PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Does early vitamin B12 supplementation improve neurodevelopment and cognitive function in childhood and into school age; a study protocol for extended follow-ups from randomized controlled trials in India and Tanzania
AUTHORS	Winje, Brita; Kvestad, Ingrid; Krishnamachari, Srinivasan; Manji, Karim; Taneja, Sunita; Bellinger, David; Bhandari, Nita; Bisht, Shruti; Darling, Anne Marie; Duggan, Christopher; Fawzi, Wafaie.; Hysing, Mari; Kumar, Tivendra; Kurpad, Anura; Sudfeld, CR; Svensen, Erling; Thomas, Susan; Strand, TA

VERSION 1 – REVIEW

REVIEWER	Eamon Laird Trinity College Dublin, Ireland
REVIEW RETURNED	18-Sep-2017

GENERAL COMMENTS	This is an interesting and timely study protocol to investigate the potential associations of vitamin B12 supplementation during pregnancy and neurocognitive effects in the infants and children. The projects aims to test a wide range of neurocognitive outcomes using data from three well described previous supplementation studies. Advantages are the diverse range of the population, the large number of participants and the comprehensive range of tests to be conducted. Major queries: 1). How will the study adjust for other factors that might influence neurocognitive/growth outcomes in these population groups as a large range of confounders may exist: (i) Even these are low to middle income countries, there could be extreme differences in wealth between individuals which could affect diet quality, access to B12 rich foods etc. Will socio-economic status be measured? (ii) What about other micronutrients that may affect neuro-cognition –e.g. 25(OH)D. Will this also be measured/analysed (vitamin D status would be varied across the three different locations) (iii) Will the blood status of the mothers be correlated with the outcomes in the infants? MMA, HCY were measure in some of the studies as was B12 though it was not stated if it was total B12 or holo-TC measured and you could get a different 'answer' depending on which biomarker is chosen. (iv) Will the blood B-vitamins status of the infants/children also be measured along with an FFQ in order to ascertain their current status?

	Will the FFQ measure intakes of other foods and micronutrients. For example, high fish intakes or other certain foods may be independently associated with neurocognition (v) What about seasonal factors? In certain areas seasonal food shortages/disease or parasitic infections may impact on status or growth outcomes/B12 status –how will this be factored in the analysis? What about contamination of water or local foods – for example arsenic contamination of groundwater is common in certain places in India and may impact on the outcomes (Rosado JL, Ronquillo D, Kordas K, Rojas O, Alatorre J, Lopez P, Garcia-Vargas G, del Carmen Caamaño M, Cebrián ME, Stoltzfus RJ. Arsenic exposure and cognitive performance in Mexican schoolchildren. Environmental health perspectives. 2007 Sep;115(9):1371). (vi) Will there be any measure to see if the children are learning to be bilingual as this ability could enhance cortex development – see 'Bilingual Language Control Mechanisms in Anterior Cingulate Cortex and Dorsolateral Prefrontal Cortex: A Developmental Perspective' Dean D'Souza and Hana D'Souza Journal of Neuroscience. This could be a potential confounder. 2). Is it the authors hypothesis that mothers who were supplemented with B12 will have children with better neurocognitive or growth outcomes? This is not firmly stated in the aims or objectives –please clarify. 3). It is stated that the mothers from the Dar es Salaam cohort were HIV negative, is this true for the other cohorts as well from Delhi or Bangalore? 4). Will this study investigate the impact of folate status on the associations of B12 and growth/neurocognitive outcomes? Is the study powered to do this/will this be another secondary analysis after the main project? Minor Queries: 1). Page 6, lines 52-57: Please be more explicit for the reader why you are including references/studies about macrobiotic diet and cognitive tests when the study is about vitamin B12 and cognition outcomes 2). Page 7, lines 12-15: Reference 15 –please state what doses were used

REVIEWER	Renae Fernandez University of Adelaide, Australia
REVIEW RETURNED	10-Oct-2017

GENERAL COMMENTS	Under the 'Specific objectives and objectives of the separate studies' section, please indicate the length of follow up for the study of South Indian children.

REVIEWER	Dr Elaine McCarthy University College Cork, Ireland
REVIEW RETURNED	11-Oct-2017

