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The effects of Menaquinone-7 supplementation in patients with severe coronary calcifications: study protocol for a randomised controlled trial

Acronym: The DANish COronary DEcalcification (DANCODE) trial

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1. Abstract

Introduction

Coronary artery calcification (CAC) and especially progression in CAC is a strong predictor of acute myocardial infarction and cardiovascular mortality. Supplementation with vitamin K2 and D has been suggested to have a protective role in the progression of CAC. In this study, we will examine the effect of vitamin K2 and D in men and women with severe CAC. We hypothesize that supplementation with vitamin K2 and D will slow down the calcification process.

Method and analysis

In this multicenter and double-blinded placebo-controlled study, 400 men and women with CAC score ≥ 400 are randomized (1:1) to treatment with vitamin K2 (720 µg/day) and vitamin D (25 µg/day) or placebo treatment (no active treatment) for two years. Among exclusion criteria are treatment with vitamin K antagonist, coagulation disorders and prior coronary artery disease. To evaluate progression in coronary plaque, a cardiac CT-scan is performed at baseline and repeated after 12 and 24 months of follow-up. Primary outcome is progression in CAC score from baseline to follow-up at two years. Among secondary outcomes are coronary plaque composition and cardiac events. Intention-to-treat principle is used for all analyses.

Ethics and dissemination

There are so far no reported adverse effects associated with the use of vitamin K2. The protocol was approved by the Regional Scientific Ethical Committee for Southern Denmark and the Data Protection Agency. It will be conducted in accordance with the Declaration of Helsinki. Positive as well as negative findings will be reported.

Trial registration number: ClinicalTrials.gov Identifier: NCT05500443.

Key words: coronary artery calcification; randomized controlled trial; vitamin K2.

Strengths and limitations of this study:

- This is a randomised, double-blind, multicenter study to assess the effect of menaquinone-7 supplementation on progression of coronary artery calcification (CAC) as the primary outcome measure
- Both adult men and women will be included and followed for up to 2 years
- Only patients with severe baseline CAC score (>400) will be included

2. Introduction

Ischemic heart disease causes 19% and 20% of all deaths among men and women, respectively, thus prevention is of outmost importance.[1] Ischemic heart disease is often silent until symptoms of myocardial infarction. However, subclinical coronary artery disease is easily detected by non-contrast cardiac CT scans as coronary artery calcifications (CAC). CAC increases with age, and men have higher CAC scores than women.[2] In a population, in which CAC is absent, there is a very low risk of future CVD, but as the CAC score increases, so does the risk of ischemic heart disease.[3,4] Thus, to prevent CVD, identification and treatment of individuals with severe CAC is important.

Vitamin K and the calcification process

Vascular calcification is a slowly progressive process and caused by an imbalance between the mechanisms that promote and inhibit the deposition of calcium in the vessel wall, and vitamin K-dependent proteins play an essential role in this inhibition. The most familiar K vitamin is phylloquinone (vitamin K1), as it is essential in activation of several coagulation factors. Menaquinone-7 (MK-7), also known as vitamin K2, is another very important vitamin K species. Vitamin K2 is deemed necessary for γ-carboxylation of proteins related to the inhibition of arterial calcification, i.e., matrix-Gla proteins (MGP).[5–8] Without these activated proteins, the balance of cellular calcium uptake and the mineralization process in bone and blood vessels is impaired. The inhibiting process of the vitamin K-dependent proteins was originally showed by Luo et al. in 1997.[9] In a mice model they described activated (carboxylated) MGP to be an important inhibitor of vascular calcification. Likewise, observational human studies suggest that long-term use of vitamin K antagonist (which inhibits carboxylation of MGP) is associated with both increased coronary- and extra-coronary vascular calcification.[10–13] Furthermore, combined low vitamins K and D status has been associated with increased all-cause mortality risk compared with adequate vitamins K and D status.[14]

No recommendations of vitamin K2 supplementation are available; however, as demonstrated in a randomized controlled trial there is a dose-dependent decrease of uncarboxylated MGP concentrations by vitamin K2 supplementation (180 µg/day, 360 µg/day or placebo).[15] Thus, we know that the daily intake in the Western world is not sufficient to meet the request for a complete activation of MGP. Additionally, there is no documented toxicity for vitamin K1 or vitamin K2, and the WHO has set no upper tolerance level for vitamin K intake.[16]

The effect of supplementation with high-dose vitamin K2 (720 µg/day) and vitamin D (25 µg/day) was examined in the very recent Danish AVADEC (Aortic Valve DECalcification) Trial.[17] Aortic valve calcification progression was non-significantly decreased.[18] However, the supplementation appeared to slow down the progression of CAC, especially in patients with severe CAC (score >

400), and to slow down the progression of the non-calcified coronary plaque volume. Very importantly, the number of cardiac events and all-cause death was significantly lower (unpublished data). As these findings were secondary outcomes, the results are only hypothesis generating, and a confirmatory trial is requested.

2.1. Hypothesis

In a randomized setup, we test the hypothesis that supplementation with vitamin K2 (720 µg/day) and vitamin D (25 µg/day) in comparison to placebo will reduce the progression of CAC in patients with severe CAC.

3. Methods

3.1. Trial design

The DANish COronary DEcalcification (DANCODE) trial is a double-blinded, randomized, placebo-controlled study.

3.2. Participants

The Danish Heart Registry will be used to identify patients who underwent a cardiac-CT in Western Denmark within the past three years. Patients who are living nearby the including centres and have a CAC score of 400 or above are eligible for DANCODE.

Exclusion criteria are:

- History of coronary revascularization
- History of venous thrombosis including pulmonary embolism
- Coagulation disorders
- Vitamin K antagonist use
- Disorders of calcium and phosphate metabolism (as primary hyperparathyroidism)
- Women of childbearing age (due to radiation issues)
- A life-expectancy < 5 years
- Age under 18 years

The study will take place at three Danish hospitals (Odense University Hospital in Odense and in Svendborg and Vejle Hospital) from 2023 to 2026.

3.3. Intervention

Patients are randomly assigned in a 1:1 ratio to either daily oral supplementation with MK-7 (2 pills containing 360 ug MK-7 and 12.5 µg Vitamin D each (K2VITAL®Delta), thus a total of 720 µg/day

of MK-7 and 25 µg/day of Vitamin D) or matching placebo pills. Treatment of both groups will last for at least 24 months.

3.3.1 Trial visits and procedures

Table 1 provides an overview of the trial visits and procedure. Throughout the course of the study, participants will come to our research facility five times at intervals of six months (Table 1). At baseline, before randomization, and after 12 and 24 months of follow-up, we will conduct a non-contrast CT scan to assess CAC score. A contrast-CT scan will also be performed on included study participants at baseline and after 24 months of follow-up.

Participants with CAC score below 400 at baseline or those fulfilling the exclusion criteria will be excluded from the study. The investigator can decide to withdraw a subject from the study for urgent medical reasons or in case of poor adherence. If subjects are required to take vitamin K antagonist during the course of the study, they will be withdrawn from the study.

The participant will be evaluated for side effects, adverse events, and compliance with the study intervention at each visit. Adherence to treatment will be monitored by interview and pill count at the visits. Phone calls and study reminders are used for participation retention and to increasing compliance.

	Month								
	0	3	6	9	12	15	18	21	24
Informed consent and randomisation	X								
Medical interview	X		X		X		X		X
Web-based survey		X		X		X		X	
Biochemical measurements	X		X		X		X		X
Biobank	X				X				X
Non-contrast CT	X				X				X
Contrast CT	X								X

Table 1: Timeline and applied tests during the trial

3.4. Outcome

The *primary endpoint* is the change in CAC score from baseline to 24-months follow-up.

