TO: Dr Hiromichi Kumagai
BMC Nutrition Journal

FROM: Dr. Denise Evans (devans@witshealth.co.za or devans@heroza.org)
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DATE: 10 July 2013

REF: MS 1552731278982454

Dear Hiromichi Kumagai

We have received the journal’s response to our manuscript, “Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy” for publication in BMC Nutrition Journal.

We received the reviewer’s comments and appreciate the opportunity to revise and resubmit the manuscript at this stage. We have been through each comment thoroughly and have responded in the attached letter below. We feel this revision has improved the manuscript and look forward to your positive response. We thank the reviewers for their valuable comments and suggestions.

I will continue to act as corresponding author on this manuscript and all authors have reviewed and approved the final contents as submitted.

This manuscript has not been submitted, nor is it being considered elsewhere, for publication. The authors have declared that no competing interests exist.

We look forward to your response.

Sincerely,

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Author's response to reviews

Title: Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy.

Author: Denise Evans (devans@witshealth.co.za)

Version: 2 Date: 08 July 2013

Reviewer's report

Title: Impact of nutritional supplementation on immune response, body mass index and bioimpedance in HIV-positive patients starting antiretroviral therapy.

Version: 1 Date: 30 May 2013

Reviewer: Andrew Hills

Reviewer's report:

Summary
The paper reports on an interesting but complex area. Poor health status, including malnutrition, compromises immunity to infectious diseases which in turn aggravates malnutrition. The malnutrition-infection interface in the presence of HIV can magnify the situation. Further, HIV/AIDS also drives household vulnerability to food insecurity which is a major contributor to food inadequacy and the inability of many individuals to meet the daily nutrient requirements necessary for an active and healthy life.

Major Compulsory Revisions
The paper provides a reasonable rationale for the study and has also included some of the recent literature in the area, including reference to the group’s protocol paper. However, there are some gaps in the literature cited and also some inconsistencies in respect of terminology and apparent understanding of anthropometry and body composition assessment.

1. Please only use abbreviations after detailing full terminology, for example ART on p.3
   The full terminology for ART was provided in the abstract but has been included again on page 3. Others have also been corrected (i.e. HIV - human immunodeficiency virus (page 3); GI - gastrointestinal (page 6); MCV – mean cell volume (page 6)

2. The authors are inconsistent in their use of many terms, particularly in anthropometry and body composition assessment. For example, in the Introduction reference is made to nutritional status and BMI - BMI is used and mis-used in many contexts - it is simply a ratio of weight and stature and provides no meaningful information regarding an individual's body composition status. Thank you for highlighting this important point – we have been through the manuscript to correct this (for example on page 5 paragraph 3; page 10 paragraph 2).
Similarly, it is inappropriate to reference BIA as 'the single best nutritional predictor of survival.' Further, what is 'cell integrity' and why/how is BIA a marker or measure of cell integrity? Lower phase angles appear to be consistent with either cell death or a breakdown of the cell membrane. Higher phase angles appear to be consistent large quantities of intact cell membranes and body cell mass. Hence the term "cellular integrity"; expressed figuratively, well nourished, "plump" cells with stable membrane potentials have a large phase angle, whereas poorly nourished "withering" cells with low membrane potentials have correspondingly small phase angles.

This sentence has been corrected as follows (page 4 last paragraph) “a low phase angle $\alpha$ (the relationship between two vector components of resistance and reactance) from bioelectrical impedance analysis has been shown to be an independent prognostic marker of clinical progression and survival in HIV-infected patients on ART.”

