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Study protocol of the InterVitaminK trial

a Danish population-based randomised double-blinded placebo-controlled trial of the effects of vitamin K (menaquinone-7) supplementation on cardiovascular, metabolic and bone health

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BMJ Open Study protocol of the InterVitaminK trial: a Danish population-based randomised double-blinded placebo-controlled trial of the effects of vitamin K (menaquinone-7) supplementation on cardiovascular, metabolic and bone health

Freja Bach Kampmann ¹, Sanne Marie Thysen,¹ Camilla Friis Bryde Nielsen ¹, Klaus Fuglsang Kofoed,^{2,3} Lars Køber,² Michael Huy Cuong Pham,² Allan Vaag,^{4,5,6} Niklas Rye Jørgensen,^{3,7} Janne Petersen,^{1,8} Rikke Kart Jacobsen,¹ Line Lund Kårhus,¹ Axel Diederichsen ^{9,10}, Marie Frimodt-Møller ⁴, Allan Linneberg^{1,3}

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Correspondence to

Dr Allan Linneberg;
allan.linneberg@regionh.dk

ABSTRACT

Introduction Vitamin K has been suggested to have protective effects against progression of vascular calcification and development of cardiovascular disease (CVD). However, few well-powered randomised controlled trials have examined whether vitamin K prevents progression of vascular calcification in individuals from the general population. The aim of the InterVitaminK trial is to investigate the effects of vitamin K supplementation (menaquinone-7, MK-7) on cardiovascular, metabolic, respiratory and bone health in a general ageing population with detectable vascular calcification.

Methods and analysis The InterVitaminK trial is a randomised, double-blinded, placebo-controlled, trial. A total of 450 men and women aged 52–82 years with detectable coronary artery calcification (CAC), but without manifest CVD, will be randomised (1:1) to receive daily MK-7 (333 µg/day) or placebo tablets for 3 years. Health examinations are scheduled at baseline, and after 1, 2 and 3 years of intervention. Health examinations include cardiac CT scans, measurements of arterial stiffness, blood pressure, lung function, physical function, muscle strength, anthropometric measures, questionnaires on general health and dietary intake, and blood and urine sampling. The primary outcome is progression of CAC from baseline to 3-year follow-up. The trial has 89% power to detect a between-group difference of at least 15%. Secondary outcomes are bone mineral density, pulmonary function and biomarkers of insulin resistance.

Ethics and dissemination Oral MK-7 supplementation is considered safe and has not been found to cause severe adverse events. The Ethical Committee of the Capital Region (H-21033114) approved the protocol. Written informed consent is obtained from all participants and the trial is conducted in accordance with the Declaration of Helsinki II. Both negative and positive findings will be reported.

Trial registration number NCT05259046.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ A major strength of the InterVitaminK trial is the population-based randomised double-blinded placebo-controlled design and the long intervention period (3 years).
- ⇒ The trial is powered for the main outcome, coronary artery calcification (CAC), which serves as an intermediate outcome of cardiovascular disease (CVD). However, with the unique Danish registers, follow-up for disease endpoints, including cardiovascular events and mortality after trial completion, is possible.
- ⇒ Vascular calcification is a slow process; thus, to limit the duration of the trial, we have limited the trial population to individuals with detectable CAC.
- ⇒ The trial will serve as proof of concept of whether vitamin K supplementation (MK-7) may serve as a measure for the prevention of vascular calcification and, thus, age-related diseases such as CVD in middle-aged and elderly people with measurable vascular calcification.

INTRODUCTION

Vitamin K acts as a co-enzyme, essential for carboxylation and activation of vitamin K-dependent γ -carboxyglutamate (Gla)-containing proteins important to coagulation.¹ In addition to its important role in coagulation, vitamin K also activates other vitamin K-dependent proteins (VKDPs),² which may protect against arterial calcification,³ promote bone formation^{2 4 5} as well as be involved in the regulation of glucose metabolism.⁶ Thus, vitamin K might play a role in the pathogenetic mechanisms of

several non-communicable age-related diseases as diverse as cardiovascular disease (CVD), osteoporosis and type 2 diabetes.

