Dietary Intake of Menaquinone Is Associated with a Reduced Risk of Coronary Heart Disease: The Rotterdam Study[1]

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ABSTRACT Vitamin K–dependent proteins, including matrix Gla-protein, have been shown to inhibit vascular calcification. Activation of these proteins via carboxylation depends on the availability of vitamin K. We examined whether dietary intake of phylloquinone (vitamin K-1) and menaquinone (vitamin K-2) were related to aortic calcification and coronary heart disease (CHD) in the population-based Rotterdam Study. The analysis included 4807 subjects with dietary data and no history of myocardial infarction at baseline (1990–1993) who were followed until January 1, 2000. The risk of incident CHD, all-cause mortality, and aortic atherosclerosis was studied in tertiles of energy-adjusted vitamin K intake after adjustment for age, gender, BMI, smoking, diabetes, education, and dietary factors. The relative risk (RR) of CHD mortality was reduced in the mid and upper tertiles of dietary menaquinone compared to the lower tertile [RR = 0.73 (95% CI: 0.45, 1.17) and 0.43 (0.24, 0.77), respectively]. Intake of menaquinone was also inversely related to all-cause mortality [RR = 0.91 (0.75, 1.09) and 0.74 (0.59, 0.92), respectively] and severe aortic calcification [odds ratio of 0.71 (0.50, 1.00) and 0.48 (0.32, 0.71), respectively]. Phylloquinone intake was not related to any of the outcomes. These findings suggest that an adequate intake of menaquinone could be important for CHD prevention. J. Nutr. 134: 3100–3105, 2004.

KEY WORDS: • *phylloquinone* • *menaquinone* • *vitamin K* • *coronary heart disease* • *population-based study*

Vitamin K is an essential cofactor for the conversion of glutamate into γ-carboxyglutamate (carboxylation) and plays an important role in hemostasis through the activation of blood coagulation and anticoagulation factors in the liver (1–3). Vitamin K–dependent proteins, however, are also present in extrahepatic tissues such as bone and vascular tissue (3–8). Matrix Gla-protein (MGP)[3] was identified in human atherosclerotic plaque, where it may prevent calcium precipitation similarly as it does in bone. This was clearly demonstrated in MGP-knockout mice that die from massive aortic and coronary calcification shortly after birth (9).

In healthy individuals dietary intake of vitamin K fills the needs for coagulation, but little is known about vitamin K requirements for extrahepatic processes (1,4). Carboxylation of vitamin K–dependent proteins conveys the ability to bind calcium ions, which is essential for their biological activity (1). Elevated levels of undercarboxylated osteocalcin, a vitamin K–dependent protein involved in bone metabolism, may result from subclinical vitamin K deficiency and are frequently observed in the elderly (7). We hypothesize that an inadequate dietary intake of vitamin K may similarly result in undercarboxylation of vascular MGP, leading to enhanced calcification of atherosclerotic lesions and, consequently, an increased risk of coronary heart disease. Vitamin K is obtained from the diet as phylloquinone (vitamin K-1) and menaquinone (MK-*n*, vitamin K-2). Phylloquinone is abundant in dark-green leafy vegetables and vegetable oils. The main dietary sources for menaquinone in Western populations are meats (MK-4) and fermented foods, especially cheese and curds (mainly MK-8 and MK-9) (8,10).

We examined the association of dietary intake of phylloquinone and menaquinone with the incidence of coronary heart disease (CHD), all-cause mortality, and aortic calcification in the population-based Rotterdam Study.

[1] Supported by a grant from the Health Research and Development Council (ZON), The Hague, The Netherlands (Grant No. 28.2388).
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[3] Abbreviations used: CHD, coronary heart disease; ECG, electrocardiogram; GP, general practitioner; ICD-10, International Statistical Classification of Diseases and Related Health Problems, 10th revision; MGP, matrix Gla-protein; MI, myocardial infarction; MK-*n*, menaquinone-*n*; OR, odds ratio; RR, relative risk; TGRLP, triacylglycerol-rich lipoprotein.

