ORIGINAL RESEARCH

# Intake of Dietary Phylloquinone and Menaquinones and Risk of Stroke

Linda E. T. Vissers, BSc; Geertje W. Dalmeijer, PhD; Jolanda M. A. Boer, PhD; W. M. Monique Verschuren, PhD; Yvonne T. van der Schouw, PhD; Joline W. J. Beulens, PhD

***Background*—**Dietary vitamin K intake is thought to decrease the risk of cardiovascular disease (CVD) by reducing vascular calcification, although vitamin K is also involved in coagulation. Studies investigating the association between phylloquinone intake and risk of stroke are scarce, and the relation with menaquinones has not been investigated to date.

***Methods and Results*—**We investigated the association between intake of phylloquinone and menaquinones and stroke in a prospective cohort of 35 476 healthy subjects. Information on occurrence of stroke was obtained by linkage to national registries, and stroke was further specified into ischemic and hemorrhagic stroke. Vitamin K intake was estimated using a validated food-frequency questionnaire. Multivariate Cox proportional hazards models adjusted for cardiovascular risk factors, lifestyle, and other dietary factors were used to estimate the associations. During a follow-up of 12.1±2.1 years, 580 incident cases of stroke were identified, 163 of which were hemorrhagic and 324 were ischemic. Phylloquinone intake was not associated with risk of stroke with a hazard ratio (HR) of 1.09 (95% CI: 0.85 to 1.40, $P_{trend}$ 0.41) for the highest versus lowest quartile. For intake of menaquinones similar results were found, with an $HR_{Q4\ versus\ Q1}$ of 0.99 (95% CI: 0.75 to 1.29, $P_{trend}$ 0.82). When specifying hemorrhagic and ischemic stroke or menaquinone subtypes, no significant associations were detected.

***Conclusion*—**In our study, neither dietary phylloquinone nor dietary menaquinones intake were associated with stroke risk. **(*J Am Heart Assoc*. 2013;2:e000455 doi: 10.1161/JAHA.113.000455)**

**Key Words:** diet • menaquinone • phylloquinone • stroke • vitamin K

Vitamin K is a fat-soluble vitamin occurring in 2 biologically active forms. Vitamin $K_1$ (phylloquinone), the most common form of vitamin K, is present in green, leafy vegetables and certain vegetable oils.[1] Vitamin $K_2$ (menaquinones; MK) occurs in animal products such as meat, eggs, and fermented foods like cheese and curd.[2] Menaquinones can be further subdivided into menaquinone-4 through menaquinone-10 based on the length of the side chain. Vitamin K affects both coagulation and vascular calcification.

Vitamin K functions first and foremost as a cofactor for the gamma-glutamyl carboxylation of certain glutamate (Gla) residues that are present in coagulation factors in the liver.[3] Vitamin K antagonists block carboxylation of coagulation factors and thereby reduce blood coagulation. Extra-hepatic Gla-proteins have been also identified in bone (osteocalcin) and the vascular wall (matrix Gla-protein [MGP]).[3] MGP is a strong local inhibitor of soft tissue calcification.[4]

Transport of phylloquinone is with triacylglycerol-rich fraction, which is mainly cleared by the liver, while menaquinones are found in both triacylglycerol-rich lipoprotein and low-density lipoprotein, which transports it to extra-hepatic tissues.[5] Therefore, phylloquinone predominantly serves as a cofactor for proteins in blood coagulation within the liver and menaquinones serve as a cofactor in extra-hepatic tissues such as the vascular wall.[3]

Vitamin K deficiency and in particular menaquinones deficiency could therefore lead to vascular calcification and perhaps cardiovascular disease.

