Author’s response to reviews

Title: EFFECT OF VITAMIN D FORTIFIED FOODS ON BONE MARKERS AND MUSCLE STRENGTH IN WOMEN OF PAKISTANI AND DANISH ORIGIN LIVING IN DENMARK: A RANDOMISED CONTROLLED TRIAL

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Author’s response to reviews:

Reviewer reports:

Reviewer #1: The authors have done a nice job studying the effect of fortified foods on BTMs and muscle strength, though the article needs some corrections before it could be published:

- The introduction section is too long compared with other parts of the article.

Thank you. The introduction has now been shortened and is now more focused.

- As the article is based on a larger project (ODIN-FOOD), the reference to that project or its protocol is needed. The methods section is also not well explained. More details and reference to the published protocol (if available) would be beneficial.

We have added more information and reference to the larger project several places. No protocol on the ODIN-Food project is published.

- More information on the participants is needed for instance their age range, how long have they been living in Denmark.
Details on the baseline characteristics have been added to Table 1 (how many years lived in Denmark if not born in Denmark and age range in each study group).

- Is taking vitamin D supplement also an exclusion? If not how have the authors reduced its effect in their analysis

Yes, taking vitamin D supplement during the trial was an exclusion criteria as described in the methods (lines 93-96). Also travelling to countries below 47°N and several other criteria described in detail in a previous publication (Title: Vitamin D fortified foods improve wintertime vitamin D status in women of Danish and Pakistani origin living in Denmark: A randomized controlled trial).

- More information on the fortified foods and their consumption protocol is needed? Their vitamin D content, with which vitamin D were they fortified, how much were they used per day, how did the researchers made sure that they were actually used.

Thank you for this comment. We have added more information on the above points in the method section (see lines 98-110) and provided a reference to the previous paper published on the study. In this paper nutritional composition, vitamin D content, portion sizes and compliance has been described in the methods on fortification vehicles as well as in Table 1. The compliance was monitored by a self-reported questionnaire (user-friendly including pictures of every food item) (see also lines 127-128).

- One of the main concerns is the confounding factors, the authors should discuss it in the limitation and also explain how they have managed to deal with these factors.

Thank you for raising this valid point. We have been advised by a statistician and chosen not to discuss confounding factors specifically due to the nature of the design – A randomized placebo-controlled trial. Our argument is that this gives enough spread of any confounding between the study groups. The covariates added to the linear models in addition add to the avoidance of confounding.

- I also agree with the authors that 12 weeks has not been enough to have a significant change in the result

- On line 262, the authors discuss only the pakistani women. What about the Danish women.

Thank you for this observation. Low vitamin D intake and status is also a problem among Danish women, we have added information on the Danish population according to the most recent population study (lines 237-239).
- In the same paragraph, they mention that the idea was to study at risk women. I cant find this objective in the methods section where the authors describe the study.

Thank you for this comment. For clarity, we have now highlighted this. Risk groups are now mentioned in line 57-61 (aim and hypothesis) and line 71 (methods).

- I assume the references mentioned in this paragraph are all results from the same study. Maybe it should be highlighted. In the whole discussion section, its hard to figure out the phrases are based on the results of the same study or just a Danish study. These should be made clear.

We agree with this comment. We have now improved the paragraph by adding more information on the present paper and the previous paper published on the main outcomes of the presents study. Generally, the ODIN Food study was inspired by the design of a previous project (OPTIFORD). The OPTIFORD study (baseline and intervention) was published in 2008 is also inserted as a reference in this section.

- In the discussion section, the authors should provide more reasons explaining the reason behind the results they found specially the differences between the ethnicities.

We have removed the results on the ethnic difference in baseline characteristics as a result of the comments described below.

- In the limitation section, the authors correctly mention the shortcoming of BTMs due to preanalytical variability and lack of standardized assay but if they have used a single protocol for reducing the modifiable factors like taking blood on special hour in a fasting status and used a single kit to do the measurements then this would be actually their strength!

Thank you. This is a valid point as we made some efforts to overcome the preanalytical variability and we will look more upon our protocol on the BTM as a strength. The strength and limitations have been changed accordingly.

