Author's response to reviews

Title: Multiple micronutrient supplementation improves vitamin B12 and folate concentrations of HIV infected children in Uganda: a randomized controlled trial

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Author's response to reviews: see over
Responses to reviewer 1_Angel Remacha

Reviewer's report
Title: Multiple micronutrient supplementation improves vitamin B\textsubscript{12} and folate concentrations of HIV infected children in Uganda: a randomized controlled trial
Version: 1 Date: 20 January 2011
Reviewer: Angel F. F Remacha

Reviewer's report:
This is an interesting article studying vitamin B\textsubscript{12}/folate supplementation in African Children. Prevalence of low vitamin B\textsubscript{12} and low serum folate is similar to pre-HAART era. Some comments should be addressed.

Major Compulsory Revisions

Reviewer's comment 1: Homocysteine and methylmalonic acid metabolites of vitamin B\textsubscript{12} were not measured indicators of deficiency states. Low vitamin B12 is not synonymous with vitamin B12 deficiency. In fact, in previous works, most cases with low vitamin B12 serum levels in HIV patients this vitamin deficiency was not demonstrated using tests distinguishing low levels from real vitamin B12 deficiency, such as homocysteine or d-Uridine suppression test. This work does not use these tests, therefore it is impossible to assess this aspect.

Response: It is correct that we did not measure homocysteine and methyl malonic acid which is a limitation to the interpretation of our findings. The main reason was the limited amount of blood that was possible to draw from these children and the multiple micronutrients that were listed for analysis. We made this trade-off: measuring B\textsubscript{12} but not homocysteine or MMA. We have amended the text deleting ‘deficiency’ where appropriate and instead referring to low or marginal vitamin B\textsubscript{12} concentrations. It is clear, however, that supplementation improved vitamin B\textsubscript{12} and folate status.

Reviewer's comment 2: However, as Hb does not change in cases with low vit B\textsubscript{12} after supplementation, this finding supports that there is not a real vitamin B\textsubscript{12}.

Response: In a landscape of multiple deficiencies, such as HIV-infected children in a low-income country, there is always the potential for other deficiencies to alter the response, for instance, iron deficiency (iron was not supplied) could have been the rate limiting factor for increasing the haemoglobin and the explanation for the non-increase.

Reviewer's comment 3: Low vitamin B\textsubscript{12} could be related to folate deficiency or, more probably, to low vitamin B\textsubscript{12}-binding proteins (cobalophilin, transcobalamin I or R-Binders) as a consequence of neutropenia.

Response: We also agree that low vitamin B\textsubscript{12} could be related to folate deficiency or to low vitamin B\textsubscript{12} binding proteins. Although we did not find an association
between low vitamin B<sub>12</sub> and white blood cell count this does exclude the possibility that low vitamin B<sub>12</sub> concentrations could be related to neutropenia.

**Reviewer’s comment 4:** On the other hand, Hb increases in low folate children after supplementation, suggesting that the folate deficiency is present and is inducing anaemia. In previous studies, folate deficiency was common in HIV patients in pre-HAART era and this deficiency was demonstrated using the above mentioned tests. After folate treatment, for instance, hyperhomocysteinemia was normalized in adults. With the design of this work is impossible to differentiate whether anaemia is caused by folate or vitamin supplementation. A study of vitamin B<sub>12</sub>/folate metabolites (homocysteine, methyl malonic acid) could help to solve this point.

**Response:** In both treatment groups the haemoglobin improved compared to baseline levels. Although baseline low folate concentrations were associated with a low haemoglobin level we could not conclusively attribute the anaemia to low folate concentrations since we did not measure red blood cell folate concentrations. We acknowledge this as a weakness and have included it in the discussion.

**Reviewer’s comment 5:** HAART group is small and, therefore, it is difficult to obtain conclusions, curiously the prevalence of low vitamin B<sub>12</sub> and low folate is similar to HIV children not in HAART therapy. In adults, this was not the case. CD4 levels in HAART and non-HAART patients could help to understand this discrepancy.

**Response:** It is true that the HAART stratum consisted of very few children and inevitably this limits the conclusions. A paragraph has been added on the results section (Comparison between baseline and 6 months) to explain the differences between the HAART and non-HAART strata. The following sentence has been inserted in the discussion: ‘The lack of differences in baseline vitamin B<sub>12</sub> or folate concentrations could be explained by the short duration of HAART compared to other studies.’

**Reviewer’s comment 6:** To evaluate better evaluate folate metabolism, red cell folate should have been determined.

**Response:** Unfortunately we were unable to perform the suggested studies, mainly for the reasons mentioned above. However, we still think sub-normal serum folate is a useful indicator of folate status that warrants reporting.

