Author’s response to reviews

Title: Low Vitamin D Level Was Associated With Metabolic Syndrome And High Leptin Level In Subjects With Nonalcoholic Fatty Liver Disease: A Community-based Study

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Author’s response to reviews:

Dear Professor Renée Blaauw, editor, BMC Gastroenterology

Thank you very much for your kind letter of 3rd August, 2018 and for the constructive comments on our manuscript. My colleagues and I greatly appreciate the suggestions offered by you and the reviewers as well as the opportunity to improve our manuscript.

We have made the following changes according to the suggestions made by you and the reviewers:
Reviewer 1 provided 28 comments:

Congratulations on a comprehensive study, for which I would recommend a change in focus (according to the aim of the study). Language editing is highly recommended, although a certificate was provided.

Q1. The title: 'Low Vitamin D Level Increases the Risk of Metabolic Syndrome in Subjects with Nonalcoholic Fatty Liver Disease: A Community-based Study’ does not align with the aim of the study "to evaluate the association between serum vitamin D levels and nonalcoholic fatty liver disease (NAFLD) parameters, including metabolic syndrome risk and hepatic steatosis”. The classic question of which came first - the chicken or egg needs to be answered to prevent confusion between cause and symptom when not providing sufficient scientific evidence. A more 'conservative' title could be considered based on the findings of this study.

Reply: Thank you for your valuable comments. We agree that the study aimed to evaluate the association between serum vitamin D levels and NAFLD parameters, including metabolic syndrome risk and hepatic steatosis. We found that low serum vitamin D was associated with metabolic syndrome in all subjects, including the NAFLD group. We further compared the mean levels of vitamin D between the NAFLD group and normal group (Page 4, lines 69-71). Subjects in the NAFLD group presented a lower mean serum vitamin D value than did those in the control group. Hence, we changed the title to “Low Vitamin D Levels are Associated with Metabolic Syndrome but not with Hepatic Steatosis Severity in Subjects with Nonalcoholic Fatty Liver Disease: A Community-based Study” (Page 1, lines 1-3)

Q2. Revisit second sentence of methods in abstract - "All subjects participated in a demographic survey to exclude alcohol abuse, blood testing and abdominal ultrasonography (US)" - does not make sense without necessary verbs added.

Reply: We revised this sentence as follows: “All subjects were administered a demographic survey, blood testing and abdominal ultrasonography (US)” (Page 4, lines 62-63).
Q3. The results: "Subjects with serum vitamin D deficiency or insufficiency had a higher risk for metabolic syndrome than those with sufficient vitamin D levels [deficiency vs. sufficiency, adjusted odds ratio (OR) =1.860 (95% CI=1.234-2.804), P=0.003; insufficiency vs. sufficiency, adjusted OR=1.669 (95% CI=1.237-2.251), P=0.001]. Similarly, subjects in the lowest quartile of vitamin D had a higher risk for metabolic syndrome than those in the highest quartile of vitamin D (OR= 2.792, 95% CI=1.719-4.538, P<0.001)" would make more sense if reported objectively as the difference in Vitamin D levels between the two groups. Cause is implicated, which is not explicitly indicated and explained as the case.

Only the last sentence of the results is linked to the aim - consider language editing. I observed that a language certificate was issued, but the manuscript is still difficult to read because of language errors and sentence construction.

Reply: Thank you for the constructive comments. This section described the odds ratio of metabolic syndrome between low and adequate vitamin D levels according to logistical regression analysis. We compared the mean levels of vitamin D between the NAFLD group and normal group (Page 4, lines 69-71). Subjects in the NAFLD group had a lower mean serum vitamin D levels than those in the control group. We changed the world “risk” to “odds” to match the statement from the regression analysis (Page 4, line 72; Page 5, 76). We have sent this manuscript for English editing again.

Q4. The conclusion cannot be made from the results (first part of the conclusion) - can be seen as the other way around if biochemical background is not provided.