Several studies have indeed shown that vitamin K deficiency leads to vascular calcification in mice and human arteries.[6,7] Animal studies also showed that vitamin K antagonists increase aortic calcification, which could be reversed by treatment with vitamin K.[8] Similarly, an increase in arterial calcification and formation of more vulnerable plaques is seen in humans on vitamin K antagonists.[9–12]

To date, observational studies showed that a high intake of menaquinones is associated with reduced arterial calcifica-

*From the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands (L.E.T.V., G.W.D., Y.T.S., J.W.J.B.); National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (J.M.A.B., W.M.M.V.).*

**Correspondence to:** Geertje W. Dalmeijer, PhD, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, STRT 6.131, PO Box 85500, 3508GA Utrecht, the Netherlands. E-mail: g.w.dalmeijer@umcutrecht.nl

Received September 23, 2013; accepted November 11, 2013.

tion[13,14] and coronary heart disease (CHD).[14,15] Although observational studies did not detect an association between phylloquinone intake and calcification or CHD, a recent randomized, controlled trial showed that phylloquinone supplementation reduced progression of coronary calcification in healthy older adults with preexisting coronary arterial calcification (CAC).[16] So far, only the Health Professionals' Follow Up study and the Nurses' Health study have investigated the relationship between phylloquinone intake and (ischemic) stroke risk, but did not find an association.[17,18] To the best of our knowledge, the relationship between the intake of menaquinones and risk of stroke has not been investigated to date.

In this study, we therefore investigated the relationship between intake of phylloquinone and menaquinones and risk of stroke.

Because studies suggested that long-chain menaquinones have a longer halftime, making them more bioavailable,[2,19–21] we also explored the associations of short-chain menaquinones and long-chain menaquinones on stroke risk separately.

Because vitamin K affects both coagulation and vascular calcification,[22–24] we additionally specified stroke risk in ischemic and hemorrhagic stroke risk.

## Methods

### Study Population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-NL consists of the Prospect-EPIC and MORGEN-EPIC cohorts, the 2 Dutch contributions to the EPIC study. Both cohorts were set up simultaneously during the years from 1993 to 1997. The MORGEN-EPIC cohort consists of 22 654 adults aged 21 to 64 years, selected from random samples of 3 Dutch towns. The Prospect-EPIC cohort includes 17 357 women aged 49 to 70 years living in Utrecht or its vicinity and were recruited through a breast cancer screening program, providing a total study population of 40 011 persons. Details on the design and recruitment have been described elsewhere.[25] All participants provided informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the institutional review board of the University Medical Centre Utrecht (Prospect) and the Medical Ethical Committee of TNO nutrition and Food Research (MORGEN). After exclusion of individuals with prevalent stroke or CHD (n=3101), missing nutritional data (n=130), individuals without informed consent for linkage to municipal population registries for fatal endpoints or the hospital discharge register (n=977), loss to follow up (n=1), and individuals with abnormal energy intake (highest and lowest 0.5% of energy intake divided by predicted basal metabolic rate) (n=326), 35 476 participants were left for data analysis.

### Baseline Measurements

At baseline, participants filled out a general questionnaire, containing questions on demographics, presence of chronic diseases, and risk factors for chronic diseases. A physical examination was performed and non-fasting blood was drawn. Smoking was categorized into current, past, and never smoker. Oral anticontraceptive use was categorized into <1 year, 1 to 5 years, 5 to 10 years, 10 to 15 years, 15 to 20 years, or >20 years. Total and HDL cholesterol were measured using a homogeneous assay with an enzymatic method performed on an autoanalyser.[25] Systolic and diastolic blood pressure measurements were performed twice on the right arm with the participant in the supine position using a Boso Oscillomat (Prospect) or on the left arm using a random zero sphygmomanometer (MORGEN) and the mean of these 2 measurements was taken. Hypertension was defined as being present based on diastolic blood pressure >90 mm Hg or systolic blood pressure >140 mm Hg or self-reported use of antihypertensive medication, or self-reported presence of hypertension. Height and weight were measured and BMI was calculated. Education was categorized into low (primary education to intermediate vocational education), average (higher secondary education), and high (higher vocational education or university). Physical activity was assessed using a questionnaire validated in an elderly population[26] and the Cambridge Physical Activity Score was calculated and used to categorize physical activity.[27] Because we could not calculate the Cambridge physical activity score for 14% of all participants, we imputed missing scores by means of single linear regression modelling (SPSS MVA procedure).

