Author’s response to reviews

Title: Effects of vitamin D supplementation on liver fibro-genic factors in non-alcoholic fatty liver patients with steatohepatitis: study protocol for a randomized clinical trial

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Author’s response to reviews:

Dear Dr. Hiemstra,

Thank you very much for your letter informing us of your decision regarding the manuscript entitled “Effects of vitamin D supplementation on liver fibrogenic factors in non-alcoholic fatty liver patients: study protocol for a randomized clinical trial”. The manuscript has been revised according to the editor’s comments. Responses to the reviewer’s comments have been provided below. Revised texts have been provided in red font. Thank you so much in advance.

Yours Sincerely,

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Response to Reviewer 1 #
Comments to the Author

The authors present the design of a randomised controlled proof of concept trial. In principle the submission is acceptable for publication and the study is of interest. However, a number of factors deserve attention before it can be recommended for publication:

1) There are multiple linguistic and grammatical errors which need to be addressed. The manuscript would benefit from review by a native English speaker.

Authors: Thank you. We tried our best to revise the linguistic and grammatical errors.

2) The protocol proposes to assess safety by measuring PTH. A change in PTH in either direction will not inform safety; for this, the authors would need to monitor calcium concentrations in blood.

Authors: Thank you. Calcium concentrations in blood are regulated by hormones and the body maintains its levels in normal ranges by several compensatory mechanisms. It has been shown that blood calcium levels just can change under pathologic condition and its levels rarely change during both mild hypo- and hyper-vitaminosis D (Feher, J. Calcium and Phosphorus Homeostasis I. Quantitative Human Physiology. 2017; 924–932.). Most studies have suggested monitoring the PTH levels as a good measure of safety in studies administering vitamin D supplements (Mithal A, Wahl DA, Bonjour JP & et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009; 20(11):1807-20). This is particularly important in our study population who suffer from hypovitaminosis D, in which increased levels of PTH is evident (Mithal A, Wahl DA, Bonjour JP & et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009; 20(11):1807-20).

3) Sample size calculations are alluded to based on laminin as a candidate outcome measure. However, the data from which these sample size calculations have been derived are not provided, making it impossible to reproduce the authors' sample size estimates.

Authors: Agreed. Added (Page 5). We calculated required sample size based on data from a previous study by considering serum laminin as a key dependent variable, type I error of 0.05, and study power of 90%. Considering the serum laminin mean of 136.7 ng/mL, based on the suggested formula for parallel clinical trials, we reached the sample size of 18 patients in each group. Taking into account a possible drop out of 30%, 23 patients will be enrolled in each group.

4) The statistical analysis plan requires more detail. There is a description of comparison of baseline variables by t-test or chi-square. First, given the randomised design, baseline variables should not be compared in this manner and p-values should not be reported.

Thank you. This part was omitted.
Second, although the authors describe the use of a test for normality they proceed to describe only a parametric test. Some variables are already known to be non-parametrically distributed, for example PTH. How will these be compared?

Authors: Thank you. This part was expanded to cover the point you mentioned. (Page 10).

5) Greater clarity is required regarding the randomisation procedure. It is not clear to this reviewer whether the authors are proposing stratified randomisation, minimisation or block randomisation. Furthermore the authors state that randomisation will be 'carried out by a blinded researcher'. Is this randomisation or allocation? If the former, how will randomisation be carried out?


Response to Reviewer 2 #

Comments to the Author

The research protocol under review, is for double blind RCT to examine the effects of vitamin D supplementation on liver fibrogenic factors in non-alcoholic fatty liver patients. I have following comments:

1) Patient population: The study is planned to include all NAFLD patients aged 20-60 year, who has been diagnosed with NAFLD for at least six months prior to enrolment in the study; diagnosis of fatty liver will be done based on findings from ultrasound reports. Inflammation and fibrosis are not of much relevant in those with bland NAFLD, usually detected on USG; inflammation and fibrosis are of use in subset of NAFLD patients, known as NAFLD with steatohepatitis (NASH). Hence to make the results more relevant, I will suggest including only those NAFLD who have NASH.

Authors: Thank you. We replaced NAFLD with NAFLD with NASH throughout the manuscript.

2) Sample size: SS calculation is done on the basis of meta-analysis data on serum laminin; the objective of the study is not related to laminin hence it is not appropriate such sample
size calculation; further the term laminin is not used anywhere in the manuscript which is referred for sample size calculation (J Gastroenterol Hepatol. 2016; 31(4):848-55). Hence SS need clarification

Authors: Thank you. Actually, we calculated required sample size based on an original study (Santos VN & et al. Serum laminin, type IV collagen and hyaluronan as fibrosis markers in non-alcoholic fatty liver disease. Braz J Med Biol Res. 2005, 38(5):747-53.) in which concentrations of laminin were provided. There was a mistake in the order of references in the initial version of the manuscript, which was corrected in the revised file (Page 5).

3) Randomization (i) as the authors have planned for stratified randomization based on sex and BMI, they will need calculated sample size for each subgroup of gender and BMI categories (ii) block randomization of two is inappropriate specially if you have more than two BMI subcategories; if block of two, after assigning treatment to the first person, assigned group for the second person is obvious, hence the block size shall be at least 4 or 6.

Authors: Thank you for your valuable comments. We clarified all these points in the revised manuscript (page 6-7).

4) Long sentences which be shortened and several sentences could be joined into one to reduce the manuscript word count.

Authors: Thank you. We tried our best to shorten the manuscript.

5) Frequent spelling mistakes

Authors: Agreed. Done.