ORIGINAL RESEARCH

OUTCOMES AND QUALITY

Effects of Vitamin K2 and D Supplementation on Coronary Artery Disease in Men

A RCT

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ABSTRACT

BACKGROUND Extent and progression of coronary artery calcification (CAC) are strong predictors of myocardial infarction and mortality.

OBJECTIVES This study aims to investigate if vitamin K2 and D supplementation can reduce CAC progression.

METHODS A total of 389 participants were randomized to supplementation with vitamin K2 (720 μg/day) and D (25 μg/day) vs placebo in a multicenter double-blinded randomized controlled trial. The primary endpoint (progression of aortic valve calcification) has been reported. This study reports CAC progression in participants with no ischemic heart disease. CT scans were performed at baseline, 12, and 24 months. ΔCAC and coronary plaque volume were evaluated in the entire group and in 2 subgroups. A safety endpoint was the composite of myocardial infarction, coronary revascularization, and all-cause mortality.

RESULTS In total, 304 participants (male, mean age 71 years) were identified. The intervention and placebo group both increased in mean CAC scores from baseline to 24-month follow-up (Δ203 vs Δ254 AU, $P = 0.089$). In patients with CAC scores ≥400 AU, CAC progression was lower by intervention (Δ288 vs Δ380 AU, $P = 0.047$). Plaque analyses showed no significant difference in progression of noncalcified plaque volume (Δ-6 vs Δ46 mm³, $P = 0.172$). Safety events were fewer in participants receiving supplementation (1.9% vs 6.7%, $P = 0.048$).

CONCLUSIONS Patients with no prior ischemic heart disease randomized to vitamin K2 and D supplementation had no significant reduction in mean CAC progression over a 2-year follow-up compared to placebo. Although the primary endpoint is neutral, differential responses to supplementation in those with CAC scores ≥400 AU and in safety endpoints are hypothesis-generating for future studies. (JACC Adv 2023;2:100643) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

AVC = aortic valve calcification

CAC = coronary artery calcification

CCTA = coronary computed tomography angiography

MGP = matrix Gla protein

Coronary artery calcification (CAC) and aortic valve calcification (AVC) are important precursors for myocardial infarction (MI) and aortic stenosis.[1,2] Both are increasing with age, and scores are higher among men compared to women.[3,4] Even though CAC and AVC share common risk factors, the pathogenesis of the 2 diseases is complex, and it has been suggested that they represent distinct pathways.[4] Various dietary and pharmaceutical interventions have been investigated for the reduction of the risk.[5] A growing body of evidence suggests that not only vitamin K2 but also vitamin D play a significant role in protection against vascular calcification. This is due to their stimulating effect on matrix Gla protein (MGP), which is considered the strongest inhibitor of the calcification processes in the vascular wall.[6-8] Both arterial and aortic valve diseases are initiated with lipid deposition, followed by a calcification process regulated by MGP.[7,9] While lipid-lowering treatment is commonly used for the prevention of arterial diseases like MI, treatments targeting the calcific process in both arterial and aortic valve diseases remain to be clarified.

To investigate whether supplementation with vitamin K2 and D may reduce the progression of CAC and AVC, we conducted the randomized, double-blinded, placebo-controlled AVADEC (Aortic Valve Decalcification) trial. There was no significant effect of the intervention on our primary endpoint, which was AVC progression during a 2-year follow-up.[10] The aim of this study on secondary findings was to investigate the effect of supplementation with vitamin K2 and D on CAC progression, as well as changes in plaque composition and coronary artery stenosis, in a randomized controlled setting.

METHODS

STUDY DESIGN. The AVADEC trial is an investigator-initiated randomized, double-blinded, placebo-controlled multicenter trial with the primary aim to investigate progression in AVC. It was conducted at 4 Danish hospitals (Odense, Svendborg, Vejle, and Silkeborg). The trial design and the primary results on AVC have been reported previously.[10,11] The current study is investigating the prespecified secondary endpoints concerning coronary artery disease.[11] The trial protocol was approved by the Regional Scientific Ethical Committee for Southern Denmark (S-20170059) as well as the Data Protection Agency (17/19,010), and was performed in accordance with the principles of the Declaration of Helsinki. Written and oral informed consents were obtained from each participant. The study protocol is available (NCT03243890). The authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial and this report to the protocol.[11] The data that supports the findings of this study are available from the corresponding author on reasonable request.

PATIENTS. Participants were recruited to AVADEC from the DANish CArdioVAscular Screening (DANCAVAS) trial, which included men from the general population.[12] Eligible patients in AVADEC were men between the ages of 65 and 74 years with an AVC score of ≥300 AU (>90th percentile). Patients with previous heart valve surgery, moderate aortic stenosis (peak aortic jet velocity >3.0 m/s), treatment with vitamin K antagonists, calcium and phosphate metabolism, or coagulation system disorders were excluded from AVADEC.[11] In this study, we additionally excluded participants with previous MI, percutaneous coronary intervention, or coronary artery bypass graft surgery at baseline. The purpose of this exclusion from the primary population was to optimize the CAC evaluations. Moreover, participants with missing baseline cardiac computed tomography (CT) scans were excluded.

RANDOMIZATION AND MASKING. Therapeutic randomization was performed by the pharmacy at Odense University Hospital. On the basis of a computer-generated assignment scheme, the tablets had a random number according to the sequential order of the study site. The randomization was stratified according to center and AVC score (300-599 AU or ≥600 AU). The placebo tablet had an identical appearance to the intervention tablet, and they were matched for taste, color, and size. The randomization list was available to the data and safety monitoring board, but patients, nurses, physicians, and other data collectors were kept blinded to the allocation

until the last patient completed the study and all analyses were finalized.[11]

PROCEDURE. Patients were randomly assigned in a 1:1 ratio to either daily oral supplementation with vitamin K2 (720 μg/d, K2VITALDELTA) and vitamin D (25 μg/d) or placebo for 24 months. Patients were followed for 24 months undergoing clinical examination with withdrawal of blood samples every 6 months. A biobank was established with blood samples at baseline and after 1 and 2 years of follow-up. Analysis of plasma dp-ucMGP in the biobank samples was used as a proxy for vitamin K status.[10] Participants underwent both noncontrast electrocardiogram-gated CT for calcium scoring at baseline, 1-year, and 2-year follow-up, as well as contrast-enhanced electrocardiogram-gated coronary CT angiography (CCTA) at baseline and 2-year follow-up. Imaging was performed at different scanners at the 4 sites: Siemens Somatom Force, Siemens Somatom Definition Flash 128 slice Dual Source, Toshiba Aquilion One 320 slice scanner, and GE Healthcare Revolution scanner. Tube current, voltage, and contrast volume were adjusted individually based on body mass index.

