PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of Vitamin K2 and D3 supplementation in Patients With Severe Coronary Artery Calcification: A study protocol for a randomised controlled trial
AUTHORS	Hasific, Selma; Øvrehus, Kristian; Hosbond, Susanne; Lambrechtsen, Jess; Kumarathurai, Preman; Mejldal, Anna; Ravn, Emil; Rasmussen, Lars; Gerke, Oke; Mickley, Hans; Diederichsen, Axel

VERSION 1 – REVIEW

REVIEWER	Kaesler, Nadine Rheinisch-Westfälische Technische Hochschule Aachen University Hospital
REVIEW RETURNED	24-Mar-2023

GENERAL COMMENTS	To study the effect of MK7 plus vitamin D in patients with a high CAC score is interesting and the study proposal is of general interest to the community. Some questions remain open. Abstract- Strength and limitations This is rather a study summary than pointing at strengths and limitations. Introduction Any introduction on vitamin D and calcification is missing. It should be mentioned that K1 and K2 metabolism is not fully separated, as described. K1 can be endogenously converted to K2 and can also activate MGP. How long was the treatment period of the initial AVADEC study? Methods: How was the dosage of 720µg MK7 chosen? Which form of vitamin D will be given (D2? D3?)? How will the coronary plaque composition be analyzed? Will sex stratified analyses be performed? The authors mention the different baseline CAC scores between men and women. What does the placebo pill contain? Are any advices given to the participants how/ when to take the pills/ in addition to any fat containing meal? Participants Why is vitamin D supplementation not excluded? Sample size Which software was used for the power analysis? Laboratory assessment Vitamin D measurement should be 25-OH vitamin D (?)

REVIEWER	Bo, Pang Guanganmen Hospital Department of Acupuncture, Guang'anmen hospital

REVIEW RETURNED	09-Apr-2023

GENERAL COMMENTS	This study protocol is well designed. The authors showed an urgent necessity of slowing down the coronary artery calcification process. And the hypothesis that supplementation of vitamin K2 and D could potentially achieve this goal is supported by the existing data. And the Danish AVADEC Trial showed promising results, as the protocol described, of slowing down the progression of the non-calcified coronary plaque volume and decreasing the number of cardiac events and all-cause death as well. Here are some concerns regarding this manuscript: 1 In part 11.2 Labortary assessment, Vitamin D and dp-ucMGP will be measured in the biobank samples after the last patient visit. My suggestion is that, if necessary, the authors might consider of Vitamin K measurement, although Vitamin K2 supplement is sufficient and dp-ucMGP will be measured. 2 In part 11.3 Multi-Slice Computed Tomography Scans, Scans are analysed by an experienced cardiologist. Maybe more than two experienced cardiologists or radiologists are better. But if the results are straight forward enough after being analyzed by the software, there is no need to add more researchers.

REVIEWER	Kremer, Daan Universitair Medisch Centrum Groningen, Internal Medicine, Division of Nephrology
REVIEW RETURNED	11-Apr-2023

GENERAL COMMENTS	In their manuscript, Hasific et al. Have provided a study protocol for a randomised controlled trial to study the effects of menaquinone-7 supplementation in patients with severe coronary artery calcifications. Overall, the planned study seems promising and could potentially provide valuable insights into the effects of vitamin K2 and D supplementation on CAC progression in a high-risk population. However, I have several major and minor suggestions that may help to further improve the study. Major points: The vitamin D intervention comes out of the blue sky, without sufficient introduction or pathophysiological rationale. Vitamin D is also unmentioned in the title. I would strongly advise the authors to better explain why this intervention is added on top of the vitamin K-supplementation. Also, I feel that it is necessary to explain how vitamin D/K may interact, whether they may work synergistically on regulating calcium metabolism, etc. Please discuss/review this in the introduction section (for some inspiration, see e.g. doi: 10.1155/2017/7454376). One may also argue that this combined intervention may blur the effects of vitamin K, rendering it impossible to distinguish the potential beneficial effects of vitamin D vs. vitamin K supplementation. Please address this potential problem in interpretation, or explain how this may be solved in the planned analyses.

