding by healthier dietary patterns and lifestyles associated with vitamin $K_1$ intake might explain the lack of association [11,12]. The low relative validity of vitamin $K_1$ as estimated by our FFQ may be involved also.

The concept of proposing beneficial effect to vitamin $K_2$ seems to have different basis as for vitamin $K_1$. Vitamin $K_1$ has been associated with a heart-healthy dietary pattern in the earlier work in the USA and this attenuated their associations with CHD [11,12]. Vitamin $K_2$ has different sources and relate to different dietary patterns than vitamin $K_1$. This suggests that the risk reduction with vitamin $K_2$ is not driven by dietary patterns, but through biological effects. Indeed, the different effects of vitamin $K_2$ and vitamin $K_1$ seem to reflect differences in metabolism

as a result of different distributions over plasma lipoproteins. Vitamin $K_1$ is primarily transported with the triacylglycerol-rich lipoprotein fraction, which is mainly cleared by the liver [29]. Therefore, vitamin $K_1$ is very effectively cleared from circulation by the liver to function as a cofactor for proteins in blood coagulation [30]. Hence, accumulation and utilization of vitamin $K_1$ in extrahepatic tissues such as the vessel wall is lower.

In contrast to vitamin $K_1$, however, vitamin $K_2$ is found in both triacylglycerol-rich lipoprotein and low-density protein, the latter forming a major transport system to extrahepatic tissues [29]. This has been confirmed by animal experiments showing that extrahepatic tissues, such as the vessel wall, preferentially accumulate vitamin $K_2$ [9]. Menaquinone could therefore more successfully influence MGP and coronary calcification. Moreover, an animal study showed that MK-4 but not phylloquinone inhibited warfarin-induced coronary calcification [9].

Thus, although our findings may have important practical implications on CVD prevention, it is important to mention that in order to increase the intake of vitamin $K_2$, increasing the portion vitamin $K_2$ rich foods in daily life might not be a good idea. Vitamin $K_2$ might be, for instance more relevant in the form of a supplement or in low-fat dairy. More research into this is necessary.

In our population, the total supply of vitamins $K_1$ and $K_2$ was sufficient for optimal carboxylation of the coagulation enzymes in the liver. However, prospective studies, including randomized trials, evaluating the relationship of vitamin K supplementation with vascular calcification are needed to define optimal intake levels of vitamin K intake for the inhibition of vascular calcification.

Of the subtypes of vitamin $K_2$, it appeared that particularly MK-7, MK-8 and MK-9 affected the risk of CHD. A stronger effect of the longer subtypes could be due to a slower hepatic clearance of these subtypes, making them longer available for carboxylation reactions [2].

In conclusion, the findings in this prospective study among elderly women suggest that a high intake of vitamin $K_2$ might protect against CHD. This may particularly reflect an effect of the higher subtypes of vitamin $K_2$, mainly MK-7, MK-8 and MK-9.

## Conflict of interest

The authors have no conflict of interest.

## Funding

None.

## Disclosure statement

None.

## Acknowledgements

## References

[1] Furie B, Bouchard BA, Furie BC. Vitamin K-dependent biosynthesis of y-carboxyglutamic acid. Blood 1999;93:1798—808.

[2] Vermeer C, Braam L. Role of K vitamins in the regulation of tissue calcification. J Bone Miner Metab 2001;19:201—6.

[3] Shanahan CM, Proudfoot D, Farzaneh-Far A, Weissberg PL. The role of Gla proteins in vascular calcification. Crit Rev Eukaryot Gene Expr 1998;8:357—75.

[4] Spronk HM, Soute BA, Schurgers LJ, Cleutjens JP, Thijssen HH, De Mey JG, et al. Matrix Gla protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. Biochem Biophys Res Commun 2001;289:485—90.

[5] Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78—81.

[6] Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arterioscler Thromb Vasc Biol 1998;18:1400—7.

[7] Schurgers LJ, Teunissen KJ, Knapen MH, Kwaijtaal M, van DR, Appels A, et al. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. Arterioscler Thromb Vasc Biol 2005;25:1629—33.

[8] Jie K-SG, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K intake and osteocalcin levels in women with and without aortic atherosclerosis: a population-based study. Atherosclerosis 1995;116:117—23.

[9] Spronk HM, Soute BA, Schurgers LJ, Thijssen HH, De Mey JG, Vermeer C. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. J Vasc Res 2003;40:531—7.

[10] Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam study. J Nutr 2004;134:3100—5.

[11] Erkkila AT, Booth SL, Hu FB, Jacques PF, Manson JE, Rexrode KM, et al. Phylloquinone intake as a marker for coronary heart disease risk but not stroke in women. Eur J Clin Nutr 2005;59:196—204.

[12] Erkkila AT, Booth SL, Hu FB, Jacques PF, Lichtenstein AH. Phylloquinone intake and risk of cardiovascular diseases in men. Nutr Metab Cardiovasc Dis 2007;17:58—62.

[13] Boker LK, van Noord PA, van der Schouw YT, Koot NV, de Mesquita HB, Riboli E, et al. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. European Prospective Investigation into Cancer and Nutrition. Eur J Epidemiol 2001;17:1047—53.

[14] Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen A, Pols MA, Van Staveren WA, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26:S37—48.

[15] Nederlands Voedingsstoffenbestand. Den Haag, Dutch: Voorlichtingsbureau voor de Voeding; 1996.

[16] Nederlands Voedingsstoffenbestand. Den Haag, Dutch: Voorlichtingsbureau voor de Voeding; 2001.

[17] Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Haemostasis 2000;30:298—307.

[18] Suttie J. Vitamin K and human nutrition. J Am Diet Assoc 1992;92:585—90.

[19] Shearer MJ, Bach A, Kohlmeier M. Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. J Nutr 1996;126:1181S—6S.

[20] Booth SL, Madabushi HT, Davidson KW, Sadowski JA. Tea and coffee brews are not dietary sources of vitamin K-1 (phylloquinone). J Am Diet Assoc 1995;95:82—3.

[21] Ferland G, MacDonald DL, Sadowski JA. Development of a diet low in vitamin K-1 (phylloquinone). J Am Diet Assoc 1992;92:593—7.

[22] Booth SL, Sadowski JA, Weihrauch JL, Ferland G. Vitamin K-1 (phylloquinone) content of foods: a provisional table. J Food Comp Anal 1993;6:109—20.

[23] Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26:S49—58.

[24] Herings RM, Bakker A, Stricker BH, Nap G. Pharmaco-morbidity linkage: a feasibility study comparing morbidity in two pharmacy based exposure cohorts. J Epidemiol Community Health 1992;46:136—40.

[25] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S—8S.

[26] Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR. Relationship between osteoporosis and cardiovascular disease in postmenopausal women. J Bone Miner Res 2005;20:1912—20.

[27] Danesh J, Saracci R, Berglund G, Feskens E, Overvad K, Panico S, et al. EPIC-heart: the cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. Eur J Epidemiol 2007;22:129—41.

[28] Beulens JWJ, Bots ML, Atsma F, Bartelink MLEL, Prokop M, Geleijnse JM, et al. High dietary menaquinone intake is associated with reduced coronary calcification. Atherosclerosis 2008. doi:10.1016/j.atherosclerosis.2008.07.010.

[29] Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. Biochim Biophys Acta 2002;1570:27—32.

[30] Cranenburg EC, Schurgers LJ, Vermeer C. Vitamin K: the coagulation vitamin that became omnipotent. Thromb Haemost 2007;98:120—5.