CARDIAC CT. Noncontrast CT. All CAC scores in the 304 participants were assessed by 2 trained physicians at Odense University Hospital using the Agatston method in clinically available software (syngo.CT CaScoring-Siemens Healthcare). All data were transferred to a server, where they were analyzed after the end of the follow-up. The readers were blinded to all clinical data and allocation while measuring the CAC score of the baseline, 1-year, and 2-year CT scans in one session.

Contrast CT. Quantitative assessment of coronary plaque subtypes was performed by the use of a dedicated deep learning system (AutoPlaque, Version 3.0, Cedars-Sinai Medical Center) and was done by 1 of 2 trained physicians.[13] Image quality of the contrast CT scans for the purpose of plaque analysis was graded from 0 to 4, starting from not analyzable, poor, reasonable, good, to excellent quality. To ensure valid results, only participants with contrast CT scans of good to excellent image quality (image quality 3 and 4) were included in the plaque analyses. Quantitative analysis was performed for patients who had one or more segments of nonobstructive or obstructive plaque in vessels with a distal normal reference of ≥2.0 mm. For all patients with normal coronary arteries, the plaque volumes were set to 0.
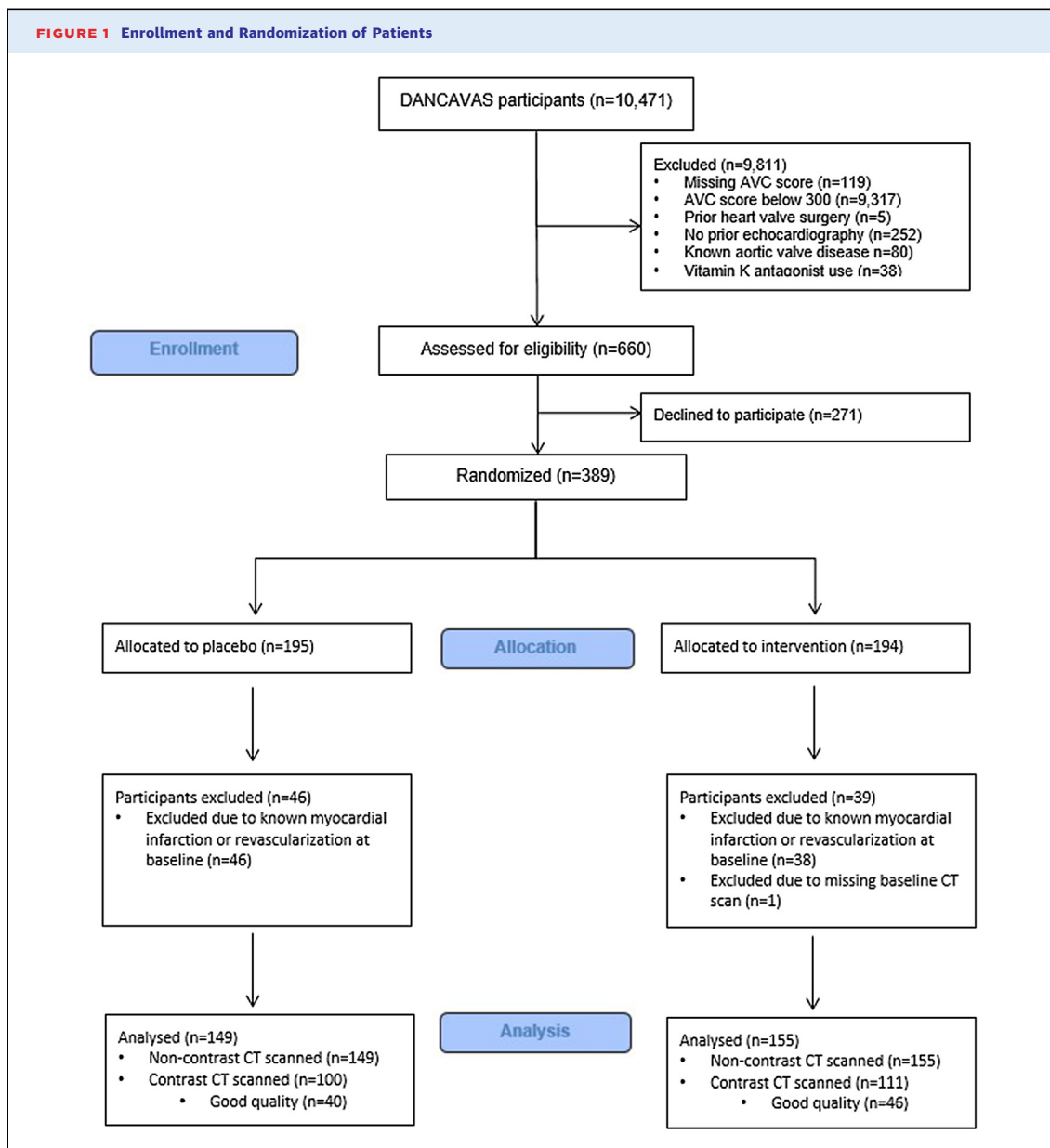
The proximal and distal aspects of coronary artery segments with atherosclerotic plaque were manually defined. Vessel wall, lumen, and plaque constituents were automatically identified by artificial intelligence, with manual adjustments performed if required. Scan-specific thresholds for plaque constituents were generated as described previously.[13-15] Plaque volumes (mm^3) were measured for the following plaque subtypes: total plaque, calcified plaque, and noncalcified plaque. Plaque volume on per-patient level was used as our primary assessment for each plaque type.

Coronary artery stenosis was visually assessed on a 17-segment basis on contrast CCTA by 2 expert cardiologists. The luminal stenosis was classified as normal, nonobstructive, and obstructive (≥50% in left main stem or ≥70% in other segments ≥2.0 mm). Obstructive coronary artery disease was defined as the presence of one or more coronary vessels with obstructive stenosis. Left main stem stenosis was defined as a 2-vessel disease.

OUTCOME. All outcomes were prespecified in the statistical analysis plan (NCT03243890). The primary outcome was the absolute change in CAC score assessed by noncontrast CT scan from baseline to 24 months. The secondary endpoints were changes in CAC score in 2 prespecified subgroups (CAC score <400 AU and ≥400 AU). CAC score progression was also evaluated from baseline to 12 months and from 12 months to 24 months. Additional secondary outcomes were changes in plaque volume (mm^3) of total plaque, calcified plaque, and noncalcified plaque on per-patient level assessed by CCTA from baseline to 24 months. The change in presence of normal, nonobstructive, and obstructive coronary artery stenosis in contrast CT scans from baseline to 24 months was also a secondary endpoint. Lastly, the safety endpoint was prespecified as the combined number of patients with MI, coronary revascularization, and all-cause mortality during the follow-up period. All events were adjudicated by the safety committee. The first author and investigator of this study was blinded through the complete process of data analyses and statistics. A statistician performed the statistical analyses, and the investigator remained blinded to allocation.