What is a bioimpedance index? This has been corrected

BIA provides the opportunity to predict body water status and as a function (and assuming a 2-compartment model), predict FFM and FM compartments. Significant work is required to better understand and describe the nature and relevance of body composition changes, including the strengths and limitations of BIA as a body composition technique. The following paragraph has been included in the Introduction (page 4 paragraph 2) “A variety of assessment tools can be used to determine nutritional status; these include anthropometric methods (i.e. skinfold measurement and mid-upper-arm circumference), BMI and bioelectrical impedance analysis (BIA). BIA allows researchers to determine the specific component of weight loss (fat-free mass [FFM], body cell mass [BCM] or fat mass [FM]) that is biologically relevant to the development of adverse outcomes. BCM refers to the cellular and metabolically active components of the body, including muscles, solid organs and intracellular water while FFM includes all the components of BCM plus additional components that are not metabolically active (extracellular water and tissue support)\textsuperscript{20}. BIA is a rapid, affordable, non-invasive method for measuring changes in body composition and by doing so can predict poor prognosis in HIV-infected patients\textsuperscript{20}. For example a low phase angle $\alpha$ (the relationship between two vector components of resistance and reactance) from bioelectrical impedance analysis has been shown to be an independent prognostic marker of clinical progression and survival in HIV-infected patients on ART\textsuperscript{21,22}. Lower phase angles appear to be consistent with either cell death or a breakdown of the cell membrane whereas higher phase angles appear to be consistent large quantities of intact cell membranes and body cell mass. A study amongst 539 HIV-positive adults with pulmonary tuberculosis adults from Kampala Uganda showed lower BMI, lower ICW:ECW, lower phase angle $\alpha$ and loss of body cell mass (BCM) and fat mass were associated with more advanced HIV infection (CD4+ lymphocyte count < 200 cells/mm3)\textsuperscript{20}. Depletion of BCM has been associated with an increased risk of death among adults with HIV infection. BIA is a good tool for detecting body cell mass loss in HIV wasting and compares favorably with gold standard methods (total body potassium and dual energy x-ray absorptiometry) however its use has been limited by the lack of standardized
methods and quality control procedures. Furthermore certain conditions must exist for measurements, for example, subjects should not have exercised or consumed alcohol within 8 - 12 hours of the test, subjects should not be moist from sweat or lotion and should not have a fever or be in shock”

3. BIA measures were not completed on pregnant women - why? The RJL Body Composition 2.1. User’s Guide states “Women who use this device should not be pregnant or suspected of being pregnant. Besides increased inaccuracy of readings in this condition, the effects of measurement on the fetus are unknown” (page 5; http://www.rjlsystems.com/pdf-files/bc-manual.pdf). Therefore, if a woman was already pregnant at baseline (study entry) they were excluded from the study whereas if a women became pregnant on study she was only excluded from the BIA analysis (page 6 paragraph 3).

4. On page 5 authors provide a list of anthropometric measures - most are not anthropometric measures. Thank you for highlighting this – the sentence has been corrected as follows (page 7 paragraph 3): “Height and weight together with bioelectrical impedance was collected for each patient at each monthly visit for 6 months. BMI, body fat percentage (% fat), FFM, lean dry mass (LDM), total body water (TBW), intra- and extra-cellular water (ICW, ECW), phase angle α, basal metabolic rate (BMR; measure of resting energy expenditure) and daily energy expenditure (DEE) were estimated using the RJL Body Composition 2.1 software from the resistance, reactance and patient details that were recorded (i.e. gender, height, weight, age, daily activity level and body frame size).”

5. In order to achieve the maximum benefit of ARV treatment, adequate nutrition is essential. Whist some reference is made to dietary intake this is inadequate. For example, it is common for HIV-infected individuals to have reduced food intake due to the effect of the HIV infection and this is compounded by secondary opportunistic infection. This can have a marked effect on appetite which leads to further reductions in food intake and consequent weight loss. Any comments? Thank you for this point – we have added the following paragraph to the Introduction (page 3 paragraph 2): “Adequate diet is believed to be important for adherence to antiretroviral therapy (ART) as inadequate nutrient intake is known to favour opportunistic infections and contributes to wasting. Individuals with HIV/AIDS require greater protein and micronutrient intake to support a weakened immune system4 however they are also more vulnerable to malnutrition since they may have impaired nutrient absorption (due to diarrhoea/intestinal tract damage), reduced food intake (due to symptoms such as vomiting or pain on swallowing), food insecurity and medication side effects such as loss of appetite, depression or abdominal pain5. HIV and malnutrition may compound or cause severe immuno-deficiency which ultimately increases susceptibility to opportunistic infections3. Opportunistic infections can affect food intake, absorption and metabolism and so cause weight loss. Hence a cycle of infection, malnutrition and immuno-deficiency has been described6.”