### Dietary Intake

Daily dietary intake was obtained by a validated food frequency questionnaire (FFQ) containing questions on the usual frequency of consumption of 79 main food items during the year preceding enrollment. This questionnaire allows the estimation of the mean daily consumption of 178 foods and was validated against twelve 24-hour dietary recalls.[28] The 1996 Dutch food composition table[29] was used to calculate energy and nutrient intakes. However, this table does not contain information on the vitamin K content of foods. Therefore, the concentrations of phylloquinone and menaquinones (MK-4 through 10) were assessed in a series of 52 Dutch foods with 4 to 15 samples tested per food item at the Biochemistry Laboratory Maastricht University.[2] For several other foods, published data by others were used to update the dietary database for vitamin K.[2,30–34] Vitamin K contents of 260 foods in total were collected and tabulated to estimate intake of phylloquinone and menaquinones. We validated vitamin K intake estimated by the FFQ against twelve 24-hour

recalls in 58 women and 63 men.[28,31] We observed a low relative validity of phylloquinone and MK10 intakes with Spearman correlations of the FFQ against 24-hour recalls of 0.24 and 0.23, respectively. Relative validity for intakes of MK-4 through MK-9 was reasonable to good with Spearman correlation coefficients ranging from 0.51 for MK-7 to 0.72 for MK-5. Intakes of nutrients were adjusted for energy intake by the regression residual method.[28]

## Stroke

Information on the occurrence of stroke during follow-up was obtained from causes of death and from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. In this register, admission files have been entered continuously from all general and university hospitals in the Netherlands from 1990 onwards. All diagnoses were coded according to the ICD-9-CM. Our cohort was linked to the database with a validated probabilistic method, based on birth date, gender, postal code, and general practitioner.[35] Vital status of participants was obtained through linkage with the municipal population registries and causes of death were obtained through linkage with the causes of death registry from Statistics Netherlands. For our analysis, stroke (ICD-9; 430-434, 436, ICD-10; I60-I66), subdivided into hemorrhagic (ICD-9; 430-432, ICD-10; I60-I62) and ischemic (ICD-9; 433, 434, ICD-10;I63, I65) stroke was the endpoint of interest. Follow-up was complete until January 1, 2008.

## Data Analysis

Baseline characteristics were inspected by quartiles of energy-adjusted dietary phylloquinone and menaquinones intake. We calculated person-years of follow-up for each participant from the date of return of the questionnaire to the date of stroke, the date of death, emigration, or January 1, 2008, whichever came first. We used Cox regression to estimate hazard ratios (HRs) of stroke for quartiles of energy-adjusted phylloquinone or menaquinones intake and for each 50 μg increment of energy-adjusted phylloquinone intake and for each 10 μg increment of energy-adjusted menaquinone intake, coinciding with an approximate half SD for both phylloquinone (SD 98) and menaquinones (SD 17). We calculated a *P* for trend over the quartiles by adding the median of phylloquinone and menaquinones intake in each quartile as a linear covariate. For the estimation of the HRs for ischemic and hemorrhagic stroke the participants were divided into tertiles of energy-adjusted intake of phylloquinone and menaquinones because of fewer events.

We adjusted for cardiovascular risk factors, lifestyle and dietary factors using 3 models, all stratified for cohort. The first model was adjusted for age and sex. In the second model, we included smoking status (non/current/former), waist circumference, physical activity (4 categories), and use of oral anticontraceptives. In the final multivariate model, we also adjusted for diet by including total energy intake and energy-adjusted intake of fat, protein, glycemic index, alcohol, vitamin C, and fiber (all continuous, except for alcohol; divided into categories [on average 0 to 0.5, 0.5 to 3, 3 to 6, 6 to 12, and >12 glasses of alcohol per week]). In a separate model, we also checked whether adjusting for hypertension and blood cholesterol ratio influenced the results.

The interaction between phylloquinone and menaquinone intake with fat intake was tested by including interaction term into the model. Presence of a nonlinear association of phylloquinone or menaquinones intake with stroke was explored by including the quadratic term of the linear term of phylloquinone and menaquinones in the model with the linear term, but no evidence of nonlinear associations was found.