Manuscript received 1 April 2004. Initial review completed 28 April 2004. Revision accepted 25 August 2004.

METHODS

The Rotterdam Study. The Rotterdam Study is a prospective, population-based study to assess the occurrence of diseases of the elderly and to clarify their determinants (11). The cohort comprises 7983 men and women aged 55 y and over (78% of the eligible population) who live in a defined district of Rotterdam. From August 1990 until June 1993, trained research assistants collected data on current health, use of medication, medical history, lifestyle, and risk indicators for chronic diseases during an extensive home interview. The participants subsequently visited the study center for clinical examination and assessment of diet.

Assessment of diet and vitamin K intake. The participants indicated on a checklist all foods and beverages that they consumed more than once a month during the preceding year. The completed checklist formed the basis for an interview at the study center by a trained dietician. A validated, semiquantitative food-frequency questionnaire was used (12). Intake of total energy, alcohol, macronutrients, and a large number of micronutrients was computed using Dutch food composition tables (13).

Concentrations of phylloquinone and menaquinone (MK-4 through MK-10) in a large series of Dutch foods were assessed at the laboratory of one of the authors (C.V., Cardiovascular Research Institute Maastricht, University of Maastricht), as described previously (10,14). Briefly, food items were supplemented with internal standard (2′,3′-dihydrophylloquinone) and homogenized in water/propanol-2 (1:2, v:v). The homogenates were extracted with hexane, prepurified on Silica Sep-pak cartridges (Millipore), and analyzed by HPLC using an Econosphere C-18 reversed phase column (Alltech) with fluorescence detection after postcolumn electrochemical reduction. Isocratic elution was performed using a mixture of methanol-isopropanol-water-tetramethylammonium octahydrotriborate (88.5:10:1.5:0.045 by volume). The effluent of the column was reduced with a Coulochem 5010 analytical cell (Environmental Sciences Associates) maintained at a potential of −1.5 V and analyzed with a Jasco 821-FP spectrofluorometric detector (Separations Analytical Instruments). The excitation wavelength was 246 nm and the emission wavelength was 430 nm. Because of the long retention times for the long-chain menaquinones (MK-7 through MK-10) the flow was increased from 0.5 to 1.0 mL/min at 11 min after injection. Phylloquinone and menaquinones were recorded in the same run. Authentic menaquinones (Hoffmann-La Roche) served as reference materials.

For some foods, we also used published data by others to update our dietary database for vitamin K (3,8,15–18). Main dietary sources of phylloquinone in our study were green leafy vegetables and vegetable oils. Menaquinone was present in meats and eggs (MK-4 only), fish, sauerkraut, cheese, and other dairy produce (MK-5 through MK-10) (19).

Clinical examination. Height and weight were measured with the subject wearing indoor clothing without shoes. BMI was computed as weight (kg) divided by height squared (m). Sitting systolic and diastolic blood pressure were measured twice with a random-zero sphygmomanometer by a trained research assistant after a 5-min rest, and values were averaged. A standard electrocardiogram was obtained, which was interpreted by the Modular ECG Analysis System (20). Diabetes mellitus was considered present when the subject reported use of antidiabetes medication or insulin. Serum total cholesterol level (mmol/L) was determined by an automated enzymatic procedure (21). HDL cholesterol was measured similarly after precipitation of the non-HDL fraction.

Follow-up procedures. The present analysis is based on follow-up data collected from baseline (1990–1993) until January 1, 2000. Informed consent for collection of follow-up data was obtained from 7802 participants (98%). Information on vital status was obtained at regular intervals from the municipal population registry. Fatal and nonfatal events were reported by general practitioners (GPs) in the research area (covering 85% of the cohort) by means of a computerized system. Information from GPs outside the research area was obtained by paper forms. In the Netherlands, the GP forms the link to all specialized medical care and it is therefore unlikely that clinical events are missed by our follow-up procedure. Research physicians verified information using GP records and hospital discharge letters. Events were coded independently by 2 physicians according to the *International Statistical Classification of Diseases and Related Health Problems, 10th revision* (ICD-10) (22). If there was disagreement, consensus was reached in a separate session. Coded events were reviewed by a medical expert in the field. The judgment by the expert was considered definite in case of discrepancy.