Secondary endpoints are:

- Change in CAC score from baseline to 24 months in men and women, respectively
- Change in CAC score from baseline to 24 months in two pre-specified subgroups (baseline CAC score <1000 and ≥ 1000)

- Change in coronary plaque composition by contrast CT from baseline to 24 months
- Cardiac events (non-fatal myocardial infarction, coronary revascularization, and cardiac death) during the follow-up period
- Change in calcifications in the aortic valve by non-contrast CT from baseline to 24 months
- Change in quality of life assessed using EuroQol-5D from baseline to 24 months.[19]

An exploratory endpoint is:

- Change in dephosphorylated-undercarboxylated Matrix Gla-Protein (dp-ucMGP)

Safety endpoints are:

- Death
- Cardiovascular events (myocardial infarction, coronary revascularization, heart valve surgery, stroke, significant aortic disease (dissection, rupture, and surgery) and significant peripheral artery disease (thromboembolisms and surgery)
- Venous thromboembolism including pulmonary embolism
- Bleeding (including intracranial bleeding and haemorrhage associated with a drop in haemoglobin of ≥ 2mmol/l)
- Cancer, including solid and hematologic
- Significant deterioration in laboratory measurements (haemoglobin, creatinine (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, or prothrombin time-international normalized ratio (PT-INR)).

3.5 Sample size

We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control per experimental subject. In the AVADEC trial, the mean (standard deviation) two-year CAC progression among 182 men with CAC score ≥ 400 was 380 AU (330 AU) in the placebo group and 288 AU (280 AU) in the intervention group. The joint standard deviation was 311 AU. If this is true in a population of men and women, inclusion of 180 experimental subjects and 180 control subjects are needed to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05 (two-sided). Accordingly, 360 subjects are needed. However, to comply with the uncertainty and to account for drop-out of 10%, 400 patients will be included. The sample size is based on two years of treatment.

3.6 Randomization and blinding

Randomization is performed by the pharmacy at Odense University Hospital. Based on a computer-generated assignment scheme the tablet plastic bottles will have a random number according to the sequential order. The randomisation will be stratified by sex. The treatment allocation will be concealed in sealed opaque envelope. The data and safety monitoring board has access to the randomization list, but patients, nurses, doctors, and other data gatherers are unaware of the allocation until end-of-study for all patients and all analyses are completed. The study is not a medical trial (see Safety and Ethics), and accordingly unblinding is only possible if a patient is excluded from the study.

The taste, colour, and size of the active and matching placebo pills are all the same.

3.8 Statistical methods

We will use the intention-to-treat principle for all analyses. The primary endpoint (change in CAC score) will be presented as continuous variable. Additionally, the changes are analysed in men and women, respectively, and in two pre-specified patient subgroups (CAC score 400-999 AU and ≥ 1000 AU, respectively). Primary hypothesis testing will be done hierarchically to maintain a closed testing procedure: only if the overall treatment effect is statistically significant, testing in CAC strata will be performed with confirmatory intent, otherwise solely for explorative reasons. Secondary endpoints include 1) change in coronary plaque composition by contrast CT; 2) cardiovascular events and mortality; 3) change in calcifications in the aortic valve by non-contrast CT; and 4) change in quality of life (see also Section 3.4).

We use linear mixed models (employing group, time point, and group x time point interaction) for the primary and for secondary endpoints as well as potential harms. Supplementary sensitivity analyses making use of imputed values under the missing at random assumption will be conducted for the primary analysis if more than 5% of expected data points are missing. There will be no interim analyses.

3.9. Patient and Public Involvement

Patients and public were not involved in the design of study.

4. Organization

The Steering Committee will consist of Professor Axel Diederichsen (PI, Department of Cardiology, OUH), PhD Kristian Øvrehus (Department of Cardiology, OUH), MD Selma Hasific (Department of Cardiology, OUH), Professor Lars Melholt Rasmussen (LMR, Department of Clinical

Biochemistry and Pharmacology, OUH), and one from each screening site. All practical issues concerning the treatment, data sampling and publication will be handled by *The Steering Committee*. The study is investigator initiated, and the data is owned by *The Steering Committee*. Data will be available to other researchers upon reasonable request.

The data and safety monitoring board (DSMB) consists of the following experts: Professor in Cardiology Hans Mickley and Professor in Clinical Biostatistics in Diagnostic Research Oke Gerke. DSMB is independent of *The Steering Committee*. During the study, the DSMB will have access to the complete database including the randomization-list. The DSMB will advise *The Steering Committee* to end the study if safety issues arise (see also Safety and Ethics).

The data registration is performed via REDCap (Research Electronic Data Capture) with logging and secure storage directly on a server under Odense Patient data Explorative Network (OPEN), Region of Southern Denmark.

5. Publication

Project results reporting the primary endpoint will be published in peer reviewed international journals. We used the SPIRIT checklist when writing our report. After the primary publication the results are communicated to the participants and the public. Positive as well as negative findings will be reported.

6. Feasibility

Every year, approx. 13,000 patients with suspected angina pectoris are examined by cardiac-CT in the Western Denmark. All data, including baseline cardiovascular risk factors, history of ischemic heart disease and symptoms, scan characteristics, and results, including measurements of the CAC score, are collected prospectively in the Danish Heart Registry. In 2021, 1525 men and women had a cardiac CT, and a CAC score above 400. In 2022 1860 men and women had a CAC score above 400. Thus, more than 3300 patients fulfil the inclusion criteria to participate in DANCODE. An invitation to participate in DANCODE is send by mail to these patients. If a patient is interested, he/she is invited to the local site to discuss the trial with a study nurse. If he/she is willing to participate in the study, informed consent is obtained, and he/she is randomly assigned to the vitamin K2 or placebo group. Thus, we are able to identify enough participants.

7. Safety and Ethics

The study will utilize the same active and placebo pills as the AVADEC trial, which demonstrated great safety and tolerability.[18] In addition, no difference in quality of life and no difference in laboratory safety measurements were found. In line with the AVADEC trial, a Belgian dose-finding study using 360, 720 or 1080 µg of vitamin K2 thrice weekly for 8 weeks in chronic haemodialysis patients found no severe adverse effects.[20] Vitamin K2 was well tolerated and did not cause a hypercoagulable state.[21] Thus, there are no reported adverse effects associated with the use of vitamin K2.[16]

Each patient will undergo three CT scans during the DANCODE study. Epidemiological studies do suggest that radiation exposure is associated with a slightly increased risk of cancer.[22] No large studies involving medically exposed adult cohorts are available, but a linear no-threshold model has been considered. The average dose of one non-contrast cardiac CT scan is 1 mSv. Two additional contrast cardiac CT scans are performed (baseline and 24 months) with an average dose of 3 mSv each, thus at average the participants in DANCODE will receive 9 mSv (baseline: 4 mSv, 12 months: 1 mSv and 24 months: 4 mSv). For comparison, the annual background radiation dose in Denmark is 3 mSv, and the average annual limit for radiation workers is 20 mSv.[23]

An independent DSMB are established to perform ongoing safety surveillance. None of the DSMB-members are directly or indirectly involved in the coordination, execution, or analysis of the study. On a monthly basis, the following is assessed: 1) severe adverse events (death, myocardial infarction, coronary revascularization, stroke, heart valve surgery and venous thromboembolism), and 2) laboratory measurements (creatinine (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, and prothrombin time-international normalized ratio (PT-INR)). If there is a reason for concern, the DSMB can advise to interrupt the study for further analysis, and the study can be terminated prematurely if the number of severe adverse events is significantly higher in the treatment group versus the placebo group. This will be discussed in a meeting with the investigators and DSMB. The investigator will inform the subjects in case of interruption or termination of the study.