Similar models were used for the analysis of intake of total vitamin K intake and menaquinones subtypes and stroke, that is, short-chain menaquinones (MK-4 through MK-6) intake and long-chain menaquinones (MK-7 through MK-10) intake.

Results were considered statistically significant at 2-sided $P \leq 0.05$. Data analysis was performed using PASW statistics (version 17.0 for Windows).

## Results

At baseline, the study population was on average 49±12 years and consisted of 74.1% women. Mean intake of phylloquinone and menaquinones was 199.9±97.8 μg/day, and 30.7±13.8 μg/day, respectively. With higher intakes of phylloquinone and menaquinones participants were more often female, had a higher age, higher protein intake and higher prevalence of hypertension. With high phylloquinone intake, participants smoked less often and had higher intake of energy-adjusted fiber and vitamin C whereas energy-adjusted alcohol intake was lower. With higher menaquinone intake, participants were more often physically active and had higher energy-adjusted alcohol consumption and intakes of calcium and saturated fat (Table 1).

During an average follow-up of 12.1±2.1 years, we documented 580 cases of incident stroke (1.6% of the total cohort), of which 163 cases were hemorrhagic, 324 cases were ischemic, and 93 were unspecified. Phylloquinone intake was not associated with age- and sex-adjusted risk of stroke in any of the models (Table 2). When comparing the highest quartile with the lowest quartile, the HR was 1.02 (95% CI [0.81 to 1.28]). Further adjustment for cardiovascular risk factors ($HR_{Q4\ versus\ Q1}$ 1.02, 95% CI [0.81 to 1.29]) and dietary factors ($HR_{Q4\ versus\ Q1}$ 1.09, 95% CI [0.85 to 1.40]) did not affect these results. Intake of menaquinones was not associated

**Table 1.** Baseline Characteristics According to Quartiles of Energy-adjusted Intake of Phylloquinone and Menaquinones of 35 476 Dutch Adults