In the present study, we examined risk of nonfatal myocardial infarction (MI, ICD-10 code I21), CHD (I20–I25 and I46), and overall mortality during follow-up. If reinfarctions occurred during follow-up, only the first event was included in the analysis. Events were considered fatal if death occurred within 28 d after the onset of symptoms.

Assessment of aortic calcification. At baseline, a lateral radiographic film of the lumbar spine was made from a fixed distance while the participant was seated. Calcification was diagnosed off-line by detecting calcified deposits in the abdominal aorta parallel and anterior to the lumbar spine (L1–L4), as described in more detail elsewhere (23). The extent of abdominal aortic atherosclerosis was scored on a scale of 0 to 5, according to the length of the involved area. For the present analysis, the severity of aortic calcification was graded as "absent or mild" (≤1 cm calcification), "moderate" (>1 and <5 cm), or "severe" (≥5 cm).

Study population. Noninstitutionalized subjects who visited the study center at baseline (n = 6521, 82% of the cohort) were eligible for a dietary interview. Diet was not assessed in 271 subjects who participated in the pilot phase of the Rotterdam Study. Also, dietary data were not obtained in 122 subjects suspected of dementia because of expected difficulties in dietary recall and in a random group of 481 subjects due to logistic reasons. Furthermore, 212 dietary reports were considered unreliable by the dietician and therefore excluded. Dietary data were thus available for 5435 subjects. We excluded 613 subjects with a history of MI, as diagnosed on the baseline electrocardiogram (ECG), leaving 4807 subjects for the present analysis. Within this cohort, 4683 subjects had baseline radiographic films for the assessment of calcification in the abdominal aorta. The X-ray of 210 subjects did not allow proper scoring, leaving 4473 subjects for the analysis of aortic calcification.

Statistical methods. Data analyses were performed using SPSS 11.0.1 for Windows. Two-sided P values < 0.05 were considered statistically significant. Descriptive data are presented as means ± SD or, for categorical variables, as percentages. All statistical analyses were performed for phylloquinone and menaquinone separately. Vitamin K intakes were first adjusted for total energy intake and divided into tertiles on the basis of the total cohort with dietary data. Pearson correlations (r) for the association of vitamin K intake with lifestyle factors and nutrient intakes were computed to identify potential confounders.

The risk of coronary events and all-cause mortality in tertiles of vitamin K intake was studied in a Cox regression model with adjustment for age, gender, and total energy intake (model 1), using the lower tertile as the reference. Survival time was calculated as the number of days from entry into the study until death, occurrence of nonfatal MI (in analyses of MI only), or January 1, 2000, whichever occurred first. Hazard ratios, subsequently referred to RR, are presented with 95% CI and P for linear trend across the median values of vitamin K tertiles.

The association between tertiles of vitamin K intake and aortic calcification was studied in a multivariate logistic regression model with adjustment for age, gender, and total energy intake (model 1). Odds ratios (OR) are presented with 95% CI and P for trend. Associations with moderate calcification (i.e., length of calcified area >1 cm and <5 cm) and severe calcification (≥5 cm) were examined separately. In both analyses, the control group comprised subjects with no or mild calcification (calcified area ≤ 1 cm).

All analyses were repeated with additional adjustment for BMI (kg/m^2), diabetes mellitus (i.e., treatment with oral antidiabetes medication or insulin), smoking status (current, past, or never), pack-years of cigarette smoking, education (3 categories), and intake of alcohol (g/d), calcium (g/d), flavonols (total of quercetin, kaempferol, and myricetin; mg/d), SFA (g/d), and PUFA (g/d) (model 2). In an additional analysis, further adjustment was made for intake of vitamin E (mg/d), vitamin C (mg/d), β-carotene (mg/d), and fiber (g/d).