Reply: Thank you for your valuable comments. We revised the conclusion as follows “Subjects with low vitamin D levels presented higher odds of metabolic syndrome than did those with high vitamin D levels. However, the distribution of fatty liver severity according to abdominal US was similar among the subjects with NAFLD who exhibited different vitamin D levels” (Page 5, line 83-85).
Q5. line 88-110: The explanation of a causal relationship between vitamin D and MS is not provided to support the motivation for the study.

Reply: Previous studies have shown that vitamin D levels are inversely related to fasting glucose concentrations (Ortlepp JR, 2003; Scragg R, 2004), adiposity (Buffington C, 1993; Parikh SJ, 2004), lipids (Challoumas D, 2014), and blood pressure (Lind L, 1989; Pfeifer M, 2001). Fasting blood sugar, adiposity (central obesity), lipid levels and blood pressure are the criteria of metabolic syndrome. Several studies have further confirmed that vitamin D deficiency can significantly increase the risk of metabolic syndrome [Gagnon C, 2012; Ju SY, 2014; Chen LW, 2016]. In patients with NAFLD, vitamin D influences hepatocytes and nonparenchymal hepatic cells (hepatic stellate cells, Kupffer cells) via metabolic, anti-inflammatory and anti-fibrotic effects [Tilg H, 2010; Eliades M, 2015]. Vitamin D deficiency and NAFLD have been shown to engender similar risks for cardiovascular disease, insulin resistance and MS in epidemiologic studies [Kwok RM, 2010; Sakkby T, 2014; Eliades M, 2015]. These findings were the motivation for the current study, which aimed to evaluate the association between serum vitamin D levels and nonalcoholic fatty liver disease (NAFLD) parameters, including metabolic syndrome risk and hepatic steatosis. We added all the relevant data in the Introduction section (Page 6, lines 98-108; Page 7, lines 109-116).

Q6. Line 114: add reference, method or accept normal aging?

Reply: The reason for including subjects of more than 30 years old in the present study was based on a previous study (JAMA 2002). In Ford’s study, the prevalence of metabolic syndrome in subjects aged 20 to 29 years old was only 6.7 percent (see figure below). Because the majority of metabolic syndrome develops in people in middle age, the current study included subjects aged more than 30 years. We added the Ford’s study to the references (reference 24).

Q7. line 116: serum study - please indicate for what?

Reply: Serum analysis indicated completion of blood testing. Blood tests included complete blood cell counts, liver and renal biochemistry tests and determination of fasting sugar and insulin, total vitamin D, calcium, intact-parathyroid hormone (i-PTH), adiponectin, leptin, TNF-α and HS-CRP levels and lipid profiles. We revised “serum study” as “and completion of blood testing” (line 116). We added pregnancy to the exclusion criteria.

Q8. line 116-125: - consider to rather list

Reply: We revised the exclusion criteria concisely (lines 116-123?).
Q9. Please check language (single, plural, addition of words, etc), line 133: glucose? Total Vitamin D?

Reply: We corrected “fasting sugar” to fasting “glucose” and changed “total vitamin D” to “vitamin D” (Page 7, line 127). The revised manuscript has been corrected for English.

Q10. line 137: Consider to move to after the sentence starting in 138.

Reply: We moved the sentence in line 137 to after the sentence in line 138, as per your suggestion (Page 9, lines 150-151).

Q11. line 140-142 - first part of the study methods.

Reply: We moved the statement about Institutional Review Board approval to the first part of the Materials and Methods section (Page 7, lines 119-123).

Q12. line 169: Were DM included/ excluded in study - indicate earlier in methods

Reply: DM was included in this study in the metabolic syndrome survey. However, DM was excluded when insulin resistance was evaluated by HOMA-IR according to the original definition (Matthews DR, 1985). Subjects who had DM or were under oral hypoglycemic agent (OHA) control were excluded when using HOMA-IR as a tool for insulin resistance evaluation (Page 10, lines 179-184; page 11, line 185).

Q13. line 172 - how was this applied in the study - quartiles or specific values used?