	It appears biologically plausible that vitamin K supplementation is only useful in patients with proven vitamin K insufficiency. This is consistent with studies e.g. in kidney transplant recipients, where there were beneficial effects on CAC in a population with proven vitamin K-insufficiency at baseline (doi: 10.1016/j.ajt.2022.12.015), but not in a population where vitamin K-insufficiency was not proven (doi: 10.1111/ajt.16566). This may have to do with the very major improvement in vitamin K-status in the first, but a much smaller improvement in the second population (see discussion section of doi: 10.1016/j.ajt.2022.12.015). The authors do consider dp-ucMGP in exploratory analyses, but I would suggest to put more emphasis on this outcome. Ideally, it may be added as an inclusion criterion, but I realize that this may be difficult for many reasons. However, I would suggest to at least add subgroup analyses based on dp-ucMGP (taking into account kidney function, see for example https://doi.org/10.3390/nu13093069 / https://doi.org/10.3390/nu14122440). Also please mention the potential inclusion of vitamin K-sufficient subjects as a limitation of the current study, and elaborate on the potential implications of this limitation on the study results. In fact, the same holds true for the vitamin D-intervention – vitamin D-status is not considered at all in the study currently. I would advise the authors to elaborate on this. Minor points: Title: from the title only it is unclear what effects the authors aim to study. Only the population is clear, but not the outcome. I understand that it may seem a bit redundant, but for searchability and clarity the authors may still consider to add that they aim to study the effects of MK-7 on coronary artery calcifications. Also, for consistency, I would suggest to use the terminology 'coronary artery calcifications' consistently, rather than 'coronary calcifications' (only used in the title). Intro: There is accumulating evidence for vitamin K supplementation in many different populations. Yet, the 'knowledge gap' is not identified very clearly by the authors. I think the manuscript may be improved by briefly summarizing what the current clinical evidence is for vitamin K (and vitamin D, If that is indeed part of the intervention) supplementation in different populations, and what the current study adds to the existing body of evidence. Notably, the added value of the study may also be emphasized in other parts of the manuscript (e.g. strengths and limitations section, discussion section). Intro, p4, line 12-14: "men have higher CAC scores than women", this is not always the case. I would suggest to change the wording. Intro: p4, line 14-20. Please guide the authors on the differences between vitamin K1 and K2. Do they bind to the same receptors/how do their effects compare? Intro: p4, line 23: "the inhibiting process" > e.g. "the calcification-inhibiting properties" Intro p4 line 28 > remove '-' after coronary Throughout manuscript: some sentences are very long and contain many commas (e.g.: p4 line 11-13). For ease of reading, I would advise the authors to separate some overly long sentences. Methods, trial design: please mention whether this is a single-center or multi-center study. The BMJ Open guidelines specifically mention that dates of (planned) study initiation etc. should be included in the protocol. Please add these dates for transparency.

	The trial registration number is now only in the abstract. Please add it to the methods. Overall, quite some minor grammatical errors (several plural vs. singular form mismatches between subject and verb, e.g. p10 “an independent DSMB are” and several other mismatches in intro/methods; feasibility paragraph: ‘in the Western Denmark’ > ‘in Western Denmark’; some double spaces; please carefully check the entire manuscript e.g. using the Microsoft Word grammar check. Regarding the review checklist, I answered ‘No’ to several questions. Most have already been addressed in the review, but as an overview: 3. Is the study design appropriate to answer the research question? > Although the objective is clear, there is a mismatch between the objective and the methods. The rationale of adding vitamin D is not clearly explained. In fact, such a combined intervention may blur the actual vitamin K-effects. 8. Are the references up-to-date and appropriate? > I would suggest to add more clinical studies for the current body of evidence of vitamin K-supplementation in different populations. Please address the ‘knowledge gap’, and what the current study will add. 12. Are the study limitations discussed adequately? > I would suggest the authors to mention the previously mentioned limitations (vitamin D-effects, potential inclusion of vitamin D- and/or vitamin K-sufficient subjects who may have limited benefit of the interventions.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Nadine Kaesler, Rheinisch-Westfälische Technische Hochschule Aachen University Hospital
Comments to the Author:

To study the effect of MK7 plus vitamin D in patients with a high CAC score is interesting and the study proposal is of general interest to the community. Some questions remain open.
Abstract- Strength and limitations
This is rather a study summary than pointing at strengths and limitations.
Thank you for the comment. As noted in the editorial request above, we have revised this section.