6. Reference is made to the importance of understanding physical activity and energy expenditure yet there is no clear indication of the measure of physical
activity used! The following was added (page 7 paragraph 4): “Physical activity was assessed at enrolment, 3 months and again at 6 months using a physical activity recall questionnaire. This included 16 multiple choice questions to assess an individual’s level of activity and calculate a physical activity score. In addition a 5 day physical activity recall diary using activity codes25 and pedometer log were used to calculate calorie expenditure and verify physical activity level (PAL)30.”

Further, reference is made to RMR - was it measured, predicted and how was this undertaken? BMR was obtained from BIA using a Quantum BIA analyzer. The following was added: “Height and weight together with bioelectrical impedance was collected for each patient at each monthly visit for 6 months. BMI, body fat percentage (% fat), FFM, lean dry mass (LDM), total body water (TBW), intra- and extra-cellular water (ICW, ECW), phase angle \( \alpha \), basal metabolic rate (BMR; measure of resting energy expenditure) and daily energy expenditure (DEE) were estimated using the RJL Body Composition 2.1 software from the resistance, reactance and patient details that were recorded (i.e. gender, height, weight, age, daily activity level and body frame size).”

7. Greater attention is needed to highlight the key findings at the front end of the Discussion. In too many places throughout the text the presentation of material could be improved. The following text has been included at the beginning of the Discussion (page 11 last paragraph) “We demonstrate that nutritional supplementation taken concurrently with ART for 6 months resulted in an increase in BMI, CD4 count, haemoglobin, red blood cell and white blood cell count and improvement in physical activity when compared to Controls. In addition, serum ferritin was the only biochemical marker that was significantly different between the two arms. Patients in the NS arm showed an increase in FFM, TBW, ICW, ECW and BMR when compared to Controls. Phase angle \( \alpha \) was also higher in patients in the NS arm (26% vs. -1.5%; \( p=0.063 \)) which may suggest improved health in these patients which may result in improved treatment outcomes. By 12 months after ART initiation patients in the NS arm continued to show a significant increase in BMI but not CD4 count when compared to the Control arm. The greatest gain in CD4 count and improvement in physical activity was observed in patients in the NS arm with a CD4 count <100 cells/mm\(^3\) at study entry.”

8. Table 2 references a discrepancy in numbers with intervention and control groups identified as 19 and 19, respectively then completers and non-completers, 26 and 12, respectively. This has been checked. To clarify, 45 patients were screened and 38 patients were randomized (19 nutritional supplement/NS arm and 19 Control arm). 26 patients completed the 6 month follow-up visit (completers) while 12 patients did not complete the 6 month follow-up (non-completers). The Table heading has been changed to reflect this (Table 2) “Patient demographics and baseline clinical characteristics of those that completed (completers) and those that did not complete the 6 month follow-up (non-completers) were compared.”

Table 3 indicates that using BMI, participants were not malnourished using the stated criterion of (<18 kg/m\(^2\)). This is correct. The initial protocol included BMI < 18.5kg/m\(^2\) as one of the
Inclusion criteria however these patients were often very sick and were subsequently hospitalized within a few days of initiating ART. This inclusion criteria was amended so that any patient with a self-reported unintentional weight loss (defined as a drop in 2 dress sizes or loss of 5 – 10% of normal body weight in the last 3 months) were included in the study – regardless of BMI. This has been included in the study limitation and the following sentence has also been added [page 15 paragraph 2] “Our findings are not only applicable to malnourished HIV-positive patients (less than 30% of our patients had a BMI < 18.5kg/m²) but also HIV-positive patients that present at ART initiation with self-reported weight loss.”

