Response to Reviewers
A Randomized Trial of Multivitamin Supplementation in Children with Tuberculosis in Tanzania
Reviewer 1: Marianne Visser
Reviewer's report:
Major Compulsory Revisions:
1. Baseline comparability of the 2 treatment groups: In the multivitamin group 39% were HIV-infected vs. 29% of the placebo group. It would be useful to know how many HIV-infected children were enrolled in each age stratum in the 2 treatment groups? No comment is made regarding the higher baseline CD8 count in the multivitamin group?

Dear Dr. Visser,

Thank you for your review of this manuscript. We have added the number of HIV-infected children enrolled in each age stratum to Table 1.

HIV-infected 37 (29.13%) 50 (39.06%)
By Age categories; # 6 months 4 (44.44%) 4 (30.77%)
6 months to # 3 years 23 (24.73%) 29 (34.12%)  
> 3 years 10 (40.00%) 17 (56.67%)  

Though the baseline CD8 count was higher in the multivitamin group compared to the placebo group, the difference was not statistically significant.

2. 
The conclusion made regarding significant weight gain among infants less than 6 months should be made with caution, since it involved only 22 children? Could you please comment on the baseline comparability of these children in terms of weight? How many children < 6months in each treatment group were HIV-infected?

We agree and have added the number of children to the text of our conclusion. The median (IQR) weight of children in the 6weeks-6months age group in the placebo and treatment groups was 6.70 (5.00, 7.41) and 6.10 (5.58, 6.92) respectively. There were 4 HIV-infected children in both the placebo and the multivitamin group.

3. 
The conclusion regarding height gain among HIV-infected children also requires caution due to the fact that the trial was not primarily designed to assess changes in height. Again the number of HIV-infected children used in the analysis is unclear.

We have added the number of HIV-infected children included in this analysis (n=79) in the results section. We have also added a statement in the limitations section stating that the trial was not designed to measure effects within subgroups and hence, these results cannot be treated as conclusive evidence.

4. 
Serum albumin is a negative acute phase protein and therefore it is reduced in the presence of active infection/inflammation. It is expected that with the administration of anti-tubercular treatment, serum levels will increase. The authors report a statistical difference in the increase in serum albumin concentrations among supplemented vs. non-supplemented HIV-infected children. In my opinion this is likely to be related to the reduction in the inflammatory response in the 2 groups and not directly to multivitamin supplementation. Fleck A. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutr Soc 1989; 48: 347-354.

We have removed references to albumin on the suggestion of Reviewer 4.

5. 
The authors report that the CD8 count increased among children aged > 3 years who were supplemented. The significance of this finding is not discussed?

We can only speculate about the potential explanations for this finding and are
not aware of any known age-specific effects of multivitamins on CD8 cells in this age group. We have added a sentence about this to the text in the manuscript.

Minor Essential Revisions:
Table 1 can be improved by providing the reader with absolute numbers. Percentages could be given in brackets. The number of HIV-infected children per age stratum should be provided.

We have made the suggested changes to Table 1 and added the number of HIV-infected children in each age group.

Discretionary Revisions:
It is a pity that this trial had to be terminated prematurely. This could be mentioned in the abstract, since it may be the main reason why the trial failed to show any overall beneficial effect on weight gain. Only 64% of the calculated sample size was enrolled, resulting in less statistical power.

We have added this statement to the abstract.

Reviewer 2: Lovett Lawson
Reviewer’s report:

Reviewers’ comments

A randomized Trial of Multivitamin Supplementation in Children with Tuberculosis in Tanzania

This is a very important manuscript especially since it looked at supplementation in children with tuberculosis, a group where studies have been few.

Abstract

1. In the results section, since the children were grouped by age, it is important to state (line 18, p3) that the significant improvement in haemoglobin levels at the end of follow-up was at all ages.

Dear Dr. Lawson,

Thank you so much for reviewing this manuscript. We have made this change in the manuscript.

2. Also in the result section, the authors should include the results of the effect of supplementation on the primary outcome.

The first sentence of the results section in the abstract – page 3 line 15 – states that there was no significant effect of multivitamin supplementation on the primary outcome of the trial.

3. A statement should be included in the conclusion to reflect the observation.

We have made this change.
Introduction

1. Authors should clarify if TB cannot be associated with weight loss and at the same time protein and calorie malnutrition (line 18, p5)

We have corrected this statement to reflect that TB can be associated with both weight loss and protein and calorie malnutrition.

2. Authors should endeavour to mention few more micronutrient supplementation trials in the introduction in a way that more information is given on what is known in the field of micronutrient supplementation.

