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High dietary menaquinone intake is associated with reduced coronary calcification

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ABSTRACT

Background: Dietary vitamin K is thought to decrease risk of cardiovascular disease by reducing coronary calcification, but inconsistent results are reported. This may be due to different effects of vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone, MK), but few studies included both.
Methods: We investigated the association of intake of phylloquinone and menaquinone, including its subtypes (MK4–MK10), with coronary calcification in a cross-sectional study among 564 post-menopausal women. Phylloquinone and menaquinone intake was estimated using a food-frequency questionnaire.
Results: Sixty-two percent ($n = 360$) of the women had coronary calcification based on 1.5-mm thick slices. Phylloquinone intake was not associated with coronary calcification with a relative risk (RR) of 1.17 (95%-confidence interval: 0.96–1.42; $p_{trend} = 0.11$) of the highest versus lowest quartile. Menaquinone intake was associated with decreased coronary calcification with an RR of 0.80 (95%-CI: 0.65–0.98; $p_{trend} = 0.03$).
Conclusion: This study shows that high dietary menaquinone intake, but probably not phylloquinone, is associated with reduced coronary calcification. Adequate menaquinone intakes could therefore be important to prevent cardiovascular disease.

1. Introduction

Vitamin K is a fat-soluble vitamin that occurs in two biologically active forms; vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone; MK-4 through MK-10). Phylloquinone, the most common form, is present in green, leafy vegetables and certain vegetable oils [1], while menaquinones mostly occur in animal products like meat, eggs, and cheese [2]. Vitamin K functions as a cofactor in the gamma-glutamyl carboxylation of certain glutamic acid (Gla) residues of vitamin K-dependent proteins for their activation [3]. Such Gla-proteins include coagulation factors prothrombin, Factor VII, IX, and X [3]. Phylloquinone is effectively cleared from the circulation by the liver, the main site for clotting factor synthesis and is therefore thought to be particularly important for blood coagulation [4].

Vitamin K also functions as a cofactor for activation of Gla-proteins in extrahepatic tissues such as bone (osteocalcin) and the vessel wall (matrix Gla-protein) (MGP). Matrix Gla-protein is a powerful inhibitor of vascular calcification [5]. Vascular vitamin K deficiency could therefore increase the amount of undercarboxylated, non-functional MGP and lead to increased calcium deposition. Coronary calcification is a strong, independent predictor of coronary events [6] and this is an actively regulated process [7] that could be regressed by vitamin K, carbonic anhydrase, and bisphosphonates [8]. Vitamin K deficiency could therefore lead to cardiovascular disease (CVD) [3,9]. Indeed, MGP-knock out mice develop severe coronary calcification [10]. In addition, the drug warfarin, inhibiting Gla residue formation, was shown to increase coronary calcification in rats and humans [11,12]. Studies showed that vitamin K rich diets could prevent these effects in warfarin-treated rats [13], but these effects on coronary calcification were particularly due to menaquinone and not phylloquinone [13].

Results from human, observational studies investigating relations between vitamin K intake and cardiovascular diseases are inconsistent. The Nurses' Health Study showed a modest risk reduction of coronary heart disease (CHD) for high phylloquinone intakes [14], while no significant associations were observed in the Health

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Professionals Follow-up Study and the Rotterdam Study [15,16]. On the other hand, in the Rotterdam Study a strong inverse association between menaquinone intake and coronary heart disease mortality and severe aortic calcification was observed [16]. These inconsistencies may relate to different effects of phylloquinone and menaquinone on coronary calcification. So far, only the Rotterdam Study investigated both phylloquinone and menaquinone intake, while relations of different menaquinone subtypes with coronary calcification have not been studied. We investigated the association between dietary intake of both phylloquinone and menaquinone, including its subtypes, with coronary calcification in a cross-sectional study of 564 Dutch women.

2. Materials and methods

2.1. Study population

We used data from a cross-sectional study among 564 post-menopausal healthy women as has been detailed earlier [17]. In short, these women were selected from participants of the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). In PROSPECT 17,357 healthy participants of a nationwide population-based breast-cancer screening programme, aged 49–70 years, living in Utrecht and surroundings were enrolled between 1993 and 1997. Between October 2002 and April 2004, 1996 women were randomly selected from 5844 participants of the PROSPECT study who were post-menopausal and did not use contraceptives or hormone replacement therapy, and 1000 agreed to participate. Of these 1000 women, a random selection of 573 underwent a multislice CT examination at a second visit between January and December 2004. The Medical Ethical Committee of the University Medical Center Utrecht approved the study and written informed consent was obtained from all participants before enrolment. The study complied with the Declaration of Helsinki. We excluded 8 subjects with missing information on coronary calcification and 1 subject with missing vitamin K intake, leaving 564 subjects for analysis.