Introduction
Any introduction on vitamin D and calcification is missing.
Thank you – you are right. We have added a brief introduction to the effect of vitamin D (p3):”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… A synergistic effect of the two vitamins on bone and cardiovascular health has been suggested.18”

It should be mentioned that K1 and K2 metabolism is not fully separated, as described. K1 can be endogenously converted to K2 and can also activate MGP.
Thank you for this comment. As vitamin K2 is the most potent activator of MGP, partly because of its higher extra-hepatic concentrations, we made this separation in the description of their effects. However, we have tried to elaborate by changing the text to (p3): “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 “

How long was the treatment period of the initial AVADEC study?
We have now added the treatment period to the description of AVADEC (p4): “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”

Methods:
How was the dosage of 720µg MK7 chosen?
Currently, there are no official recommendations on MK7 intake. In the AVADEC study, the intervention with 720 µg MK7 and 25 µg vitamin D was not only found to be efficient in patients with CAC > 400, it also had no safety concerns. As we wanted to test the hypothesis generated from the AVADEC results, we also decided to use the completely same intervention with 720 µg MK7 in this study. We have added the following text to the section concerning intervention (p5): “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”

Which form of vitamin D will be given (D2? D3?)?
The participants are supplemented with the patented product K2VITAL®Delta, which contains vitamin K2 and vitamin D3. It is now added to the protocol, page 5.

How will the coronary plaque composition be analyzed?
In section 11.3, the use of AutoPlaque is described. AutoPlaque is a semi-automated software using artificial intelligence to characterize and quantify coronary plaque. We have added some further information to clarify that this is used for the secondary endpoint about coronary plaque composition (p12): “All coronary segments ≥2 mm in diameter with plaque will be analysed using the semi-automated software AutoPlaque that measures coronary plaque composition and volume.”

Will sex stratified analyses be performed? The authors mention the different baseline CAC scores between men and women.
Thank you for the very relevant question. Because of the difference between baseline CAC scores in men and women, we have also defined a secondary outcome concerning sex. Please see section 3.4: “Secondary endpoints are:

- Change in CAC score from baseline to 24 months in men and women, respectively”

What does the placebo pill contain?
The placebo pills contains the following ingredients:
Calciumphosphate E341, Microcrystalline cellulose E460, Hydroxypropyl methylcellulose E464, Cross-linked sodium carboxymethylcellulose E468, Magnesium salts of fatty acids E470b, Acacia gum E414, Polyfructose, Talc E553b, Titanium dioxide E171, Fatty acids E570, Carnauba wax E903.
We will include the list as a supplemental material.
The active tablets will contain the same ingredients in addition to vitamin K2 and D3.
Are any advices given to the participants how/ when to take the pills/ in addition to any fat containing meal?
Even though we are aware, that dietary fat increases the absorption of both vitamin K and D, the participants were not specifically advised to take the pills with fat containing meals. They are advised to take them after their dinner, though. Also, just the combination of vitamin K and D themselves increases the absorption of both vitamins.
Participants

Why is vitamin D supplementation not excluded?
The participants were not restricted in their normal daily life, meaning that they were allowed to eat and consume fruits, vegetables and other supplements, as they like. The vitamin D levels are not only sensitive to diet and supplementation but also sun exposure. The vitamin D dose of the intervention pill corresponds to a standard recommended daily dose and we do not suspect any additional supplementation to have a negative effect on our results as long as the patient is within the normal range of 25-OH vitamin D during the study. Also, previous randomised studies on the effect of vitamin D on calcifications did not show any significant result, suggesting that vitamin D itself does not affect the calcium metabolism in the arteries (Manson et al. Calcium/vitamin D supplementation and coronary artery calcification in the Women's Health Initiative. Menopause. 2010 Jul;17(4):683-91). We have elaborated further on this, please see page 11.

Sample size
Which software was used for the power analysis?
We have added the following to the Sample Size section 3.5:
The sample size is based on two years of treatment and was assessed with Stata/MP 17 (StataCorp, College Station, Texas 77845 USA).

Laboratory assessment
Vitamin D measurement should be 25-OH vitamin D (?)
Yes, that is correct. We have corrected the text to be more specific in section 11.2:
“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.”