| | Phylloquinone | | | | Menaquinones | | | |
|---|---|---|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 |
| N | 8869 | 8869 | 8869 | 8869 | 8869 | 8869 | 8869 | 8869 |
| Female sex | 66.8 | 71.6 | 77.4 | 83.7 | 63.4 | 73.3 | 79.4 | 83.5 |
| Age, y | 46.4±12.4 | 48.4±11.8 | 49.9±11.6 | 52.3±10.9 | 44.9±12.9 | 49.2±11.9 | 50.7±11.2 | 52.2±10.1 |
| BMI, kg/m$^2$ | 25.3±3.9 | 25.5±3.9 | 25.7±3.9 | 26.2±4.1 | 25.3±3.9 | 25.7±4.0 | 25.8±4.0 | 25.8±4.0 |
| Waist-hip ratio | 0.83±0.09 | 0.82±0.09 | 0.82±0.08 | 0.82±0.08 | 0.83±0.09 | 0.83±0.08 | 0.82±0.08 | 0.81±0.08 |
| Current smoker | 32.9 | 29.2 | 28.6 | 29.6 | 35.4 | 30.7 | 27.7 | 38.6 |
| Physically inactive* | 34.7 | 30.4 | 30.2 | 32.0 | 34.1 | 32.2 | 30.5 | 30.6 |
| Higher education | 23.6 | 21.2 | 20.4 | 16.6 | 17.7 | 18.4 | 21.0 | 24.7 |
| Diabetes | 1.4 | 1.4 | 1.9 | 2.4 | 1.2 | 1.4 | 2.0 | 2.5 |
| Use of oral anticontraceptives† | 30.7 | 32.2 | 33.8 | 34.6 | 30.4 | 32.2 | 34.7 | 33.9 |
| Hypertension | 33.8 | 35.0 | 37.9 | 40.8 | 32.6 | 37.6 | 38.3 | 39 |
| Diastolic blood pressure, mm Hg | 78 (11) | 78 (10) | 78 (10) | 78 (11) | 77 (10) | 78 (10) | 78 (11) | 78 (11) |
| Systolic blood pressure, mm Hg | 125 (19) | 126 (18) | 127 (19) | 128 (78) | 124 (18) | 127 (19) | 127 (19) | 128 (19) |
| Ratio total/HDL cholesterol | 4.2±1.5 | 4.3±1.4 | 4.2±1.5 | 4.4±1.5 | 4.3±1.6 | 4.3±1.6 | 4.3±1.5 | 4.2±1.4 |
| Dietary intake‡ | | | | | | | | |
| Energy, kcal/day | 2062 (630) | 2098 (614) | 2064 (598) | 1981 (571) | 2094 (652) | 2061 (603) | 2037 (578) | 2014 (582) |
| Phylloquinone, μg/day | 96.6±24.6 | 156.6±15.1 | 213.7±19.0 | 332.7±81.7 | 187.8±96.6 | 201.0±96.7 | 205.8±99.2 | 205.0±97.5 |
| Menaquinones, μg/day | 29.5±14.5 | 30.4±13.6 | 31.0±13.3 | 31.7±13.5 | 15.6±3.7 | 24.7±2.2 | 33.1±2.7 | 49.3±10.8 |
| Protein, g/day | 46.6±12.3 | 48.0±11.4 | 49.1±11.7 | 50.9±12.2 | 40.9±10.8 | 46.7±10.0 | 50.5±10.3 | 56.3±11.3 |
| Saturated fat, g/day | 32.0±5.9 | 32.6±5.7 | 32.8±5.7 | 33.2±6.1 | 29.5±5.4 | 31.8±5.2 | 33.2±5.1 | 36.0±5.8 |
| Glycemic index, g/day | 0.53±0.04 | 0.53±0.04 | 0.52±0.03 | 0.52±0.04 | 0.53±0.04 | 0.53±0.04 | 0.52±0.03 | 0.52±0.04 |
| Alcohol§, g/day | 5.2 (15) | 4.7 (13) | 4.6 (13) | 4.1 (13) | 3.8 (13) | 4.7 (14) | 4.7 (13) | 5.3 (15) |
| Fiber, g/day | 21.2±4.6 | 22.9±4.4 | 23.9±4.4 | 25.5±4.7 | 23.0±5.3 | 23.4±4.8 | 23.6±4.5 | 23.5±4.6 |
| Calcium, mg/day | 1007±368 | 1055±346 | 1080±341 | 1132±347 | 809±295 | 993±284 | 1130±280 | 1343±320 |
| Vitamin C, mg/day | 94.7±39.4 | 107.0±42.5 | 113.9±44.2 | 123.1±49.4 | 101.8±47.1 | 108.8±44.1 | 113.2±43.9 | 114.9±44.6 |

Data are means±SD or % unless otherwise indicated. BMI indicates body mass index; HDL, high-density lipoprotein.
*Inactive or moderately inactive according to the Cambridge physical activity index.
†Use of oral anticontraceptives for >5 years.
‡All nutrients are energy adjusted.
§Median (interquartile range).

with stroke risk ($HR_{Q4\ versus\ Q1}$ 0.99, 95% CI [0.75 to 1.29]) in the final multivariate adjusted model (Table 2). When specifying our analyses to ischemic stroke or hemorrhagic stroke, we did not observe significant associations for both phylloquinone and menaquinones as well (Tables 3 and 4). Finally, we repeated our analyses for the intake of total vitamin K, short-chain menaquinones (MK-4 through MK-6) and long-chain menaquinones (MK-7 through MK-10) but none were associated with total, ischemic, or hemorrhagic stroke (data not shown). Adjusting for hypertension of blood-cholesterol ratio did not change our results (data not shown). We did not observe interaction between phylloquinone and menaquinones intake and fat intake ($P$=0.68 and $P$=0.36, respectively).