Only the independent data and safety monitoring board will have access to the whole database, including the randomization list, during the course of the research. Research electronic data capture (REDCap) is used to register the data,[24] and it is logged and securely stored on a server under the Odense Patient Data Explorative Network, Region of Southern Denmark.

The protocol was approved by the Regional Scientific Ethical Committee for Southern Denmark (S-20220030) and the Data Protection Agency (22/28984), and it will be conducted in accordance with the Declaration of Helsinki. According to the Danish Medicines Agency, vitamin K is a dietary supplement, and accordingly DANCODE is not a medical trial.

The study information is given by study staff. Oral and written informed consent is obtained from each participant. Subjects can leave the study at any time for any reason if they wish to do so, without any consequences. The patients are covered by "Lov om Klage- og Erstatningsadgang indenfor Sundhedsvæsenet /Patienterstatningen". The study is registered at clinicaltrials.gov Identifier: NCT05500443.

8. Funding

Private foundations and companies are sought for funding. Study tablets, including placebo, are provided free of charge by Kappa Bioscience, Norway and Orkla Care, Denmark. The companies are not involved in the design, execution of the study, analysis of the data or reporting of results.

9. Discussion

CAC and especially progression in CAC is a strong predictor of acute myocardial infarction and cardiovascular mortality.[25] This study will examine the effect of vitamin K2 supplementation on progression of CAC in a randomized, placebo-controlled study. We hypothesize that vitamin K2 supplementation will slow down the progression of CAC. If positive effects are shown, a new treatment option may be available to prevent not only progression of CAC, but also ischemic heart disease. The results of this study are expected in 2026.

10. Competing interest statement

None declared.

11. Applied tests during the study

11.1. Medical interview

Baseline data will be obtained at first visit. At the subsequent visits, an interview is conducted, and the following is evaluated: incident cardiovascular disease, chest pain, dyspnoea, and quality of life (EuroQol-5D). A web-based survey is performed 3 months after each visit to support compliance and to evaluate possible side effects.

11.2. Laboratory Assessment

Blood samples are obtained at every visit. Routine parameters include haemoglobin, creatinine, urea, sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, and prothrombin time-international normalized ratio (PT-INR).

Additionally, leukocytes, thrombocytes, lipid profile, haemoglobin A1c, alanine transaminase, lactate dehydrogenase, bilirubin, creatinine kinase, troponin T and c-reactive protein are measured at first and last visit.

As a part of the study, 40 mL of blood from each of the participants will be collected at baseline, 12 and 24 month visit and centrifuged, labelled, and stored at -70°C in a biobank until serial testing. Vitamin D and dephosphorylated-undercarboxylated Matrix Gla-Protein (dp-ucMGP) are measured in the biobank samples after the last patient visit.

11.3. Multi-Slice Computed Tomography Scans

Cardiac CT scans will be performed using a dedicated cardiac CT- scanner. A standard non-contrast as well as contrast scan is performed according to usual clinical care. In patients with a stable heart rate above 60 beats per minute, orally or intravenously β-blocker are administered until the heart rate is appropriate (if possible below 60). Sublingual nitrates are administered to all patients prior to the scan. Regarding the non-contrast scan, 120 kV tube voltage (mandatory) are used. In patients with stable heart rate below 65 beats per minute, a prospectively diastolic scan (70% phase) are used. Otherwise a prospectively scan 300 ms after the QRS-complex. The scanning protocol during the contrast scan depends on the local CT scanner and the patient heart rate. In patients with stable heart rate below 65 beats per minute, a prospectively diastolic scan (65-75% phase) are used. Otherwise a prospectively scan 200-400 ms after the QRS-complex. 50-70 mL of contrast agent are injected into an antecubital vein at a rate of 6.0 mL/s followed by 50-70 mL intravenous saline (6.0 mL/s) using a dual-head power injector. Data acquisition parameters depends on the local CT scanner, but slice collimation will be below 0.6mm, gantry rotation time as fast as possible and a tube voltage of 70 or 120 kV depending on patients' weight.

All scans are sent to and analysed at end-of-study by the core lab in Odense University Hospital. CAC scores are measured using the Agatston method by summing-up all spots of calcifications in the coronaries. The coronary artery tree will be analysed for the presence and severity of CAD, according to the classification of the American Heart Association 16-segment model. All coronary segments ≥2 mm in diameter with plaque will be analysed using a semi-automated software (Autoplaque). Coronary plaques are defined as visible structures within or adjacent to the coronary artery lumen, which can be clearly distinguished from the vessel lumen and the surrounding pericardial tissue. Scans are analysed by an experienced cardiologist.

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Skriftlig deltagerinformation

Engelsk titel: *The DANish COronary DEcalcification (DANCODE) trial*

Dansk titel: Hæmning af forkalkninger i kranspulsårerne (DANCODE)

Region Syddanmark

OUH
Odense Universitetshospital

Indledning

Vi vil hermed spørge dig, om du er interesseret i at deltage i et forskningsprojekt, hvor formålet er at undersøge om K-vitamin-tilskud kan forsinke eller stoppe yderligere forkalkninger.
Vi skriver til dig, fordi du tidligere har fået foretaget en hjerte-CT skanning, hvor lægerne fandt, at du havde udtalte forkalkninger i hjertes kranspulsårer.

Hvad er baggrunden for undersøgelsen?

Et nyt dansk studie tyder på, at tilskud med højdosis K2-vitamin og D-vitamin forebygger forkalkning af kranspulsårerne. Med DANCODE ønsker vi at afklare om tilskuddet kan forebygge yderligere forkalkninger hos personer, der har udtalte forkalkninger. Der inkluderes i alt 400 mænd og kvinder.

Hvad går undersøgelsen ud på?

Undersøgelsen er et lodtrækningsforsøg: halvdelen af deltagerne vil få K2-vitamin (720 µg dagligt) samt D-vitamin (25 µg dagligt) og halvdelen vil få placebobehandling (uvirksomt stof). Samlet varighed er planlagt til to år. I løbet af denne periode vil vi:

1. **Hver 6. måned tale med dig om dit helbred**
2. **Hver 6. måned tage blodprøver**
   - Blodprøvetagningen indebærer et stik i armen, og der kan efterfølgende komme en lille blodansamling. Vi vil måle din nyrefunktion, elektrolytbalance, kalkstofskifte, blodstørkning, samt nogle forskningsanalyser vedrørende årsager til forkalkning.
   - Ved start og årligt de følgende to år vil vi tage en ekstra blodprøver (40 ml blod hver gang) til en forskningsbiobank, hvorfra eventuelt tiloversbleven materiale ved forsøgets afslutning bliver overført til biobank til fremtidig forskning. Prøverne vil maksimalt blive opbevaret i 10 år og vil herefter blive destrueret. Du kan til enhver tid kontakte den projektansvarlige og bede om at få dit materiale destrueret.
3. **Hvert år foretage CT skanning af dit hjerte**
   - Denne skanning gennemføres 3 gange (ved start og årligt de følgende to år). Med denne kan vi måle mængden af kalk i kranspulsårerne.
   - Derudover vil vi ved første og sidste CT skanning også give røntgenkontrast. Med denne skanning kan vi måle mængden af fedt i pulsårerne.

Hvor lang tid tager undersøgelsen?