The associations of vitamin K intake with serum total and HDL cholesterol and systolic and diastolic blood pressure were obtained by multiple linear regression analysis with adjustment for age, gender, and other confounders as indicated above. The analysis of serum cholesterol comprised 4581 subjects who were not treated with lipid-reducing agents, and the analysis of blood pressure comprised 3370 subjects who were not treated with antihypertensive drugs.

We investigated whether serum HDL or total cholesterol could mediate the effect of dietary vitamin K on coronary events, all-cause mortality, or aortic calcification by adding these parameters to the multivariate models and examining changes in risk estimates.

RESULTS

Characteristics of the study population are shown in **Table 1**. Men had higher intakes of vitamin K than women, but the associations were reversed after adjustment for total energy intake (253.5 vs. 241.1 μg/d for phylloquinone and 29.2 vs. 26.9 μg/d for menaquinone, respectively). All further associations were adjusted for total energy intake. Phylloquinone intake was not correlated with menaquinone intake ($r = -0.02$). Phylloquinone intake was positively associated with dietary fiber, calcium, vitamin antioxidants, flavonols, and BMI and inversely associated with smoking (all $P < 0.001$). Menaquinone intake was positively associated with intake of total fat and SFA, dietary calcium, BMI, and diabetes mellitus and inversely associated with intake of PUFA (all $P < 0.001$). Additional adjustment for age and gender did not change these associations.

TABLE 1

Baseline characteristics of 4807 Dutch men and women aged 55 y and over with no history of MI[1]

	Men (n = 1836)	Women (n = 2971)
Age, *y*	66.9 ± 7.4	67.7 ± 8.0
BMI, *kg/m²*	25.7 ± 2.9	26.6 ± 4.0
Smoking status, %		
Current	29.8	19.1
Former	61.5	28.8
Never	8.7	52.0
Alcohol use, %	88.1	74.4
Educational level,[2] %		
Low	23.3	41.1
Intermediate	57.1	52.1
High	19.7	6.8
Blood pressure,[3] *mm Hg*		
Systolic	138.5 ± 21.9	138.7 ± 22.2
Diastolic	74.8 ± 11.4	73.1 ± 11.0
Serum cholesterol level,[4] *mmol/L*		
Total	6.3 ± 1.1	6.9 ± 1.2
HDL	1.2 ± 0.3	1.5 ± 0.4
Diabetes mellitus,[5] %	3.1	3.6
Dietary intake		
Total energy, *kJ/d*	9459 ± 2130	7494 ± 1693
Total fat, *g/d*	92.7 ± 28.6	73.0 ± 23.5
SFA, *g/d*	36.3 ± 12.4	29.1 ± 10.5
PUFA, *g/d*	17.9 ± 8.3	13.5 ± 6.8
Fiber, *g/d*	18.0 ± 5.3	16.1 ± 5.6
Flavonols,[6] *mg/d*	26.7 ± 11.7	29.8 ± 12.4
Calcium, *g/d*	1.15 ± 0.4	1.10 ± 0.4
Vitamin C, *mg/d*	115.1 ± 50.7	124.1 ± 55.6
Vitamin E, *mg/d*	15.3 ± 6.6	12.8 ± 5.6
β-Carotene, *mg/d*	1.6 ± 0.7	1.5 ± 0.8
Phylloquinone, *μg/d*	257.1 ± 116.1	244.3 ± 131.9
Menaquinone, *μg/d*	30.8 ± 18.0	27.0 ± 15.1
MK-4	7.7 ± 3.4	6.3 ± 2.8
MK-5 through MK-10	23.1 ± 16.3	20.7 ± 13.8

[1] Values are means ± SD or percentages.
[2] Low, primary education or less; intermediate, secondary general or vocational education; high, higher vocational education, university.
[3] Data on blood pressure were missing for 26 subjects.
[4] Data on serum lipids were missing for 27 subjects.
[5] Defined as treatment with oral antidiabetes medication or insulin.
[6] Total of quercetin, myricetin, and kaempferol.