## Discussion

In this study among 35 476 men and women, we observed no association between intake of phylloquinone or menaquinones and stroke risk. These results, did not change when ischemic and hemorrhagic stroke were separately analyzed. The results were also similar when short chain and long chain menaquinones were individually analyzed. Strengths of this study include the complete information on cardiovascular risk

**Table 2.** Energy-adjusted Intake of Phylloquinone and Menaquinones and Risk of Stroke Among 35 476 Dutch Men and Women

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | $P_{trend}$ | Per 50 μg |
|---|---|---|---|---|---|---|
| Phylloquinone | | | | | | |
| Number of cases | 131 | 127 | 143 | 179 | | |
| Mean intake, μg/day | 101 | 157 | 213 | 308 | | |
| Age and sex adjusted | 1 | 0.88 (0.69 to 1.13) | 0.93 (0.73 to 1.17) | 1.02 (0.81 to 1.28) | 0.70 | 1.01 (0.97 to 1.05) |
| Multivariate adjusted* | 1 | 0.91 (0.71 to 1.16) | 0.95 (0.75 to 1.21) | 1.02 (0.81 to 1.29) | 0.71 | 1.01 (0.97 to 1.05) |
| Multivariate adjusted† | 1 | 0.94 (0.74 to 1.21) | 1.00 (0.78 to 1.28) | 1.09 (0.85 to 1.40) | 0.41 | 1.01 (0.97 to 1.06) |
| | | | | | | Per 10 μg |
| Menaquinones | | | | | | |
| Number of cases | 136 | 148 | 137 | 159 | | |
| Mean intake, μg/day | 16 | 25 | 33 | 46 | | |
| Age and sex adjusted | 1 | 0.86 (0.70 to 1.12) | 0.78 (0.61 to 0.99) | 0.86 (0.69 to 1.09) | 0.17 | 0.96 (0.90 to 1.02) |
| Multivariate adjusted* | 1 | 0.91 (0.72 to 1.14) | 0.81 (0.64 to 1.03) | 0.91 (0.72 to 1.14) | 0.33 | 0.97 (0.91 to 1.03) |
| Multivariate adjusted† | 1 | 0.94 (0.74 to 1.20) | 0.86 (0.67 to 1.12) | 0.99 (0.75 to 1.29) | 0.82 | 0.99 (0.99 to 1.06) |

Data are HRs (95% CI). CI indicates confidence interval; HR, hazard ratio.
*Adjusted for age, sex, waist circumference, smoking status, physical activity, and use of oral anticontraceptives.
†Adjusted for confounders in footnote "*" and energy intake+energy-adjusted intake of protein, fat, glycemic index, alcohol, vitamin C, and fiber.

factors, the large study population, the small degree of loss to follow-up and long duration of follow-up. However, there are some limitations that should be addressed. The main limitation of this study is the relative validity of our FFQ to estimate intake of phylloquinone and menaquinones. Relative validity compared with twelve 24-hour recalls was low for phylloquinone intake, which can lead to bias towards the null, which should be acknowledged when interpreting our results for phylloquinone intake. However, relative validity was reasonable for intake of menaquinones with Spearman correlations for intake of menaquinones varying from 0.51 to 0.72.[28] Therefore, especially our results

**Table 3.** Energy-adjusted Intake of Phylloquinone and Menaquinones and Risk of Ischemic Stroke Among 35 476 Dutch Men and Women

| | Quartile 1 | Quartile 2 | Quartile 3 | $P_{trend}$ | Per 50 μg |
|---|---|---|---|---|---|
| Phylloquinone | | | | | |
| Number of cases | 105 | 85 | 134 | | |
| Mean intake, μg/day | 107 | 184 | 307 | | |
| Age and sex adjusted | 1 | 0.72 (0.54 to 0.96) | 0.97 (0.75 to 1.26) | 0.97 | 1.00 (0.95 to 1.06) |
| Multivariate adjusted* | 1 | 0.74 (0.56 to 0.99) | 0.98 (0.76 to 1.27) | 1 | 1.00 (0.95 to 1.05) |
| Multivariate adjusted† | 1 | 0.77 (0.57 to 1.03) | 1.01 (0.77 to 1.34) | 0.82 | 1.00 (0.95 to 1.06) |
| | | | | | Per 10 μg |
| Menaquinones | | | | | |
| Number of cases | 101 | 115 | 108 | | |
| Mean intake, μg/day | 17 | 29 | 46 | | |
| Age and sex adjusted | 1 | 0.92 (0.71 to 1.21) | 0.81 (0.61 to 1.07) | 0.13 | 0.93 (0.86 to 1.01) |
| Multivariate adjusted* | 1 | 0.95 (0.73 to 1.25) | 0.85 (0.64 to 1.12) | 0.23 | 0.94 (0.67 to 1.03) |
| Multivariate adjusted† | 1 | 0.99 (0.75 to 1.31) | 0.90 (0.66 to 1.24) | 0.55 | 0.96 (0.87 to 1.06) |

