Author’s response to reviews

Title: Clinical and metabolic response to vitamin D plus probiotic in schizophrenia patients

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Author’s response to reviews:

Dear Dr Mantere,

Thank you very much for your letter informing us of your decision regarding the manuscript no. BMC Psychiatry - BPSY-D-18-00828 entitled "Clinical and metabolic response to vitamin D plus probiotic in schizophrenia patients". The manuscript has been revised according to the referees' comments. Responses to the reviewers' comments have been provided below.

Yours Sincerely,

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First of all, we would like to thank the reviewers for their great comments to improve the quality of the manuscript. We tried our best to revise the manuscript according to the reviewers' comments.
Reviewer reports:

Darryl Eyles (Reviewer 2): I gather this paper was previously reviewed and I have been drafted as an additional external reviewer? This is a small randomised placebo controlled trial to see if a supplement could affect well described metabolic syndrome in schiz patients. Met syndrome is a major problem in both the onset and treatment of schiz particularly affected by the use of anabolising 2nd gen antipsychotics. The primary data that these supplements attenuated some of the metabolic syndrome in these patients is not that surprising. Indeed the rising evidence that Vit D modulates gut biome and therefore probably patient immune responsivity may represent an interesting therapeutic potential.

Q1 However the reduction (although quite small) in PANSS is definitely a new outcome. Could the authors explain why the BPRS in that case remained unaffected? Also I (and the authors agree) note that BPRS scores and PANSS were substantially lower in the active arm at baseline in table 3? Surely this totally biases study outcome? I am not sure "adjustment for baseline" is what is required rather a better randomisation” I think this is THE MAJOR ISSUE in this whole manuscript. Could the authors comment?

Authors: Thank you for this important comment. This point has been addressed in the revised version:

“Lack of significant effect of vitamin D and probiotic co-supplementation on BPRS and PANSS Positive subscales may be due to baseline values of measured PANSS subscales and BPRS, baseline levels of 25-OH-vitamin D, different dosages and type of vitamin D and probiotic used. In order to improve BPRS and PANSS Positive subscales, individuals might need higher doses of vitamin D plus probiotic supplementation for a longer period of time to provide appropriate circulating levels for improving BPRS. Therefore, further studies are required to confirm the validity of our findings” (Lines 287-93).

“It should be taken into account that there was a significant difference in positive PANSS score and BPRS between vitamin D plus probiotic and placebo groups at study baseline. There are several reasons to explain this difference. In the current study, we did not randomize participants based on their PANSS score and BPRS due to all participants being diagnosed with chronic schizophrenia. Random allocation into two groups was done after stratification for age (<65 vs. ≥65 y), gender (male vs. female) and pre-intervention BMI (<25 vs. ≥25 kg/m²) by using computer-generated random numbers. Therefore, the difference in positive PANSS score and BPRS existing between two groups occurred by chance. In addition, when we adjusted the analyses for baseline values, age and baseline BMI, changes difference in negative PANSS score and BPRS became statistically significant” (Lines 267-76).

“In addition, when we adjusted the analyses for baseline values, age and baseline BMI, the difference in changes of negative PANSS score and BPRS became statistically significant” (Lines 405-7).
Q2 So which one was it? The probiotic or the D that affected PANSS so positively? This should be discussed in the light of the work cited from Jones et al.

Authors: This point clarified in the revised version:

“There is growing evidence suggesting synergistic impact of combined vitamin D and probiotic administration on clinical symptoms and metabolic disorders of patients diagnosed with schizophrenia, especially those with vitamin D deficiency. This combination might alleviate mental health parameters, and biomarkers of inflammation and oxidative stress in patients with chronic schizophrenia. The basis of this approach relies on probiotic's effect on increasing serum vitamin D levels [46]. In addition, probiotics might have synergistic effects with vitamin D, through improving the expression of vitamin D receptors [47]. Therefore, modulating microbiota-gut-brain axis plus improving vitamin D concentrations by probiotics might provide a novel approach to treat mental and metabolic disorders” (Lines 328-37).

Q3 Body weight did not vary over the 12 weeks? Was there an expectation it would give the other metabolic outcomes? Would this take longer?

Authors: This point clarified:

“In the current study, there was no significant difference between two intervention groups in terms of weight and BMI after 12-week intervention. Further studies are required to examine the effects of vitamin D and probiotic co-supplementation on weight, BMI and metabolic outcomes in subjects with schizophrenia, considering longer duration, which may affect clinical symptoms and metabolic profiles” (Lines 276-81).

Q4 Ref 19 Jones et al used L. reuteri NCIMB 30242 to increase 25OHD. Is that anything like the probiotic use here?

Authors: Yes. This point discussed in Discussion section. Please, see our answer in comment 2.

Statement: - One real opportunity missed here is an analysis of 16s RNA of fecal matter pre and post intervention.

It would be fascinating to know what microflora change had occurred in the probiotic group and correlate this with individual clinical outcome. Hopefully this will be done in future studies.

