Author's response to reviews

Title: Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial

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Author's response to reviews: see over
Dear Dr. Lee,

**Re: Responses to reviewers of ‘Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial’**

Thank you for the opportunity to respond to the reviewers of our manuscript. I am pleased that you found two highly qualified reviewers for this work. They have made a number of useful suggestions which have improved the manuscript. Our detailed responses are below. We have also followed editorial requests to include the trial registration date and to move sections on Competing Interests, Author Contributions, and Acknowledgements to just after the conclusion.

I am not clear whether you wanted revisions to the manuscript highlighted since there were also instructions to make detailed formatting changes according to journal style which presumably does not involve highlighted text. I have therefore uploaded the non-highlighted version but can provide the highlighted one if needed.

Please let us know if you require further information.

Sincerely,

Suzanne Filteau

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**Reviewer #1, Sera Young**

Thank you for the opportunity to read this well written manuscript based on a rigorous clinical study.

The low rate of adherence to supplement should be included in the abstract.

*We have included this.*

Do you have a sense of baseline micronutrient levels, in order to understand if there was a potential to benefit from micronutrient supplementation?

*We do not have baseline measures of micronutrient status, only haemoglobin which was very low but that could reflect the patients’ severe infection. We have conducted considerable previous work at both trial sites which suggests that micronutrient status is low even in the absence of HIV infection. For example, Lusaka women have low plasma levels of some micronutrients (Gitau et al., Pub Health Nutr, 8: 837-843); micronutrient status of young Zambian children improved following provision of a multimicronutrient-fortified food (Gibson et al. J Nutr 2011; 141: 935-943); and multiple micronutrient supplements improved health of Mwanza tuberculosis patients (PrayGod et al., J Nutr 2011; 141: 685-691). We have included mention of this and the references in Methods under Setting. Furthermore, there is evidence (cited Cochrane review by Irlam et al., 2010) that HIV patients may require additional micronutrients and can benefit from supplementation.*

How long did the recruitment stage typically last?
The time taken for consent was very short (1-2 days). Total recruitment for those completing the trial was a median of 15 weeks, that is, a median of 3 weeks between referral for ART and starting ART plus 12 weeks of ART. This has been clarified in the second paragraph of Results.

The readers really need more information about adherence—how assessed, distribution of supplement consumption, how 75% was determined as cut-off for adherence/compliance. I assume predictors of adherence will be explored in a follow-on paper, but we really need to know more about the distribution. Further analyses would not be uncalled for either, e.g. did you see “dose response” in mortality, CD4 count when greater number of categories of supplement uptake were regarded (instead of just dichotomous)?

We have added mention in Methods under Adherence that patients were given sachets at each visit to last to the next visit. Median adherence was 66%. 75% was pre-specified as a level where we would expect benefit from the supplement without leaving too small a sample.

Although we agree that studying predictors of adherence might be interesting, we did not conduct these or other extensive analyses, nor are we planning any, because we are aware of the limitations of our adherence estimates. Estimates were based on returned sachets and patients often forgot to return empty ones or returned a lot of empty ones several visits later. In consequence, we believe we have actually underestimated adherence. As mentioned, we did a sensitivity analysis for patients who had died but this did not affect our results.

How was the caloric composition of the LNS determined?
This was calculated by the manufacturer based on the ingredients included and using energy content from their ingredient database. These energy contents are based on Atwater energy values, i.e. 17 kJ/g (4.0 kcal/g) for protein, 37 kJ/g (9.0 kcal/g) for fat and 17 kJ/g (4.0 kcal/g) for carbohydrates.

Another potential impact of supplementation could be adherence to ART regimen. Was there any measurement of ART adherence, or even viral load?
Adherence to ART was very high and not different between groups. This information has been added to results, right after mention of the poor adherence to supplementation. We did not measure viral load, in part because it is not used diagnostically at the sites and in part because of financial constraints.

The second paragraph of the introduction is very information dense—and quite informative. But dividing into several paragraphs with clear topic sentences for each would make it much more informative. In that same passage, a further explanatory sentence should come after the sentence ending in “: young children.”

We have divided the long paragraph into three.

We believe that the sentences already following the one ending ‘young children’ are the further explanation requested.

Hypothesis should be stated at end of intro.
Done

Insert comma on p 13 after “for the whole study cohort”
Done

On Table 1, if the denominator is constant across rows, the n’s can be dropped in each column. It distracts from interpretation of results.
We do not understand this comment since Table 1 is the diet description and has no n values.