Data are HRs (95% CI). CI indicates confidence interval; HR, hazard ratio.
*Adjusted for age, sex, waist circumference, smoking status, physical activity, and use of oral anticontraceptives.
†Adjusted for confounders in footnote "*" and energy intake+energy-adjusted intake of protein, fat, glycemic index, alcohol, vitamin C, and fiber.

**Table 4.** Energy-adjusted Intake of Phylloquinone and Menaquinones and Risk of Hemorrhagic Stroke Among 35 476 Dutch Men and Women

| | Quartile 1 | Quartile 2 | Quartile 3 | $P_{trend}$ | Per 50 μg |
|---|---|---|---|---|---|
| Phylloquinone | | | | | |
| Cases: 163 | 46 | 58 | 59 | | |
| Mean intake, μg/day | 107 | 184 | 309 | | |
| Age and sex adjusted | 1 | 1.13 (0.77 to 1.67) | 1.01 (0.67 to 1.50) | 0.98 | 1.03 (0.95 to 1.11) |
| Multivariate adjusted* | 1 | 1.16 (0.80 to 1.72) | 1.02 (0.69 to 1.51) | 0.97 | 1.02 (0.95 to 1.10) |
| Multivariate adjusted† | 1 | 1.21 (0.81 to 1.80) | 1.11 (0.73 to 1.69) | 0.66 | 1.05 (0.97 to 1.13) |
| | | | | | Per 10 μg |
| Menaquinones | | | | | |
| Cases: 163 | 49 | 49 | 65 | | |
| Mean intake, μg/day | 17 | 29 | 46 | | |
| Age and sex adjusted | 1 | 0.83 (0.56 to 1.24) | 1.04 (0.71 to 1.52) | 0.76 | 1.00 (0.89 to 1.12) |
| Multivariate adjusted* | 1 | 0.87 (0.58 to 1.29) | 1.09 (0.75 to 1.59) | 0.6 | 1.01 (0.90 to 1.13) |
| Multivariate adjusted† | 1 | 0.92 (0.60 to 1.39) | 1.21 (0.78 to 1.87) | 0.36 | 1.04 (0.91 to 1.18) |

Data are HRs (95% CI). CI indicates confidence interval; HR, hazard ratio.
*Adjusted for age, sex, waist circumference, smoking status, physical activity, and use of oral anticontraceptives.
†Adjusted for confounders in footnote "*" and energy intake+energy-adjusted intake of protein, fat, glycemic index, alcohol, vitamin C, and fiber.

for phylloquinone intake should be interpreted with caution. In addition, we used an FFQ at baseline to assess dietary intake, and this may not be representative for long-term dietary exposure. However, a German EPIC study showed fairly high-ranking agreement between FFQ derived dietary intakes 6 years apart, suggesting confidence of using baseline dietary data as long-term exposures.[36]

Furthermore, our hospital discharge register was not validated for stroke diagnoses but studies that validate discharge registers of stroke diagnoses concluded that administrative registers can be used for monitoring of stroke incidence.[37,38] Additionally, we have 93 unspecified cases of stroke in our cohort. It has been shown that ICD-9 codes are not always accurate in the diagnosis of ischemic stroke.[39] It is possible that the majority of the unspecified cases of stroke are in fact ischemic. However, adding these 93 cases of unspecified stroke to the 324 cases of ischemic stroke did not affect our results. Therefore, we do not think this influenced our results to a large extent. Furthermore, as in any observational study, our results could at least in part be influenced by differences in factors other than vitamin K intake. Although we simultaneously adjusted for several lifestyle and dietary cardiovascular risk factors in our analyses, residual confounding may be present.