Reply: The cut-off point of HOMA-IR for insulin resistance was initially set as 2.5. Because there was no fixed value of HOMA-IR for insulin resistance analysis, we also applied the values of HOMA-IR 2 and 3 for further analysis. The use of 2, 2.5 or 3 as the cut-off point of HOMA-IR for insulin resistance analysis produced similar results. The results revealed that the percentage of insulin resistance in the NAFLD group was greater than the percentage in the control group. We revised the section on HOMA-IR and the results (Page 10, line 184; Page 11, line 185; Page 14, lines 243-245).
Q14. line 176 - just recheck recommendations - 88/89 cm / 35 in ?, line 191 - did the study include African American participants? - if the case, waist circumference reference value also needs to adapt.

Reply: We rechecked the NCEP ATPIII criteria for metabolic syndrome, in which the waist size criteria for Asian people are 90 cm (35.5 inches) for men and 80 cm (31.5 inches) for women (Page 11, line 189). The waist size for men was corrected to 35.5 inches for Asian people. This study was performed in the northeastern part of Taiwan, where no African American participants were included.

Q15. line 210 - motivate and explain why adjustments were made for the specific confounders

Reply: The hypothesis addressed in this study is that vitamin D influences metabolic syndrome and NAFLD via inflammatory cytokines [such as TNF-α and highly sensitive C reactive protein (HS-CRP)] and adipokines (such as adiponectin and leptin). These cytokines may participate in the reactions of liver inflammation and fibrosis. We performed bivariate correlation between these cytokines and age, sex, body mass index and vitamin D levels. Age and male sex were positively correlated with vitamin D levels. However, leptin was negatively correlated with vitamin D levels (Page 14, lines 248-251).

In the multivariate logistical regression analysis, the factors age, sex, BMI and NAFLD status were adjusted for the metabolic syndrome odds ratio survey. Age and sex were adjusted in this study because age and male sex were positively correlated with vitamin D levels. Vitamin D levels were correlated with adiposity in previous studies (Snijder MB 2005; Freedman BI 2010), and the factor BMI was entered as a common adjusted factor. In the analysis of the whole group of participants (NAFLD and control group), we added the factor NAFLD as an adjusted factor. The result of the analysis revealed that low vitamin D was associated with metabolic syndrome in both all participants and the NAFLD group. However, low vitamin D was not associated with the status of NAFLD in this study (Page 14, lines 252-260; page 15, lines 261-269).

Q16. line 211 - Sure about SPSS version - it is quite old?

Reply: We used the old version SPSS 16 because we have not bought the new version of SPSS.

Q17. line 214-218 - relate to information in inclusion/ exclusion criteria, please correct language.

Reply: We have sent the manuscript for English-language correction again.
Q18. line 224 - control or non-NAFLD group?

Reply: In this study, the control group was equal to the non-NAFLD group.

Q19. line 225-233 - not expected? Participants grouped according to symptoms will look different if looking at a syndrome with interrelated symptoms.

Reply: We wish to indicate that the prevalence of metabolic syndrome in the NAFLD group is higher than the prevalence in the control group. We agree that this finding is expected and is not a novel finding. However, this finding could help to demonstrate the accuracy of our data.

Q20. line 240-245 - the study corrected for factors included in the classification of MS - important to explain the interaction and role. Important to explain the production of vitamin D in the body with relation to adiposity to clarify results.

Reply: It is important to explain the production of vitamin D in the body in relation to adiposity. Reports from Rosenstreich SJ and Mawer EB in the 1970s suggested that the association between serum vitamin D and obesity could be explained by increased storage of vitamin D in adipose tissue in obese subjects. Decreased release of stored vitamin D in the circulation induces lower serum vitamin D levels in obese subjects (Snijder MB, 2005). We added a sentence to explain why serum vitamin D levels were lower in obese people (Page 17, lines 316-317; Page 18, lines 318-320).

Q21. line 289 - Important to discuss the link between leptin, body fat and vitamin D to prevent confusion if the study focus on this association.

