Phylloquinone Concentrations and the Risk of Vascular Calcification in Healthy Women

Geertje W. Dalmeijer, Yvonne T. van der Schouw, Sarah L. Booth, Pim A. de Jong, Joline W.J. Beulens

Objective—To investigate the association of plasma phylloquinone concentrations with coronary artery calcification (CAC) and vascular calcification.

Approach and results—In a prospective cohort of 508 postmenopausal women, plasma phylloquinone concentrations were measured by high-pressure liquid chromatography. Calcification was measured in the coronary arteries, aortic valve, mitral valve, and thoracic aorta by multidetector computed tomography. To combine these calcification scores, we dichotomized each of the 4 areas into present or absent. Because of the continuous measurement of CAC, we categorized this as calcification present if Agatston score was >0, and calcification score was calculated as the sum of the calcified areas. Multivariate-adjusted prevalence ratios and odds ratios were estimated using Poisson regression and multinomial logistic regression. After 8.5 years of follow-up, 22% of the women had no calcification, whereas 5% had calcification in all measured areas. Detectable phylloquinone concentrations were associated with increased CAC compared with nondetectable phylloquinone concentrations with a prevalence ratio of 1.34 (95% confidence interval, 1.01–1.77). When dividing women with detectable phylloquinone concentrations into low detectable (0–0.70 nmol/L) and moderate to high detectable (>0.70 nmol/L) phylloquinone concentrations versus nondetectable phylloquinone concentrations, both were associated with increased CAC with a prevalence ratio of 1.32 (95% confidence interval, 0.99–1.76) and 1.36 (95% confidence interval, 1.02–1.81), respectively. Detectable phylloquinone concentrations were not associated with the number of calcified areas with an odds ratio of 1.60 (95% confidence interval, 0.65–3.99; P=0.31).

Conclusions—Detectable phylloquinone concentrations are not associated with reduced vascular calcification but seemed to be associated with an increased prevalence of CAC. (Arterioscler Thromb Vasc Biol. 2014;34:1587-1590.)

Key Word: epidemiology • nutrition • phylloquinone • vascular calcification
intake than phylloquinone intake measured by food frequency questionnaires. A recent case–cohort study showed that low phylloquinone concentrations could be associated with greater CAC progression. Based on these results, we hypothesized that high phylloquinone concentrations are associated with less calcification. Mechanistically, there seems to be some overlap between coronary and aortic calcifications with heart valve calcification because both pathologies share some risk factors. Theoretically, treatment for aortic and coronary calcifications would be effective for valve calcifications as well.

We, therefore, investigated the association of plasma phylloquinone concentrations with CAC and vascular calcification among 508 postmenopausal healthy women.

**Materials and Methods**

Materials and Methods are available in the online-only Supplement.

**Results**

Baseline characteristics of the study population dichotomized by detectable and nondetectable phylloquinone concentrations are presented in Table 1. The mean (±SD) follow-up time was 8.5 (±1.3) years. Of these women, 42% had CAC, 22% had aortic valve calcification, 11% had mitral valve calcification, and 62% had aortic calcification. No calcification measures were available at baseline. Women with detectable phylloquinone concentrations (>lower limit of detection) at baseline were more likely to be older and had a higher body mass index, cholesterol ratio, and phylloquinone intake but were less likely to be smokers. No significant differences were noted in baseline blood pressure, total cholesterol levels and energy, or menaquinone intake between the 2 groups.

Detectable phylloquinone concentrations were associated with more CAC at follow-up compared with those with nondetectable phylloquinone concentrations with a prevalence ratio of 1.42 (95% confidence interval [CI], 1.7–1.87; P=0.01) in crude analyses. This association attenuated to a prevalence ratio of 1.34 (95% CI, 1.01–1.77; P=0.04) after adjustment for follow-up time, age, smoking, and body mass index. Adding non–high-density lipoprotein (HDL) ratio and cholesterol-lowering drug use into the model did not materially change the results (prevalence ratio, 1.33; 95% CI, 1.01–1.77; P=0.04).