Finally, the evidence presented does appear to indicate no or little relationship between vitamin K intake and stroke risk. However, it should be noted that based on the wide confidence intervals, relatively large hazard ratios cannot be ruled out.

The research on vitamin K and stroke risk has so far been restricted to the relationship between phylloquinone intake and risk of (ischemic) stroke. To date, 2 studies investigated this relationship, and neither of these studies observed an association between phylloquinone intake and total stroke or ischemic stroke risk.[16,17] These results are consistent with our findings.

Previous observational studies observed associations between high intake of menaquinones and reduced coronary calcification and coronary heart disease risk, but not for phylloquinone.[13–15] Similarly, research on fermented dairy products, the main source for menaquinones, and risk of stroke also suggested a reduced risk of stroke with high intakes of fermented dairy products. Larsson et al[40] showed a modest inverse association between cheese consumption and stroke risk. Goldbohm et al[41] showed a weak inverse association between fermented milk and yogurt and stroke risk. Finally, in our EPIC-NL cohort, fermented dairy also tended to be associated with reduced risk of stroke.[42] We therefore expected stronger associations for intake of menaquinones and stroke risk than for intake of phylloquinone. Also, no significant associations for menaquinones were found in our study.

These results could be explained by the following factors. The effect of menaquinones on cardiovascular diseases is presumably caused by the effect it has on calcification of the vessel wall through carboxylation of MGP.[43] The coronary calcium score, based on CT-scan imaging of coronary arteries[44] indeed proved to be a strong predictor

of incident CHD.[45] However, for stroke a recently published systematic review has concluded that carotid plaques with less severe plaque calcification were more likely to result in a TIA or stroke.[46] In other words, the assumption that carotid calcification, as caused by a vitamin K deficiency, is a strong predictor for cerebral ischemia may be incorrect. Even though there are some differences between studies included in this systematic review, it does indeed suggest that the relationship between carotid calcification and risk of stroke is weaker than between CAC and CHD. A second explanation could perhaps be the regulatory mechanisms of the brain to protect itself against ischemic damage, for instance through neuronal protection during ischemic injury by protein S, a hepatic vitamin K-dependent protein.[47,48] Moreover, this neuroprotective effect of protein S was already obtained at lower doses of protein S than necessary for the anticoagulant effect.[48] Because such regulatory mechanisms are not present in the coronary arteries, this may explain why vitamin K is more strongly associated with CHD than stroke.

When we split up stroke into ischemic and hemorrhagic stroke, we could also not detect significant associations. Human and rat studies showed that hepatic vitamin K-dependent proteins have preferential utilization of phylloquinone in response to a low phylloquinone dietary intake over extra-hepatic vitamin K-dependent proteins like MGP.[23,24] Therefore, a diet low in phylloquinone and menaquinone leads to undercarboxylation of extra-hepatic Gla-proteins, such as MGP, rather than undercarboxylation of hepatic Gla-proteins, such as coagulation factors V, VII, X, prothrombin, and fibrinogen. We therefore hypothesized a stronger association with ischemic stroke than hemorrhagic stroke. However, we could not confirm this hypothesis with results from our study.

In conclusion, in this prospective cohort of Dutch men and women, we did not find an association of intake of phylloquinone and menaquinones with stroke risk. These results persisted both for ischemic and hemorrhagic stroke and when specifying menaquinones into short-chain and long-chain menaquinones.

## Sources of Funding

The EPIC-NL study was funded by "European Commission: Public Health and Consumer Protection Directorate 1993–2004; Research Directory-General 2005"; Dutch Ministry of Public Health, Welfare and Sports (WVS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), and World Cancer Research Fund (WCRF) (The Netherlands). This research was supported by a personal Dr. Dekker postdoctoral grant (2008T062) from the Netherlands Heart Foundation (J. W. J. Beulens).

## Disclosures

None.

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