There are two forms of vitamin K which are obtained from very different dietary sources: vitamin K1 (phylloquinone) and vitamin K2 (menaquinones—MKs), with the latter being a complex group of isomers differing from one another by the length of their side chain, which ranges from 4 to 13 isoprenoid unit repeats (MK-4–MK-13). In the InterVitaminK trial, we will use (MK-7), which is believed to have greater effects in peripheral tissues compared with phylloquinone, and due to MK-7's longer half-life and greater bioavailability in peripheral tissues compared with short-chain MKs.⁷

Vitamin K and cardiovascular health

CVD is one of the leading causes of death worldwide and has been estimated to account for 18.6 million deaths in 2019.⁸ Coronary artery calcification (CAC) is a strong predictor of future cardiovascular events, independent of traditional cardiovascular risk factors⁹ and progresses more rapidly in individuals with pre-existing CAC.¹⁰

Progression of vascular calcification is caused by an imbalance between inhibition and promotion of calcium deposition in the endothelial wall of arteries.¹¹ Vitamin K is required to activate Matrix Gla Protein (MGP), which is considered the most potent inhibitor of soft-tissue calcification. High levels of dephosphorylated-uncarboxylated MGP (dp-ucMGP) reflect low vitamin K status.^{25,12} Recently, we found low vitamin K status (defined as dp-ucMGP >500 pmol/L) to be associated with several CVD risk factors in a general population.¹³ A meta-analysis of observational studies found low vitamin K status to be associated with 70% increased all-cause mortality (95% CI 1.44 to 2.18), 85% increased CVD mortality (95% CI 1.44 to 2.18) and 41% increased risk of CVD (95% CI 0.94 to 2.12).¹⁴ However, results from randomised controlled trials (RCTs) have been conflicting and primarily conducted in high-risk populations, for example, patients with chronic kidney disease or aortic stenosis.^{15–17} At present, few RCTs have been performed in individuals without manifest CVD.^{18–20} One RCT in healthy older adults found no overall effect of a 3-year intervention with vitamin K1 on progression of CAC, while among adherent individuals with mild pre-existing CAC, vitamin K reduced CAC progression with 6%.¹⁸ Similar to these findings, 3 years supplementation with 180 µg menaquinone-7 (MK-7) per day significantly improved arterial stiffness in healthy postmenopausal women, with the most pronounced effect seen in women with increased arterial stiffness at baseline.²⁰ Thus, subanalyses from these RCTs indicate that vitamin K supplementation could have beneficial effects on CVD risk in individuals without manifest CVD but with detectable CAC at baseline.^{18, 20} However, these findings need to be investigated in long-term intervention studies with sufficient power and well-recognised endpoints for CVD risk.

Vitamin K and bone health

Another VKDP, osteocalcin, regulates bone mineral accretion and protects against bone loss. A meta-analysis of observational studies found low vitamin K intake to be associated with bone fractures.²¹ This is also supported by a study that found high dp-ucMGP (low vitamin K status) associated with bone fractures.²² Although several observational and intervention studies have examined the relationship between vitamin K and bone metabolism, findings are conflicting.²³

Vitamin K and metabolic health

Experimental animal studies have shown that obesity and glucose intolerance can be induced by a vitamin K-depleted diet, paralleled by increased levels of uncarboxylated osteocalcin. This suggests that a vitamin K-dependent activation of osteocalcin could play a role in glucose homeostasis. Consistent with this, osteocalcin knockout mice exhibit an obese and glucose-intolerant phenotype.²⁴ Moreover, studies in humans suggest that vitamin K supplementation may improve insulin sensitivity²⁵ and glucose and lipid metabolism^{26, 27} and may be associated with anti-inflammatory properties.⁶ Generally, the RCTs performed so far have been relatively small and of short duration (2 years or shorter).^{25–27}