Vi vil gerne se dig hver 6. måned i gennem 2 år. Første gang vil vi fortælle dig om undersøgelsen. Hver gang vil vi måle puls, blodtryk og tage blodprøver. Derudover vil vi foretager CT-skanninger af hjertet. Samtalen og undersøgelserne tager op mod 2 timer per gang.

Hvad får du ud af at deltage?

Hver anden deltager får K2-vitamin tilskud, mens hver anden får placebobehandling (uvirksomt stof). Når alle resultater er analyseret, kan vi fortælle dig, om du har fået K2-vitamin og om dine forkalkninger er ændret.
I løbet af de to år vil vi undersøge dit hjertet tre gange, men screener ikke efter andet som f.eks. kræft. Vi forventer, at enkelte i løbet af de to år vil udvikle hjertesygdomme. Er du blandt dem, da vil vi tilbyde dig standard medicinsk behandling.

Hvis du ikke deltager vil vi ikke gøre yderligere, men anbefale dig at fortsætte behandling i samråd med din læge.

Er der nogle ulemper/risici for mig som forsøgsdeltager?

Halvdelen får højdosis K2-vitamin tilskud. Der er ikke tidligere rapporteret bivirkninger til K2-vitamin, men der kan være uforudsete risici og belastninger forbundet med forsøget. Indtager du kosttilskud kan du fortsætte med dette, men du bør ikke tage mere, imens du deltager i projektet pga. risiko for overdosering. Der foretages halvårlige blodprøvekontroller for at observere dette.

I forbindelse med CT skanningen benyttes røntgenstråling. Der er planlagt 3 CT skanninger og den samlede stråledosis bliver op mod 9 mSv. Baggrundsstrålingen, det vil sige den stråling du udsættes for fra omgivelserne (fra jorden, luften, mad og byggematerialer) er 3 mSv pr år i Danmark. Det betyder, at man i løbet af de to år undersøgelsen varer udsættes for, hvad der svarer til tre gange den årlige baggrundsstråling. Dette vil øge risikoen for at få kræft med 0,045%, hvilket bør ses i forhold til den generelle risiko for at udvikle kræft i Danmark på 25%, og således resultere i en samlet kræftrisiko på 25,045%.

Røntgenkontrast kan medføre nyreproblemer, hvis man har dårlig nyrer. Vi vil derfor ikke give dig røntgenkontrast, hvis du har nedsat nyrefunktionen.

Undersøgelsen vil blive afbrudt, hvis der findes uventede bivirkninger til højdosis K2-vitamin tilskud.

Hvorfor er det vigtigt at du deltager i undersøgelsen?

På trods af sund sundlivsstil og forebyggende medicin vil nogen alligevel få en blodprop, så vores aktuelle behandling er ikke helt tilstrækkelig. Det kunne tyde på, at K2-vitamin tilskud kan forebygge forkalkninger, og med dette studie vi vil nu afklare om K2-vitamin tilskud er en relevant forebyggende behandling.

Du kan ikke deltage i undersøgelsen, hvis du har fået foretaget en hjerteoperation, eller hvis du får blodfortyndende Marevan.

Samtykke

I forbindelse med informationen om studiet er du velkommen til at medbringe en ægtefælle eller ven. Efter at den skriftlige og mundtlige information er givet, kan du umiddelbart sige ”ja” til undersøgelsen eller vælge betænkningstid på minimum et døgn. Du kan når som helst trække dit tilsagn tilbage uden at det vil have nogen konsekvenser for dig.

Personfølsomme oplysninger

Hvis du vælger at deltage i studiet, samtykker du også til at de projektansvarlige må få adgang til din journal, således at vi ved behov kan indhente information om, hvilken medicin du tager, og hvilke sygdomme du fejler mv. De oplysninger vi får fra dig, fra din journal samt fra undersøgelserne vil alene blive brugt i forbindelse med projektet, så vi kan passe bedst muligt på dig. Databeskyttelsesforordningen og databeskyttelsesloven overholdes.

Resultaterne af undersøgelsen vil blive offentliggjort i videnskabelige tidsskrifter, og i den sammenhæng vil alle dine oplysninger blive helt anonymiseret, således at det ikke er muligt at identificere dig eller andre enkeltpersoner.

Økonomi

Der vil blive søgt støtte til lønninger og laboratorieundersøgelser fra offentlige og private fonde, men også fra private firmaer. Det forventes at tabletter doneres fra producenterne (Kappa Bioscience og Orkla Care). Professor Axel Diederichsen (hjertemedicinsk afdeling B, Odense Universitetshospital) har taget initiativ til forsøget. Der er ingen økonomisk tilknytning mellem støttegivere og de forsøgsansvarlige.

Du får som forsøgsdeltager ikke et vederlag, men vi kan tilbyde transportgodtgørelse.

Som yderligere information vedlægges følgende materiale:

”Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt” udgivet af Den Videnskabsetiske Komité.

Kontaktoplysninger

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i forsøget og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Hvis du vil vide mere, er du meget velkommen til at kontakte den projektansvarlige:

Overlæge
X X
Hjertemedicinsk Afdeling X,
X hospital
Mobil xx, e-mail: x@x.dk

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 7-8

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7-8
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	4

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	5
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	5, 8, 10-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	5
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7, SAP
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7, SAP

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7, SAP
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8, 9
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8-10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	7
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	8
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	8
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	14-16
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	5, 11

Notes:

- 18a: 5, 8, 10-11 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 27. February 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Effects of Vitamin K2 and D3 supplementation in Patients With Severe Coronary Artery Calcification: A study protocol for a randomised controlled trial

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Effects of Vitamin K2 and D3 supplementation in Patients with Severe Coronary Artery Calcification: A study protocol for a randomised controlled trial

Acronym: The DANish COronary DEcalcification (DANCODE) trial

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1. Abstract

Introduction

Coronary artery calcification (CAC) and especially progression in CAC is a strong predictor of acute myocardial infarction and cardiovascular mortality. Supplementation with vitamin K2 and D3 has been suggested to have a protective role in the progression of CAC. In this study, we will examine the effect of vitamin K2 and D3 in men and women with severe CAC. We hypothesize that supplementation with vitamin K2 and D3 will slow down the calcification process.

Method and analysis

In this multicentre and double-blinded placebo-controlled study, 400 men and women with CAC score ≥ 400 are randomised (1:1) to treatment with vitamin K2 (720 µg/day) and vitamin D3 (25 µg/day) or placebo treatment (no active treatment) for two years. Among exclusion criteria are treatment with vitamin K antagonist, coagulation disorders and prior coronary artery disease. To evaluate progression in coronary plaque, a cardiac CT-scan is performed at baseline and repeated after 12 and 24 months of follow-up. Primary outcome is progression in CAC score from baseline to follow-up at two years. Among secondary outcomes are coronary plaque composition and cardiac events. Intention-to-treat principle is used for all analyses.

Ethics and dissemination

There are so far no reported adverse effects associated with the use of vitamin K2. The protocol was approved by the Regional Scientific Ethical Committee for Southern Denmark and the Data Protection Agency. It will be conducted in accordance with the Declaration of Helsinki. Positive as well as negative findings will be reported.

Trial registration number: ClinicalTrials.gov Identifier: NCT05500443.

Key words: coronary artery calcification; randomised controlled trial; vitamin K2.