In 4581 subjects not treated with lipid-reducing agents, positive associations of phylloquinone intake with serum total cholesterol ($0.044\ \text{mmol}\cdot\text{L}^{-1}\cdot 100\ \mu\text{g}^{-1}$, $P = 0.003$) and serum HDL ($0.016\ \text{mmol}\cdot\text{L}^{-1}\cdot 100\ \mu\text{g}^{-1}$, $P < 0.001$) were found. Menaquinone intake was inversely associated with serum total cholesterol ($-0.025\ \text{mmol}\cdot\text{L}^{-1}\cdot 10\ \mu\text{g}^{-1}$, $P = 0.055$), and positively associated with serum HDL ($0.015\ \text{mmol}\cdot\text{L}^{-1}\cdot 10\ \mu\text{g}^{-1}$, $P < 0.001$). Intakes of phylloquinone and menaquinone were not associated with blood pressure.

Incidence of CHD and all-cause mortality. From baseline until January 1, 2000, 144 first nonfatal MI and 99 fatal coronary events (54 of which were fatal MI) occurred in this cohort of 4807 older men and women. A total of 602 subjects died from other causes during follow-up. The study had a mean duration of follow-up of 7.2 y (SD 1.9) and comprised 34,645 person-years.

Energy-adjusted intake of phylloquinone was not associated with risk of nonfatal MI, incident CHD (fatal and nonfatal events combined), CHD mortality, and all-cause mortality (**Table 2**). For menaquinone intake (**Table 3**) there was an inverse relationship with nonfatal MI in the upper vs. lower tertile after adjustment for confounders, but findings were not statistically significant. Risk of incident CHD, however, was strongly and significantly reduced in the upper tertile of menaquinone intake (RR = 0.59), as were risk of CHD mortality (RR = 0.43) and all-cause mortality (RR = 0.74). Additional adjustment for intake of fiber, vitamin C, vitamin E, and β-carotene did not change these results.

RR for coronary events and all-cause mortality with phylloquinone and menaquinone intake remained essentially the same after inclusion of serum total cholesterol and HDL cholesterol in the multivariate models.

Atherosclerosis. Aortic calcification was mild or absent in 2874 subjects (64.3%), moderate in 1359 subjects (30.4%), and severe in 240 subjects (5.4%). Categories of aortic calcification were about equally distributed in men and women. Moderate aortic calcification at baseline was strongly and positively associated with risk of CHD mortality after adjustment for age and gender [RR = 1.95 (95% CI: 1.23, 3.10)], as was severe aortic calcification [RR = 2.75 (1.39, 5.43)]. Similar positive associations of aortic calcification with nonfatal coronary events and overall mortality were observed during follow-up.

Mean intake of phylloquinone intake was similar in categories of aortic calcification (249.2, 249.0, and 245.0 μg/d for mild, moderate, and severe stages, respectively), after adjustment for age, gender, and total energy intake. Menaquinone intake was lower in subjects with severe aortic calcification (25.6 μg/d) than in subjects with moderate or mild calcification (28.6 and 28.8 μg/d, respectively; $P = 0.001$).

