





BMJ Open Effects of vitamins K2 and D3 supplementation in patients with severe coronary artery calcification: a study protocol for a randomised controlled trial

Selma Hasific ¹, Kristian A Øvrehus¹, Susanne Hosbond,² Jess Lambrechtsen,³ Preman Kumarathurai,¹ Anna Mejldal,^{4,5} Emil Johannes Ravn ¹, Lars Melholt Rasmussen,^{6,7} Oke Gerke ⁸, Hans Mickley,¹ Axel Diederichsen ¹

To cite: Hasific S, Øvrehus KA, Hosbond S, *et al.* Effects of vitamins K2 and D3 supplementation in patients with severe coronary artery calcification: a study protocol for a randomised controlled trial. *BMJ Open* 2023;**13**:e073233. doi:10.1136/bmjopen-2023-073233

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2023-073233>).

Received 27 February 2023
Accepted 03 July 2023



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Axel Diederichsen;
axel.diederichsen@rsyd.dk

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 vitamins K2 and D3 in men and women with severe CAC. We hypothesise that supplementation with vitamins 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 2 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 2 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 NCT05500443.

INTRODUCTION

Ischaemic heart disease accounts for 19% and 20% of all deaths among men and women, respectively, underscoring the critical importance of prevention.¹ Ischaemic heart disease is often silent until symptoms of myocardial infarction. However, during subclinical stages, ischaemic heart disease can be detected as coronary artery calcifications (CAC) on non-contrast cardiac CT scans. CAC increases with

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, 2 years of follow-up might be too short to find a difference.

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 ischaemic 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 phyloquinone (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, that is, 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 mineralisation 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 vitamins K and D status has been associated with increased all-cause mortality risk compared with adequate vitamins 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.

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.

METHODS

Trial design

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

Participants

The Danish Heart Registry will be used to identify patients who underwent a cardiac-CT in Western Denmark within the past 3 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 revascularisation.
- ▶ 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 8 February 2023 to March 2026.

Intervention

Patients are randomly assigned in a 1:1 ratio to either daily oral supplementation with vitamins K2 and D3 (two pills containing 360 µg MK-7 and 12.5 µg vitamin D3 each (K2VITALDelta), 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 vitamins 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 online supplemental appendix 1.

Trial visits and procedures

Online supplemental 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 6 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.

Online supplemental appendix 2 shows the patient information leaflet (in Danish).

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 prespecified 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 revascularisation 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 revascularisation, 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 ≥2 mmol/L).
- ▶ Cancer, including solid and haematologic.
- ▶ Significant deterioration in laboratory measurements (haemoglobin, creatinine, estimated glomerular filtration rate (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone or prothrombin time-international normalised ratio (PT-INR)).

Sample size

We are planning a study of a continuous response variable from independent control and experimental subjects with one control per experimental subject. In the AVADEC trial, the mean (SD) 2-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 SD 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 2 years of treatment and was assessed with Stata/MP V.17 (StataCorp, College Station, Texas, USA).

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 (DSMB) 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.

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 prespecified 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 the section ‘Outcome’).

We use linear mixed models (employing group, time point and group × 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.

Patient and public involvement

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

Organisation

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 are owned by *The Steering Committee*.

Data will be available to other researchers on reasonable request.

The 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.

Publication

Project results reporting the primary endpoint will be published in peer-reviewed international journals. We used the Standard Protocol Items: Recommendations for Interventional Trials (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.

Feasibility

Approximately, 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 ischaemic 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 sent 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.

Ethics and dissemination

Safety

The study will utilise 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 three times per week 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 revascularisation, stroke, heart valve surgery and venous thromboembolism), and (2) laboratory measurements (creatinine, eGFR, sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone and 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 DSMB will have access to the whole database, including the randomisation list, during the course of the research.

Data management

REDCap is used to register the data,²⁸ and it is logged and securely stored on a server under the OPEN, 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'.

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.