Word Count: Abstract 243; Manuscript without references: 3572

Strengths and limitations of this study:

- A major strength of this study is the multicentre, double-blinded, randomised design to assess the effect of vitamin K2 and vitamin D3 supplementation on progression of coronary artery calcification (CAC)
- Another strength is inclusion of both men and women
- Patients with baseline CAC ≥ 400 are included in the study which ensures detectable CAC progression over 2 years of follow-up

- Although the study is unique and based on our previous findings, two years of follow-up might be too short to find a difference“

2. Introduction

Ischemic heart disease accounts for 19% and 20% of all deaths among men and women, respectively, underscoring the critical importance of prevention.[1] Ischemic heart disease is often silent until symptoms of myocardial infarction. However, during subclinical stages, ischemic heart disease can be detected as coronary artery calcifications (CAC) on non-contrast cardiac CT scans. CAC increases with age, and men tend to have higher CAC scores on average than women.[2] In a population with no CAC, the risk of future cardiovascular disease (CVD) is very low; however, as CAC score increases, so does the risk of ischemic heart disease.[3,4] Thus, to prevent CVD, identification and treatment of individuals with severe CAC is important.

Vitamin K and the calcification process

The most familiar K vitamin is phylloquinone (vitamin K1), as it is essential for the activation of several coagulation factors. Menaquinone-7 (MK-7), also known as vitamin K2, is another very important vitamin K species with mostly extra-hepatic effects due to higher concentrations outside the liver. Even though some pathways are shared, vitamin K2 is thought to be the primary activator of non-hepatic proteins related to the inhibition of arterial calcification, i.e., matrix-Gla proteins (MGP).[5–8] The activation of these important proteins is, however, dependent on their synthesis, which again is stimulated by vitamin D3.[9] Without both synthesis and activation of relevant proteins, the balance of cellular calcium uptake and the mineralization process in bone and blood vessels is impaired. The inhibiting effect of the vitamin K-dependent proteins on calcification was originally showed by Luo et al. in 1997.[10] In a mice model they found activated (carboxylated) MGP to be an important inhibitor of vascular calcification. More recent randomised clinical trials have tested this theory with vitamin K2 supplementation in different populations finding only a discreet reduction in CAC.[11,12] Contrarily, observational studies suggest that long-term use of vitamin K antagonists is associated with increased vascular calcification.[13–16] Furthermore, combined low vitamin K and D status has been associated with increased all-cause mortality risk compared with adequate vitamin K and D status.[17] A synergistic effect of the two vitamins on bone and cardiovascular health has been suggested.[18] Currently, no recommendations of vitamin K2 supplementation are available. As demonstrated in a randomised controlled trial there is a dose-dependent decrease of uncarboxylated MGP concentrations by vitamin K2 supplementation (180 µg/day, 360 µg/day or placebo).[19] Thus, we know that the daily intake in the Western world is not sufficient to meet the request for a complete activation of MGP. Additionally, there is no

documented toxicity for vitamin K1 or vitamin K2, and the WHO has set no upper tolerance level for vitamin K intake.[20]

The effect of supplementation with high-dose vitamin K2 (720 µg/day) and vitamin D (25 µg/day) over 2 years was examined in the very recent Danish AVADEC (Aortic Valve DECalcification) Trial.[21] Aortic valve calcification progression was non-significantly decreased.[22] However, the supplementation appeared to slow down the progression of CAC, especially in patients with severe CAC (score > 400). It also reduced progression of the non-calcified coronary plaque volume. Very importantly, the total number of cardiac events and all-cause death was significantly lower (unpublished data). As these findings were secondary outcomes, the results are only hypothesis generating and a confirmatory trial is requested.

2.1. Hypothesis

In a randomised setup, we test the hypothesis that supplementation with vitamin K2 (720 µg/day) and vitamin D3 (25 µg/day) in comparison to placebo will reduce the progression of CAC in patients with severe CAC.

3. Methods

3.1. Trial design

The DANish COronary DEcalcification (DANCODE) trial is a multicentre, double-blinded, randomised, placebo-controlled study. Trial registration number is ClinicalTrials.gov Identifier: NCT05500443.

3.2. Participants

The Danish Heart Registry will be used to identify patients who underwent a cardiac-CT in Western Denmark within the past three years. Patients who are living nearby the including centres and have a CAC score of 400 or above are eligible for DANCODE.

Exclusion criteria are:

- History of coronary revascularization
- History of venous thrombosis including pulmonary embolism
- Coagulation disorders
- Vitamin K antagonist use
- Disorders of calcium and phosphate metabolism (as primary hyperparathyroidism)
- Women of childbearing age (due to radiation issues)
- A life-expectancy < 5 years

- Age under 18 years

The study will take place at three Danish hospitals (Odense University Hospital in Odense and Svendborg and at Vejle Hospital) from February 8th 2023 to March 2026.

3.3. Intervention

Patients are randomly assigned in a 1:1 ratio to either daily oral supplementation with Vitamin K2 and D3 (2 pills containing 360 ug MK-7 and 12.5 µg Vitamin D3 each (K2VITAL®Delta), thus a total of 720 µg/day of MK-7 and 25 µg/day of Vitamin D3) or matching placebo pills. Treatment of both groups will last for at least 24 months. The selected dosage of Vitamin K2 and D3 is based on the AVADEC trial, which demonstrated efficacy in patients with CAC > 400 and exhibited no safety concerns.[22] The ingredients in the placebo tablets are listed in Appendix 1.

3.3.1 Trial visits and procedures

Supplementary Table 1 provides an overview of the trial visits and procedure. Throughout the course of the study, participants will come to our research facility five times at intervals of six months. At baseline, 12 and 24 months of follow-up, we will conduct a non-contrast CT scan to assess CAC score. A contrast-CT scan will also be performed on included study participants at baseline and after 24 months of follow-up.

Participants with CAC score below 400 at baseline or those fulfilling the exclusion criteria will be excluded from the study. The investigator can decide to withdraw a subject from the study for urgent medical reasons or in case of poor adherence. If subjects are required to take vitamin K antagonist during the course of the study, they will be withdrawn from the study.

The participants will be evaluated for side effects, adverse events, and compliance with the study intervention at each visit. Adherence to treatment will be monitored by interview and pill count at the visits. Phone calls and study reminders are used for participation retention and to increasing compliance.

Appendix 2 shows the patient information leaflet (in Danish).

3.4. Outcome

The *primary endpoint* is the change in CAC score from baseline to 24-months follow-up.
Secondary endpoints are:

- Change in CAC score from baseline to 24 months in men and women, respectively
- Change in CAC score from baseline to 24 months in two pre-specified subgroups (baseline CAC score <1000 and ≥ 1000)
- Change in coronary plaque composition by contrast CT from baseline to 24 months

- Cardiac events (non-fatal myocardial infarction, coronary revascularization, and cardiac death) during the follow-up period
- Change in calcifications in the aortic valve by non-contrast CT from baseline to 24 months
- Change in quality of life assessed using EuroQol-5D from baseline to 24 months.[23]

An exploratory endpoint is:

- Change in dephosphorylated-undercarboxylated Matrix Gla-Protein (dp-ucMGP)

Safety endpoints are:

- Death
- Cardiovascular events (myocardial infarction, coronary revascularization, heart valve surgery, stroke, significant aortic disease (dissection, rupture, and surgery) and significant peripheral artery disease (thromboembolisms and surgery)
- Venous thromboembolism including pulmonary embolism
- Bleeding (including intracranial bleeding and haemorrhage associated with a drop in haemoglobin of ≥ 2mmol/l)
- Cancer, including solid and hematologic
- Significant deterioration in laboratory measurements (haemoglobin, creatinine (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, or prothrombin time-international normalized ratio (PT-INR)).