Reply: In the current study, vitamin D levels were negatively correlated with leptin in a bivariate correlation analysis. Previous studies have revealed vitamin D-mediated inhibition of leptin (Hajimohammadi M, 2017). Another study showed that an increased leptin level was linked to decreased HDL-C levels (Rainwater DL, 1997). In the current study, subjects with vitamin D deficiency and NAFLD exhibited increased leptin levels but lower HDL-C levels, which could partially explain the association between low vitamin D levels and MS in subjects with NAFLD (Page 17, lines 300-306).
Q22. line 300-301 - Please consider the link between adiposity and dermal Vitamin D production - in other words result vs risk?

Reply: According to Wortsman J et al., obesity-associated vitamin D insufficiency is likely due to decreased bioavailability of vitamin D3 from cutaneous and dietary sources. Because most vitamin D is produced by sunlight exposure in humans, the decreased bioavailability of cutaneously synthesized vitamin D3 in obese subjects may have altered the release of vitamin D3 from the skin to the circulation. Thus, obese patients exhibit lower serum vitamin D levels (Wortsman J, 2000). One meta-analysis study associated vitamin D deficiency with obesity, irrespective of age, latitude and cut-offs for defining vitamin D deficiency (M. Pereira-Santos, 2015). The other possible explanation for the lower serum vitamin D levels observed in obese patients is that obese patients may take part in fewer outdoor activities or exercises. Dermal vitamin D production depends on UV light exposure. Thus, inadequate sun light exposure might be responsible for lower serum vitamin D levels in obese people than in nonobese people. Hence, adiposity (obese) may be a risk factor for low vitamin D. We added a description about decreasing dermal synthetic vitamin D in the circulation (Page 18, lines 318-319).

Q23. line 282-285 - This should be explained in the introduction to set the reason for the study.

Reply: The hypothesis of this study has been presented in the Introduction section (Page 7, lines 114-116).

Q24. line 329-330 - Should mention in methods section.

Reply: We added a statement indicating that the serum vitamin D test was performed throughout the year, without avoiding the winter time. Additionally, we explained that sunshine is adequate in our study area (Page 9, lines 158-159). We also explained why both traditional and quartile classification of vitamin D are applied in this study (Page 17; lines 307-315).

Q25. line 334-337 - Consider again the conclusion based on risk or result.

Reply: We revised the Conclusion based on the findings and results of this study. Low vitamin D was associated with the status of metabolic syndrome and high leptin levels. The vitamin D level was not associated with fatty liver severity by abdominal US. (Page 18, lines 348-351)
Q26. References - 2010 Institutes of Medicine recommendations for Vitamin D can be included.

Reply: We included this reference (reference 41).


Q27. Tabel 1 - careful for selecting two groups based on differences and then indicate p-values.

Reply: We revised Table 1 as per your suggestion. We omitted some items, such as SBP, DBP, i-PTH, and calcium, because these factors may be less important in the pathogenesis of NAFLD.

Q28. A different focus of this study would be of more value - reporting on the original aim of the study. The introduction should include much more background (biochemical) if the current focus of the publication is followed.

Reply: The original hypothesis was that vitamin D levels may be associated with inflammatory factors (CRP, TNF-α) and adipokines (adiponectin, leptin), which have been reported to be involved in the pathogenesis of metabolic syndrome or hepatic steatosis. Bivariate correlation revealed a negative correlation between vitamin D levels and leptin levels. Multivariate analysis revealed that low vitamin D was associated with metabolic syndrome after adjusting for the confounding factors of age, sex and BMI. We revised the Background and Discussion sections. We added a discussion about vitamin D, adiposity, leptin and metabolic syndrome. Some references were added based on the hypothesis and results.
Reviewer 2 has one comment:

Please overwrite this text when adding your comments to the authors.

I read the article carefully. The article was very interesting. A lot of work was done. Although it has already worked, I accepted.

Enclosed please find our revised manuscript. We hope that it is now acceptable for publication in BMC Gastroenterology.

Thank you very much for your generous attention, patience and help.

We look forward to hearing from you at your earliest convenience.

Best Regards,

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