We have added a couple of sentences to the introduction but the details of micronutrient supplementation trials are covered in the Discussion section to keep the Introduction section concise.

3. It is important to include information on the level of micronutrient deficiency in the region.

There is no publically available information for the levels of vitamins B-complex, C, and E deficiencies in children with tuberculosis in sub-Saharan Africa that we are aware of.

Method

1. The statement on line 13, p6, should read “with probable TB infection” as most of the cases are not confirmed TB.

We have made this change.

2. The probable number of TB patients was 275. It will help to give some information why only 255 were enrolled.

4 patients refused to participate in the study. 16 patients were lost to follow-up before any information could be collected.

3. The name and address of the manufacturer of the regimen bottles should be added.

We have added this (Nutriset, France).

4. The authors should state whether the active regimen and the placebos capsules were identical. My understanding is that the regimen bottles are the ones similar.

Yes, the capsules were identical in shape, size, and color. We have clarified this in the text.

5. Line 16, p8 should explain which of the treatment arm or arms received one capsule daily.

We have clarified this.
6. Indicate if pre and post counselling to the parents/guidance of the patients were done.

Pre- and Post-test counseling was offered to parents/guardians of all patients before HIV testing.

Results
1. Having referred to table 1 in the text, except for any significant information in the table, there is no need for repetition in the text.

We have removed most of the repetition of Table 1 findings from the text.

2. Line 16, p12 should read “both the placebo and the supplement groups..........”

We have made this change.

3. It is obvious that both the placebo and supplement groups had similar weight gain of 0.84 kg as such the next statement is not necessary.

We have deleted the next statement.

4. The change in triceps skin-fold thickness does not tell me if the change was an increase or a decrease in thickness.

We have corrected this and added the word increase to the statement.

Discussion
1. It should be stated that the improvement in haemoglobin was in all groups (line 5, p15)

We have made this change.

2. The beneficial effects of multivitamin supplementation were not shown in this study, as such the statement starting on Line 16 p17 should be modified.

We have edited this statement to state that multivitamin supplementation has the potential to have several beneficial effects.

Reviewer 3: Christian Wejse

Reviewer’s report:

Overall
This study reports the finding of a randomised clinical trial testing the effect of multivitamin vs placebo in 255 children with presumed TB in Tanzania. The paper deals with a very important subject and the study provides important new information. The research question is novel and well defined, there is limited knowledge in the field and this type of trial is difficult to recruit patients for and to undertake in a resource limited setting. The randomised trial appears to be well conducted although more detailed information about the trial is needed in order
to be able to replicate the work. It is not clear if the manuscript adheres to the relevant RCT standards, and a CONSORT statement would be helpful. The conclusion is sound and based on the presented data, but needs further clarification and more data should be presented.

Major comments

1. A secondary outcomes at the time of trial registration was clearance of chest x-ray which is not reported. Standard TB outcomes (cure, completed treatment, default, relapse, transfer, death) should be reported.

Dear Dr. Wejse,

Thank you so much for reviewing this manuscript!

There was no difference in the clearance of chest x-ray or mortality in the two treatment arms. 199 children had a chest X-ray available at the end of follow-up and 100 of them showed complete resolution. The follow-up in the study ended after two months of initial intensive phase of anti-tuberculous treatment, and therefore it was not feasible to assess outcomes such as cure and default after the period of two months.

There was no difference in the clearance of chest x-ray or mortality in the two treatment arms. 199 children had a chest X-ray available at the end of follow-up and 100 of them showed complete resolution. The follow-up in the study ended after two months of initial intensive phase of anti-tuberculous treatment, and therefore it was not feasible to assess outcomes such as cure and default after the period of two months.

2. The authors study a large number of outcomes with no adjustment for multiple outcome assessment which should be done (Bonferroni). The question whether some of the findings are chance findings resulting from the large number of analyses conducted should be discussed more thoroughly. It is also critical to know whether the authors were testing a priori hypotheses or generating hypotheses.

Tables 2 and 3 present results from testing a priori hypotheses for this primary manuscript for the study – the effect of multivitamin supplements on changes in weight, anthropometric indices, hemoglobin, albumin, CD4, and CD8. There are 10 comparisons in the two tables – the outcomes are the rows with the change in each variable. The supplemental comparisons at baseline and intermediate follow-up points are presented to make it easier for the readers to get a visual snapshot of how the children progressed through the two months of follow-up. There is only one significant result and that is the effect of multivitamins on the change in hemoglobin over the duration of follow-up. The p-value for this comparison is <0.0001, and therefore, even if we used the Bonferroni correction, this result will be significant. We have changed the p-value in the table to reflect precision to four decimal values.