STATISTICAL ANALYSIS. Statistical analyses were performed according to the statistical analysis plan. Numerical characteristics are presented as mean ± SD or median (25th and 75th percentiles) where appropriate. Means were compared by 2-sample t-test and medians by Wilcoxon rank-sum test. Categorical variables were presented as n (%) and compared by the chi-square test or Fisher's exact test.

FIGURE 1 Enrollment and Randomization of Patients

DANCAVAS participants (n=10,471)

Excluded (n=9,811)
- Missing AVC score (n=119)
- AVC score below 300 (n=9,317)
- Prior heart valve surgery (n=5)
- No prior echocardiography (n=252)
- Known aortic valve disease n=80)
- Vitamin K antagonist use (n=38)

Enrollment

Assessed for eligibility (n=660)

Declined to participate (n=271)

Randomized (n=389)

Allocated to placebo (n=195)   **Allocation**   Allocated to intervention (n=194)

Participants excluded (n=46)
- Excluded due to known myocardial infarction or revascularization at baseline (n=46)

Participants excluded (n=39)
- Excluded due to known myocardial infarction or revascularization at baseline (n=38)
- Excluded due to missing baseline CT scan (n=1)

Analysed (n=149)   **Analysis**   Analysed (n=155)
- Non-contrast CT scanned (n=149)
- Contrast CT scanned (n=100)
  - Good quality (n=40)

- Non-contrast CT scanned (n=155)
- Contrast CT scanned (n=111)
  - Good quality (n=46)

The primary outcome and numeric secondary outcomes were compared using mixed-effects linear models including bootstrapped standard editions where needed to take into account deviations from normality assumptions. The mixed-effects linear models included a fixed effect for time points (baseline, 12, and 24 months), a fixed effect for treatment, a fixed effect interaction between treatment and time point, and a random intercept for each included patient. The random effects were assumed to be normally distributed with a mean of zero and an unstructured covariance matrix.

This analysis was performed for the total group as well as separately for the 2 subgroups (CAC score 0-399 and ≥400 AU). Moreover, we conducted these models for the subgroup of participants with good to excellent CT image quality defined as image quality ≥3. The rate of adverse events was reported as counts and proportions and compared between groups using Fisher's exact test.

Supplementary investigation of the primary outcome was performed by repeating the main mixed-effect linear analysis by stratifying by age (<70 or ≥70 years at baseline), diabetes, hypertension,

TABLE 1 Characteristics of the Total Population at Baseline

	Placebo Group (n = 149)	Vitamin K2+D Group (n = 155)	P Value
Age, y	71.16 (2.24)	70.75 (6.12)	0.44
Body mass index, kg/m^2	28 (26-31) (n = 142)	29 (26-32) (n = 145)	0.75
Coexisting condition			
Diabetes	22 (14.8)	28 (18.1)	0.44
Hypertension	95 (63.8)	105 (67.7)	0.46
Atrial fibrillation	17 (11.5)	15 (9.7)	0.61
Renal impairment, eGFR <60 mL/min 1.73 m^2	11 (7.4)	21 (13.6)	0.08
Family history of premature CVD	10 (6.7)	22 (14.4)	0.046
Smoking status			0.83
Active smokers	20 (13.4)	17 (11.1)	
Former smokers	83 (55.7)	88 (57.5)	
Nonsmokers	46 (30.9)	48 (31.4)	
HDL, mmol/L	1.4 (1.2-1.6) (n = 140)	1.3 (1.1-1.6) (n = 145)	0.70
LDL, mmol/L	2.1 (1.6-2.8) (n = 140)	2.2 (1.8-2.7) (n = 145)	0.51
Total cholesterol, mmol/L	4.1 (3.6-4.8) (n = 140)	4.2 (3.7-4.8) (n = 145)	0.45
Estimated GFR, ml/min/1.73 m^2	81 (70-88) (n = 148)	81 (67-89) (n = 154)	0.48
Dp-ucMGP, pmol/L	717.5 (634.0-863.5) (n = 140)	736.0 (641.0-859.0) (n = 145)	0.76
Systolic blood pressure, mm Hg	148 (135-161) (n = 146)	144 (133-154) (n = 153)	0.13
Diastolic blood pressure, mm Hg	87 (79-94) (n = 146)	83 (78-90) (n = 153)	0.032
Medications			
ACE inhibitor or ARB	79 (53.0)	80 (51.6)	0.81
Beta-blocker	26 (17.4)	32 (20.6)	0.48
Mineralocorticoid-receptor antagonist	8 (5.4)	4 (2.6)	0.21
Antiplatelet therapy	96 (64.4)	100 (64.5)	0.99
DOAC	14 (9.4)	14 (9.0)	0.91
Statin therapy	109 (73.2)	106 (68.4)	0.36
Baseline CAC score, AU	655 (182-1,380)	636 (200-1,443)	0.95
CAC score group			0.96
<400 AU	60 (40.3)	62 (40.0)	
≥400 AU	89 (59.7)	93 (60.0)	

Values are n (%) or median (IQR). Means were compared by 2-sample *t*-test, medians by Wilcoxon rank-sum test. Categorical variables were compared by chi-squared test or Fisher's exact test.

ACE = angiotensin-converting enzyme; ARB = angiotensin-receptor blocker; CAC = coronary artery calcification; CVD = cardiovascular disease; DOAC = direct oral anti-coagulant; dp-ucMGP = dephosphorylated-uncarboxylated Matrix Gla protein; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

atrial fibrillation, renal failure, smoking status, statin therapy, and serum concentration of median dp-ucMGP, respectively. In the supplementary material, the analysis of the primary outcome was repeated on those with nonzero CAC scores, with a square root transformation of the CAC scores as recommended by Budoff et al.[16]

All analyses followed the intention-to-treat principle. Two-sided *P* values of 0.05 or less were considered to indicate statistical significance. Analyses were performed with Stata/SE 17.0.