2.2. Classical cardiovascular risk factors

At the first re-examination visit, smoking behaviour and family history of CHDs were assessed by a questionnaire. Age was calculated from birth date and date of investigation. Height and weight were measured and body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Systolic and diastolic blood pressures (SBP and DBP) were measured at both arms with an automated and calibrated blood pressure device (DINAMAP^{TM} XL, Critikon, Johnson & Johnson, Tampa, Florida, USA) with the subject in supine position. A venous blood sample was drawn after an overnight fast of at least 8 h. Plasma total cholesterol, plasma triglycerides, and plasma glucose were measured using standard enzymatic procedures. High-density lipoprotein (HDL) cholesterol was measured by the direct method (inhibition, enzymatic). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. We defined hypertension as either using antihypertensive therapy or a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg.

2.3. Vitamin K intake

Energy and nutrient intake were estimated from a validated food-frequency questionnaire (FFQ) [18,19]. 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. The FFQ has been validated in pilot studies prior to the start of the study [18,19]. Nutrient intake was calculated using the 1996 version of the Dutch national food composition table. This table does not contain information on vitamin K contents of foods. Therefore the concentrations of phylloquinone and menaquinone (MK-4 through MK-10) in a large series of Dutch foods were assessed at the Biochemistry Laboratory, Maastricht University [2]. For some foods, published data by others were used to update the dietary database for vitamin K [20–24]. 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 [18,19]. We observed a low relative validity of phylloquinone and menaquinone-10 intake with Spearman correlations of 0.24 and 0.23, respectively. Relative validity for intake of menaquinone and MK4 to menaquinone-9 was reasonable with Spearman correlation coefficients ranging from 0.51 for menaquinone-7 to 0.72 for menaquinone-5. In our study population of 564 post-menopausal women, vegetables contributed 82% of phylloquinone intake, while cheese contributed 54%, milk products 22% and meat 15% of menaquinone intake.

2.4. Coronary calcium measurements

The participants underwent a multi-detector computed tomography (MDCT) examination for the assessment of CAC. The amount of calcium in the coronary arteries was assessed with a MDCT scanner (Mx 8000 IDT 16, Philips Medical Systems, Best, The Netherlands). Subjects were positioned within the gantry of the MDCT scanner in supine position. During a single breath hold, images of the heart, from the level of the tracheal bifurcation to below the base of the heart, were acquired using prospective ECG triggering at 50–80% of the RR-interval, depending on the heart rate. Scan parameters were 16 mm $\times$ 1.5 mm collimation, 205 mm field of view (FOV), 0.42 s rotation time, 0.28 s scan time per table position, 120 kVp and 40–70 mA s (patient weight <70 kg: 40 mA s; 70–90 kg: 55 mA s; >90 kg: 70 mA s). Scan duration was approximately 10 s, depending on heart rate and patient size. Scan duration was approximately 10 s, depending on heart rate and patient size. Quantification of coronary calcium was performed on a separate workstation with software for calcium scoring (Heartbeat-CS, EBW, Philips Medical Systems, Best, The Netherlands). All regions with a density over 130 Hounsfield units were identified as potential calcifications. After completing a training-program, a trained scan reader, blinded for other results of the women, manually selected only the calcifications within the coronary arteries (left main, left anterior descending, left circumflex, right coronary artery, or posterior descending artery). To reduce the influence of noise, the minimum size of a calcified lesion was set at 0.5 mm^2. The peak density in Hounsfield units and the area in mm^2 of each selected region were calculated. The Agatston [25] calcium score was obtained by multiplying the area by a weighting factor that is dependent on the peak signal anywhere in the lesion. The scores of individual lesions were added to obtain the Agatston calcium score for the entire coronary tree. Calcium presence was defined as score >0. We performed reproducibility studies in which 199 scans were read in duplicate, showing intraclass correlation coefficients (ICCs) of >0.95 for the duplicate readings. Another reproducibility study in which in 73 women a duplicate MDCT scan was made within 3