3. The authors state the results of a subgroup analysis of weight gain in the youngest age group as their main finding, but this analysis was not stated in the protocol as a pre-specified analysis and the result should be interpreted as such and may only be hypothesis-generating.

We agree and have taken care to emphasize in the manuscript that this is the result of a small subgroup analysis. Further, we did not include it in the conclusions section of the abstract.
4. It is not clear from the introduction that the aim was to study HIV-infected as well as HIV-uninfected individuals. Both groups are mentioned in the trial registration, but they may not be comparable, and the evidence on this matter should be referred to as well as discussed further.

We intended to study both HIV-infected and HIV-uninfected children as part of this trial. We agree that the two groups may not be comparable and therefore, have presented results in both the groups separately.

5. What were the considerations on choice of content of micronutrients as well as dose? The authors only state why zinc and iron were not added, but more details on the choice of interventions should be provided. Why was vitamin D not included? There is a long history of vitamin D treatment for TB and recent epidemiological and laboratory data suggest an effect on MTB infection.

The major nutrients that were not included in the supplement and are not referred to in the manuscript are vitamins A and D. Vitamin A was not included as all children received periodic vitamin A supplementation as recommended by the Tanzanian Ministry of Health once they reached six months of age.

Re: vitamin D, the field activities of the trial started in the summer of 2005. This was before the explosion of vitamin D research in this area that has happened in the last five years, particularly after P.T. Liu and others’ Science paper in 2006. Further, we are of the opinion that more research – both observational and from dose-response studies – is needed to define the optimal dose and duration for a vitamin D supplement that can be used in such studies, particularly for the age-group in this trial.

6. How many of the children had microbiologically confirmed TB?

We had results for culture of sputum or gastric aspirate for 146 children – only 3 of them had confirmed tuberculosis. We have added this to the results section.

7. What is the reason for the skewed distribution of HIV infected and did this have any effect on outcomes?

We analyzed the effect of the supplement in both HIV-infected and HIV-uninfected groups separately and the effect on hemoglobin levels was consistent across the two groups. Significant effect on other outcomes was mostly in sub-group analyses and as you mentioned, can only be hypothesis-generating.

8. The estimated sample size was not met, the authors should state what difference in the primary outcome would be possible to show with the sample reached.

Our original power calculations had assumed a 10% loss to follow-up, leaving 180 patients in both the treatment groups; a mean weight gain of 0.65 kg (SD=0.60) in the placebo arm; and used a two-sided test with a 0.05 level of
significance. If we had reached the planned sample size, we would have had adequate power to detect a 27.4% higher weight gain in the multivitamin group (0.83 kg). This is stated in the statistical analysis section in the Methods in the manuscript.

In the trial, a median weight gain of 0.84 kg was observed in both arms.

9. In the trial profile or in the text please add more information on how many patients participated in all follow-up visits, and how many were missing one or more visits/information on primary outcomes. How did you deal with missing information?

We have included all the follow-up numbers in the tables and not repeated these in the text. For example, 237 children had information on weight, the primary endpoint, at the end of the study and were included in the analysis. We used a complete case approach for the primary outcomes in this manuscript.

10. In the conclusion it should be stated specifically that there was no effect on weight, or that the study was not able to assess the primary outcome sufficiently.

We have clarified this.

Minor comments:

1. It is not clear whether "HIV infection" covers only HIV-1 (supposedly since in TZ) or HIV1 and/or 2. Were there any dual infected patients?

HIV-infection refers to HIV-1 in the manuscript and we have now clarified this.

2. There is available evidence from adults which should be described, eg. the recently published major similar (and larger) trial in adults from Malawi (INT J TUBERC LUNG DIS 11(8):854–859)

This trial doesn’t describe the effect on weight or hemoglobin; instead we have added another recent trial from Tanzania, which examined the effect of micronutrient supplementation on weight gain at the end of the intensive phase of TB treatment.

3. It would be relevant to add information about the prevalence of micronutrient deficiencies in the study area.

We are not aware of any published papers examining the deficiencies of vitamins B-complex, C, and E in children with tuberculosis in sub-Saharan Africa that we can compare our study population to.

4. Was the success of the blinding evaluated? If not done already, we recommend that the authors fill in the CONSORT check list.

We assessed compliance with the study regimen using a combination of direct questioning and capsule counting. Self-reported compliance was very high with more than 98% of the children’s guardians claiming that no dose was missed in the past four weeks.
We have indeed filled out the CONSORT check list – and the CONSORT diagram is the basis for Figure 1 included in the manuscript.