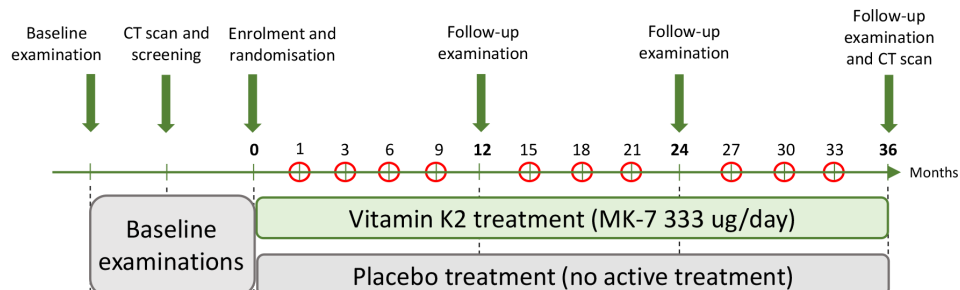
Vitamin K and respiratory health

It has been hypothesised that vitamin K dependent activation of calcification-inhibiting proteins, for example, MGP, in peripheral lung tissue could play a role in the prevention of lung diseases such as chronic obstructive pulmonary disease and acute COVID-19. This is mainly based on a few studies in smaller cohorts of patients with chronic obstructive pulmonary disease,^{28, 29} COVID-19^{30, 31} and cystic fibrosis.³² All studies found lower vitamin K status in patient populations compared with controls. To date, no intervention studies have been performed.

Objectives and hypotheses

The primary objective is to investigate the effect of 3 years of intervention with daily vitamin K supplementation on the progression of vascular calcification in terms of CAC score (change from baseline to 3-year follow-up in CAC) in individuals with detectable vascular calcification. Secondary objectives are to evaluate changes in bone mineral density (BMD), pulmonary function and biomarkers of insulin resistance. We hypothesised that vitamin K supplementation would reduce the progression of CAC by 15% compared with placebo which we consider a clinically relevant difference.

Findings of the InterVitaminK trial will inform the design of future large-scale studies and may contribute to new dietary recommendations for the intake of vitamin K. From a public health perspective, there is an urgent need for simple and feasible interventions to inhibit the progression of vascular calcification and reduce the risk of CVD. If vitamin K supplementation is found to slow progression of vascular calcification, improve glucose metabolism and/or promote bone



Visit	V0-A	V0-B	V0-C	V1	V2	V3
Participant information						
Written participant information		x				
Inclusion and exclusion criteria		x	x			
Informed content			x			
Registration of compliance and medicine						
Compliance phone calls				x	x	x
Registration of medication			x	x	x	x
Health examinations						
Cardiac CT scans		x				x
Arterial stiffness (SphygmoCor)	(x)			x	x	x
Blood pressure	(x)			x	x	x
Blood sampling and biochemical measurements	x		x	x	x	x
Urine sample	(x)			x	x	x
Anthropometrics	(x)			x	x	x
Body composition (bioelectrical impedance)	(x)			x	x	x
Pulmonary function (Spirometry)			x	x	x	x
Physical performance and handgrip strength	(x)			x	x	x
Questionnaires						
General health, chronic diseases, lifestyle factors	(x)		x	x	x	x
FFQ to assess intake of vitamin K			x			x

Figure 1 Trial design and time schedule: Baseline health examinations are conducted at three visits (V0-A, V0-B and V0-C). V0-A: Baseline examination (conducted as part of the Inter99 20-year follow-up study), V0-B: Baseline examination (Cardiac CT scan and screening prior to invitation), V0-C: Enrolment visit and randomisation, V1: 1-year follow-up visit, V2: 2 years follow-up visit, V3: 3 years follow-up visit. Examinations marked with (x) will be applied as baseline measurements for the InterVitaminK trial. Red circles illustrate phone calls to register treatment compliance. FFQ, food frequency questionnaire.

mineralisation, even modest effects in the population on average, could lead to the prevention of a significant number of disease events on a population level.

METHODS AND ANALYSES

Trial design and setting

The InterVitaminK trial is a population-based, double-blinded, placebo-controlled, randomised intervention trial (figure 1). The InterVitaminK trial will be carried out at the Centre for Clinical Research and Prevention, Denmark. The cardiac CT scans will be performed at the department of cardiology, Rigshospitalet, Copenhagen, Denmark.

Study population, recruitment and enrolment

Participants will be recruited from the 20-year follow-up examination of the Inter99 cohort (ClinicalTrials.gov identifier: NCT05166447). The Inter99 cohort was initiated in 1999 and designed as a population-based intervention study investigating the effects of a lifestyle intervention on the incidence of CVD.³³ All individuals who participated in the health examination at baseline