Intake of phylloquinone was not significantly associated with moderate or severe aortic calcification after adjustment for age and gender (model 1) or after further adjustment for confounders. In fully adjusted analysis (model 2), ORs for severe calcification were 0.86 (0.61, 1.22) and 1.03 (0.72, 1.48) in the mid and upper tertiles of phylloquinone, respectively, compared to the lower tertile. Menaquinone intake showed no significant association with moderate calcification (**Table 4**). For severe calcification, however, a strong inverse

TABLE 2

Association of coronary events and all-cause mortality with intake of phylloquinone in 4807 Dutch men and women aged 55 y and over[1]

	Energy-adjusted phylloquinone intake (μg/d)			
	<200	200–278	>278	*P* for trend
n	1588	1608	1611	
Median intake, *μg/d*	154.6	236.5	336.9	
Nonfatal MI				
Person-years	11323	11556	11766	
Events, *n*	48	56	40	
RR, model 1[2]	1	1.19 (0.81, 1.75)	0.86 (0.56, 1.30)	0.44
RR, model 2[3]	1	1.14 (0.77, 1.70)	0.84 (0.54, 1.31)	0.42
Incident CHD[4]				
Person-years	11323	11556	11766	
Events, *n*	82	81	70	
RR, model 1	1	1.01 (0.74, 1.38)	0.89 (0.64, 1.22)	0.45
RR, model 2	1	1.00 (0.73, 1.37)	0.89 (0.63, 1.25)	0.48
CHD mortality[5]				
Person-years	11502	11764	11880	
Events, *n*	36	30	33	
RR, model 1	1	0.86 (0.53, 1.40)	0.98 (0.67, 1.57)	0.94
RR, model 2	1	0.90 (0.55, 1.48)	1.02 (0.61, 1.69)	0.93
All-cause mortality				
Person-years	11502	11764	11880	
Events, *n*	246	240	215	
RR, model 1	1	0.98 (0.82, 1.17)	0.91 (0.75, 1.09)	0.28
RR, model 2	1	1.02 (0.85, 1.23)	0.94 (0.77, 1.14)	0.53

[1] RR obtained by Cox proportional hazard analysis, with 95% CI in parentheses and *P* for linear trend across the tertiles.
[2] Model includes age, gender, and total energy intake.
[3] Model includes age, gender, total energy intake, BMI, smoking status, pack-years of cigarette smoking, diabetes, education (3 categories), and intake of alcohol, SFA, PUFA, flavonols (quercetin, myricetin, and kaempferol), and calcium.
[4] CHD comprises fatal and nonfatal MI, sudden cardiac death, and other forms of acute and chronic ischemic heart disease (ICD-10 codes I20–I25 and I46).
[5] CHD events followed by death within 28 d after the onset of symptoms.

relationship with menaquinone intake persisted after adjustment for BMI, smoking, education, diabetes, and intake of alcohol, PUFA, SFA, flavonols, and calcium (Table 4). Additional adjustment for intake of fiber, vitamin C, vitamin E, and β-carotene did not change these results.

Interaction terms between gender and intake of phylloquinone or menaquinone were not significant in the multivariate models (all $P > 0.15$). Additional adjustment for serum total cholesterol or HDL cholesterol did not change the associations of dietary phylloquinone or menaquinone with aortic calcification.

DISCUSSION

The results of this study suggest a protective effect of dietary menaquinone intake against CHD in older men and women. As indicated by the inverse association with all-cause mortality, high intake of menaquinone does not increase the risk for other major diseases, such as cancer. There was no consistent association of phylloquinone intake with CHD, mortality, or aortic calcification.

In contrast to phylloquinone, intake of menaquinone (mainly MK-4 from eggs and meat, and MK-8 and MK-9 from cheese) (19) is not related to a healthy lifestyle or diet, which makes it unlikely that the observed reduction in coronary risk is due to confounding. Subjects with a history of MI were excluded from the analysis to avoid bias that may arise from intentional changes in diet. Intake of menaquinone was inversely associated with severe aortic calcification in asymptomatic subjects, which provides additional support for a causal relationship between menaquinone and CHD. Aortic atherosclerosis was assessed during the baseline examination of the Rotterdam Study by detection of calcified deposits in the aorta on abdominal X-rays (23). The method shows good agreement with coronary calcification scores obtained by electron beam tomography (24) and is predictive for cardiovascular mortality (25).