Table 2 shows the association of detectable phylloquinone concentrations divided into 2 equal groups versus nondetectable phylloquinone with follow-up CAC. The low detectable phylloquinone group was associated with increased CAC with a prevalence ratio of 1.32 (95% CI, 0.99–1.76; P=0.06) and the high detectable phylloquinone group with a prevalence ratio of 1.36 (95% CI, 1.02–1.81; P=0.04; P for trend, 0.07) after full adjustment. Similar, but slightly attenuated results were observed additionally adjusting for non-HDL cholesterol, cholesterol-lowering drug use, and hypertension, with prevalence ratios of 1.29 (95% CI, 0.97–1.73; P=0.09) and 1.30 (95% CI, 0.98–1.71; P=0.07; P for trend, 0.13) for the 2 detectable phylloquinone concentration groups, respectively.

In addition, phylloquinone concentration was associated with more calcification at follow-up with an odds ratio (OR) (no versus Agatston score >400) of 1.83 (95% CI, 1.09–3.08; P=0.02) and an OR (no versus Agatston score ≥2400) of 3.74 (95% CI, 0.82–16.99; P=0.09) after adjustment.

Baseline phylloquinone concentrations were not associated with the number of calcified areas at follow-up with an OR (n=89) of 1.60 (95% CI, 0.65–3.99; P=0.31) after adjustment (Table 3). This association attenuated to an

### Table 1. Baseline Characteristics of Study Participants by Nondetectable and Detectable Plasma Phylloquinone (Vitamin K₁) Concentrations (nmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Phylloquinone=0*</th>
<th>Phylloquinone &gt;0*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
</tr>
<tr>
<td>n</td>
<td>(n=89)</td>
<td>(n=419)</td>
</tr>
<tr>
<td>Phylloquinone, nmol/L†</td>
<td>508</td>
<td>0±0.0</td>
</tr>
<tr>
<td>Follow-up time†</td>
<td>508</td>
<td>8±1.2</td>
</tr>
<tr>
<td>Age, y†</td>
<td>508</td>
<td>56±4.6</td>
</tr>
<tr>
<td>BMI, kg/m²†</td>
<td>508</td>
<td>24.3±3.9</td>
</tr>
<tr>
<td>Waist–hip ratio, cm²</td>
<td>506</td>
<td>0.77±0.05</td>
</tr>
<tr>
<td>Smoking, pack-years†</td>
<td>493</td>
<td>8.0±11.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>508</td>
<td>128±20.9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>508</td>
<td>78.7±10.1</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>485</td>
<td>6.0±1.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L†</td>
<td>484</td>
<td>1.64±0.47</td>
</tr>
<tr>
<td>Cholesterol ratio*</td>
<td>484</td>
<td>3.99±1.45</td>
</tr>
<tr>
<td>Dietary intakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>507</td>
<td>173±426</td>
</tr>
<tr>
<td>Vitamin C, mg/d‡</td>
<td>507</td>
<td>122±43</td>
</tr>
<tr>
<td>Vitamin K, µg/d†‡</td>
<td>507</td>
<td>241±94</td>
</tr>
<tr>
<td>Phylloquinone, µg/d‡</td>
<td>507</td>
<td>204±94</td>
</tr>
<tr>
<td>Menaquinones, µg/d†</td>
<td>507</td>
<td>35±13.8</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD). BMI indicates body mass index; and HDL, high-density lipoprotein.

*Zero is used to depict lower limit of detection, which for this analysis was 0.1 nmol/L.
†P<0.05 between nondetectable and detectable phylloquinone concentrations.
‡Energy-adjusted intake.