Reviewer: 2
Dr. Pang Bo, Guanganmen Hospital Department of Acupuncture Comments to the Author:

This study protocol is well designed. The authors showed an urgent necessity of slowing down the coronary artery calcification process. And the hypothesis that supplementation of vitamin K2 and D could potentially achieve this goal is supported by the existing data. And the Danish AVADEC Trial showed promising results, as the protocol described, of slowing down the progression of the non-calcified coronary plaque volume and decreasing the number of cardiac events and all-cause death as well.

Here are some concerns regarding this manuscript:

1 In part 11.2 Labortary assessment, Vitamin D and dp-ucMGP will be measured in the biobank samples after the last patient visit. My suggestion is that, if necessary, the authors might consider of Vitamin K measurement, although Vitamin K2 supplement is sufficient and dp-ucMGP will be measured.
Thank you for the comment. Tests for vitamin K2 are not easy or readily available. Therefore, we decided to use the dp-ucMGP as both a marker of our interest due to its effect on arterial calcification but also as a surrogate measure of the vitamin K2 levels. Our description of the biobank samples has already been changed to (p12): “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.”

2 In part 11.3 Multi-Slice Computed Tomography Scans, Scans are analysed by an experienced cardiologist. Maybe more than two experienced cardiologists or radiologists are better. But if the results are straight forward enough after being analyzed by the software, there is no need to add more researchers.

Thank you. Actually, this has been changed. Two experienced cardiologists have trained four experienced technicians to analyse the CT scans, both the non-contrast and contrast CT scans. The descriptions are continuously supervised and monitored. The text has now been corrected (page 12): "Scans are analysed by four trained and experienced technicians under continuous monitoring by two cardiologists."

Reviewer: 3
Dr. Daan Kremer, Universitair Medisch Centrum Groningen Comments to the Author:

In their manuscript, Hasific et al. Have provided a study protocol for a randomised controlled trial to study the effects of menaquinone-7 supplementation in patients with severe coronary artery calcifications. Overall, the planned study seems promising and could potentially provide valuable insights into the effects of vitamin K2 and D supplementation on CAC progression in a high-risk population.
However, I have several major and minor suggestions that may help to further improve the study.

Major points:
The vitamin D intervention comes out of the blue sky, without sufficient introduction or pathophysiological rationale. Vitamin D is also unmentioned in the title.
I would strongly advise the authors to better explain why this intervention is added on top of the vitamin K-supplementation. Also, I feel that it is necessary to explain how vitamin D/K may interact, whether they may work synergistically on regulating calcium metabolism, etc. Please discuss/review this in the introduction section (for some inspiration, see e.g. doi: 10.1155/2017/7454376).
Thank you very much for the suggestion. We have changed the title and added a brief introduction to the role of vitamin D and the synergistic effects in the introduction. Please also see our comments to reviewer 1.
One may also argue that this combined intervention may blur the effects of vitamin K, rendering it impossible to distinguish the potential beneficial effects of vitamin D vs. vitamin K supplementation. Please address this potential problem in interpretation, or explain how this may be solved in the planned analyses.
Thank you for your comments. We agree that it may not be possible to distinguish between the potential effects of vitamin K2 and vitamin D3. However, we do believe that the main effect is mediated by vitamin K. We have added the following to the Discussion section (page 11):
"…However, previous randomised trials on vitamin D supplementation alone have failed to show any effect on progression on coronary artery calcium (Manson E. Calcium/vitamin D supplementation and coronary artery calcification in the Women's Health Initiative. Menopause. 2010 Jul;17(4):683-91). In addition, the combination of vitamin D and vitamin K showed lower increase in carotid intima-media thickness compared to vitamin D alone (I Kurnatowska. Pol Arch Med Wewn. 2015;125(9):631-40). 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…"
It appears biologically plausible that vitamin K supplementation is only useful in patients with proven vitamin K insufficiency. This is consistent with studies e.g. in kidney transplant recipients, where there were beneficial effects on CAC in a population with proven vitamin K-insufficiency at baseline (doi: 10.1016/j.ajt.2022.12.015), but not in a population where vitamin K-insufficiency was not proven (doi: 10.1111/ajt.16566). This may have to do with the very major improvement in vitamin K-status in the first, but a much smaller improvement in the second population (see discussion section of doi: 10.1016/j.ajt.2022.12.015).
Thank you for the suggestions. Unfortunately, neither of the mentioned studies really found a significant effect on arterial calcification. In general, it has been suggested that there is a high prevalence of vitamin K2 deficiency and thereby high levels of inactive MGP in the Western population. In the AVADEC study, we did not include participants based on their vitamin K or D levels