3.5 Sample size

We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control per experimental subject. In the AVADEC trial, the mean (standard deviation) two-year CAC progression among 182 men with CAC score ≥ 400 was 380 AU (330 AU) in the placebo group and 288 AU (280 AU) in the intervention group. The joint standard deviation was 311 AU. If this is true in a population of men and women, inclusion of 180 experimental subjects and 180 control subjects are needed to be able to reject the null hypothesis (H_0). The H_0 of this study is that the progression means of the experimental and control groups are equal, with probability (power) 0.8. The Type I error probability associated with this H_0 test is 0.05 (two-sided). Accordingly, 360 subjects are needed. However, to comply with the uncertainty and to account for drop-out of 10%, 400 patients will be included. The sample size is based on two years of treatment and was assessed with Stata/MP 17 (StataCorp, College Station, Texas 77845 USA).

3.6 Randomisation and blinding

Randomisation is performed by the pharmacy at Odense University Hospital. Based on a computer-generated assignment scheme, the tablet plastic bottles will have a random number

according to the sequential order. The randomisation will be stratified by sex. The treatment allocation will be concealed in sealed opaque envelope. The data and safety monitoring board has access to the randomisation list, but patients, nurses, doctors, and other data gatherers are unaware of the allocation until end-of-study for all patients and until all analyses are completed. The study is not a medical trial (see Safety and Ethics), and accordingly unblinding is only possible if a patient is excluded from the study.

The taste, colour, and size of the active and matching placebo pills are all the same.

3.8 Statistical methods

We will use the intention-to-treat principle for all analyses. The primary endpoint (change in CAC score) will be presented as a continuous variable. Additionally, the changes are analysed in men and women, respectively, and in two pre-specified patient subgroups (CAC score 400-999 AU and ≥ 1000 AU, respectively). Primary hypothesis testing will be done hierarchically to maintain a closed testing procedure: only if the overall treatment effect is statistically significant, testing in CAC strata will be performed with confirmatory intent, otherwise solely for explorative reasons. Secondary endpoints include 1) change in coronary plaque composition by contrast CT; 2) cardiovascular events and mortality; 3) change in calcifications in the aortic valve by non-contrast CT; and 4) change in quality of life (see also Section 3.4).

We use linear mixed models (employing group, time point, and group x time point interaction) for the primary and for secondary endpoints as well as potential harms. Supplementary sensitivity analyses making use of imputed values under the missing at random assumption will be conducted for the primary analysis if more than 5% of expected data points are missing. There will be no interim analyses.

3.9. Patient and Public Involvement

Patients and public were not involved in the design of study.

4. Organization

The Steering Committee will consist of Professor Axel Diederichsen (PI, Department of Cardiology, OUH), PhD Kristian Øvrehus (Department of Cardiology, OUH), MD Selma Hasific (Department of Cardiology, OUH), Professor Lars Melholt Rasmussen (LMR, Department of Clinical Biochemistry and Pharmacology, OUH), and one from each screening site. All practical issues concerning the treatment, data sampling and publication will be handled by *The Steering*

Committee. The study is investigator initiated, and the data is owned by *The Steering Committee*. Data will be available to other researchers upon reasonable request.

The data and safety monitoring board (DSMB) consists of the following experts: Professor in Cardiology Hans Mickley and Professor in Clinical Biostatistics in Diagnostic Research Oke Gerke. DSMB is independent of *The Steering Committee*. During the study, the DSMB will have access to the complete database including the randomisation-list. The DSMB will advise *The Steering Committee* to end the study if safety issues arise (see also Safety and Ethics).

The data registration is performed via REDCap (Research Electronic Data Capture) with logging and secure storage directly on a server under Odense Patient data Explorative Network (OPEN), Region of Southern Denmark.

5. Publication

Project results reporting the primary endpoint will be published in peer reviewed international journals. We used the SPIRIT checklist when writing our report. After the primary publication the results are communicated to the participants and the public. Positive as well as negative findings will be reported.

6. Feasibility

Approx. 13,000 patients with suspected angina pectoris are examined by cardiac-CT in Western Denmark every year. All data, including baseline cardiovascular risk factors, history of ischemic heart disease and symptoms, scan characteristics, and results, including measurements of the CAC score, are collected prospectively in the Danish Heart Registry. 1525 men and women had a cardiac CT, and a CAC score above 400 in 2021. In 2022, 1860 men and women had a CAC score above 400. Thus, more than 3300 patients fulfil the inclusion criteria to participate in DANCODE. An invitation to participate in DANCODE is send by mail to these patients. If a patient is interested, he/she is invited to the local site to discuss the trial with a study nurse. If he/she is willing to participate in the study, informed consent is obtained, and he/she is randomly assigned to the vitamin K2 or placebo group. Thus, we are able to identify enough participants.

7. Ethics and dissemination

SAFETY

The study will utilize the same active and placebo pills as the AVADEC trial, which demonstrated

great safety and tolerability.[22] In addition, no difference in quality of life and no difference in laboratory safety measurements were found. In line with the AVADEC trial, a Belgian dose-finding study using 360, 720 or 1080 µg of vitamin K2 thrice weekly for 8 weeks in chronic haemodialysis patients found no severe adverse effects.[24] Vitamin K2 was well tolerated and did not cause a hypercoagulable state.[25] Thus, there are no reported adverse effects associated with the use of vitamin K2.[20]

Each patient will undergo three CT scans during the DANCODE study. Epidemiological studies do suggest that radiation exposure is associated with a slightly increased risk of cancer.[26] No large studies involving medically exposed adult cohorts are available, but a linear no-threshold model has been considered. The average dose of one non-contrast cardiac CT scan is 1 mSv. Two additional contrast cardiac CT scans are performed (baseline and 24 months) with an average dose of 3 mSv each, thus at average the participants in DANCODE will receive 9 mSv (baseline: 4 mSv, 12 months: 1 mSv and 24 months: 4 mSv). For comparison, the annual background radiation dose in Denmark is 3 mSv, and the average annual limit for radiation workers is 20 mSv.[27]

SAFETY MONITORING

An independent DSMB is established to perform ongoing safety surveillance. None of the DSMB-members are directly or indirectly involved in the coordination, execution, or analysis of the study. On a monthly basis, the following is assessed: 1) severe adverse events (death, myocardial infarction, coronary revascularization, stroke, heart valve surgery and venous thromboembolism), and 2) laboratory measurements (creatinine (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, and prothrombin time-international normalized ratio (PT-INR)). If there is a reason for concern, the DSMB can advise to interrupt the study for further analysis. The study can be terminated prematurely if the number of severe adverse events is significantly higher in the treatment group versus the placebo group. This will be discussed in a meeting with the investigators and DSMB. The investigator will inform the subjects in case of interruption or termination of the study.

Only the independent data and safety monitoring board will have access to the whole database, including the randomisation list, during the course of the research.

DATA MANAGEMENT

Research electronic data capture (REDCap) is used to register the data,[28] and it is logged and securely stored on a server under the Odense Patient Data Explorative Network, Region of Southern Denmark.

ETHICAL APPROVALS

The protocol is approved by the Regional Scientific Ethical Committee for Southern Denmark (S-20220030) and the Data Protection Agency (22/28984), and it will be conducted in accordance with the Declaration of Helsinki. According to the Danish Medicines Agency, vitamin K is a dietary supplement, and accordingly DANCODE is not a medical trial.

The study information is given by study staff. Oral and written informed consent is obtained from each participant. Subjects can leave the study at any time for any reason if they wish to do so, without any consequences. The patients are covered by "Lov om Klage- og Erstatningsadgang indenfor Sundhedsvæsenet /Patienterstatningen". The study is registered at clinicaltrials.gov Identifier: NCT05500443.