Vegetables and vegetable oils are the main dietary sources for phylloquinone (8,17,19). The intake of phylloquinone of around 250 μg/d in our study is higher than in the U.S. population aged 55 and over, where intakes are 80–210 μg/d (26). This may be explained by the relatively high consumption of vegetables in the Netherlands, which was around 200 g/d in the Rotterdam Study. For menaquinone (MK-*n*), dietary data are scanty in the literature. We therefore analyzed foods and beverages frequently consumed in the Netherlands for MK-4 through MK-10 and linked these data to the dietary database of the Rotterdam Study (19). Intake of menaquinone comprised 10% of the total vitamin K intake, but its bioavailability is probably higher than for phylloquinone that is strongly bound to vegetable fiber (14). Menaquinone in our Dutch population was mainly derived from dairy products, especially cheese (19). Interestingly, in this respect, cheese has not been established as a dietary risk factor for cardiovascular disease in epidemiological studies, despite its high levels of saturated fat and salt. We hypothesize that menaquinones in cheese (MK-8 and MK-9) could exert a beneficial effect in the cardiovascular system and that the high cheese consumption

TABLE 3

Association of coronary events and all-cause mortality with intake of menaquinone in 4807 Dutch men and women aged 55 y and over[1]

	Energy-adjusted menaquinone intake (μg/d)			
	<21.6	21.6–32.7	>32.7	*P* for trend
n	1578	1605	1624	
Median intake, μg/d	15.1	26.9	40.9	
Nonfatal MI				
Person-years	11181	11549	11915	
Events, *n*	51	57	36	
RR, model 1[2]	1	1.15 (0.79, 1.69)	0.74 (0.48, 1.14)	0.18
RR, model 2[3]	1	1.08 (0.73, 1.62)	0.67 (0.41, 1.09)	0.12
Incident CHD[4]				
Person-years	11323	11556	11766	
Events, *n*	86	89	58	
RR, model 1	1	1.05 (0.78, 1.42)	0.71 (0.51, 1.00)	0.048
RR, model 2	1	0.96 (0.70, 1.31)	0.59 (0.40, 0.86)	0.007
CHD mortality[5]				
Person-years	11356	11747	12043	
Events, *n*	41	35	23	
RR, model 1	1	0.84 (0.54, 1.33)	0.59 (0.35, 0.99)	0.045
RR, model 2	1	0.73 (0.45, 1.17)	0.43 (0.24, 0.77)	0.005
All-cause mortality				
Person-years	11356	11747	12043	
Events, *n*	258	248	195	
RR, model 1	1	0.97 (0.82, 1.16)	0.81 (0.67, 0.98)	0.030
RR, model 2	1	0.91 (0.75, 1.09)	0.74 (0.59, 0.92)	0.007

[1] RR obtained by Cox proportional hazard analysis, with 95% CI in parentheses and *P* for linear trend across the tertiles.
[2] Model includes age, gender, and total energy intake.
[3] Model includes age, gender, total energy intake, BMI, smoking status, pack-years of cigarette smoking, diabetes, education (3 categories), and intake of alcohol, SFA, PUFA, flavonols (quercetin, myricetin, and kaempferol), and calcium.
[4] CHD comprises fatal and nonfatal MI, sudden cardiac death, and other forms of acute and chronic ischemic heart disease (ICD-10 codes I20–I25 and I46).
[5] CHD events followed by death within 28 d after the onset of symptoms.

in France and the Mediterranean countries may possibly account for lower prevalences of CHD. Menaquinone is also produced by the intestinal flora, but the absorption seems to be limited (27). In our study, however, it was not possible to quantify endogenous menaquinone synthesis. Dietary intake of menaquinone is reflected in serum levels. In healthy Japanese subjects who consumed fermented soybean (natto) high in menaquinone (especially MK-7), serum concentrations of

TABLE 4

Association of aortic calcification with intake of menaquinone in 4473 Dutch men and women aged 55 y and over[1,2]