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 hypothesise that vitamins 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 vitamins 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 with vitamin D alone.¹² Although, the population (and dosage of vitamin D) are different in these trials compared with 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 of that, a new treatment option may be available to prevent not only progression of CAC, but also ischaemic heart disease. The results of this study are expected in 2026.

Applied tests during the study

Medical interview

Baseline data will be obtained at first visit. At the subsequent visits, an interview is conducted, and the following is evaluated: incident CVD, 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.

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 PT-INR.

Additionally, leukocytes, thrombocytes, lipid profile, haemoglobin A1c, alanine transaminase, lactate

dehydrogenase, bilirubin, creatine 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. 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.

Multislice CT 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 min, 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 min, 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 min, 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 0.6 mm, 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 laboratory 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 semiautomated 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.

Author affiliations

¹Department of Cardiology, Odense University Hospital, Odense, Denmark

²Department of Cardiology, Sygehus Lillebalt, Vejle, Syddanmark, Denmark

³Department of Cardiology, Svendborg Hospital, Svendborg, Denmark

⁴Department of Clinical Research, University of Southern Denmark, Odense, Denmark

⁵Open Patient Data Explorative Network, Odense University, Odense, Denmark

⁶Department of Clinical Biochemistry and Pharmacology, Odense Universitetshospital, Odense, Denmark

⁷Centre for Individualised Medicine in Arterial Diseases, Odense Universitetshospital, Odense, Denmark

⁸Department of Nuclear Medicine, Odense University Hospital, Odense, Denmark

Twitter Selma Hasific @SelmaHasific and Axel Diederichsen @ADiederichsen

Contributors Conceptualisation: SH, KAØ and AD; data curation and analysis: SH, AM and AD; funding acquisition: AD; investigation: SH, KAØ, SH, JL, PK, EJR, LMR, OG, HM and AD; project administration: SH and AD; writing—original draft: SH, PK and AD; writing—review and editing: SH, KAØ, SH, JL, PK, AM, EJR, LMR, OG, HM and AD.

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.

Competing interests None declared.

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.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Selma Hasific <http://orcid.org/0000-0001-7654-8492>