- In the same section, they also mention the discrepancy because of the subjects becoming familiar with the tests at the end of the study, I thougt they mentioned there was an introductory session for the participants.

Thank you for this comment. We have now clarified that despite a familiarization session of the instrument, we cannot rule out that there may be a further learning curve influencing the final measurement. We have changed the section to clarify this (lines 315-317).

- the conclusion section should be rewritten. The absence of change in knee extension after intervention is more important than the baseline difference between the women of the two ethnicities.
Thank you for this comment. The conclusion has been rewritten to make it clearer.

- On line 275, they discuss the vitamin D status of Pakistani women living in Denmark for 10 years, was this an inclusion criteria.

The sentence has been removed as a result of another comment.

- updating the references could be beneficial

The references have been updated with some new studies on the vitamin D status in the Danish population.

- Table 1- reporting BMI is more useful than weight alone

Thank you. BMI has been added to Table 1

- Table 1, it is also useful to have the p-values to decide on the significance of the differences both between ethnicities and the intervention groups

Thank you for this comment. Since the study is randomized, we chose, based on statistical advice, not to show P-values between the study groups as these are randomized.

- Table 2, what is the definition of self-rated health

Table 2 shows the self-reported answers to the health questions. The self-rated health is based on the question: “how do you perceive your own health status on a scale?” The answers were on a scale from 1-4 (1: very good-4: very bad). The questionnaires have been used in the previous intervention study by Andersen et al. 2008. No overall score for health status is reported, only the questions in Table 2.

- Table 2, the authors shouold divide the weight bearing and not weight bearing exercises and activities

We agree that it would be beneficial to have exercise separated into weight bearing and not. Unfortunately, the questionnaire that we chose to use did not provide us with this information.

- Table 2, I dont understand whats the difference between no job and leisure? as they are two different groups
The only reason for this division was the formulation of the questions to be aimed at those that had a job and also those that did not have a job. Overall, there were no difference in the questions except a small formulation change.

- Table 2, again the p-values could be helpful

Thank you for the comment. Since the study is randomized, it has been an active choice not to show P-values between the study groups as these are adequately randomized.

- Table 2- sun exposure is an important factor unless the authors claim that the radiation in Denmark in winter is no good for vitamin D production. In this case it should be clearly stated

Due to a high latitude of 56°N, the UV radiation in Denmark between October and April is too low for the production of vitamin D in the skin. See addition to the strength and limitations in lines 360-361.

- Table 3 and 4, p-value for the change is interesting?

We agree that the p-values in Tables 3 and 4 is less relevant since the adjusted comparison happens in Table 5.

- Table 5 and 6 are not well discussed in the text

In order to avoid too many double descriptions of the table information, we have chosen to only discuss the tables minimally in the text.

Reviewer #2: Comments to the Author:

The paper do not follow the author instructions for Nutrition research. Both the abstract and the paper are too long and the paper is not structured with numbered sections.

There are too many keywords and some of the keywords are not compatible with Index Medicus MeSHTerms. The abstract do not include the hypothesis for the study. The authors hypothesis for the research is not clear in the paper. The terminology used in the paper is not correct, measurement of 25(OH)D result in 25(OH)D concentrations, not levels. Vitamin D status can be deficient but it should not be described as "low".

Thank you for these comments. We have addressed them at our best knowledge, with use of the author guidelines provided by the editorial office. The abstract do not contain the hypothesis, but
the aim due to the word limit of 350 words. The hypothesis is written in the final lines of the introduction (lines 57-61).

Abstract

Line 3 Vitamin d status cannot be "low".

Thank you. We have changed the terminology to deficient and insufficient vitamin D status instead and both are defined at first use.

Line 7 Specify the placebo-controlled line, was it the same food but without vitamin D content?

Thank you for this comment, a description of the placebo foods has been added. The placebo foods were the same products produced without adding vitamin D3.

Line 9 Specify the method of vitamin D analyses.

The method (LC-MS/MS) have now been added.

Line 20 Study of ethnic differences in knee extension strength was not the aim of the study and do not need to be discussed in the abstract.

Thank you. This has now been removed.

Line 26-27 This can be removed.