	Energy-adjusted menaquinone intake (μg/d)			
	<21.6	21.6–32.7	>32.7	*P* for trend
n	1468	1493	1512	
Median intake, μg/d	15.1	26.9	40.9	
Moderate calcification				
Controls, *n*	916	958	1000	
Cases, *n*	454	452	453	
OR, model 1[3]	1	0.93 (0.79, 1.10)	0.94 (0.80, 1.11)	0.49
OR, model 2[4]	1	0.91 (0.77, 1.09)	0.93 (0.76, 1.12)	0.45
Severe calcification				
Controls, *n*	916	958	1000	
Cases, *n*	98	83	59	
OR, model 1	1	0.75 (0.54, 1.03)	0.56 (0.39, 0.80)	0.001
OR, model 2	1	0.71 (0.50, 1.00)	0.48 (0.32, 0.71)	<0.001

[1] Aortic calcification was graded according to the length of the calcified area, i.e., no/mild (reference), ≤1 cm; moderate, >1 and <5 cm; severe, ≥5 cm.
[2] OR obtained by multivariate logistic regression, with 95% CI in parentheses and *P* for linear trend across the tertiles.
[3] Model includes age, gender, and total energy intake.
[4] Model includes age, gender, total energy intake, BMI, smoking status, pack-years of cigarette smoking, diabetes, education (3 categories), and intake of alcohol, SFA, PUFA, flavonols (quercetin, myricetin, and kaempferol), and calcium.

MK-7 and carboxylated osteocalcin were significantly increased (28).

Vascular tissue and calcified plaques contain MGP, a vitamin K–dependent protein known to prevent excessive calcium deposition in bone (1–4,7,8). Lack of vascular MGP resulted in excessive aortic and coronary calcification in knockout mice (9). The inverse association of menaquinone with aortic calcification and CHD in our study may be explained by undercarboxylation of vascular MGP and consequently enhanced calcification of atherosclerotic lesions. Calcified plaques are more prone to rupture, which will elicit a thrombotic response, thereby increasing the risk of a coronary event (29). Another vitamin K–dependent protein found in the vessel wall is protein S (30). Together with activated protein C, this anticoagulant plays an important role in preventing clot formation at the inner surface of the vessel wall. However, the quantitative contribution of extrahepatic protein S synthesis to hemostasis is probably small.

There is evidence for a differential effect of vitamin K subtypes in the cardiovascular system. Rats at our laboratory were fed diets containing phylloquinone, menaquinone, or both after treatment with high doses of warfarin. Despite similar in vitro cofactor activity for γ-carboxylase, menaquinone but not phylloquinone supplementation prevented warfarin-induced arterial calcification (31). The tissue-specific use of phylloquinone and menaquinone in rats was assessed by measuring the ratios of quinone over epoxide (K:KO ratios) during warfarin treatment. In the arterial vessel wall, K:KO ratios were substantially lower for phylloquinone than for menaquinone whereas the reverse was observed for the liver, suggesting a tissue-specific utilization of vitamin K subtypes (31). In a recent trial in humans, phylloquinone was almost exclusively incorporated into the triacylglycerol-rich lipoprotein (TGRLP) fraction after intestinal absorption, whereas a substantial part of the menaquinones was recovered from the LDL fraction (32). The TGRLP fraction is mainly cleared by the liver, whereas LDL forms a transport system to extrahepatic tissues. Menaquinone supplementation lowered serum cholesterol levels in a study of 17 dialysis patients (33). In our population of healthy older subjects, we confirmed the favorable effect of menaquinone on blood lipids but effects were small and could not explain the inverse relation that we observed between dietary menaquinone and CHD.

In conclusion, our findings suggest a protective effect of menaquinone intake against CHD, which could be mediated by inhibition of arterial calcification. Adequate intake of foods rich in menaquinones, such as curds and (low-fat) cheese, may contribute to CHD prevention.

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