Table 4 includes a good deal of the material presented in the previous Table. Suggest a significant rationalisation of Tables. Thank you for this suggestion – however since Table 4 represents the data stratified by CD4 count (< 100 vs. > 100 cells/mm³) we would like to present these values (median and IQR for baseline, 6 months and % change) separately.

9. Major changes are required in the interpretation and representation of body composition findings. For example, what is the reason for utilising multiple staff to take responsibility for measurements and further, multiple pieces of some equipment. This study was conducted in a clinic setting where staff members share study responsibilities and may work on multiple studies (i.e. 2 nurses may be assigned to 1 study to take the BIA measurements). Staff may also leave during the course of an ongoing study so new staff have to be recruited and trained to take over those responsibilities. The following sentence has been added [page 15 last paragraph] “We limited this by training experienced nurses and study coordinators to administer questionnaires, measure height, weight, wrist circumference and to record the BIA. Study staff were trained using standardized procedures and measurements were repeated twice and the average used for analysis.”

No mention is made of the reliability of measures and testers. We used two BIA devices (the RJL Quantum X analyzer and the Omron BF400 body fat monitor) - this is a scientifically validated procedure with over 2000 published research studies that have used this technology. For both devices we trained experienced study staff using standardized procedures and measurements were repeated twice and the average used for analysis. [page 15 last paragraph] We compared the measurements from each device (i.e. % fat, BMI, weight) for accuracy. Patient files were also checked regularly for discrepancies.

Why compare 2 BIA devices - RJL and Omron? This sentence has been removed since we only present the results from the RJL Quantum X analyzer and do not present or compare any results from the Omron BF400 body fat monitor in this manuscript [page 15 last paragraph].

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** 'I declare that I have no competing interests'
Reviewer's report

Title: Impact of nutritional supplementation on immune response, body mass index and bioimpedance in HIV-positive patients starting antiretroviral therapy.

Version: 1 Date: 29 May 2013

Reviewer: Maria Cristina Cristina Marazzi

Reviewer's report:

This is a very simple and interesting paper on the impact of nutritional supplementation on People Living with HIV/AIDS who are receiving HAART. The study I well designed and clearly described. Some weakness that can be improved are in the results and discussion sections

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

a. The limited number of patients involved in the study represents a limit that should be highlighted in the discussion. Many of the results can be considered more suggestions for further study than evidences because a sample of 19 patients against 19 is really small.

Thank you for this point – We have included the following sentence under the Strengths and Limitations (page 15 paragraph 2; page 16 paragraph 1): “Fourth, a limitation of the study was the relatively small sample size. Consistent with other pilot studies, wider investigation with a larger sample size, increased duration of intervention and longer follow-up to determine the impact on patient treatment outcomes such as mortality is recommended”.

b. The authors stated in the conclusion that the described intervention is “feasible and affordable”. In the text there is nothing neither about the cost effectiveness of the intervention nor about the Incremental cost per DALY (and it is understandable because of the limited number of patients). What do they mean with “feasible and affordable”? They could conclude the intervention appears to be effective bearing in mind the limits of the study. otherwise they should explain clearly what they mean for “feasible and affordable” and what are the preliminary data on which they seem to base their statement.

Thank you for this point – we have removed this sentence (page 16 paragraph 1) since we did not evaluate the cost effectiveness of the intervention. Cobb and Bland however studied the cost of managing children infected with HIV in resource-constrained settings using the same supplement (FutureLife-porridge®) and reported that providing a nutritional supplement cost an additional 11.6% to that of supplying 26-week ART to the same children. Their study illustrates the low proportional costs of procuring FutureLife-porridge® for HIV-infected children.

1. Is the question posed by the authors new and well defined? Yes
2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work? Yes
3. Are the data sound and well controlled? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? Not completely
6. Do the title and abstract accurately convey what has been found? Yes
7. Is the writing acceptable? Yes

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interest