Methods

Participants

Details of the MESA study design and protocol have been published(1). In brief, between July 2000 and August 2002, 6,814 adults free from clinically apparent cardiovascular disease, between the ages of 45 to 84 years, were recruited from 6 representative communities and participated in the baseline examination. The cohort was comprised of 38% Caucasian (N=2,622), 28% African-American (N=1,893), 22% Hispanic (N=1,496) and 12% Chinese (N=803) participants. Individuals underwent extensive clinical, laboratory, and radiographic examination as part of the baseline examination. The institutional review boards at all participating centers approved the study, and all participants gave informed consent.

For this analysis, we used a weighted cohort design drawing from two previous MESA studies, as seen in Figure 1. In a previous study, serum phylloquinones were measured in a random subcohort of MESA (n=780), after excluding warfarin users (n=24) and a random subcohort due to high sample usage (n=995)(2). In a second case-cohort study, DCP concentrations were measured in 104 participants (cases) defined as an ankle brachial index >=1.4, while the cohort (n=624) was a random sampling of the phylloquinone subcohort. Our cohort includes the cases and a random sampling of the phylloquinone cohort. To account for the oversampling of cases in the study design, Barlow weights were used in the Kaplan-Meier estimates and Cox regression models(3). Since the random cohort was drawn from a previously established random cohort, the subcohort sampling fraction of 11% was calculated as a proportion of the original cohort after exclusions. Sensitivity analysis showed little impact of using this value in the Barlow weighting method. Six extreme outliers were excluded from analysis, and two patients were missing follow up data, leaving a total weighted cohort of 709 participants for primary analysis; results that winsorized these individuals at the 99th percentile yielded essentially identical results.

Plasma VKDP activity

The primary exposure was DCP concentrations measured at the baseline MESA examination, using a commercially available ELISA kit (Asserachrom DCP-II, Stago, France) that uses murine monoclonal antibodies against the under-carboxylated form of prothrombin. The detectable range for this assay was 0.335 - 207 ng/mL. Based on 4 controls, the intra-assay CVs were 6.5%, 16.1%, 5.2%, and 12.2%, and the inter-assay CVs were 10.2%, 32.3%, 9.1%, and 12.5%. Higher DCP concentrations suggest lower VKDP activity.

Ischemic cardiovascular disease

Follow-up took place through December 2011, for a median of 11.0 years of follow-up. CVD events were adjudicated by the MESA mortality and morbidity review committee. A full description of MESA event ascertainment is available at http://www.mesa-hhlbi.org. We defined incident ischemic cardiovascular disease as definite and probable myocardial infarction, coronary percutaneous angioplasty, coronary artery bypass grafting surgery, fatal or nonfatal stroke, or coronary related death. Details of CVD ascertainment have been published(4).

Covariates

Age, gender, race/ethnicity (white, Chinese, African-American, or Hispanic), level of education, income, smoking history, physical activity, and medication usage were self-reported through standard questionnaires. Body mass index was calculated from height and weight measurements obtained without shoes. Diabetes was defined by either a fasting glucose >=126 mg/dl or the use of either oral hypoglycemic medications or insulin. Fasting blood was collected and stored at -70 until needed for the appropriate assays. High density lipoprotein (HDL) cholesterol was measured using the cholesterol oxidase cholesterol method (Roche Diagnostics), and low density lipoprotein was calculated using the Friedewald equation. The estimated glomerular filtration rate (eGFR) was calculated by using the Chronic Kidney Disease Epidemiology Collaboration formula based on serum creatinine measurements. Urine albumin and creatinine were measures in a single morning sample by nephelometry and the rate Jaffe equations, and expressed as albumin-creatinine ratio (ACR) in mg/g. Serum phylloquinone was measured by reversed-phase HPLC followed by fluorometic detection.

Statistical analysis

We describe participants' baseline characteristics according to quartiles of DCP. We calculated incident event rates per quartile of DCP accounting for the case-cohort study design by using Barlow weights, and describe the log-rank p-value across the 4 strata using the Kaplan-Meier product-limit estimator. Weighted Cox proportional hazard models using robust standard errors were used to describe the association between baseline DCP concentrations and incident ischemic CVD. Model I included adjustment for age (years), gender and race/ethnicity (Black, Hispanic, Chinese, white). Model II added adjustment for body mass index (BMI), cigarette smoking (never, former, current), intentional physical activity (MET-min/day), current alcohol use, high school graduation, diabetes, systolic blood pressure (SBP), low-density lipoprotein cholesterols (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, high-sensitivity C-reactive protein (hs-CRP), lipid-lowering medication, hypertension medication, estimated glomerular filtration rate (eGFR), and albumin/creatinine ratio. Model III added adjustment for serum phylloquinone and dihydrophylloquinone concentrations. The proportional hazards assumption for Cox regression models was tested using Stata's 'phtest' command to evaluate whether the log hazard ration function was constant over time, and showed no evidence of non-proportionality in the global test of the full model.

To determine the dose-response relationship, we applied locally weighted scatterplot smoothing (LOWESS) relating DCP concentrations with cumulative incidence. Based on these results, and because DCP concentrations were skewed, we also present hazard ratios using a log base 2 transformation; hazard ratios from this model can be interpreted as the risk per doubling of DCP concentrations. In addition, given that DCP>2 ng/ml has been considered as a threshold of VKDP inactivity(5), we also investigated it as a binary variable.

To examine the robustness of our findings within strata, we tested multiplicative interaction terms between log base 2 DCP concentrations and diabetes, hypertension (anti-hypertensive medication usage

or SBP > 140 mm Hg), renal function (continuous eGFR and eGFR<60 ml/min), cohort median hs-CRP, and serum phylloquinone < 1 nmol/L in adjusted analyses (Model III).

Finally, we also examined the association of DCP concentrations with incident ischemic cardiac disease, excluding stroke events from the primary endpoint.

1. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002;156(9):871-81.
2. Shea MK, Booth SL, Miller ME, Burke GL, Chen H, Cushman M, et al. Association between circulating vitamin K1 and coronary calcium progression in community-dwelling adults: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2013;98(1):197-208.
3. Sharp SJ, Poulaliou M, Thompson SG, White IR, Wood AM. A review of published analyses of case-cohort studies and recommendations for future reporting. PLoS One. 2014;9(6):e101176.
4. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, et al. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Arch Intern Med. 2008;168(12):1333-9.
5. Holden RM, Morton AR, Garland JS, Pavlov A, Day AG, Booth SL. Vitamins K and D status in stages 3-5 chronic kidney disease. Clin J Am Soc Nephrol. 2010;5(4):590-7.