On Table 2, were there any significant differences? If not, indicate in a footnote. Since significance testing is designed to determine the probability that a difference occurred by chance, we believe it is inappropriate for the baseline description of patients in a well-conducted randomised controlled trial for which chance can be the only reason for any apparent differences. We believe that there is a consensus among statisticians on this point.

Table 3, the n’s for women and men do not sum to 1815, as they do across other sub-category analyses. Many thanks for spotting an important typo. The number of men in the LNS-VM group has been amended to 471.

In Figure 3, include footnote about cutoffs. Cut-offs, with a reference, have been added to the footnote.

Why was enrollment stopped early? (p 12) The reasons were described in Methods under Sample Size Justification. The DSMB conducted a planned interim analysis and recommended stopping recruitment. They based this recommendation on the fact that the mortality was higher than expected so we already had power — this was obviously know by the research team — and – unknown to the research team – the likely lack of effect of the intervention on mortality.

In that same paragraph, it isn’t clear to me why the difficulties of following up very ill patients for long term care would contribute to low compliance with the supplement. The participants needed to attend follow-up visits both to receive their supplements and to return sachets for compliance estimation; this has been clarified in the paragraph mentioned.

Was substitution of LNS for other foods that would have normally been consumed assessed? Were there any 24 h recall dietary data collected? This is an important concern in nutritional intervention studies, but data are difficult to capture. We felt that detailed dietary assessment using, for example, 24 hour recalls, was too time-consuming for an RCT with a mortality primary outcome and would detract from staff’s efforts to recruit and follow patients to get data on the primary outcome. Furthermore, it is very difficult to get accurate or precise estimates of energy intake or intake of staples likely to be displaced by LNS in free-living adults. We did collect dietary diversity measures on a subset, mainly to have some record of usual diets at the sites, but these are unable to capture information about amounts of foods which might be displaced by LNS.

Was change in appetite during the study assessed? Appetite was assessed at several time points during the study using two methods. Detailed analysis showing that a questionnaire appeared more valid than a porridge consumption test among Tanzanians and that neither technique appeared valid among Zambians is published in ‘Rehman AM et al. Appetite testing in HIV-infected African adults recovering from malnutrition and given antiretroviral therapy (ART). Pub Health Nutr, 2014; doi.org/10.1017/S1368980014000718.’ We have also submitted a paper regarding effects of treatment arm on appetite and anthropometry elsewhere, but do not feel the information adds much to this paper concerned with mortality.

Reviewer #2, Wafaie Fawzi
Reviewer’s report: The paper reports the findings of a randomized controlled trial to examine the effect of nutritional interventions among undernourished individuals who were ART-naïve and
beginning to be initiated on ART. The trial addresses an important research question given the high mortality among patients with low BMI.

- The Introduction focuses primarily on the role of macronutrients in the context of ART and HIV/AIDS management. But the trial is in essence examining the additional effect of micronutrients vs not on top of a base that includes varying quantities of macronutrients. The rationale for examining the efficacy of micronutrients is not clearly stated. We have added to the last paragraph of Background a statement supporting the rationale for looking at micronutrients. In addition, the hypothesis statement requested by Reviewer #1 reinforces this rationale.

- It would be good to report that duration that each of the two nutritional regimen/phases of the trial (mean, Median, standard deviation). We have added this information to the Results section.

- It would be important to present the results of the two phases of the trial, recognizing there may limited statistical power to examine them separately. Thus, the results could be presented comparing mortality and outcomes in the two treatment arms from recruitment until 2 weeks post-ART; and separately from 2 weeks of ART when the second nutrition regimen was introduced until the end of follow up. We hesitate to over-analyse the data by subdividing the mortality analysis at too many different time points. The reviewer also asks below for the analysis to be divided into pre- and post-ART. We believe the latter time division is more informative for most readers and so have elected to do only that one. We have added the overall pre- and post-ART mortality and the rate ratios for effects of the supplements to Table 3 and mentioned the lack of effect of the intervention in the Results.

- Magnesium levels were reported to have been examined in a subset of specimens but the results were not of clinical interest with the other data. The reason for exclusion of the results is not clear, and the results may be of interest to some readers. There were two problems with the magnesium analyses. First, when the Optima machines were experiencing problems, which they did at both sites, the alternative local labs we used could provide potassium and phosphate results but not magnesium results. Secondly, when the Optimas were working, both Lusaka and Mwanza labs routinely got values for the external QC which agreed between our two labs but which were both slightly below the expected values provided by the QC manufacturer (Seronorm). We have no explanation for this and decided we should not use the results, citing merely technical reasons.