5. Did the authors conduct the analyses by sex? There are important sex-differences with regard to TB and HIV – and in the response to micronutrients – therefore the main outcomes should be analysed by sex. It should be stated in the results section that all analyses were done separately for each sex, and important findings should also be presented.

We examined sex as an effect modifier in all analyses and did not find any significant interactions with the treatment regimen. However, in the analysis with change in hemoglobin as the outcome, the effect of multivitamins was not significant in female children with tuberculosis (p=0.11). We have added this to the results and the discussion sections.

6. The number of tables is high and they all report multiple outcomes. Table 4 might be omitted and the results mentioned in the text.

We have removed Table 4 and just refer to the results in the text.

Reviewer 4: Paul Kelly

Reviewer’s report:

Major compulsory revisions

1. It is not stated what the ‘standard anti-TB treatment’ was. Presumably it contains isoniazid. I am concerned that the children allocated to placebo were being denied standard care in that they were not given pyridoxine and presumably some of them developed isoniazid-related neuropathy. This must be discussed and justified.

Dear Dr. Kelly,

Thank you for your review of this manuscript.

All patients participating in this study were treated according to the guidelines of the National Tuberculosis and Leprosy program of Tanzania (NTLP). The guidelines at the time of the trial recommended a six-month course of anti-tuberculous drugs (Isoniazid 50 mg, Rifampicin 200 mg, Ethambutol 10-15 mg/kg, and Pyrazinamide 20-30 mg/kg daily for 2 months, followed by Isoniazid 50 mg and Rifampicin 200 mg daily for 4 months) using DirectlyObserved Therapy (DOT). Therapy with anti-TB drugs was provided under direct supervision of the DOT providers in the health clinics and the study staff maintained a close liaison with the NTLP officials and DOT providers to ensure that all the enrolled participants received the therapy as recommended. We have added this information to the methods section.

The NTLP guidelines did not recommend routine vitamin B6 treatment along with anti-tuberculous therapy. However, vitamin B6 was provided in suspected cases
of peripheral neuropathy.

2 It is not clear what happened to the 192 excluded children. It would be helpful if the first paragraph on study design and population (p6, lines 12-22) could be re-worked to clarify what the inclusion and exclusion criteria were. Presumably the ineligible children did not have any features of TB, but this should be made more explicit.

We have made the inclusion criteria more explicit in the Methods section.

3 The problem of multiple comparisons is prominent in this paper. Just using the tables, there are 53 comparisons of active vs placebo using statistical tests, and there are more if you include comparisons based on age groups. Of 53 comparisons, 2-3 would be expected to have P<0.05 even if drawn from a random numbers dataset. The authors are therefore insufficiently discriminating about the inferences they make on the basis of some borderline P values, and should weight those sub-group analyses they performed in favour of comparisons which were pre-specified or have highly significant P values. Were all the sub-group analyses on p11 pre-specified? I think the statements on albumin (p13, lines 18-22) should be deleted, and the same applies to the albumin results on p14. The first paragraph of the Discussion needs to be re-worked to remove the undue emphasis on sub-group analyses or borderline significance.

As mentioned in the response to Dr. Wejse’s question #2 (Major comments), there are only 10 comparisons of active vs. placebo that we are presenting in Tables 2 and 3. Instead of just presenting the outcome, i.e. change in weight or hemoglobin for example, we have also given a snapshot of how the children progressed during follow-up at different time points. Considering that the p-value for the only significant result in those tables – viz. change in hemoglobin – is 0.0001, even if we used a correction factor, it will still remain statistically significant. We have tried to project results from subgroup analyses as hypothesis generating rather than conclusive, especially considering that this whole trial was conceptualized and conducted as a proof of concept study as very little is known about tuberculosis and safety of vitamin supplements in this age group.

We have reworked the first paragraph of the Discussion section and also deleted the statements about albumin, as per your suggestion.

Minor compulsory revisions

4 The inclusion of hypoalbuminaemia in the Discussion appears unwarranted – the mechanism of hypoalbuminaemia in liver disease is complex. Given the comments above, this should be removed.

Done.

5 I am concerned that there are errors in the references and some names should be checked.
We have rechecked the references to ensure that they are correct.

6 In Table 2, what does “Triceps” refer to? Is it triceps skin fold thickness?
Yes, and we have clarified this in the table.

Discretionary revisions

7 No attempt was apparently made to improve diagnostic accuracy using gastric lavage. This is commonly done in children. I would like to see some discussion and justification why this was not done.

We were able to obtain gastric aspirate samples in 145 children for culture. However, only 3 of the children had mycobacteria isolated in the culture.