RESULTS

CHARACTERISTICS OF PATIENTS. A total of 389 participants from 4 centers were included in the AVADEC trial. Due to known ischemic heart disease

at baseline, 84 participants were excluded, while 1 was excluded because of a missing noncontrast CT scan at baseline. A total of 304 participants, 155 in the control group and 149 in the intervention group, fulfilled the inclusion criteria and completed the trial (**Figure 1**). **Table 1** describes the baseline characteristics of the study participants receiving placebo and intervention. Overall, the participants had a mean age of 71 years, and the 2 groups were comparable with respect to baseline characteristics. However, the intervention group had a significantly higher portion of participants with family history of cardiovascular disease (7% vs 14%, *P* = 0.046) and a lower diastolic blood pressure (87 mmHg vs 83 mm Hg, *P* = 0.032). Use of statin was common in both groups (73.2% vs 68.4%, *P* = 0.36). At baseline, the median CAC score was 655 AU (95% CI:

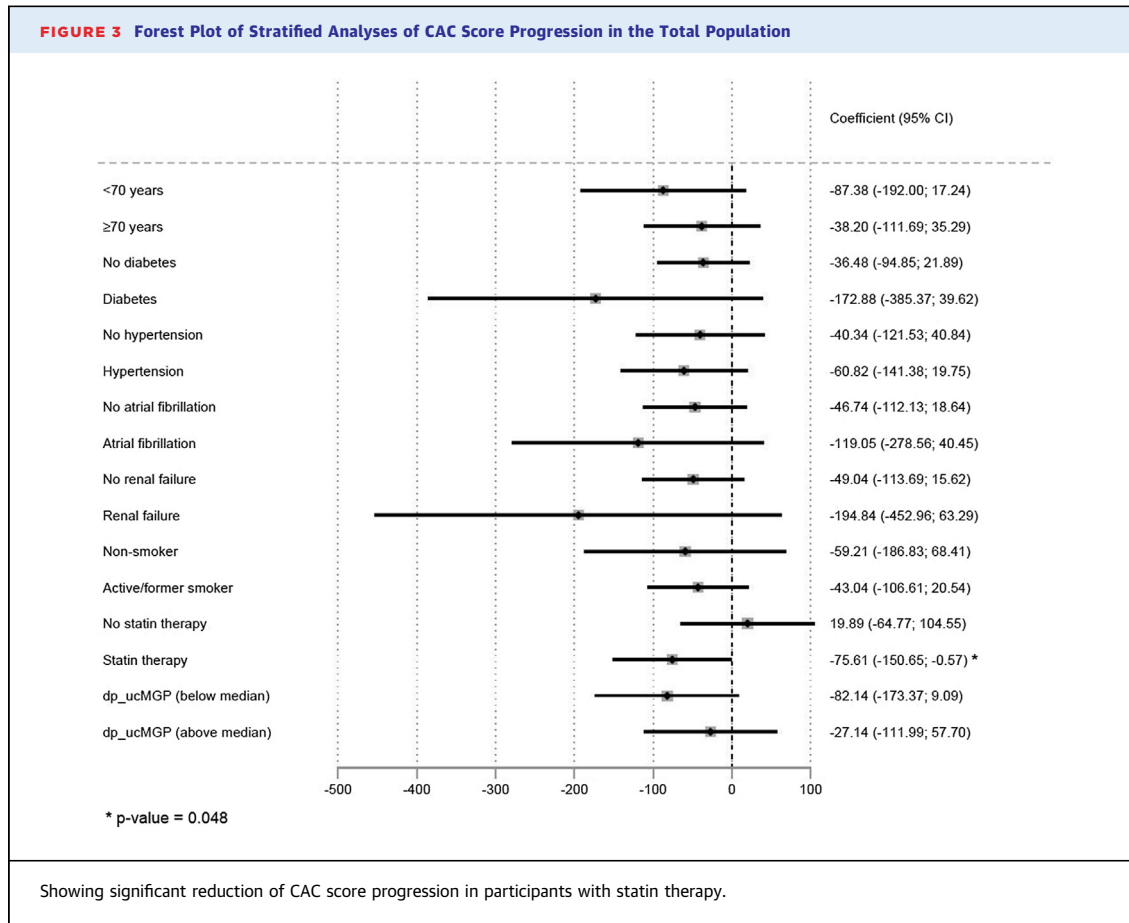**TABLE 2 CAC Score Progression in Primary and Secondary Analyses**

	Mean Change From 0 to 24 Months (95% CI)				Mean Change From 0 to 12 Months (95% CI)				Mean Change From 12 to 24 Months (95% CI)				
	Placebo Group (n = 149)	Vitamin K2+D Group (n = 155)	Treatment Effect From 0 to 24 Months (95% CI)	P Value	Placebo Group	Vitamin K2+D Group	Treatment Effect From 0 to 12 Months (95% CI)	P Value	Placebo Group	Vitamin K2+D Group	Treatment Effect From 12 to 24 Months (95% CI)	P Value	P Value[a]
CAC score (all participants), AU	254 (209-299)	203 (163-242)	−51 (−110 to 8)	0.089	104 (67-142)	95 (64-126)	−9 (−59 to 40)	0.716	150 (102-198)	108 (80-135)	−42 (−97 to 13)	0.133	0.454
CAC score (baseline CAC score <400 AU)	81 (56-105)	77.12 (53-102)	−4 (−40 to 33)	0.846	37 (22-52)	95 (64-126)	−5 (−27 to 17)	0.666	43 (22-64)	45 (28-61)	1 (−26 to 28)	0.930	0.724
CAC score (baseline CAC score ≥400 AU)	380 (312-448)	288 (231-345)	−92 (−183 to −1)	0.047	151 (91-211)	137 (89-186)	−13 (−91 to 64)	0.736	229 (151-307)	151 (108-193)	−79 (−168 to 11)	0.084	0.363

Mixed-effects linear model testing the difference in treatment effect in different time periods. The model included bootstrapped SEs where needed, fixed effect for time point, fixed effect for treatment, fixed effects interaction between treatment and time point and a random intercept for each participant. [a]P value Difference in treatment effect from the first 12 months to last 12 months.

CAC = coronary artery calcification.

FIGURE 2 CAC Score Progression According to Treatment Allocation
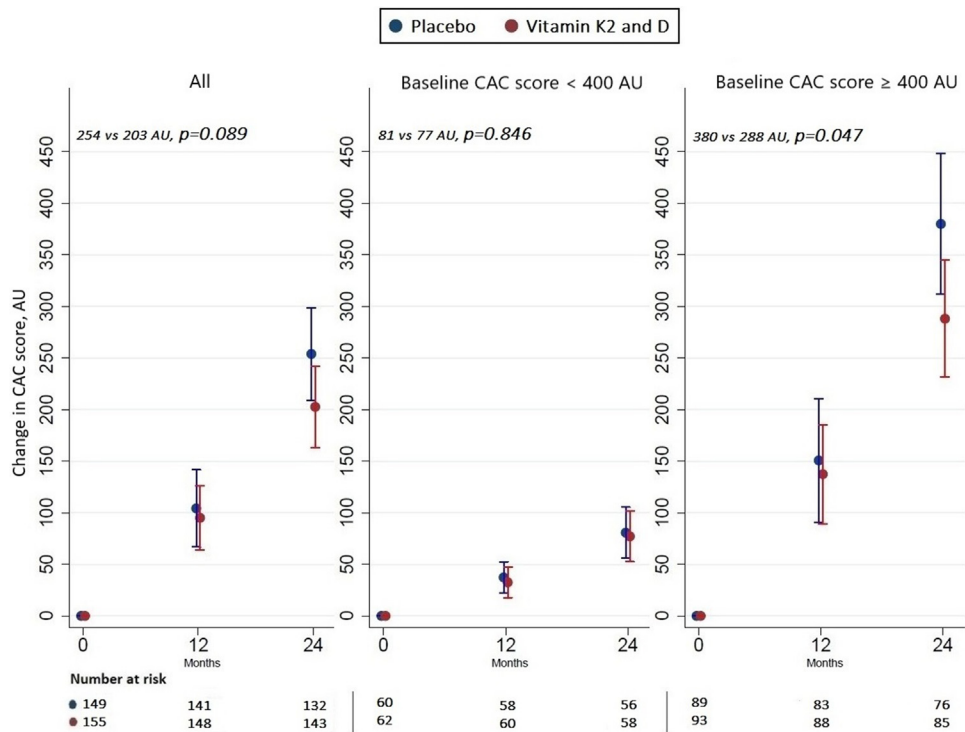
Showing no significant difference in CAC score progression in the total population as well as in participants with baseline CAC score <400 AU. However, in the subgroup of participants with baseline CAC score above 400 AU, a significant difference at 24-month follow-up is shown (P = 0.047).