Minor Essential Revisions
**Reviewer’s comment 7:** Tables using mean logs are difficult to follow, this table should be rebuilt using mean or not included.

**Response:** Thank you for this comment. Table 3 has been deleted.

**Reviewer’s comment 8:** Figures have not been received.

**Response:** One figure to explain the study profile (participants enrolled in the main study and those who had measurements for B12 and folate) has been included in this revised version.
Reviewer's report 2_ Siyazi Mda
Title: Multiple micronutrient supplementation improves vitamin B12 and folate concentrations of HIV infected children in Uganda: a randomized controlled trial
Version: 1 Date: 21 February 2011
Reviewer: Siyazi Mda

Reviewer's report:
Minor Essential Revisions:
Reviewer's comment 1. In the Methods section, under Participants (third paragraph, last sentence) the researchers state that there were no significant differences among those who had results for vitamin B12 and folate compared to those who did not have results.
What differences are they referring to? Are these differences in age, gender, weight, height, receipt of HAART, CD4+ cell count?

Response: These were no differences in demographic and clinical characteristics such as age, sex, anthropometric measurements and other laboratory measurements like CD4 + cell count. We have clarified this in the manuscript.

Reviewer’s comment 2: Intervention: The amounts in the supplement were based on twice the recommended dietary allowance (RDA) for a 4 year old child. The subjects were children aged 1-5 years. What informed their decision to choose the age 4 years?

Response: We decided to use 2 RDA based on the fact that most children were malnourished despite routine supplementation with multivitamins. Secondly some previous studies of HIV infected adults had indicated that HIV infected persons may require multiples of RDA in order to achieve normal serum concentrations of micronutrients (Baum 1994). Thirdly looking at the tables of the nutritional requirements for children we noted that there are 2 age bands where our participants would fall 1-3 and 4-8 years of age. We noticed that there were minor variations in dosages of some micronutrients while others like iodine and vitamin did not vary. We therefore decided to use the 4 year old category for our study. In addition it was easier to administer a uniform intervention. There were no similar studies in the region and there was no literature to guide us on the micronutrient status of Ugandan HIV infected children. Also there were no food composition tables based on the local foods so we could not estimate how much micronutrients children get from the usual diet. We have inserted this explanation in the manuscript under the section on intervention.

Reviewer's comment: How were the supplements administered to the children? Was the powder mixed with food, water or milk?

Response: The powder was mixed with 10 to 20 mls of milk or water. We have added this explanation to the intervention section in the manuscript.

Reviewer’s comment: How did they ensure that the child consumed the complete dose? How did the researchers ensure compliance?
**Response:** The first dose of the supplement was administered at the study clinic following a demonstration and under observation by the study nurse. At the time of administering the first dose we counselled the mother on the importance of completing the dose and ensuring that the daily dose was given. Whenever the child vomited during or within 30 minutes of administering the dose a repeat dose was given. Mothers were given calendar charts and instructed to tick on the appropriate date whenever a dose was given. They would return to the clinic on follow up visits with the container/remaining supplement together with the calendar charts. The remaining amount of supplement was measured using a light weight scale and the level of compliance determined using the proportion of the supplement consumed against the expected. Each dose/scoop measured 4 grams and the supply for one month was 140 grams and the expected dose for 30 days was 120 grams. The remaining 20 grams was to cater for vomited doses or in case there was spillage. The mothers administered the subsequent doses from home and it was not possible to observe them. This section has been added to the manuscript.

**Reviewer’s comment 3:** Randomisation and blinding:
At what stage was the treatment assignment revealed?

**Response:** The treatment assignment was revealed upon completion of the study. The randomisation code was kept at Geneva by RB who generated it and by the manufacturers at NUTRISET, France.

**Reviewer’s comment 4:** Laboratory measurements:
What were the coefficients of variation for the assays?

**Response:** The coefficient of variation for the assays was less than 5%.

Discretionary revisions:

**Reviewer’s comment 5:** In the Methods section, under Participants (second sentence), the researchers indicate that they excluded children who had enrolled in other studies. Were there other micronutrient supplementation studies being conducted in the same clinics at the time? If the reason for exclusion was receipt of folic acid and/or vitamin B\textsubscript{12} this should be stated.

**Response:** There were no other micronutrient supplementation studies going on at the time so the exclusions were done to avoid participant fatigue and interference with procedures. Multivitamin supplementation was routinely practiced at the study clinics but these were not excluded. This explanation has also been included in the manuscript.