GENERAL COMMENTS	Overall, this is a well written protocol of an important study. I just have a small number of suggestions aimed to help improve the

	manuscript further. 1. Introduction - I thought it would be more logical to put the section on "why vitamin B12 may be important for the developing brain" before the section on "vitamin B12 and nueordevelopment" that details the human data. 2. Study objectives (page 8/9) - these would be more clear if you could ensure that the objectives of the separate studies are specific ie. include age of exposure, age of outcome, duration of supplementation etc. As it stands currently, some of the objectives seem to have more specific details than others. 3. Methods and analysis (page 9 line 24) - can you please add in what the RDA for vitamin B12 is so that readers that are not familiar with the nutrient will understand when you say "at least two recommended dietary allowances". 4. Methods and analysis (page 11) - in the explanation of the studies in Delhi and Dar es Salaam, it is important to add in the dose of nutrients given during the trial and the duration of supplementation as you have done on page 9 for the summary of the study in Bangalore. 5. Methods and analysis (Neurodevelopment section page 12) - a little more detail on the assessments used apart from just their name would be helpful as these are the main outcomes of the study, especially a brief description of how ERPs are measured is needed. It's important to emphasise that these are all different types of assessments (neurophysiologic, global assessments, screeners etc) as this is a strength of your study design. Some indication of how the assessments are administered is required ie. psychologist assessed, parent-report etc. 6. Table 2 - please give full titles for the biomarkers mentioned on the table, only the abbreviations are given currently that not all readers will be familiar with.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Major queries:
1. How will the study adjust for other factors that might influence neurocognitive/growth outcomes in these population groups as a large range of confounders may exist: Thank you for pointing this out.

We agree that these factors are important when interpreting our results and acknowledge that we have not sufficiently addressed this in the manuscript. In this project, we follow up children from four RCTs with the lowest sample size being 400 participants. The participants were individually randomized and with these sample sizes significant baseline differences are unlikely and the risk of confounding accordingly small. However, many factors may have profound impact on our outcomes and may potentially dilute the effect of B12 supplementation in early life. Thus, we may underestimate the effect of B12 supplementation. We have added a sentence to clarify on this under “Relevance and benefit to society”

a. Even these are low to middle income countries, there could be extreme differences in wealth between individuals which could affect diet quality, access to B12 rich foods etc. Will socio-economic status be measured?

Response: Yes, we will measure socio-economic status across all studies and populations. A description of this is now included in the analysis plan

b. What about other micronutrients that may affect neuro-cognition –e.g. 25(OH)D. Will this also be measured/analysed (vitamin D status would be varied across the three different locations)

Response: Thank you for this comment. We believe that this is an excellent idea. We have measured vitamin D status in the cohort in north-India and will measure to what extent vitamin D status in early life is associated with neurodevelopment too. We have included this in the presentation of the north-Indian study.

c. Will the blood status of the mothers be correlated with the outcomes in the infants? MMA, HCY were measure in some of the studies as was B12 though it was not stated if it was total B12 or holo-TC measured and you could get a different 'answer' depending on which biomarker is chosen.

Response: We will not use holo-TC. We apologize for not being sufficiently clear on the included biomarkers. We have updated Table 2 with more detailed information. Describing the vitamin status in mothers and children is not the scope of this study. We are, however, addressing this in the described studies and other projects. (see Srinivasan K, et al., Matern Child Nutr. 2017;13(2))

d. Will the blood B-vitamins status of the infants/children also be measured along with an FFQ in order to ascertain their current status? Will the FFQ measure intakes of other foods and micronutrients. For example, high fish intakes or other certain foods may be independently associated with neurocognition.

Response: Thank you for highlighting this. We will have good dietary intake data from the Indian studies that we may measure against blood vitamin B12 status. This is clarified in the analysis plan in the updated manuscript. Fish intake is however, negligible in these areas of India.

e. What about seasonal factors? In certain areas seasonal food shortages/disease or parasitic infections may impact on status or growth outcomes/B12 status –how will this be factored in the analysis? What about contamination of water or local foods – for example arsenic contamination of groundwater is common in certain places in India and may impact on the outcomes (Rosado JL, Ronquillo D, Kordas K, Rojas O, Alatorre J, Lopez P, Garcia-Vargas G, del Carmen Caamaño M, Cebrián ME, Stoltzfus RJ. Arsenic exposure and cognitive performance in Mexican schoolchildren. Environmental health perspectives. 2007 Sep;115(9):1371).

Response: We will capture seasonal factors in each site and include this as a separate exposure in the analysis. This is now mentioned in the analysis plan. We do not, however, have data on arsenic exposure or other environmental contaminants that may affect neurodevelopment.

f. Will there be any measure to see if the children are learning to be bilingual as this ability could enhance cortex development – see 'Bilingual Language Control Mechanisms in Anterior Cingulate Cortex and Dorsolateral Prefrontal Cortex: A Developmental Perspective' Dean D'Souza and Hana D'Souza Journal of Neuroscience.

Response: Because of the RCT designs we believe the risk of confounding due to this phenomenon is unlikely, see clarification under “Relevance and benefit to society”. However, we find the comment very interesting. It is not feasible to include this across studies, but we will explore whether it is possible to include this as a secondary outcome if the collected data allows such analyses in certain studies.

2. Is it the authors’ hypothesis that mothers who were supplemented with B12 will have children with better neurocognitive or growth outcomes? This is not firmly stated in the aims or objectives –please clarify.