FIGURE 3 Forest Plot of Stratified Analyses of CAC Score Progression in the Total Population

	Coefficient (95% CI)
<70 years	-87.38 (-192.00; 17.24)
≥70 years	-38.20 (-111.69; 35.29)
No diabetes	-36.48 (-94.85; 21.89)
Diabetes	-172.88 (-385.37; 39.62)
No hypertension	-40.34 (-121.53; 40.84)
Hypertension	-60.82 (-141.38; 19.75)
No atrial fibrillation	-46.74 (-112.13; 18.64)
Atrial fibrillation	-119.05 (-278.56; 40.45)
No renal failure	-49.04 (-113.69; 15.62)
Renal failure	-194.84 (-452.96; 63.29)
Non-smoker	-59.21 (-186.83; 68.41)
Active/former smoker	-43.04 (-106.61; 20.54)
No statin therapy	19.89 (-64.77; 104.55)
Statin therapy	-75.61 (-150.65; -0.57) *
dp_ucMGP (below median)	-82.14 (-173.37; 9.09)
dp_ucMGP (above median)	-27.14 (-111.99; 57.70)

* p-value = 0.048

Showing significant reduction of CAC score progression in participants with statin therapy.

182-1,380 AU) and 636 AU (95% CI: 200-1,443 AU) in patients receiving placebo and vitamin K2 plus D, respectively. Plaque analyses could be performed in 86 participants with contrast CT scans of image quality ≥3. The 86 participants differed from the rest by having lesser comorbidity and a significantly lower CAC score at baseline (384 AU vs 765 AU) (Supplemental Table 1).

PRIMARY OUTCOME. We found no difference in CAC score progression between the intervention and placebo groups from baseline to 2-year follow-up. The mean CAC score progression was 254 AU (95% CI: 209-299 AU) in the placebo group vs 203 AU (95% CI: 163-242 AU) in the intervention group (**Table 2**, **Figure 2**, Supplemental Table 2). This results in a nonsignificant mean difference of 51 AU ($P = 0.089$). In participants with compliance of at least 90%, there was no significant effect of vitamin K2 and D (247 AU vs 210 AU, $P = 0.80$) (Supplemental Table 3). In a stratified analysis looking into treatment-by-subgroup interaction, participants in statin treatment had a significant reduction of CAC score progression by

intervention ($P = 0.048$) (Supplemental Table 4, **Figure 3**). Also, there was a significant reduction of progression in the treatment group compared to placebo when applying the square root method on CAC score ($P = 0.042$) (Supplemental Table 5).

SECONDARY OUTCOME. In a stratified analysis, no significant difference in CAC score progression from baseline to 24 months follow-up was found in participants with baseline CAC score <400 AU (81 vs 77 AU, $P = 0.85$). In participants with baseline CAC score of ≥400 AU, there was a significant difference of 92 AU in CAC progression (380 vs 288 AU, $P = 0.047$) (**Table 2**, **Figure 2**). Similarly, there was a significant difference in the participants with baseline CAC score of ≥400 AU when using the square root method ($P = 0.007$) (Supplemental Table 5). No difference in CAC progression was observed between the groups from baseline to 12 months and from 12 months to 24 months, both for the full cohort and for the 2 subgroups (**Table 2**).
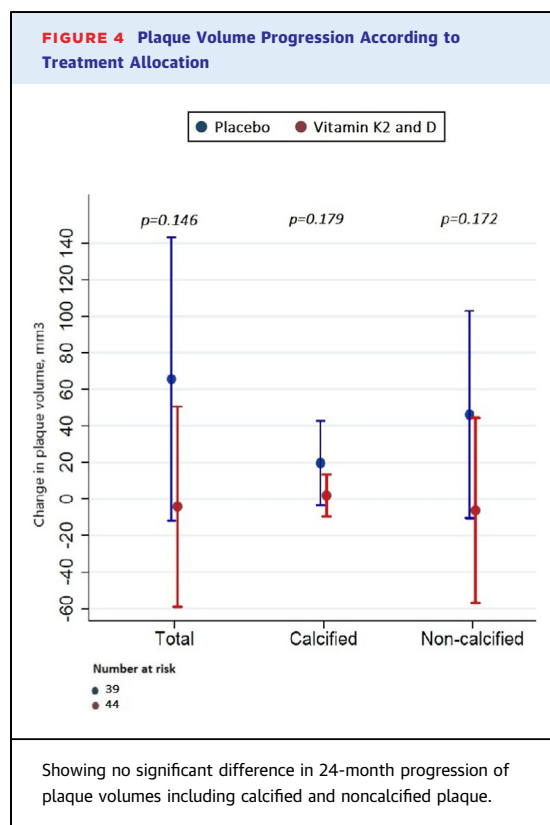
Table 3 and **Figure 4** show the progression in plaque volume from baseline to 2-year follow-up. Eighty-six

TABLE 3 Progression in Plaque Volume in Participants With Good-Quality CCTA

	Placebo Group (n = 40)	Vitamin K2+D Group (n = 46)	Group Difference	
	Mean Change From Baseline (95 % CI)	Mean Change From Baseline (95 % CI)	Treatment Effect (95 % CI)	P Value
Plaque volume and composition				
Total plaque (mm³)	66 (−12 to 143)	−4 (−59 to 51)	−70 (−164 to 24)	0.146
Calcified plaque (mm³)	20 (−4 to 43)	2 (−10 to 13)	−18 (−44 to 8)	0.179
Noncalcified plaque (mm³)	46 (−11 to 103)	−6 (−57 to 45)	−53 (−128 to 23)	0.172

Participants with poor image quality of CCTAs are excluded from the analysis. Mixed-effects linear model testing the difference in treatment effect on plaque volumes.