(n=6784) will be invited to a 20-year follow-up examination between September 2021 and December 2023. The Inter99 participants are between 52 and 82 years old (mean age: 67) at the time of the 20-year follow-up. Approximately 4000 (~66%) are expected to participate in the Inter99 20-year follow-up study, which includes a health examination and measurements of arterial stiffness, diabetic retinopathy, liver stiffness, glucose and lipid metabolism, and biochemical measurements. In addition, all participants are invited for a cardiac CT scan, which will provide measures of CAC and BMD.³⁴ At the time of the CT scan (V0-B, figure 1), subjects will receive written (online supplemental material 1) and oral information about the InterVitaminK trial and will be screened for eligibility according to the inclusion and exclusion criteria. Subjects who agree to participate will be invited for an enrolment visit (V0-C) at the Centre for Clinical Research and Prevention. At the enrolment visit, subjects will be rescreened according to the eligibility criteria and final oral and written informed consent is obtained for all participants (online supplemental material 2). The

participants are asked for separate consent for the storage of biological material in the biobank for future research (online supplemental material 3). Participants can withdraw their consent from the study at any time and request that their data be deleted.

Eligibility criteria

Participants in the Inter99 20-year follow-up study with a CAC score ≥ 10 (Agatston unit) are eligible.

Exclusion criteria are as follows:

- ▶ Manifest CVD (prior cerebral infarct, prior myocardial infarct, prior percutaneous coronary intervention or prior coronary artery bypass surgery).
- ▶ Noise on the CT scan complicates an accurate assessment of CAC and interpretation of the CT scan. An example is a pacemaker.
- ▶ Current treatment with vitamin K antagonist (VKA).
- ▶ History of coagulation disorders (haemophilia, von Willebrand disease, sickle cell anaemia).
- ▶ Active malignant disease (ongoing treatment).
- ▶ Previous surgical removal of the thyroid gland or one or more of the parathyroid glands.
- ▶ Regular use of vitamin K supplements other than trial tablets.
- ▶ Pregnancy or breast feeding.

Criteria for withdrawal and exclusion after enrolment as follows:

- ▶ If participants start to take non-trial vitamin K supplements during the study period and are not willing to cease the intake during the trial
- ▶ Initiation of VKA treatment during the study period.
- ▶ Unacceptable side effects or other safety concerns—judged by the medical doctor responsible for the project safety.

Intervention, randomisation and blinding

Participants will be randomised 1:1 to receive one daily tablet with MK-7 (333 $\mu\text{g}/\text{day}$, K2VITALDelta—active treatment) or matching placebo tablet (no active treatment) for 3 years (figure 1).

Randomisation was performed using computer-generated random numbers in blocks of 6. The randomisation list is generated by a statistician not involved in the trial and sent directly to Kappa Bioscience AS, which is responsible for producing study tablets and labelling tablet containers with unique study IDs. Trial participants, investigators and data collectors are blinded to the allocation throughout the study period. MK-7 and placebo tablets will be identical in appearance, taste, and composition, except for the active MK-7 component.

Baseline and follow-up

The baseline health examinations are conducted as part of the Inter99 20-year follow-up study (visit V0-A), and cardiac CT scans are performed at baseline (visit V0-B) prior to enrolment. At enrolment (visit V0-C), participants are randomised, and tablets for 1 year of intervention are handed out. Participants will be invited to a health

examination, including completion of online questionnaires on general health and diet at 1-year follow-up (V1), 2-year follow-up (V2) and 3-year follow-up (V3, post intervention). Phone calls will be made at 1 and 3 months after enrolment and every third month thereafter to monitor compliance (figure 1). In addition, compliance will be registered at each follow-up visit, where visits will replace the phone call. Counting of leftover tablets will be used as a proxy for compliance. Adherence to trial treatment is defined as a compliance of $\geq 80\%$.

Medicine and use of dietary supplements will be registered at enrolment (V0-C) and each follow-up visit. All follow-up health examinations include measurements of arterial stiffness, body composition, anthropometry, pulmonary function, blood pressure, handgrip strength, physical function (using a sit-to-stand test) and blood and urine sampling. Cardiac CT scans will be repeated at 3-year follow-up (V3, postintervention) (figure 1). Vitamin K status (dp-ucMGP) will be measured at baseline (V0-C) and at each follow-up visit (V1, V2, V3). For a detailed description of the health examinations, see online supplemental material 4.

Timeline

The first participant was enrolled in the InterVitaminK trial in June 2022, and we expect to enrol the last participant by December 2023. Thus, we expect to complete the follow-up of the last participant in December 2026 (figure 2).

Outcomes

The effect of vitamin K supplementation on all prespecified endpoints will be assessed as the between-group (active vs placebo) difference in change from baseline to 3-year follow-up.