Response: Yes. We have now clarified on this in the general objective “To provide evidence for the role of vitamin B12 supplementation in pregnancy or early childhood on neurodevelopment in vulnerable children in low and-middle income countries (LMICs)”

3. It is stated that the mothers from the Dar es Salaam cohort were HIV negative, is this true for the other cohorts as well from Delhi or Bangalore?

Response: Women in the Bangalore study were HIV-negative and this is now mentioned in the manuscript. Information on HIV-status was not collected in Delhi. Since women were included from a general population, some of them may be HIV-positive. We have highlighted that the Delhi study was population-based, under “Study designs and interventions”

4. Will this study investigate the impact of folate status on the associations of B12 and growth/neurocognitive outcomes? Is the study powered to do this/will this be another secondary analysis after the main project?

Response: Thank you for this comment. The study in north-India is powered to measure the effect of folic acid on the neurodevelopmental outcomes and growth. We have updated the text regarding this objective under “Study designs and interventions”

Minor Queries:
1. Page 6, lines 52-57: Please be more explicit for the reader why you are including references/studies about macrobiotic diet and cognitive tests when the study is about vitamin B12 and cognition outcomes

Response: We have included references on macrobiotic diets as these are low on vitamin B12 and thus may be important causes of vitamin B12 deficiency. Please see the updated manuscript under “Vitamin B12 and neurodevelopment”.

2. Page 7, lines 12-15: Reference 15 –please state what doses were used.

Response: We have now added information about the doses that were used under “Vitamin B12 and neurodevelopment”.

Reviewer 2

Comment: Under the 'Specific objectives and objectives of the separate studies' section, please indicate the length of follow up for the study of South Indian children.

Response: We see that we have not been sufficiently systematic in our presentation of the original studies. The length of follow-up for children in the original study was 30 months and in the follow-up study 5-6.5 years after supplementation.

Reviewer 3

1. Introduction - I thought it would be more logical to put the section on "why vitamin B12 may be important for the developing brain" before the section on "vitamin B12 and neurodevelopment" that details the human data.

Response: Thank you for this suggestion, we have now changed the order of the two paragraphs.

2. Study objectives (page 8/9) - these would be more clear if you could ensure that the objectives of the separate studies are specific ie. include age of exposure, age of outcome, duration of supplementation etc. As it stands currently, some of the objectives seem to have more specific details than others.

Response: We see that we have not been sufficiently clear on this in the manuscript. In the revised manuscript, we have included age and duration of exposure and age range at follow-up in the specific objectives for the separate studies. This information is also presented in more detail in Table 1 and as footnotes of Table 1. Details on the doses used in the different studies have been included in the presentation of the specific sub-studies in “Study design and interventions”

3. Methods and analysis (page 9 line 24) - can you please add in what the RDA for vitamin B12 is so that readers that are not familiar with the nutrient will understand when you say "at least two recommended dietary allowances".

Response: Thanks for this suggestion, the text has been updated.

4. Methods and analysis (page 11) - in the explanation of the studies in Delhi and Dar es Salaam, it is important to add in the dose of nutrients given during the trial and the duration of supplementation as you have done on page 9 for the summary of the study in Bangalore

Response: Please also see our comment to your 2nd point. This information has been added for all four studies in the specific objectives.

5. Methods and analysis (Neurodevelopment section page 12) - a little more detail on the assessments used apart from just their name would be helpful as these are the main outcomes of the study, especially a brief description of how ERPs are measured is needed. It's important to emphasise that these are all different types of assessments (neurophysiologic, global assessments, screeners etc) as this is a strength of your study design. Some indication of how the assessments are administered is required ie. psychologist assessed, parent-report etc.

Response: Thank you for this comment. We have expanded the section on the various neurodevelopmental assessments under Outcomes/neurodevelopment. We have also added information that assessments were administered by psychologists in the Indian studies and by trained health care workers in Tanzania under Data collection.

6. Table 2 - please give full titles for the biomarkers mentioned on the table, only the abbreviations are given currently that not all readers will be familiar with.

Response: We are sorry for this inconvenience. We have provided the full titles of the biomarkers in footnotes to table 2

We hope we have responded sufficiently to the remarks from the editor and reviewers and that the revised manuscript can be accepted for publication in BMJ Open.

VERSION 2 – REVIEW

REVIEWER	Eamon Laird School of Medicine Trinity College Dublin Ireland
REVIEW RETURNED	27-Nov-2017

GENERAL COMMENTS	The authors have addressed all the comments previously raised by the reviewers

REVIEWER	Dr Elaine McCarthy University College Cork, Ireland
REVIEW RETURNED	30-Nov-2017

GENERAL COMMENTS	I am satisfied that the authors have addressed my previous comments from the first review of this manuscript. The added detail and clarity in the manuscript strengthens it considerably.