CCTA = coronary computed tomography angiography.

participants had 2 evaluable contrast CT scans from baseline to 24-month follow-up, while the rest were excluded from plaque analysis due to poor image quality. Progression of noncalcified plaque volume was 46 mm³ in the placebo group vs −6 mm³ in the intervention group (P = 0.17). When stratified for CAC score under and over 400 AU, there was still no significant difference in plaque development (Supplemental Tables 6 and 7).

FIGURE 4 Plaque Volume Progression According to Treatment Allocation

Showing no significant difference in 24-month progression of plaque volumes including calcified and noncalcified plaque.

The evaluation of coronary stenosis is shown in Table 4. A majority of the participants remained at the same stenosis status over the 2 years of follow-up (91.0% vs 91.9%). In the placebo group, 8.0% experienced a worsening of coronary obstructions, while the number was 4.5% in the vitamin K2 and D group. No difference in change between the stenosis categories was found (P = 0.28).

A total of 13 participants had a clinical safety event during the follow-up period. The event rate was 10 (6.7%) in the placebo group vs 3 (1.9%) in the vitamin K2 and D group (P = 0.048) (Table 5).

DISCUSSION

We investigated the prespecified secondary end points relating to coronary artery disease in the AVADEC trial. We found no difference in progression of mean CAC between the intervention and placebo groups over a 2-year follow-up period (Central Illustration). These final results confirm the neutral preliminary analyses presented in the primary publication.[10] However, in a subgroup analysis of participants with CAC scores above 400, the intervention significantly reduced the mean CAC progression. As calcification is thought to be a stabilization of the vulnerable soft plaque, we did additional coronary analyses to ensure that the intervention would not cause more noncalcified plaque. The analyses showed a progression of 46 μL in the placebo group while it was −6 mm³ in the intervention group with no significant difference between the groups. Moreover, we observed an unanticipated significant reduction in safety events in the intervention group. Even though the results for the whole population on both progression of AVC and CAC were neutral, here we very interestingly showed that the intervention may have an effect on CAC in high-risk patients. While AVC is a good measure for aortic valve disease, it is well described that CAC and CAC progression are independent risk factors and better predictors than traditional risk factors for cardiovascular disease and even mortality.[3,16] Thus, slowing down the progression of CAC should be considered very desirable. While different approaches are used to evaluate progression, absolute change in CAC score was used in this study. No randomized controlled trials have managed to show a reduction in CAC progression to date.[17] It has previously been described that baseline CAC score predicts progression by an annual increase of 20% to 25%.[3] As expected, the high-risk participants with CAC scores over 400 AU had the highest absolute

TABLE 4 Progression of Coronary Obstruction in Participants With CCTA								
	Baseline		24 Months Follow-Up			Group Difference		
	Placebo Group (n = 100)	Vitamin K2+D Group (n = 111)	Placebo Group (n = 100)	Vitamin K2+D Group (n = 111)		Placebo Group	Vitamin K2+D Group	P Value
Coronary obstruction								
Normal	2 (2.0)	2 (1.8)	2 (2.0)	2 (1.8)	Better	1 (1.0)	4 (3.6)	0.28
Nonobstructive	93 (93.0)	90 (81.1)	86 (86.0)	90 (81.1)	No change	91 (91.0)	102 (91.9)	
Obstructive					Worse	8 (8.0)	5 (4.5)	
Total	5 (5.0)	19 (17.1)	12 (12)	19 (17.1)				
1 vessel	3 (3.0)	11 (9.9)	9 (9.0)	12 (10.8)				
2 vessels	2 (2.0)	5 (4.5)	2 (2.0)	4 (3.6)				
3 vessels	0 (0.0)	3 (2.7)	1 (1.0)	3 (2.7)				

Values are n (%). Coronary obstruction was evaluated in participants with CCTA at baseline and 24 months follow-up. Mixed-effects linear model testing the difference in treatment effect.

CCTA = coronary computed tomography angiography.

increase in CAC score, but they also seemed to have the greatest effect of vitamin K2 supplementation. It is possible that the effect is simply most evident in the participants with the most notable progression and that the follow-up period was too short to detect a difference in the participants with lower CAC scores.

In 2009, Shea et al[18] demonstrated a 6% reduction of CAC progression in healthy older women and men with pre-existing CAC (CAC score >10 AU) when supplemented with 500 μg vitamin K1 daily in a double-blinded randomized controlled trial. They also concluded that the significant change in CAC was independent of serum MGP levels; however, the MGP was not differentiated between the active and inactive forms. Vitamin K2 is believed to be more efficient than vitamin K1 in the extrahepatic carboxylation processes, and in the AVADEC primary study, a significant decrease of the inactive dp-ucMGP was seen by supplementation with 720 μg vitamin K2.[10] Accordingly, there should be an increase in active MGP in the vascular wall explaining the reduction of CAC progression seen in the AVADEC participants with CAC over 400 AU. Noteworthy is that our study participants were also supplemented with vitamin D, but we suspect that the primary effect came from the vitamin K2, as the D dose approximately corresponds to the regular recommended daily dose for the investigated age group.[19] Yet, it cannot definitely be ruled out that upregulation of MGP production caused by higher levels of vitamin D also played a role in our results.

For further investigation of the treatment effect, we were able to adjust and stratify for the traditional risk factors with no noticeable changes in the results. We did find that participants in statin treatment had a significant effect of the vitamin K2 and D supplementation on changes in CAC. This probably relates to the fact that these participants also had higher CAC scores at baseline (numbers not presented). Importantly, the number of participants in statin treatment was equal in the placebo and intervention groups. Statin itself is believed to be a contributor to CAC progression and, thus, plaque stabilization over time.[20] With that in mind, participants at the highest risk of significant progression in CAC over time by both having a high CAC score and being in statin treatment at baseline had the best effect of vitamin K2 and D supplementation.

This contrasts with the results shown in a randomized study on 42 patients with kidney disease and thereby deficiency of active MGP. Kurnatowska et al found a tendency towards less CAC progression in a subgroup of participants with CAC score under 1000 AU. The participants were supplemented with 90 μg vitamin K2/daily, while 720 μg in our study.[21] A small sample size and a short follow-up period of 270 days may have affected the results. Even a 2-year follow-up might be too short time to track a significant change in participants at low risk with CAC <400 AU in this current AVADEC substudy. Two ongoing Danish studies will hopefully contribute to more

TABLE 5 Difference in Clinical Safety Events				
			Group Difference	
	Placebo Group	Vitamin K2+D Group	Numerical Difference	P Value
Events (MI, coronary revascularization, all-cause mortality)	10 (6.7%)	3 (1.9%)	7	0.048

Values are n (%). The rate of adverse events was reported as counts and proportions and compared between groups using Fisher's exact test.