Primary outcome

The primary endpoint is change in CAC score from baseline to 3-year follow-up assessed by non-contrast cardiac CT scan. Supportive outcomes will be used to support the conclusions of the primary/secondary endpoints for which the supportive outcome is defined; hence no separate claims will be made based on supportive outcomes. Outcomes supportive of the primary outcome are classified as secondary outcomes, while outcomes supportive of secondary outcomes are classified as exploratory outcomes.

Secondary outcomes supportive of the primary outcome

- ▶ Total coronary plaque composition (calcified, non-calcified subcomponents) assessed by contrast cardiac CT scans.
- ▶ Arterial stiffness assessed by carotid-femoral pulse wave velocity measurement.
- ▶ Blood pressure assessed by a digital blood pressure device.
- ▶ Aortic valve calcifications assessed by non-contrast cardiac CT scans.

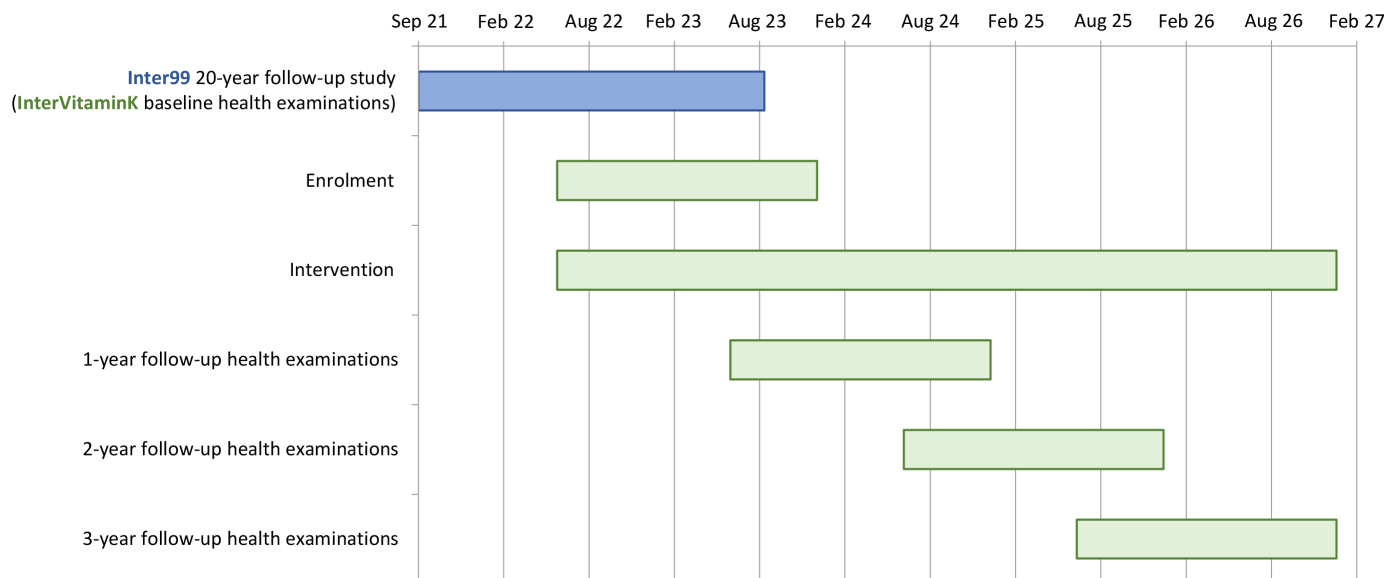


Figure 2 Timeline of the project. The Gantt chart illustrates the timeline of the entire InterVitaminK trial. Baseline health examinations for the trial are performed as part of the Inter99 20-year follow-up study. Enrolment in the InterVitaminK trial was initiated in June 2022 and is expected to run for 18 months. We expect to complete follow-up of the last participant in December 2026.

The following secondary endpoints (with supportive outcomes) will be investigated:

- ▶ BMD assessed by quantitative CT scan of the columna thoracalis.³⁴
 - Biomarkers of bone resorption and formation (supportive).
- ▶ Pulmonary function assessed by spirometry and reflected by forced expiratory volume in 1 s (FEV1).
 - Pulmonary function assessed by spirometry and reflected by expiratory forced vital capacity (FVC) (supportive).
 - Pulmonary function assessed by spirometry and reflected by FEV1/FVC ratio (supportive).
 - Lung tissue density as a measure of lung fibrosis assessed by CT scan (supportive).
 - Respiratory infections. Annual number of respiratory infectious disease episodes, including upper and lower respiratory infections and COVID-19, registered through telephone interviews four times/year during the study period (supportive).
- ▶ Insulin resistance assessed by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (HOMA-IR will be used as an estimate of insulin resistance and calculated as $((\text{Fasting plasma insulin (pmol/L)} \times \text{fasting plasma glucose (mmol/L)}) / 22.5) \times 0.144$).³⁵
 - Glucose control reflected by glycated haemoglobin A1c (supportive).