Authors: Thank you for your positive feedback. This point clarified:

“This study had few limitations. In the present study, due to funding limitations, we did not characterize the microbiota and thus cannot establish whether probiotic administration over 12 weeks changed microbiota composition. In addition, we could not evaluate an analysis of 16s
RNA of fecal matter. Therefore, analysis of 16s RNA of fecal matter is suggested in future studies” (Lines 392-6).

General comments

Although the paper is understandable there are still occasional English expression errors even in the abstract. The discussion in particular would benefit from another English speaker reviewing text.

Authors: Paper edited by native speaker.

Please remove naive statements like this in the intro "Oxidative stress and increased inflammatory cytokines play a critical role in the inflammatory processes leading to the development of schizophrenia" We have NO idea what causes schizophrenia despite many of us spending our lives working on the biology of this condition.

Authors: You are right. This section removed.

Please also drop the Patrick/Ames references to 5-HT. The comments about Vit D and 5-HT are I'm afraid also naïve. Tryptophan hydroxylase is not special in response to VDREs. Virtually every gene has VDRE sites of D activation (the authors themselves say there are 900). If you want to pick a neurotransmitter try Dopamine for instance. The enzyme for its production Tyrosine hydroxylase which is both more relevant to schiz and also has D responsive elements. Patrick and Ames have also NOT shown any brain related function in their FASEB review that is "improved". Please removes or delete this part of the discussion it detracts.

Authors: This point was done.

"One participant in the supplemented group and 4 in the placebo group dropped out for personal reasons" This is NOT what Fig 1 shows?

Authors: I apologize for this mistake. This point corrected:

“Four participants in the supplemented and placebo groups dropped out for personal reasons (Fig.1)” (Lines 214-5).

Lotje de Witte (Reviewer 3): In the study "Clinical and metabolic response to vitamin D plus probiotic in schizophrenia patients" by Ghaderi et al. the authors describe a double-blind placebo-controlled clinical trial in which they tested the effect of vitamin D in combination with probiotic supplementation on schizophrenia. They find a beneficial effect of supplementation on
general and total PANSS scores. Although the sample size is small, in light of the higher prevalence of vitamin D deficiency among patients with schizophrenia, the study is certainly of interest and the results are encouraging for follow up studies. The RCT is generally well designed and described. The manuscript could be improved by:

1. A clear description of previous clinical studies that test the effect of vitamin D supplementation in schizophrenia in the introduction. Also include "Vitamin D Supplementation in Chronic Schizophrenia Patients Treated with Clozapine: A Randomized, Double-Blind, Placebo-controlled Clinical Trial, by Krivoy et al. Describe the results of these previous studies and how their approach is different from your approach.

Authors: This point was done. Reference of Krivoy et al added to the revised version:

“In another study, vitamin D supplementation to chronic schizophrenic patients, at a dosage of 14,000 IU biweekly, for 18 weeks was associated with a trend towards improved cognition, but did not influence psychosis, mood or metabolic status [37]” (Lines 308-11).

Reference


“Current controversial evidence might be explained by different study designs, different dosages of probiotics and vitamin D used as well as participants’ characteristics of the study” (Lines 320-2).

2. A description of the distribution of 25-OH-vitamin D in both groups? Did you see a correlation between any of the PANSS score and vitamin D levels at baseline? Did patients with low vitamin D respond better to treatment then non-deficient patients?

Authors: Thank you. As all our patients had vitamin D deficiency at study baseline, we did not conduct this comparison. Meanwhile, results of linear regression analysis about association between serum 25-OH-vitamin D and the PANSS score at study baseline added to the revised version:

“Linear regression analysis revealed no association between serum 25-OH-vitamin D levels and PANSS score at study baseline (β=0.05, P=0.16)” (Lines 243-5).

3. The discussion is now more a review-like description of how vitamin D may be involved in schizophrenia pathogenesis. A better discussion of the current study and results is more relevant here and it would be interesting to read more about the next future steps that should be taken with these findings (replication of results in larger cohort, t-test effect of vitamin D and probiotic supplementation separately, etc.).
"Numerous studies have reported that vitamin D deficiency remains a widespread problem in chronic schizophrenic patients [28-31]. Several environmental risk factors for schizophrenia, including latitude, migration and season of birth, have been associated with vitamin D deficiency [28]. Furthermore, metabolic disorders such as obesity, insulin resistance, diabetes, hyperlipidaemia and cardiovascular disease, which are commonly seen in chronic schizophrenic patients, might be related to vitamin D deficiency. These findings show that patients with schizophrenia or other psychotic disorders could potentially benefit from vitamin D supplementation” (Lines 293-301).

References


“In addition, one cannot conclude if the treatment effects observed in the current study was due to the effect of which component of the combined supplementation. Therefore, further studies are required with single supplement in the current study in order to evaluate the beneficial effects of each supplement on clinical symptoms and metabolic profiles in chronic schizophrenic patients with longer duration of supplementation” (Lines 396-400).