MI = myocardial infarction.

CENTRAL ILLUSTRATION The Effect of Vitamin K2 and D vs Placebo on Change in CAC Score and Plaque Volumes Over a 2-Year Follow-Up Period
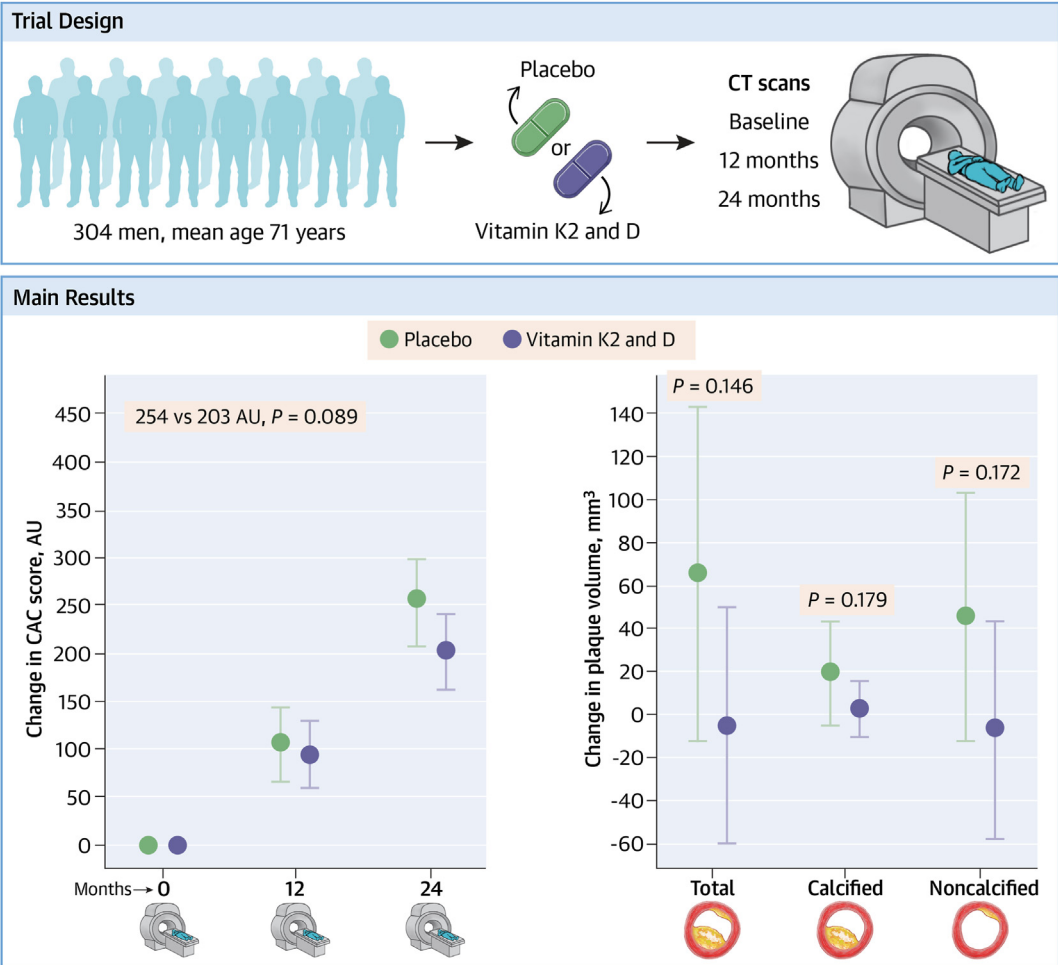
Hasific S, et al. JACC Adv. 2023;2(9):100643.

CAC = coronary artery calcification; CT = computed tomography.

knowledge about the effect of vitamin K2 supplementation. The newly started InterVitaminK trial (NCT05259046) is a randomized controlled trial aiming to investigate if supplementation with 333 µg vitamin K2 can reduce CAC progression in a background population with baseline CAC score ≥10 AU over a 3-year follow-up. On the other hand, the study group behind AVADEC has recently started a new randomized controlled study, DANCODE (DANish COronary DEcalcification) trial, including high-risk men and women with CAC score ≥400 AU based on the results from the current study (NCT05500443). In contrast to these studies, others have investigated the effect of warfarin, a vitamin K antagonist, on CAC

showing a higher degree of calcification than in participants treated with DOACs.[22,23] This further emphasizes the importance of the vitamin K metabolism in the context of coronary artery disease. The counterintuitive aim of reducing the CAC progression led to the interest in evaluation of the development of unstable noncalcified plaque. For the first time, the effect of vitamin K2 supplementation on noncalcified plaque by CCTA was evaluated in this study. Our results were limited by a relatively small proportion of good-quality cardiac CT scans for CCTA plaque analysis, but we found no significant difference in the progression of noncalcified plaque. Whether vitamin K2 affects even earlier inflammatory stages in the

vascular wall will be further investigated in an upcoming substudy from AVADEC in which pericoronary inflammation will be measured as a surrogate marker of the inflammatory processes that develop in the vessel wall.

Surprisingly, we demonstrated a lower event rate in patients supplemented with vitamin K2 and D compared to placebo. However, it must be emphasized that this finding was a safety endpoint. Thus, the study was not powered for this outcome leaving this a possible coincidental finding. There was no significant difference in outcomes in the primary publication.

STUDY LIMITATIONS. As this study investigates the secondary endpoints of AVADEC, the results are mainly hypothesis-generating but nevertheless new and significant. Yet, this study also has limitations. The AVADEC inclusion criteria entailed that the population investigated was elderly Caucasian men with AVC scores above the >90th percentile for age and sex and, accordingly, a relatively high median CAC score leaving them a highly selected population with an a priori high risk of cardiovascular disease. Also, revascularized patients were excluded for optimization of CAC analysis. An important limitation of this study is the significant proportion of poor-quality CCTAs. The project was voluntarily driven by the 4 centers, so the contrast CT scans were deprioritized in busy periods as the primary outcome was derived from the noncontrast scans. Poor image quality was a result of issues with comorbidity, low estimated glomerular filtration rate, and high CAC score, which are issues well known from clinical practice and altogether led to a reduced sample size in the plaque-related analyses. Consequently, our results might have been affected by this with the risk of type 2 error. A strength of the study and our results is that we reduced bias by keeping the investigator of this study blinded to allocation until the end.

We believe that this AVADEC substudy fills in a gap in the lack of randomized trial evidence concerning the possible beneficial role of vitamin K2 on coronary disease. The 2 upcoming randomized studies will hopefully supplement each other well and bring us closer to a conclusion on the coronary effect of vitamin K2 supplementation.