**Table 2. Prevalence Ratio (95% Confidence Interval) of Phylloquinone Concentrations With Coronary Artery Calcification Among 508 Postmenopausal Women**

<table>
<thead>
<tr>
<th>Phylloquinone</th>
<th>Crude</th>
<th>Model 1†</th>
<th>Model 2‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 nmol/L*</td>
<td>1</td>
<td>1.37 (1.02–1.84)</td>
<td>1.46 (1.09–1.95)</td>
</tr>
<tr>
<td>&gt;0–0.70 nmol/L*</td>
<td>1</td>
<td>1.35 (1.00–1.81)</td>
<td>1.43 (1.07–1.92)</td>
</tr>
<tr>
<td>&gt;0.70 nmol/L</td>
<td>1</td>
<td>1.32 (0.99–1.76)</td>
<td>1.36 (1.02–1.81)</td>
</tr>
</tbody>
</table>

*Zero used to depict lower limit of detection, which for this analysis was 0.1 nmol/L.
†Adjusted for follow-up time.
‡Adjusted for age, smoking habits, body mass index, and follow-up time.
Phylloquinone and Vascular Calcification Risk

Table 3. ORs (95% CI) of Detectable Phylloquinone Status Concentrations With Calcification Among 508 Postmenopausal Women

<table>
<thead>
<tr>
<th>Calcified Areas</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>n</td>
<td>112</td>
<td>160</td>
<td>154</td>
</tr>
<tr>
<td>Crude</td>
<td>1.07 (0.54–1.75)</td>
<td>1.83 (0.95–3.51)</td>
<td>1.96 (0.88–4.37)</td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.05 (0.52–1.71)</td>
<td>1.71 (0.89–3.30)</td>
<td>1.73 (0.77–3.91)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>1.01 (0.54–1.88)</td>
<td>1.70 (0.82–3.54)</td>
<td>1.60 (0.65–3.99)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; and OR, odds ratio.
*Adjusted for follow-up time.
†Adjusted for age, smoke habits, body mass index, and follow-up time.

Our results are inconsistent with previous intervention studies, but the phylloquinone dosages that were used were 2–4x higher than the mean habitual dietary phylloquinone intake in observational studies. It is possible that the protective effect of phylloquinone on CAC can only be reached with supplementary dosages and not with dietary intake.

In a previous study in the same study population, after full adjustment a nonsignificant trend was observed toward an increased risk with a relative risk of 1.17 (95% CI, 0.96–1.42; P for trend=0.11) for the highest versus lowest quartile of phylloquinone intake.4 This is in concordance with the results we found in the present study. Therefore, our assumption that our results on phylloquinone intake were driven by the low relative validity of phylloquinone intake as estimated by the food frequency questionnaires might be incorrect because we now observed the same direction of effect in plasma measurements.

Alternatively, participants with CAC at baseline may have had characteristics or risk factors that influenced overall nutritional status, which are reflected in the plasma phylloquinone concentrations.11 A possible explanation for this unexpected observation is that plasma phylloquinone may be a marker of an unmeasured biochemical or genetic risk factor for calcification. For example, the minor allele of VKORC1 rs8050894 (G) and the major allele of VKORC1 rs7294 (G) are associated with higher phylloquinone concentrations.12 These alleles may be linked and have been reported to be part of haplotype sequences that are associated with decreased warfarin dose requirements.13,14 Furthermore, VKORC1 haplotypes that reduce the activity of the VKORC1 enzyme have been shown to be associated with a significant higher risk of vascular calcification in rats15 and aortic calcification in humans.16 Hence, it is possible that similar polymorphisms in genes involved in the phylloquinone metabolism may result in higher phylloquinone status because of reduced phylloquinone recycling or metabolism. Furthermore, it is also possible that higher phylloquinone concentrations are caused by disturbed phylloquinone transport or cellular phylloquinone uptake. However, these potential mechanisms have not been investigated yet.