- The findings from secondary analyses related to phosphate levels are not clearly presented. It is not clear what the significance of the findings in Figure 3 is. Although the statistics related to Figure 3 are provided in Table 4, we felt these results were important enough to merit the clear visual demonstration of increased high plasma electrolytes provided by the KM curves in Figure 3. As mentioned in the Discussion, high plasma electrolytes carry some risk, may have limited the ability of the intervention to decrease mortality, and should not be used in other similar interventions for malnourished HIV patients. We have tried to be clear throughout that, since there is no DAIDS criterion for elevated phosphate, we have looked at any values over the normal range whereas for high potassium we have analysed both according to the DAIDS criterion and for any over the normal range.

- Low adherence is a concern as the authors have noted. Could there have been similarly low adherence to ART? It would be good to report that as it may also explain the high mortality noted in the study population. It is also important to discuss the generalizability of the findings – in essence the trial did not test the effect of the interventions in a large part given the low adherence.
As discussed above in response to reviewer #1, adherence to ART was remarkably good and thus not a likely cause of the high mortality. We apologise for omitting this information from the previous version. Also as discussed above, we believe our best estimate is actually an underestimate of compliance with the intervention. How much energy should be provided as LNS for such patients is unknown and we based our amount of similar studies (Ndekha et al, BMJ 2009 and Olsen et al., 2014). Patients may not be able to or wish to comply with the full amount of the intervention which represented about half of daily caloric intake and could result in a monotonous diet. However, it appears consumption of part of the supplement provided was sufficient to increase CD4 count. Brief mention of this has been added to Discussion.

- Mortality was substantially higher than expected as compared to other populations initiating ART in developing settings. The team had assumed mortality rates to be 25 per 100 person-years but they found it be more than 80 per 100 py. It would be helpful to discuss this level in absolute terms regardless of the intervention arms, and how it compares with other populations. Presenting mortality pre-ART and after ART initiation would be helpful. We believe that the KM curve in Figure 2 adequately shows the absolute mortality. We have clarified the periods pre- and post-ART in the footnote to Figure 2. We are investigating risk factors for the high mortality – an important finding – in a separate paper since it involves unplanned analyses separate from the main RCT analyses. We note that, when we recalculate mortality rates per 100-person-years from the reviewer’s own study (Liu et al., JID 2011, 204:282) post-ART mortality rates in the comparable BMI categories are remarkably similar to the rates from NUSTART. This information has been added to the Discussion.

- The discussion related to potassium could be clearer. It appears the authors are saying the dose they used is established to be safe, and actually lower than what they may have considered but the dose used was the maximum the manufacturer could include in a tablet. But then the authors speculate that the amount given ‘appeared to be more than their metabolism could handle’. How was that determined? It would be helpful to present results on potassium levels at baseline; levels as an outcome, and levels as a modifier. Our estimate that the dose was safe was based on the fact that it is lower than 1 RNI but we are aware RNIs apply to healthy people. Our statement that the amount of potassium appeared more than patients could handle is based on our own results of increased high plasma levels. We have added proportion of patients with low baseline potassium, as we did for phosphate, to Table 2 and have provided the mortality analysis stratified by baseline potassium in Table 3.

- The discussion (page 14, para 1) states that the ‘control group received LNS which contained innate vitamins and minerals …”. This point could be made clearer in the Methods section as well. Could the authors clarify what these innate nutrients are and in what quantities. The exact recipe for the study products is proprietary information with Nutriset. Table 1 provides details of levels of the micronutrients which the company measured but we do not have further information. We have added a statement to Methods to clarify that the LNS contained innate vitamins and minerals.

- The Discussion section overall could be better organized. Findings of other trials of vitamins and minerals are worth noting – what they found, how their findings differed or were in agreement with this new trial, and why. There is very little discussion of other trials in the context of HIV/AIDS(pre- and during ART) and their findings. We had decided to save space by referring mainly to the two fairly recent Cochrane reviews of nutritional interventions for people with HIV: Irlam et al. for micronutrients and Grobler et al for macronutrient interventions. However, we have now extended the parts of the Discussion comparing our trial to other related trials.
- The authors refer to ‘fairly high dose of vitamins and minerals’ (Page 12, para 1): please quantify how high.

  We have added that most were at 3 RNI.

- Figure 1 – it appears that 36 patients in one arm and 25 in another were excluded because of BMI>18.5 after randomization but were still followed up. Is that the case? If so, it seems reasonable to include the data in the analyses and report the findings per intent-to-treat principle. It is also fine to present additional results with these data excluded.

  It is correct that some patients were recruited by clinic staff but later excluded once the staff more experienced in anthropometry had found the patients had BMI>18.5 kg/m². However, since these patients were not actually eligible, we determined they did not belong in the trial at all. We feel obliged to follow the analysis plan and exclude these participants.