CONCLUSIONS

The present study showed no significant effect of vitamin K2 and D supplementation on CAC progression in a population with a high AVC and CAC score yet no known ischemic heart disease. In a subanalysis, high-risk patients with CAC ≥400 AU had a significantly lower progression of CAC. This study presents novel, interesting, and hypothesis-generating findings that need to be further investigated in future studies.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Extent and progression of CAC are strong predictors of MI and mortality.

COMPETENCY IN PATIENT CARE: Current treatment modalities of coronary artery disease include lifestyle change, blood pressure control, and lipid-lowering medication. No medication or nutritional supplementation has been shown to reduce progression of CAC in a randomized setting.

TRANSLATIONAL OUTLOOK: Vitamin K2 and D supplementation may have an interesting and beneficial effect on coronary artery disease; however, additional hypothesis-testing studies are required. If this vitamin supplementation can reduce calcified and noncalcified plaque progression, it may be a new player in the future of prevention and treatment of coronary artery disease.

REFERENCES

1. Peng AW, Dardari ZA, Blumenthal RS, et al. Coronary artery calcium (≥1000) and association with cardiovascular disease events, non-cardiovascular disease outcomes, and mortality: results from MESA. *Circulation*. 2021;143(16):1571–1583.

2. Clavel M-A, Messika-Zeitoun D, Pibarot P, et al. The complex nature of discordant severe calcified aortic valve disease grading: new insights from combined Doppler echocardiographic and computed tomographic study. *J Am Coll Cardiol*. 2013;62:2329–2338.

3. Budoff MJ, Young R, Lopez VA, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis. *J Am Coll Cardiol*. 2013;61(12): 1231–1239. https://doi.org/10.1016/j.jacc.2012.12.035

4. Diederichsen A, Lindholt JS, Møller JE, et al. Sex differences in factors associated with progression of aortic valve calcification in the general population. *Circ Cardiovasc Imaging*. 2022;15: e013165. https://doi.org/10.1161/CIRCIMAGING.121.013165

5. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2019;74(10):e177–e232. https://www.jacc.org/doi/10.1016/j.jacc.2019.03.010

6. Fraser JD, Price PA. Induction of matrix gla protein synthesis during prolonged 1,25-Dihydroxyvitamin D3 treatment of osteosarcoma cells. *Calcif Tissue Int*. 1990;46(4):270–279. https://doi.org/10.1007/BF02555007

7. Hariri E, Kassis N, Iskandar J, et al. Vitamin K2—a neglected player in cardiovascular health: a narrative review. *Open Heart*. 2021;8(2):e001715. https://doi.org/10.1136/openhrt-2021-001715

8. Schurgers LJ, Teunissen KJF, Knapen MHJ, et al. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol*. 2005;25(8):1629–1633. https://doi.org/10.1161/01.ATV.0000173313.46222.43

9. Pawade TA, Newby DE, Dweck MR. Calcification in aortic stenosis: the skeleton key. *J Am Coll Cardiol*. 2015;66:561–577. https://doi.org/10.1016/j.jacc.2015.05.066

10. Diederichsen AC, Lindholt J, Möller S, et al. Vitamin K2 and D in patients with aortic valve calcification: a randomized double-blinded clinical trial. *Circulation*. 2022;145(18):1387–1397. https://doi.org/10.1161/CIRCULATIONAHA.121.057008

11. Lindholt JS, Frandsen NE, Fredgart MH, et al. Effects of menaquinone-7 supplementation in patients with aortic valve calcification: study protocol for a randomised controlled trial. *BMJ Open*. 2018;8:e022019. https://doi.org/10.1136/bmjopen-2018-022019

12. Diederichsen AC, Rasmussen LM, Søgaard R, et al. The Danish cardiovascular screening trial (DANCAVAS): study protocol for a randomized controlled trial. *Trials*. 2015;16:554. https://doi.org/10.1186/s13063-015-1082-6

13. Lin A, Manral N, McElhinney P, et al. Deep learning-enabled coronary CT angiography for plaque and stenosis quantification and cardiac risk prediction: an international multicenter study. *Lancet Digit Health*. 2022;4(4):e256–e265.

14. Williams MC, Kwiecinski J, Doris M, et al. Low-attenuation noncalcified plaque on coronary computed tomography angiography predicts myocardial infarction: results from the multicenter SCOT-HEART trial (Scottish computed tomography of the Heart). *Circulation*. 2020;141(18):1452–1462. https://doi.org/10.1161/CIRCULATIONAHA.119.044720

15. Matsumoto H, Watanabe S, Kyo E, et al. Standardized volumetric plaque quantification and characterization from coronary CT angiography: a head-to-head comparison with invasive intravascular ultrasound. *Eur Radiol*. 2019;29:6129–6139. https://doi.org/10.1007/s00330-019-06219-3

16. Budoff MJ, Hokanson JE, Nasir K, et al. Progression of coronary artery calcium predicts all-cause mortality. *J Am Coll Cardiol Img*. 2010;3(12):1229–1236. https://doi.org/10.1016/j.jcmg.2010.08.018

17. McEvoy JW, Blaha MJ, Defilippis AP, et al. Coronary artery calcium progression: an importan

clinical measurement? a review of published report. *J Am Coll Cardiol*. 2010;56(20):1613–1622. https://doi.org/10.1016/j.jacc.2010.06.038

18. Shea MK, ODonnell CJ, Hoffmann U, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr*. 2009;89(6):1799–1807. https://doi.org/10.3945/ajcn.2008.27338

19. Vitamin D – Fact Sheet for Consumers. National Institutes of health. Office of dietary supplements. Accessed August 16, 2022. https://ods.od.nih.gov/factsheets/VitaminD-Consumer

20. Henein M, Granåsen G, Wiklund U, et al. High dose and long-term statin therapy accelerate coronary artery calcification. *Int J Cardiol*. 2015;184:581–586. https://doi.org/10.1016/j.ijcard.2015.02.072

21. Kurnatowska I, Grzelak P, Masajtis-Zagajewska A, et al. Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3-5. *Pol Arch Med Wewn*. 2015;125(9):631–640. https://doi.org/10.20452/pamw.3041

22. Osawa K, Nakanishi R, Win TT, Li D, et al. Rationale and design of a randomized trial of apixaban vs warfarin to evaluate atherosclerotic calcification and vulnerable plaque progression. *Clin Cardiol*. 2017;40(10):807–813. https://doi.org/10.1002/clc.22746

23. Lee J, Nakanishi R, Li D, et al. Randomized trial of rivaroxaban versus warfarin in the evaluation of progression of coronary atherosclerosis. *Am Heart J*. 2018;206:127–130. https://doi.org/10.1016/j.ahj.2018.08.007

KEY WORDS CAC score, cardiac CT scan, coronary artery disease, vitamin D, vitamin K2

APPENDIX For supplemental tables and figures, please see the online version of this paper.