DISSEMINATION

Positive, negative and inconclusive results from the trial will be published in international peer-reviewed journals and will be shared in the press and via social media. We used the SPIRIT checklist when writing our report.

8. Discussion

CAC and especially progression in CAC is a strong predictor of acute myocardial infarction and cardiovascular mortality.[29] This study will examine the effect of vitamin K2 and vitamin D3 supplementation on progression of CAC in a randomised, placebo-controlled study. We hypothesize that vitamin K2 and D3 supplementation will slow down the progression of CAC. The strengths of this study are the design, the large number of participants as well as their high degree of CAC at baseline with presumably significant progression over the follow-up period. The population is of special interest as it is at high risk of cardiac events. No similar randomised studies have yet been performed. A limitation is that a potential effect of the supplementation is a shared effect of vitamin K2 and D3, and no separate conclusions can be done for each of the vitamins. However, previous randomised trials on vitamin D supplementation alone have failed to show any effect on progression on coronary artery calcium.[30] In addition, the combination of vitamin D and vitamin K showed lower increase in carotid intima-media thickness compared to vitamin D alone.[12] Although, the population (and dosage of vitamin D) are different in these trials compared to ours, the currently available data suggest that any vascular effects are mediated by vitamin K and enhanced by vitamin D.

Another limitation is that the participants are not included based on their baseline vitamin levels resulting in a part of participants with normal vitamin ranges and possibly less effect of the intervention than individuals with insufficiency. If positive effects are shown despite that, a new

treatment option may be available to prevent not only progression of CAC, but also ischemic heart disease. The results of this study are expected in 2026.

9. Applied tests during the study

9.1. Medical interview

Baseline data will be obtained at first visit. At the subsequent visits, an interview is conducted, and the following is evaluated: incident cardiovascular disease, chest pain, dyspnoea, and quality of life (EuroQol-5D). A web-based survey is performed 3 months after each visit to support compliance and to evaluate possible side effects.

9.2. Laboratory Assessment

Blood samples are obtained at every visit. Routine parameters include haemoglobin, creatinine, urea, sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone, and prothrombin time-international normalized ratio (PT-INR).

Additionally, leukocytes, thrombocytes, lipid profile, haemoglobin A1c, alanine transaminase, lactate dehydrogenase, bilirubin, creatinine kinase, troponin T and c-reactive protein are measured at first and last visit.

As a part of the study, 40 mL of blood from each of the participants will be collected at baseline, 12 and 24 month visit and centrifuged, labelled, and stored at -70°C in a biobank until serial testing. Dephosphorylated-undercarboxylated Matrix Gla-Protein (dp-ucMGP), which also is a surrogate measure of vitamin K2 level, and 25-OH vitamin D are measured in the biobank samples after the last patient last visit.

9.3. Multi-Slice Computed Tomography Scans

Cardiac CT scans will be performed using a dedicated cardiac CT- scanner. A standard non-contrast as well as contrast scan is performed according to usual clinical care. In patients with a stable heart rate above 60 beats per minute, oral or intravenous β-blocker is administered until the heart rate is appropriate (if possible below 60). Sublingual nitrates are administered to all patients prior to the scan. Regarding the non-contrast scan, 120 kV tube voltage (mandatory) is used. In patients with stable heart rate below 65 beats per minute, a prospectively diastolic scan (70% phase) is used. Otherwise a prospectively scan 300 ms after the QRS-complex. The contrast scan protocol depends on the local CT scanner and the patient's heart rate. In patients with stable heart rate above 65 beats per minute, a prospectively diastolic scan (65-75% phase) is used. Otherwise a prospectively scan 200-400 ms after the QRS-complex. 50-70 mL of contrast agent is injected into an antecubital vein at a rate of 6.0 mL/s followed by 50-70 mL intravenous saline (6.0 mL/s) using

a dual-head power injector. Data acquisition parameters depends on the local CT scanner, but slice collimation will be below 0.6mm, gantry rotation time as fast as possible and a tube voltage of 70 or 120 kV depending on patients' weight.

All scans are sent to and analysed at end-of-study by the core lab in Odense University Hospital. CAC scores are measured using the Agatston method by summing-up all spots of calcifications in the coronaries. The coronary artery tree will be analysed for the presence and severity of CAD, according to the classification of the American Heart Association 16-segment model. All coronary segments ≥2 mm in diameter with plaque will be analysed using a semi-automated software (AutoPlaque) that measures coronary plaque composition and volume. Coronary plaques are defined as visible structures within or adjacent to the coronary artery lumen, which can be clearly distinguished from the vessel lumen and the surrounding pericardial tissue. Scans are analysed by four trained and experienced technicians under continuous monitoring by two cardiologists.

10. Author Contributions

Conceptualisation: Selma Hasific, Kristian Øvrehus, Axel Diederichsen; Data curation and analysis: Selma Hasific, Anna Mejldal, Axel Diederichsen; Funding acquisition: Axel Diederichsen; Investigation: Selma Hasific, Kristian Øvrehus, Susanne Hosbond, Jess Lambrechtsen, Preman Kumarathurai, Emil Ravn, Lars Melholt Rasmussen, Oke Gerke, Hans Mickley, Axel Diederichsen; Project administration: Selma Hasific, Axel Diederichsen; Writing - original draft: Selma Hasific, Preman Kumarathurai, Axel Diederichsen; Writing - review & editing: Selma Hasific, Kristian Øvrehus, Susanne Hosbond, Jess Lambrechtsen, Preman Kumarathurai, Anna Mejldal, Emil Ravn, Lars Melholt Rasmussen, Oke Gerke, Hans Mickley, Axel Diederichsen.

11. Competing interest

None declared.

12. Funding

Private foundations and companies are sought for funding. Study tablets, including placebo, are provided free of charge by Kappa Bioscience, Norway and Orkla Care, Denmark. The companies are not involved in the design, execution of the study, analysis of the data or reporting of results.

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Supplementary Table 1: Timeline and applied tests during the trial

	Month								
	0	3	6	9	12	15	18	21	24
Informed consent and randomisation	X								
Medical interview	X		X		X		X		X
Web-based survey		X		X		X		X	
Biochemical measurements	X		X		X		X		X
Biobank	X				X				X
Non-contrast CT	X				X				X
Contrast CT	X								X

Appendix 1

Ingredients of the placebo pill	Ingredients of the intervention pill
Calciumphosphate E341	Calciumphosphate E341
Microcrystalline cellulose E460	Microcrystalline cellulose E460
Hydroxypropyl methylcellulose E464	Hydroxypropyl methylcellulose E464
Cross-linked sodium carboxymethylcellulose E468	Cross-linked sodium carboxymethylcellulose E468
Magnesium salts of fatty acids E470b	Magnesium salts of fatty acids E470b
Acacia gum E414	Acacia gum E414
Polyfructose	Polyfructose
Talc E553b	Talc E553b
Titanium dioxide E171	Titanium dioxide E171
Fatty acids E570	Fatty acids E570
Carnauba wax E903	Carnauba wax E903
	Vitamin K2
	Vitamin D3

Skriftlig deltagerinformation

Engelsk titel: *The DANish COronary DEcalcification (DANCODE) trial*

Dansk titel: Hæmning af forkalkninger i kranspulsårerne (DANCODE)

Region Syddanmark

OUH
Odense Universitetshospital

Indledning

Vi vil hermed spørge dig, om du er interesseret i at deltage i et forskningsprojekt, hvor formålet er at undersøge om K-vitamin-tilskud kan forsinke eller stoppe yderligere forkalkninger. Vi skriver til dig, fordi du tidligere har fået foretaget en hjerte-CT skanning, hvor lægerne fandt, at du havde udtalte forkalkninger i hjertes kranspulsårer.