and still found a significant increase in active MGP in the intervention group – irrespectively of baseline MGP. Additionally we found an effect of the intervention on CAC progression in patients with baseline CAC score > 400. It is possible that higher baseline CAC score and thereby higher progression rates make a potential effect of vitamin K supplementation and activation of MGP easier detectable. As AVADEC generated our hypothesis that vitamin K2 and D3 supplementation may be efficient in patients at high risk with CAC score > 400, we have decided to follow the same methods to test the hypothesis. We however do agree, that it is possible that individuals with vitamin K2 deficiency may have an even higher effect of supplementation, and therefore planned to stratify outcome based on baseline MGP (please see below).

The authors do consider dp-ucMGP in exploratory analyses, but I would suggest to put more emphasis on this outcome. Ideally, it may be added as an inclusion criterion, but I realize that this may be difficult for many reasons. However, I would suggest to at least add subgroup analyses based on dp-ucMGP (taking into account kidney function, see for example https://doi.org/10.3390/nu13093069 / https://doi.org/10.3390/nu14122440
In the AVADEC population we expectedly found a significant decrease in dp-ucMGP in the intervention group compared to the placebo group. Consequently, we have also added change in dp-ucMGP as an exploratory endpoint in this study. It is also added in a stratified analysis, where the primary outcome (CAC score) will be stratified for baseline dp-ucMGP < median and >= median (Table S1 in the Statistical Analysis Plan).
Also please mention the potential inclusion of vitamin K-sufficient subjects as a limitation of the current study, and elaborate on the potential implications of this limitation on the study results. In fact, the same holds true for the vitamin D-intervention – vitamin D-status is not considered at all in the study currently. I would advise the authors to elaborate on this.
Please see the answers above. Vitamin D status is considered in the sense that blood tests include tests for 25-OH vitamin D, and change in vitamin D is tested for the two groups (Table S3, Statistical Analysis Plan).
Minor points:
Title: from the title only it is unclear what effects the authors aim to study. Only the population is clear, but not the outcome. I understand that it may seem a bit redundant, but for searchability and clarity the authors may still consider to add that they aim to study the effects of MK-7 on coronary artery calcifications.
Also, for consistency, I would suggest to use the terminology ‘coronary artery calcifications’ consistently, rather than ‘coronary calcifications’ (only used in the title).
We would like to comply, and include outcome in the title, but it is not easy. We aim to decrease progression of coronary artery calcifications (OUTCOME) in patients with severe coronary artery calcifications (POPULATION), but we think it will be clumsy if we add both. Thus we deselected outcome and included population and intervention in the title. Additionally, the terminology of coronary artery calcifications are now used consistently throughout the manuscript. The new title is: “Effects of Vitamin K2 and D3 supplementation in Patients With Severe Coronary Artery Calcification: A study protocol for a randomised controlled trial.”

Intro: There is accumulating evidence for vitamin K supplementation in many different populations. Yet, the ‘knowledge gap’ is not identified very clearly by the authors. I think the manuscript may be improved by briefly summarizing what the current clinical evidence is for vitamin K (and vitamin D, If that is indeed part of the intervention) supplementation in different populations, and what the current study adds to the existing body of evidence. Notably, the added value of the study may also be emphasized in other parts of the manuscript (e.g. strengths and limitations section, discussion section).
Thank you very much for pointing this out. We have added some important points about previous randomized studies to the introduction, as well as the strengths of this study to the Discussion section.

Intro, p4, line 12-14: “men have higher CAC scores than women”, this is not always the case. I would suggest to change the wording.
We are sorry about the vague wording. We have changed the sentence: “CAC increases with age, and men tend to have higher CAC scores on average than women.2”

Intro: p4, line 14-20. Please guide the authors on the differences between vitamin K1 and K2. Do they bind to the same receptors/how do their effects compare?
Following a similar comment from reviewer 1, we have tried to briefly clarify why the difference between vitamin K1 and K2: “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”

Intro: p4, line 23: “the inhibiting process” > e.g. “the calcification-inhibiting properties”
This has now been explained by: “The inhibiting effect of the vitamin K-dependent proteins on calcification was originally showed by Luo et al. in 1997.9”

Intro p4 line 28 > remove ‘-‘ after coronary Throughout manuscript: some sentences are very long and contain many commas (e.g.: p4 line 11-13). For ease of reading, I would advise the authors to separate some overly long sentences.
We have tried to shorten the long sentences with inserted parts.