Thank you. This has now been removed

Introduction

Line 32-35 References are studies of associations between vitamin D status and muscular symptoms, they are no proof of causality, the word consequences is not appropriate.

Thank you for this comment. We have revised the sentence to make it clearer.

Methods

Please add a detailed description of the recruitment of participants including a flow diagram. How many women of Danish and Pakistani origin ages 18-50 years live in the recruitment area?
How many were invited? What was the participation rate? How did you define "Pakistani women"? Were they born in Pakistan? This is of importance when analyzing the results. Add a flow diagram of the recruitment process and the study design.

Thank you for this comment. We have added a reference to the previously published paper on the study in which the flow diagram was shown.

Line 121 Add a subheading; Blood procurement procedures.

Thank you for suggesting this, the subheading has now been added.

Line 123-125 Add chosen measurement units.

Thank you. This has now been added.

Line 127 Specify how physical activity was measured and classified. Add a reference for the questions regarding general health.

The physical activity questions is adapted from a previous study (VitmaD), the categories consist of the mean of the self-rated responses to eight different questions on physical activity and sedentary time. The general health questions have been used in two previous studies (Optiford and VitmaD), references have been added for the two studies although these do not contain data from the questionnaires.

Line 172 Add a reference for the 30-second chair stand test.

Thank you. A reference for this test has now been added.

Line 194 Why did you not adjust for length in analyses of grip strength?

Thank you. We assume you refer to the length of the hand. We did not use different instrument settings based on hand length/hand size. We did not experience problems during the measurements that indicated large hand length differences in our population of adult women. Unfortunately, this means that we don’t have data on the hand length/size of the participants.

Results

The result section do not need to repeat all results shown in the tables.

Thank you. We have changed the text to make it shorter as well more focused.
Line 204 Add the flow diagram or remove this information.

The flow diagram has been published elsewhere, and we cannot publish the same again and thus we have referred to it instead.

Line 219-221 This should be moved to methods.

Thank you. This has now been moved to the methods.

Line 230-231, 233 This should be moved to methods.

Thank you. This has now been moved to the methods.

Line 238-240 This should be moved to methods.

Thank you. This has now been moved to the methods.

Line 246-247 This is the main result and should be in the beginning of the result section.

We agree on this point and have restructured the section accordingly.

Line 248-252 This can be removed. The participation rate is unknown, the study groups are very small and the aim of the study was not to detect ethnic differences in muscle strength.

We agree on your comment and have removed the mentioned section as well as restructured the results in general. Thank you for the suggestions, the section comes across much more clear now.

Discussion

This section is too long and needs to be reworked.

Focus on 1) The main result. 2) Strengths and limitations - Social selection bias? Low power.

The study was not designed for evaluation of the effect of vitamin D treatment on bone markers and muscle strength. There is a problem with low power and social selection bias and this is not sufficiently taken into account in the analyses, results and discussion. Mean 25(OH)D concentrations in the participants were high, just below the concentration recommended by the Institute of Medicine in the USA, which might have been crucial for the negative results.
Thank you for the suggested edits, we have taken them all into account when restructuring and shortening the discussion section. We highlight here, and in the text, that this manuscript is based on secondary outcomes.

Line 274-275 An alternative explanation might be that the high mean 25(OH)D concentrations in the Pakistani group was a result of social selection in recruitment of the Pakistani female participants.

The results of 25(OH)D concentrations in 72 Pakistani women selected from all Pakistani women living in Copenhagen and surrounding suburbs should be read with caution and is no proof of improvement of vitamin D status in the total group of Pakistani women in Denmark.

Thank you for this comment. We are aware that this is an issue for our study as well as every health/nutrition intervention study. We have addressed the issue in more detail in discussion and in the limitations. There are approx. 24000 Pakistani immigrants and descendants living in Denmark (2018) of which the vast majority lives in the Copenhagen area.

Conclusion

Conclude only that the results of the study were negative.

Thank you for the useful comments. The conclusion has now been changed to fit the new structure of the result section.

References

References are a bit old, need to be updated.

Thank you. We have updated the references.

Tables

Table 6 Add length as a variable in hand grip strength analyses.

As mentioned above, unfortunately we do not have data on the hand length.