Hvad er baggrunden for undersøgelsen?

Et nyt dansk studie tyder på, at tilskud med højdosis K2-vitamin og D-vitamin forebygger forkalkning af kranspulsårerne. Med DANCODE ønsker vi at afklare om tilskuddet kan forebygge yderligere forkalkninger hos personer, der har udtalte forkalkninger. Der inkluderes i alt 400 mænd og kvinder.

Hvad går undersøgelsen ud på?

Undersøgelsen er et lodtrækningsforsøg: halvdelen af deltagerne vil få K2-vitamin (720 µg dagligt) samt D-vitamin (25 µg dagligt) og halvdelen vil få placebobehandling (uvirksomt stof). Samlet varighed er planlagt til to år. I løbet af denne periode vil vi:

1. **Hver 6. måned tale med dig om dit helbred**
2. **Hver 6. måned tage blodprøver**
   - Blodprøvetagningen indebærer et stik i armen, og der kan efterfølgende komme en lille blodansamling. Vi vil måle din nyrefunktion, elektrolytbalance, kalkstofskifte, blodstørkning, samt nogle forskningsanalyser vedrørende årsager til forkalkning.
   - Ved start og årligt de følgende to år vil vi tage en ekstra blodprøver (40 ml blod hver gang) til en forskningsbiobank, hvorfra eventuelt tiloversbleven materiale ved forsøgets afslutning bliver overført til biobank til fremtidig forskning. Prøverne vil maksimalt blive opbevaret i 10 år og vil herefter blive destrueret. Du kan til enhver tid kontakte den projektansvarlige og bede om at få dit materiale destrueret.
3. **Hvert år foretage CT skanning af dit hjerte**
   - Denne skanning gennemføres 3 gange (ved start og årligt de følgende to år). Med denne kan vi måle mængden af kalk i kranspulsårerne.
   - Derudover vil vi ved første og sidste CT skanning også give røntgenkontrast. Med denne skanning kan vi måle mængden af fedt i pulsårerne.

Hvor lang tid tager undersøgelsen?

Vi vil gerne se dig hver 6. måned i gennem 2 år. Første gang vil vi fortælle dig om undersøgelsen. Hver gang vil vi måle puls, blodtryk og tage blodprøver. Derudover vil vi foretager CT-skanninger af hjertet. Samtalen og undersøgelserne tager op mod 2 timer per gang.

Hvad får du ud af at deltage?

Hver anden deltager får K2-vitamin tilskud, mens hver anden får placebobehandling (uvirksomt stof). Når alle resultater er analyseret, kan vi fortælle dig, om du har fået K2-vitamin og om dine forkalkninger er ændret.

I løbet af de to år vil vi undersøge dit hjertet tre gange, men screener ikke efter andet som f.eks. kræft. Vi forventer, at enkelte i løbet af de to år vil udvikle hjertesygdomme. Er du blandt dem, da vil vi tilbyde dig standard medicinsk behandling.

Hvis du ikke deltager vil vi ikke gøre yderligere, men anbefale dig at fortsætte behandling i samråd med din læge.

Er der nogle ulemper/risici for mig som forsøgsdeltager?

Halvdelen får højdosis K2-vitamin tilskud. Der er ikke tidligere rapporteret bivirkninger til K2-vitamin, men der kan være uforudsete risici og belastninger forbundet med forsøget. Indtager du kosttilskud kan du fortsætte med dette, men du bør ikke tage mere, imens du deltager i projektet pga. risiko for overdosering. Der foretages halvårlige blodprøvekontroller for at observere dette.

I forbindelse med CT skanningen benyttes røntgenstråling. Der er planlagt 3 CT skanninger og den samlede stråledosis bliver op mod 9 mSv. Baggrundsstrålingen, det vil sige den stråling du udsættes for fra omgivelserne (fra jorden, luften, mad og byggematerialer) er 3 mSv pr år i Danmark. Det betyder, at man i løbet af de to år undersøgelsen varer udsættes for, hvad der svarer til tre gange den årlige baggrundsstråling. Dette vil øge risikoen for at få kræft med 0,045%, hvilket bør ses i forhold til den generelle risiko for at udvikle kræft i Danmark på 25%, og således resultere i en samlet kræftrisiko på 25,045%.

Røntgenkontrast kan medføre nyreproblemer, hvis man har dårlig nyrer. Vi vil derfor ikke give dig røntgenkontrast, hvis du har nedsat nyrefunktionen.

Undersøgelsen vil blive afbrudt, hvis der findes uventede bivirkninger til højdosis K2-vitamin tilskud.

Hvorfor er det vigtigt at du deltager i undersøgelsen?

På trods af sund sundlivsstil og forebyggende medicin vil nogen alligevel få en blodprop, så vores aktuelle behandling er ikke helt tilstrækkelig. Det kunne tyde på, at K2-vitamin tilskud kan forebygge forkalkninger, og med dette studie vi vil nu afklare om K2-vitamin tilskud er en relevant forebyggende behandling.

Du kan ikke deltage i undersøgelsen, hvis du har fået foretaget en hjerteoperation, eller hvis du får blodfortyndende Marevan.

Samtykke

I forbindelse med informationen om studiet er du velkommen til at medbringe en ægtefælle eller ven. Efter at den skriftlige og mundtlige information er givet, kan du umiddelbart sige ”ja” til undersøgelsen eller vælge betænkningstid på minimum et døgn. Du kan når som helst trække dit tilsagn tilbage uden at det vil have nogen konsekvenser for dig.

Personfølsomme oplysninger

Hvis du vælger at deltage i studiet, samtykker du også til at de projektansvarlige må få adgang til din journal, således at vi ved behov kan indhente information om, hvilken medicin du tager, og hvilke sygdomme du fejler mv. De oplysninger vi får fra dig, fra din journal samt fra undersøgelserne vil alene blive brugt i forbindelse med projektet, så vi kan passe bedst muligt på dig. Databeskyttelsesforordningen og databeskyttelsesloven overholdes.

Resultaterne af undersøgelsen vil blive offentliggjort i videnskabelige tidsskrifter, og i den sammenhæng vil alle dine oplysninger blive helt anonymiseret, således at det ikke er muligt at identificere dig eller andre enkeltpersoner.

Økonomi

Der vil blive søgt støtte til lønninger og laboratorieundersøgelser fra offentlige og private fonde, men også fra private firmaer. Det forventes at tabletter doneres fra producenterne (Kappa Bioscience og Orkla Care). Professor Axel Diederichsen (hjertemedicinsk afdeling B, Odense Universitetshospital) har taget initiativ til forsøget. Der er ingen økonomisk tilknytning mellem støttegivere og de forsøgsansvarlige.

Du får som forsøgsdeltager ikke et vederlag, men vi kan tilbyde transportgodtgørelse.

Som yderligere information vedlægges følgende materiale:

”Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt” udgivet af Den Videnskabsetiske Komité.

Kontaktoplysninger

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i forsøget og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Hvis du vil vide mere, er du meget velkommen til at kontakte den projektansvarlige:

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 7-8

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7-8
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	4

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	5
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	5, 8, 10-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	5
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7, SAP
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7, SAP

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7, SAP
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8, 9
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8-10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	7
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	8
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	8
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	14-16
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	5, 11

Notes:

- 18a: 5, 8, 10-11 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 27. February 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai