Author's response to reviews

Title: The effect of homocysteine-lowering with B-vitamins on osteoporotic fractures in patients with cerebrovascular disease: substudy of VITATOPS, a randomised placebo-controlled trial

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The effect of homocysteine-lowering with B-vitamins on osteoporotic fractures in patients with cerebrovascular disease: substudy of VITATOPS, a randomised placebo-controlled trial.

John Gommans, Qilong Yi, John W Eikelboom, Graeme J Hankey, Christopher Chen and Helen Rodgers

As corresponding author I thank the editors and reviewers for this opportunity to submit this revised report on behalf of my co-authors. Our point by point response to the reviewer’s comments follows below.

Reviewer 1: Gaurav Garg

In this manuscript by Gommans et al, authors have evaluated homocysteine-lowering therapy with B-vitamin supplements to reduce the incidence of osteoporotic fracture in 8,164 symptomatic cerebrovascular disease patients. As per my understanding, all patients were randomly allocated to double blind treatment in two groups. One tablet daily either placebo or B-vitamins (4075 and 4089 patients respectively). Authors have analysed difference in the incidence of any osteoporotic fracture outcome between participants assigned in Placebo and B-vitamins groups after 2.8 years (median) therapy and 3.4 years follow–up. The authors have measured homocysteine levels of only 1205 participants during baseline visit and 1164 participants from the final follow-up visit. They have not found any significant impact of B-vitamin therapy on time to first fracture and baseline homocysteine levels were not found as a predictor of any osteoporotic fracture (p= 0.43).

The study is well designed with a good sample size.

Response: Thank you for the positive feedback

Minor Essential Revisions

Comment 1: Why have the authors not mentioned the protocol of sample collection, isolation of plasma/Serum and total homocysteine measurement in methodology section?

Response: We have updated the relevant section in methods – which now reads:

"Investigators were encouraged but not obligated to take blood samples from study participants who consented to these optional tests, to measure blood concentrations of tHcy, red cell folate and vitamin B12 at study entry and/or at
the time of their final follow-up visit. Fasting plasma tHcy was measured by high-performance liquid chromatography, on venous blood samples collected after an overnight fast.”

What kind of sample they have used for homocysteine measurement (plasma or serum) please clarify? The authors have given two references (22 and 23) of earlier studies in the methods section but in those publications plasma was used to measure fasting homocysteine but in this manuscript they have used serum.

Response: All samples were plasma – all previous references to serum have been corrected to read “plasma” – see above response also.

Comment 2: The authors have not compared Vitamin B-complex levels (i.e Vitamin B12, folic acid, Vitamin B6) with homocysteine levels between baseline and follow-up visit. These micronutrients are well established cofactors in methionine cycle, which have high potential to modulate homocysteine levels. Many other factors, including genetic factors, might be causing problems in absorption of vitamins, leading to vitamin deficiency constantly even after B-vitamin treatment which might be unable to modulate the level of homocysteine.

Response: The numbers of participants who volunteered to provide the optional samples for both baseline and follow up levels of folate and B12 were very small and we did not collect B6 data. The available information has been provided via a statement in results (as below) and a new Table (5).

“Table 5 compares folate and vitamin B12 levels between baseline and final follow up visits in the small subset that volunteered to provide these.”

Comment 3: The authors have not shown the significance level of total homocysteine between treated group and placebo during baseline and final follow-up visit.

Response: This has been added to paper as p <0.001

My suggestion is the authors should check difference of homocysteine levels between osteoporotic fracture outcome and without fracture in both groups (B-Vitamins and Placebo) and the result must be shown in a different table.

Response: The following sentence has been added to results section and as suggested, a new Table (4) is provided.

“Homocysteine levels at follow up were not associated with fracture outcomes in either treatment group (Table 4).”

In table 3 the result of homocysteine comparison is missing.
Response: This has been added to table 3 using the median homocysteine level of 12.6 micromol/L. We also examined using continuous homocysteine levels at baseline and this interaction was also not significant (p=0.9903).

Reviewer 2: Irena Keser

The study describes an interesting and clinically relevant topic: the effect of homocysteine on osteoporotic fractures. The study is well designed, with the large number of participants and clearly written. The research question posed by the authors is well defined. The title and abstract accurately convey what has been found. The methods are appropriate and well described. The discussion and conclusions are well balanced and adequately supported by the data. The limitations of the study are clearly stated.

Response: Thank you for the positive feedback.

Major Compulsory Revisions:
Comment 1: Methods: Why homocysteine levels were analyzed in serum and not in plasma?

Response: All samples were plasma – all previous reference to serum has been corrected to read “plasma”

It is not stated which method is used to determine homocysteine levels.

Response: Same as for reviewer 1: We have updated the relevant section in methods – which now reads:

“Investigators were encouraged, but not obligated, to take blood samples from study participants who consented to these optional tests, to measure blood concentrations of tHcy, red cell folate and vitamin B12 at study entry and/or at the time of their final follow-up visit. Fasting plasma tHcy was measured by high-performance liquid chromatography, on venous blood samples collected after an overnight fast.”

Comment 2: Results: Effect of trial medication on homocysteine levels

The authors did not state were mean homocysteine levels significantly different between the vitamin and placebo groups.

Response: This has been added to paper as p <0.001

Minor Essential Revisions:
Comment 1: References: References 6 and 26, title should be bold. Abbreviations of journals should be Italic. Page numbers are not written in a proper way. (see Instructions for authors)

Response: This has been corrected

Discretionary Revisions:
Comment 1: Background, second paragraph
Authors stated three randomised controlled trials that have examined the effect of B-vitamins on biomarkers of bone turnover. The following publication could also be cited:

Response: Thank you for bringing this paper to our attentions as it was not available when our manuscript was first submitted in January 2013. We have updated our paper to state four rather than three RCT and have added this citation to reflect this new information.

Comment 2: Discussion
The Discussion should present some more published studies on this topic. For example:

Response: Thank you. We have now included both the McLean and Gerdhem papers. We note that we have already cited/referenced another of Sato’s papers.

Reviewer 3: Nathalie van der Velde
The article is well written and given the limited publications/trials on this matter, the data add to the insight of the earlier reported association between homocysteine/B-vitamins and fractures. However, as the authors state themselves, the low fracture rate seriously limits any conclusion that may be drawn, but in agreement with the authors, publishing the data may be of value for future meta-analyses on this issue. Furthermore, changes in gathering of events over the study period may have hampered the results, e.g. dilute any potential effect, although it is not likely that this was differential between the groups.

Response: Thank you for the positive feedback and recognition that publication of these results may be of value in future meta-analysis.

Major comments:


**Comment 1:** Methods page 8/9: Although VITATOPS main outcomes have been published elsewhere; readability would improve if assessment/statistical handling of baseline characteristics is presented in the methods section.

**Response:** We have added the following to the methods regarding baseline characteristics assessed and statistical handling.

“Demographic and clinical characteristics of the participants recorded at baseline included; age, sex, ethnicity (of participants and their parents and grandparents), clinical details of the qualifying cerebrovascular event, current medications, past medical history (major vascular events, revascularisation procedures, depression), vascular risk factors (hypertension, smoking, hypercholesterolaemia, diabetes mellitus, ischaemic heart disease, atrial fibrillation, peripheral artery disease, alcohol intake), Oxford Handicap Score, Hospital Anxiety and Depression Score, and Mini Mental State Examination score. Cox’s proportional hazards models were used to adjust for differences in baseline variables.”

If the editor preferred a shorter account we would be happy to use this abbreviated version:

“Demographic and clinical characteristics of the participants recorded at baseline included; age, sex, ethnicity, clinical details of the qualifying cerebrovascular event, current medications, past medical history, vascular risk factors, alcohol intake, Oxford Handicap Score, Hospital Anxiety and Depression Score, and Mini Mental State Examination score. Cox’s proportional hazards models were used to adjust for differences in baseline variables.”

Furthermore, please add information regarding handling of potential differences between the intervention group and control group and handling of potential interaction e.g. which potential stratifications where considered.

**Response:** There were no differences; thus we did not have to take this into account.

**Comment 2:** Methods & results: please add information regarding potential different effects for gender, age-groups, and baseline levels; and potential other variables with positive interaction-terms.

**Response:** These are presented in table 3 – there were no significant interactions.

**Comment 3:** Methods: please shortly state the duration of intervention and explain differences in follow-up period.

**Response 1:** We have added the following sentence to the first paragraph in methods:

“Final follow-up was 30 June 2009 ensuring a minimum six months and up to 10.5 years on study medication.”
Response 2: we have added the following sentence to the “follow-up” section in results. “The longer duration of follow-up (0.6 years) reflects those patients who discontinued trial drugs but agreed to continue with follow-up.”

Comment 4: Methods/results: was per protocol analysis performed/considered? If results were comparable, please mention briefly; if they differed, please elucidate.

Response: The following sentences have been added to the methods and results sections:

Methods: “A secondary per protocol on-treatment analysis would exclude any patients found to be invalid after randomisation or who had cross over in treatment during follow-up.”

Results: “A secondary per protocol on-treatment analysis also showed no impact of treatment on any osteoporotic fracture outcome after removal of patients found to be invalid after randomisation or who had cross over in treatment during the follow-up; either placebo group patients taking B-vitamins or B-vitamin group patients stopping their treatment. (p=0.85).”

Comment 5: Results page 9/10: please add information regarding adverse events

Response: We are happy to include (and have done this) an adverse events section in the results - using the following taken from the original VITATOPS publication:

“Vitamin B12 deficiency was diagnosed during follow up in none of the 4089 patients in the B vitamins group compared with six (0.1%) of 4075 patients in the placebo group (p=0.02). Peripheral neuropathy suspected to be caused by vitamin B6 toxicity was diagnosed in five patients assigned to B vitamins (0.1%) compared with nine patients assigned to placebo (0.2%; p=0.30). There were no unexpected serious and non-serious adverse events and there were no significant differences in common adverse effects between the treatment groups (data not shown).”

Comment 6: Results page 10: the overall fracture rate appears to be caused for +/- 50% by the hip fracture rate. From an epidemiological point of view this is quite unlikely, and underlines the selective gathering of results during the first part of the study (as is mentioned by the authors). Since any osteoporosis-treatment/preventive measure may potentially have different effects on different bone structures (E.g. fracture sites) this may have biased the results. Please state this clearly in the limitation-section.

Response: We agree with this assessment and have added the following sentence to the relevant paragraph on study limitations - regarding incomplete identification of minor fractures:
“The relatively high proportion of all fracture outcomes due to hip fractures (48%) supports this possibility.”

Comment 7: Discussion page 11, first paragraph: Please rewrite first sentence, because of the lack of power, the conclusion needs to be written down less firm.

Response: We agree. The discussion & conclusion now both start:

“This study did not identify any effect of daily treatment with…” (Rather than “The main finding … is that daily treatment… had no significant effect…”). We have also deleted the word “significant” from the conclusion in the abstract.

Discussion page 12 second paragraph: please rewrite limitation regarding change in gathering of the primary outcome for this study as mentioned above (comment 7)

Response: We agree and have simply deleted the statement in discussion regarding prospective reporting being a potential strength of the study.

Deleted: “and the prospective reporting of fractures as a specified outcome event for the majority of participants - those who were recruited after 2004”

Discretionary comments:
Comment 1: Because of altering insights, osteoporoses/fracture research is more and more changing towards addressing not only ‘osteoporotic fracture’ but ‘any fracture’. If possible, it would be interesting to add ‘any fracture’ as secondary outcome measure’.

Response: This is not possible as investigators were only asked to report “suspected osteoporotic fractures” so ascertainment of data regarding “any fracture” would be incomplete and interpretation of this data unreliable.

Comment 2: In my opinion, adding an overall analyses of predictors of osteoporotic fracture for this particular population does not add to the insight of homocysteine-related fracture risk.

Response: While we agree to some extent with this comment, providing this data does allow readers to draw their own conclusions regarding ability to generalise our study results (in people with cerebrovascular disease) to other populations at risk of fracture.

We trust that we have adequately addressed all the reviewer’s concerns and thank you for your further consideration.

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