Strengths of this study include the prospective study design, measurement of vascular calcification, and plasma phylloquinone concentrations in healthy women. Nevertheless, our study has certain limitations to consider. Circulating phylloquinone concentrations are thought to reflect overall status but are highly correlated with triglycerides because of being transported on triglyceride lipoproteins.17 Unfortunately, data about triglyceride concentrations were only available in a subsample of 63 women. In this group, we adjusted for triglyceride concentrations that did not change the results (after adjustment, the prevalence ratio of 1.30 attenuated to a prevalence ratio of 1.27). Because we had limited data on triglyceride concentrations, we used non-HDL cholesterol as a proxy because this correlated with triglyceride concentrations (r=0.41).18 Adjusting for non-HDL in an additional analysis did not materially change our results. In addition, results did not change after excluding women with high phylloquinone concentrations (>2.0 nmol/L) as a result of high lipid levels. Furthermore, a previous study showed that circulating triglycerides were not associated with phylloquinone concentrations.19 Furthermore, phylloquinone concentrations reflect recent intakes.20,21 Although adjusting
for time since last meal did not affect our results, no information on the content of the last meal before blood sampling was available. Therefore, it could be that the use of a nonfasting plasma phylloquinone measure as a marker for long-term vitamin K status affected our results. Unfortunately, this study did not measure baseline vascular calcification, which precludes evaluation of the predictive value of phylloquinone concentrations on progression of vascular calcification.

Taken together, this study shows that a normal phylloquinone status is not associated with reduced vascular calcification but instead seemed to be associated with higher CAC evaluation of the predictive value of phylloquinone concentrations. Plasma phylloquinone measure as a marker for long-term vitamin K status affected our results. Unfortunately, this study did not consider the content of the last meal before blood sampling was taken. Time since last meal did not affect our results, no information on the content of the last meal before blood sampling was available. Therefore, it could be that the use of a nonfasting plasma phylloquinone measure as a marker for long-term vitamin K status affected our results. Unfortunately, this study did not measure baseline vascular calcification, which precludes evaluation of the predictive value of phylloquinone concentrations on progression of vascular calcification.

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Disclosures
None.

References
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Materials and methods

Study population
We used data from a sample of 508 postmenopausal healthy women as detailed previously. In brief, these women were selected from participants of the PROSPECT cohort study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). In PROSPECT, a total of 17,357 healthy breast cancer screening participants, aged 49-70 years, living in Utrecht and surrounding areas, were enrolled between 1993 and 1997. Between October, 2002 and April, 2004, 1,996 women were randomly selected from all 5,844 post-menopausal participants of the PROSPECT study who did not use contraceptives or hormone therapy. Of the 1,000 women who agreed to participate, a random selection of 573 underwent a multislice CT examination at a second visit between January and December, 2004. No blood samples were available for 56 women, eight women had no available calcification data and one woman used vitamin K antagonists. These 65 women were therefore excluded, leaving 508 women available for inclusion in these analyses. The study was approved by the Institutional Review Boards of the University Medical Center Utrecht and Tufts University, and written informed consent was obtained from all participants.

Baseline measurements
At baseline in 1993-1997, all participants filled in a general questionnaire containing questions on demographic characteristics, presence of chronic diseases, and risk factors for chronic diseases, such as hypertension, reproductive history, family history, smoking habits, drinking of alcohol and physical activity. Systolic and diastolic blood pressure were measured twice using the left arm with the participants in sitting position after 10 min of rest with an automated and calibrated oscillomat (Bosch & Son, Jungingen, Germany) and the average value was used. Body height was measured to the nearest 0.5 cm with a wall mounted stadiometer (Lameris, Utrecht, The Netherlands). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg with a floor scale (Seca, Atlanta, GA, USA). Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Non-fasting blood samples were taken to prepare citrated plasma and serum, which were subsampled and stored under liquid nitrogen at -196°C.

Vascular calcification measurements
In 2004, the participants underwent a multi-detector computed tomography (MDCT, Mx 8000 IDT 16, Philips Medical systems, Best, The Netherlands) as previously described. The amount of calcium in the coronary arteries was quantified on a separate workstation with software for calcium scoring (Heartbeat-CS, EBW, Philips Medical System, Best, The Netherlands). The Agatston calcium score was obtained by multiplying the area by a weighting factor that is dependent on the peak signal anywhere in the lesion. The score of individual lesions were added to obtain the Agatston calcium score for the entire coronary tree. Reproducibility was assessed in two ways: (1) inter-reader reproducibility by having 199 scans read by two independent observers; and (2) inter-scan reproducibility by having 58 women undergo a second scan within 3 months. In this study, the inter-reader and the inter-scan reproducibility both had intra-class correlation coefficients greater than 0.95.