Emil Johannes Ravn <http://orcid.org/0000-0002-8992-5493>

Oke Gerke <http://orcid.org/0000-0001-6335-3303>

Axel Diederichsen <http://orcid.org/0000-0002-1285-4826>

REFERENCES

- CVD Statistics report. 2017. Available: <http://www.ehnheart.org/images/CVD-statistics-report-August-2017.pdf> [Accessed 29 Mar 2022].
- Gerke O, Lindholt JS, Abdo BH, *et al*. Prevalence and extent of coronary artery calcification in the middle-aged and elderly population. *Eur J Prev Cardiol* 2022;28:2048–55.
- Erbel R, Möhlenkamp S, Moebus S, *et al*. Coronary risk stratification, discrimination, and reclassification improvement based on quantification of Subclinical coronary atherosclerosis: the Heinz Nixdorf recall study. *J Am Coll Cardiol* 2010;56:1397–406.
- Polonsky TS, McClelland RL, Jorgensen NW, *et al*. Coronary artery calcium score and risk classification for coronary heart disease prediction. *JAMA* 2010;303:1610–6.
- Danziger J, Young RL, Shea MK, *et al*. Vitamin K-dependent protein activity and incident ischemic cardiovascular disease: the multi-ethnic study of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2016;36:1037–42.
- Viegas CSB, Rafael MS, Enriquez JL, *et al*. Gla-rich protein acts as a calcification inhibitor in the human cardiovascular system. *Arterioscler Thromb Vasc Biol* 2015;35:399–408.
- Schurgers LJ, Uitto J, Reutelingsperger CP. Vitamin K-dependent carboxylation of matrix Gla-protein: a crucial switch to control ectopic mineralization. *Trends Mol Med* 2013;19:217–26.
- Rennenberg RJMW, Schurgers LJ, Kroon AA, *et al*. Arterial Calcifications. *J Cell Mol Med* 2010;14:2203–10.
- Fraser JD, Price PA. Induction of matrix Gla protein synthesis during prolonged 1,25-dihydroxyvitamin D3 treatment of osteosarcoma cells. *Calcif Tissue Int* 1990;46:270–9.
- Luo G, Ducey P, McKee MD, *et al*. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997;386:78–81.
- Shea MK, O'Donnell CJ, Hoffmann U, *et al*. Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr* 2009;89:1799–807.
- Kurnatowska I, Grzelak P, Masajtis-Zagajewska A, *et al*. Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3–5. *Pol Arch Intern Med* 2015;125:631–40.
- Rennenberg RJMW, van Varik BJ, Schurgers LJ, *et al*. Chronic coumarin treatment is associated with increased extracoronary arterial calcification in humans. *Blood* 2010;115:5121–3.
- Weijs B, Blaauw Y, Rennenberg RJMW, *et al*. Patients using vitamin K antagonists show increased levels of coronary calcification: an observational study in low-risk atrial fibrillation patients. *Eur Heart J* 2011;32:2555–62.
- Schurgers LJ, Joosen IA, Laufer EM, *et al*. Vitamin K-antagonists accelerate atherosclerotic calcification and induce a vulnerable plaque phenotype. *PLoS One* 2012;7:e43229.
- Chaikriangkrai K, Valderrabano M, Bala SK, *et al*. Prevalence and implications of subclinical coronary artery disease in patients with atrial fibrillation. *Am J Cardiol* 2015;116:1219–23.
- van Ballegooijen AJ, Beulens JWW, Kieneker LM, *et al*. Combined low vitamin D and K status amplifies mortality risk: a prospective study. *Eur J Nutr* 2021;60:1645–54.
- van Ballegooijen AJ, Pilz S, Tomaschitz A, *et al*. The synergistic interplay between vitamins D and K for bone and cardiovascular health: a narrative review. *Int J Endocrinol* 2017;2017:7454376.
- Dalmeijer GW, van der Schouw YT, Magdeleyns E, *et al*. The effect of Menaquinone-7 supplementation on circulating species of matrix Gla protein. *Atherosclerosis* 2012;225:397–402.
- Pucaj K, Rasmussen H, Møller M, *et al*. Safety and Toxicological evaluation of a synthetic vitamin K2, Menaquinone-7. *Toxicol Mech Methods* 2011;21:520–32.
- Lindholt JS, Frandsen NE, Fredgart MH, *et al*. Effects of Menaquinone-7 supplementation in patients with aortic valve calcification: study protocol for a randomised controlled trial. *BMJ Open* 2018;8:e020219.
- Diederichsen ACP, Lindholt JS, Möller S, *et al*. Vitamin K2 and D in patients with aortic valve calcification: a randomized double-blinded clinical trial. *Circulation* 2022;145:1387–97.
- Herdman M, Gudex C, Lloyd A, *et al*. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20:1727–36.
- Caluwé R, Vandecasteele S, Van Vlem B, *et al*. Vitamin K2 supplementation in Haemodialysis patients: a randomized dose-finding study. *Nephrol Dial Transplant* 2014;29:1385–90.
- Theuwissen E, Cranenburg EC, Knäpen MH, *et al*. Low-dose Menaquinone-7 supplementation improved extra-hepatic vitamin K status, but had no effect on thrombin generation in healthy subjects. *Br J Nutr* 2012;108:1652–7.
- Preston DL, Ron E, Tokuoka S, *et al*. Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res* 2007;168:1–64.
- Einstein AJ, Knuuti J. Cardiac imaging: does radiation matter? *Eur Heart J* 2012;33:573–8.
- Harris PA, Taylor R, Minor BL, *et al*. The Redcap consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- Budoff MJ, Young R, Lopez VA, *et al*. Progression of coronary calcium and incident coronary heart disease events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol* 2013;61:1231–9.
- Manson JE, Allison MA, Carr JJ, *et al*. Calcium/vitamin D supplementation and coronary artery calcification in the women's health initiative. *Menopause* 2010;17:683–91.