Author's response to reviews

Title: Gastric and intestinal barrier impairment in tropical enteropathy and HIV: limited impact of micronutrient supplementation during a randomised controlled trial

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Responses to reviewers

We are grateful to the reviewers for their thoughtful comments. We itemise our responses to these comments below and have highlighted the changes in the manuscript.

Reviewer Sturniolo

1 We have amended the title to include HIV status (p1)

2 We have clarified the method of allocation of participants to the different study groups (p4-5). The amount of work described in this paper is very considerable and it simply was not possible to conduct all these analyses at the same time. For logistic reasons these sub-studies had to be staggered. The allocation of participants to the sub-studies was largely at random, but the translocation study does include 18 participants who elected to undergo the permeability testing rather than the endoscopy. However, as these were allocated to micronutrient or placebo supplementation in a fully double-blind manner, we believe that this would have no influence on the results.

3 We have included a reference describing the science behind the sugar testing (ref 37).

4 The reviewer is quite right to criticise our language regarding reversal of hypochlorhydria on anti-retroviral therapy, and we have re-worded this to remove the implication of sequence of events (p7).

5 The statistical difference between groups is shown in Figure 1 (P=0.0005) (p22).

6 We have included the testing for salmonella and shigella in the Methods (p5). Three samples were collected from each participant for this part of the study,
hence the higher number of samples than participants. We hope that the amended text is now clear.

7 We would prefer not to describe permeability test results as ‘normal’ or ‘abnormal’ as in one sense the entire population from which our study cohort was drawn is ‘borderline abnormal’ or ‘abnormal’. Tropical enteropathy is a shift in the population norms compared to industrialised countries, and it is not clear whether it really is an abnormality or if in fact it is the European population which is ‘super-clean’ and therefore predisposed to inflammatory disease (this is the hygiene hypothesis, in a sense). It may be that different ‘norms’ in fact predispose merely to different disease profiles, and the concept of abnormality therefore is potentially misleading.

8 We have provided more details of the histological results (p9).

9 The explanations for the lack of correlation between permeability and translocation are most likely to lie in the inadequacy of lactulose permeation as a marker of barrier dysfunction, a point raised by reviewer 3. The influence of tropical enteropathy is that it may allow translocation by a different pathway. This is speculation, but these are possible explanations. We have attempted to clarify this part of the Discussion (p11).

10 As only one of the reviewers expressed the opinion that these images are redundant, and we are seeking publication in an online-only journal where space is not at a premium, we ask for them to be retained.

Reviewer Friis

1 We have dealt with this under (2) above.

2 The reviewer suggests, in his second paragraph under Major comments, that this is not a genuine randomised controlled trial. We explicitly described this work, right from the earliest sentences of the Abstract, as sub-studies nested within a randomised controlled trial. All participants were randomly allocated to micronutrient or placebo in a fully double-blind manner using eight letters of a randomly generated number sequence, and blinding was maintained throughout. The differences to which the reviewer refers, which are apparent in Table 2, are not unexpected in sub-studies within a randomised controlled trial, and we believe one should not be concerned about a difference of 1-1.5 kg/m² in BMI between the two sub-studies. This was conceived as a randomised controlled trial of micronutrient supplementation and as there were no problems encountered with the technical aspects of randomisation, blinding or determination of endpoints, we do not consider it necessary to treat this as an observational study. The principle of random treatment allocation, which is the principal tool for reducing the effect of confounders, has been adhered to and we can see no reason to doubt the lack of efficacy on primary endpoints. Consequently the lack of effect on primary endpoints should be regarded as the fully valid results of a formal randomised controlled trial. However, we do agree that it is important to make it clear that the data come from studies within a randomised controlled trial and we hope that the new wording of the title (“limited impact of micronutrient supplementation during a randomised controlled trial”) achieves this.
3 We have included a comment on the timing of the measurements (p20). These were measured at several points during the trial (baseline, 6 months, 18 months, at the cross-over, and at the close) and we have reported in the table the results just prior to the part of the trial in which the sub-study was conducted.

4 We have added additional comments on the multivariate analysis (p8). Indeed age and sex were controlled for, as one would usually expect.

5 While it is true that the original trial did include all consenting participants in the community, the reviewer is right that this does not actually apply to this trial. We have altered this comment (p2).

6 The baseline data are shown in Table 2; space does not allow these data to be included in the Abstract.

7 We have reduced the length of the Introduction from 604 to 528 words. While the latest meta-analysis of five trials on diarrhoea incidence (Aggarwal et al, Pediatrics, 2007) does support there being a 14% reduction in incidence of diarrhoea in children receiving zinc supplementation, another recent trial shows no benefit. We did not say anywhere that nutrition is not important to immunity, but we do (and we stand by this) bemoan the paucity of mechanistic data on the interaction of nutrition and immunity. We have re-worded this part of the Introduction (p3) to ensure that we do not give misleading impressions.

8 We have removed the reference to the number of participants in the main trial (p7).

9 We have corrected the number of participants in Table 3, which should have been 38.

Reviewer Mcfie

1 We fully agree that sugar probes are unlikely to reflect fully the changes in intestinal barrier function which are of most interest in understanding bacterial translocation, and they do not reflect changes in colonic permeability at all. However, there are few non-invasive alternatives currently validated, and there is a substantial literature on the use of sugar probes. We have emphasised the limitations of these techniques in the Discussion (p11).

2 We did not employ LPS and anti-LPS measurements as markers of permeability, rather as measures of the translocation which is a consequence of increased permeability. As suggested, we have discussed this in the Discussion (p11).

3 We concluded that “our data are consistent with the hypothesis that bacterial translocation drives immune activation, but at least in Africa this is probably in advanced rather than early HIV infection” both on the basis of the data currently presented taken together with the evidence assembled and presented in the last paragraph of the Discussion. We have removed this sentence (p11).