The MDCT-scans for thoracic aortic, aortic valve and mitral valve calcification were visually scored by a certificated and experienced radiologist. The radiologist was blinded for participant characteristics and outcome status. Calcification of the heart valves was anatomically subdivided into calcification of the aortic valve leaflets (AVL) and the mitral valve leaflets (MVL). AVL and MLV calcification was graded as absent, mild (one leaflet affected), severe (2 or three leaflets affected). Aortic calcification was graded as absent, mild (≤3 foci), moderate (4–5 foci or 1 calcification extending over ≥3 slices) and severe (>5 foci or 1 calcification extending over ≥3 slices).

Phylloquinone concentrations
In 2012 we obtained funding to measure phylloquinone concentrations in plasma. Plasma phylloquinone concentrations were measured by high-pressure liquid chromatography. Low and high control specimens had average values of 1.2 and 4.8 nmol/L, with intra-assay (total) coefficients of variation (CVs) of 14.5 and 8.2%, respectively. For this analysis, the lower limit of detection (LLOD) for this assay was 0.1 nmol/L.

**Data analyses**

Characteristics of the study population are presented as the mean (±SD). To handle missing data, we used multiple imputations. We assumed that the missing data occurred at random. We generated 10 imputed datasets and used Rubin’s rules to combine the estimates of the parameters. Calcium was measured in the coronary arteries (continuous), aortic valve (none, mild and severe), mitral valve (none, mild and severe) and thoracic aorta (none, mild, moderate and severe) by multi-detector computed tomography. To combine these calcification scores, we dichotomized each of the four areas into present or absent. Because of the continuous measurement of CAC, we categorized this as calcification absent if Agatston score was <0 and present if Agatston score was ≥ 0. The total calcification score was calculated as the sum of all calcified areas (grades 0-4).

We first analyzed the association between phylloquinone concentrations and presence of CAC (Agatston score was ≥ 0). We dichotomized phylloquinone concentrations in non-detectable phylloquinone concentrations (below LLOD) and detectable phylloquinone concentrations (> LLOD). In addition, women with detectable phylloquinone concentrations were divided in two equal groups categorized as low (<0.70 nmol/L) and moderate to high (>0.70 nmol/L). Because of the high prevalence of CAC, an odds ratio will overestimate the effect size. We therefore used a modified Poisson regression model to estimate prevalence ratios and 95% confidence interval (CI) of CAC, with non-detectable phylloquinone concentrations as the reference category. To test for linear trend, phylloquinone concentrations of each woman was replaced by median values of the phylloquinone status group (non-detectable, low-detectable, moderate to high-detectable) and included in the model as a continuous covariate.

In addition, we analyzed the association between phylloquinone concentrations and CAC in three categories (Agatston score 0, 0-400 and ≥ 400). Due to the categorical outcome (0-2), we used multinomial logistic regression to estimate odds ratios (OR). The groups with agatston score 0-400 and ≥ 400 were compared with the group without calcification.

Next, we analyzed the total calcification score. Due to the categorical outcome (0-4), we used multinomial logistic regression to estimate odds ratios (OR). The groups with one, two or ≥ three calcification areas were compared with the group without calcification in any area. In addition, we analyzed this association also for the areas aortic valve, mitral valve and thoracic aorta separately.

In model 1, the PRs or ORs were adjusted for follow-up time. In model 2, we additionally adjusted for age, smoking (pack years) and BMI. To explore the robustness of our associations, we adjusted for non-HDL cholesterol, cholesterol-lowering drugs use and hypertension in sensitivity analyses.

To verify that our results were not confounded very high plasma phylloquinone concentrations due to the non-fasting state and associated high lipid levels, we repeated our analyses by using only women with phylloquinone concentrations lower than 2.0 nmol/L (n=455).

A two-tailed p-value of <0.05 was considered to be statistically significant. Statistical analyses were conducted using IBM SPSS (version 20 for Windows).
Reference List