Methods, trial design: please mention whether this is a single-center or multi-center study.
It has now been added that this study is a multicenter trial: “The DANish COronary DEcalcification (DANCODE) trial is a multicenter, double-blinded, randomised, placebo-controlled study.”

The BMJ Open guidelines specifically mention that dates of (planned) study initiation etc. should be included in the protocol. Please add these dates for transparency.
The dates have been added: “The study will take place at three Danish hospitals (Odense University Hospital in Odense and in Svendborg and Vejle Hospital) from February 8th 2023 to March 2026.”

The trial registration number is now only in the abstract. Please add it to the methods.
The trial registration number has now been added to the Methods, section 3.1.

Overall, quite some minor grammatical errors (several plural vs. singular form mismatches between subject and verb, e.g. p10 “an independent DSMB are” and several other mismatches in intro/methods; feasibility paragraph: ‘in the Western Denmark’ > ‘in Western Denmark’; some double spaces; please carefully check the entire manuscript e.g. using the Microsoft Word grammar check.
We are very sorry about these mistakes. We have reread the manuscript and corrected errors.

Regarding the review checklist, I answered ‘No’ to several questions. Most have already been addressed in the review, but as an overview:
3. Is the study design appropriate to answer the research question? > Although the objective is clear, there is a mismatch between the objective and the methods. The rationale of adding vitamin D is not clearly explained. In fact, such a combined intervention may blur the actual vitamin K-effects.
Thank you. The rationale behind the combined therapy of vitamin K2 and vitamin D3 has now been explained in the introduction section.

8. Are the references up-to-date and appropriate? > I would suggest to add more clinical studies for the current body of evidence of vitamin K-supplementation in different populations. Please address the 'knowledge gap', and what the current study will add.
Thank you for the suggestion, as previously mentioned. This has also been addressed.

12. Are the study limitations discussed adequately? > I would suggest the authors to mention the previously mentioned limitations (vitamin D-effects, potential inclusion of vitamin D- and/or vitamin K-sufficient subjects who may have limited benefit of the interventions.
Thank you. We have added to limitations to section 9: "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. Moreover, the participants are not included based on their baseline vitamin levels resulting in a part of participants with normal 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 ischemic heart disease."

VERSION 2 – REVIEW

REVIEWER	Kaesler, Nadine Rheinisch-Westfälische Technische Hochschule Aachen University Hospital
REVIEW RETURNED	29-Jun-2023

GENERAL COMMENTS	The authors have addressed my questions. I also value the inclusion of the supplement contents and the comparison of the supplement and placebo. But I am surprised on 2 ingredients: 1) Calcium phosphate could negatively impact the outcome as it triggers cardiovascular calcifications 2) The EU has prohibited titanium dioxide as a food additive from January 2022 (EU 2022/63). I hereby want to encourage the authors to replace the chosen supplement in their future work.

REVIEWER	Bo, Pang Guanganmen Hospital Department of Acupuncture, Guang'anmen hospital
REVIEW RETURNED	03-Jul-2023

GENERAL COMMENTS	The author has made necessary amendments to the article according to the opinions of the reviewers. The content and format of the article have met the requirements of periodical publication. The design of the research method is rigorous and has strong enforceability. I personally look forward to seeing the publication of relevant data when the project is completed.

REVIEWER	Kremer, Daan Universitair Medisch Centrum Groningen, Internal Medicine, Division of Nephrology
REVIEW RETURNED	21-Jun-2023

GENERAL COMMENTS	Overall, the authors have satisfactorily implemented the suggestions provided by the reviewers. I wish them the best of luck with the conduction of this study, and I'm looking forward to the study results!

	(Minor remaining comment: I cannot see the 'Statistical Analysis Plan' in my reviewer center. As a result, I cannot judge whether my concerns regarding measurements of dp-ucMGP/vitamin D have been implemented. I assume that this is the case, and that it will be published alongside the article.)