The following explorative endpoints will be investigated:

- ▶ Biomarkers of lipid metabolism.
- ▶ Inflammatory biomarkers.
- ▶ Body composition (body fat percentage) assessed by bioelectrical impedance.

Sample size considerations

Sample size calculations are based on the primary endpoint (3-year progression in CAC (unit: Agatston score)). In a previous trial of vitamin K supplementation in healthy participants aged 60–80 years, the geometric mean progression in CAC over 3 years was 2.82, (SE: 0.06; SD: 0.57).¹⁸ In a Danish cohort study (DANCAVAS),³⁶ the geometric mean progression in CAC over 5 years for participants with baseline CAC values >0 and <1000 was 4.58, (SE: 0.05; SD: 1.31). Based on this, we assume that our control group's geometric mean 3-year progression in CAC will be 3.0 with an SD of 1.3. We hypothesise that vitamin K supplementation can reduce the 3-year progression in CAC by 15% in the MK-7 group compared with the placebo group. With 25% drop-out before the 3-year follow-up visits, and 450 participants (225 participants in each group) included at baseline, we will have 89% power to detect an effect of at least 15% (alpha 0.05).

Statistical methods

The effect of vitamin K supplementation on the primary outcome (CAC) will be analysed using mixed-effects linear regression. The mixed-effects linear regression will include a fixed effect for group allocation (intervention/control), a fixed effect for a time point (baseline and 3-year follow-up), a fixed effect for baseline CAC score, and an interaction between group allocation and time point. As baseline measurements are conducted before enrolment, treatment at baseline will be modelled as a common treatment category, constraining baseline measurements to no systematic treatment effect between the two arms. The mixed effects model will include a random intercept for each enrolled participant and a first-order autoregressive correlation structure allowing

correction of measurement for the same participant with higher correlation for measurements closer in time.

Likewise, secondary and supportive outcomes will be analysed using mixed-effects linear regression. For details, see the statistical analysis plan at ClinicalTrials.gov (NCT05259046). Supportive outcomes will be used to express supportive evidence and will not be used to express a claim on their own; thus, CIs and statistical tests of supportive outcomes will be descriptive, and no adjustment for multiple testing will be made.

For all outcome measures, we will evaluate the pattern of missing values and impute missing values using multiple imputations as appropriate. All main analyses will follow an intention-to-treat approach. Analyses and presentation of data will be in accordance with the CONSORT guidelines. A detailed statistical analysis plan is available at ClinicalTrials.gov (NCT05259046).

Patient and public involvement statement

No participants or the public were involved in defining the research question or the outcome measure, nor were they involved in developing plans for recruitment, design or implementation of the study. We do not plan to include participants in the interpretation of the results or writing up the results. The trial results will be disseminated to trial participants.

ETHICS AND DISSEMINATION

Ethical approvals

The trial was approved by the Ethical Committee of the Capital Region (record no. H-21033114) in December 2021. Potential future amendments will be approved by the Ethical Committee prior to the implementation. The trial is conducted in accordance with the Declaration of Helsinki II and is registered at ClinicalTrials.gov: NCT05259046. The Danish Medicines Agency has declared that MK-7, as used in the present trial, is considered a dietary supplement.

Safety

A daily dose of 333 µg MK-7 is used in this trial, and the intervention is considered safe. MK-7 supplementation is well tolerated, and similar or higher doses of MK-7 have not been reported to be associated with increased risk of thrombosis or severe adverse reactions in healthy individuals³⁷ or patients.^{15 17 38}

All health examinations are considered harmless, non-invasive and involve minimal inconvenience in addition to any discomfort related to blood sampling. Experienced health professionals conduct the health examinations.

Each participant will have two cardiac CT scans performed during the study. The total dose of radiation received from one cardiac CT scan is approximately 3–10 mSv. For comparison, Denmark's annual background radiation dose is 3 mSv, and the average annual limit for radiation workers is 20 mSv. According to the Danish National Committee on Biomedical Research Ethics,

a radiation dose of 10 mSv may increase cancer risk by 0.05%.³⁹ All participants in the Inter99 20-year follow-up study are asked about known allergic reactions to the contrast media used to perform the contrast cardiac CT scan. Participants from the Inter99 20-year follow-up study with known allergic reactions to the contrast media used to perform the CT scan are not invited for a CT scan and thus not offered participation in the InterVitaminK trial. InterVitaminK participants who experience allergic reactions to the contrast media will, at 3-year follow-up, be offered a non-contrast CT scan.

The Patient Compensation Association covers all participants according to the Danish Act on the Right to Complain and Receive Compensation within the Health Service.

Safety monitoring

A data safety and monitoring board (DSMB) has been established to oversee the trial. The DSMB consists of a professor in cardiology, a professor in clinical nutrition and a statistician. All members are appointed based on their experience, absence of conflicts of interest and knowledge of clinical trial methodology. Adverse events are registered during the trial using a case report form. The principal investigator will report potential severe adverse events to the DSMB as soon as possible. There are no planned interim analyses in the present trial. During the trial, the DSMB can request analyses should concerns regarding the safety of the trial arise.

Data management

Study information will be recorded, handled and stored safely. All data handling (input, changes and deletion) will be logged. Data from questionnaires and health examinations will be registered in the electronic data management system, REDCap. Source data from spirometry and SphygmoCor examinations will be registered on the device/the related hardware and will be uploaded to a secure drive accessible only to study staff. For CT scans, data will be stored on a PACS—picture archiving and communication system—(Sectra PACS) accessible only to study staff.

Dissemination

Scientific publications will follow Vancouver recommendations. 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. No professional writers will be engaged in the writing process.

Author affiliations

¹Center for Clinical Research and Prevention, Copenhagen University Hospital - Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark

²Department of Cardiology and Radiology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark

³Department of Clinical Medicine, University of Copenhagen Faculty of Health and Medical Sciences, Copenhagen, Denmark

⁴Steno Diabetes Center Copenhagen, Copenhagen University Hospital, Herlev, Denmark

⁵Department of Clinical Sciences, Lund University Diabetes Center, Malmö, Sweden
⁶Department of Endocrinology, Skåne University Hospital, Malmö, Sweden
⁷Department of Clinical Biochemistry, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark
⁸Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark
⁹Department of Cardiology, Odense University Hospital, Odense, Denmark
¹⁰Elitary Research Centre of Individualized Medicine in Arterial Disease (CIMA), Odense University Hospital, Odense, Denmark

Twitter Axel Diederichsen @ADiederichsen

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Contributors For the InterVitaminK trial, AL is the principal investigator, and FBK and SMT are coinvestigators. The InterVitaminK Trial Steering Committee consists of FBK, SMT, KFK, LK, JP, AD, MF-M and AL. In addition to the trial steering committee, the InterVitaminK project group consists of CFBN, AV, NRJ, RKJ and LLK. AL and AV are principal investigators in the Inter99 20-year follow-up study. AL conceived the idea for the trial. FBK, SMT and AL designed the trial with inputs from KFK, AD and MF-M. FBK was responsible for setting up the trial, assisted by CFBN, SMT and AL. LK, KFK and MHCP are responsible for cardiac CT scans. SMT wrote the first draft of the analysis plan with input from FBK, JP, RKJ and AL. FBK wrote the first draft of the manuscript with input from SMT and AL. All authors contributed to the revision of the manuscript and approved the final version.

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Disclaimer The funders are not involved in the planning, execution, and management of the trial, analysis and interpretation of data, writing of the papers, and the decision to submit papers for publication.

Competing interests FBK, SMT and AL have received funds and tablets for the trial from Kappa Bioscience AS. AL discloses the application of a patent on vitamin K on lung function for prognostic and therapeutic purposes. Aside from this, the authors declare no conflicts of interest.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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ORCID iDs

Freja Bach Kampmann <http://orcid.org/0000-0002-6106-3115>

Camilla Friis Bryde Nielsen <http://orcid.org/0000-0002-8814-2104>
 Axel Diederichsen <http://orcid.org/0000-0002-1285-4826>
 Marie Frimodt-Møller <http://orcid.org/0000-0002-8486-1515>

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