Author's response to reviews

Title: Body composition among Sri Lankan infants by 18O dilution method and the validity of anthropometric equations to predict body fat against 18O dilution

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Author's response to reviews: see over
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Dr. Andreas Nydegger  
Editor  
BMC Pediatrics  

Dear Dr. Nydegger:

Thank you very much for the opportunity to respond to the constructive comments of the two reviewers.

Our responses to their comments are as follows:

    **Responses to Reviewer's reports**

**Reviewer:** Philippe Steenhout  
**Responses to reviewer's report:**

Line 81 we would appreciate to have more information on how the « infants with chronic anomalies, chronic diseases and any illnesses » were excluded. Was by example, some biological tests done to excluded those with anemia?

> The paragraph has been modified as follows: Infants with any congenital anomalies, diseases or any illnesses were excluded based on the infant’s previous medical histories, drug treatments and medical tests performed since birth and on the medical examination by the physician. No additional biological tests were done to exclude infants with anemia.”

Concerning the statistical part of this report:

Line 79 it is mentioned that 25 infants were enrolled. On which statistical basis was this number selected? Was this number sufficient to obtain a reasonable statistical power for the results?

> The sentence has been modified as follows: Due to difficulties of enrolling infants to take part in the very first stable isotope study in Sri Lanka, we adopted the purposive and convenient sampling technique without sample size consideration for this cross-sectional study.

Line 160 The birth weight reported in 2.9 (2.0 - 4.0) kg and obviously then some of this infants must be considered as SGA. Was it an influence of the birth weight on the Fat Mass results at 4.5 months?

> The sentence has been modified and two new sentences have been added as follows: Mean birth weight was 2.9 kg (0.6) with two girls having birth weights at 2.0 kg and 2.1 kg, respectively. These two baby girls were enrolled into the study because at the time of recruitment, they were 5.5 months and 4.25 months of age, respectively. Both of their
body weights were above 6 kg at the time of recruitment suggesting satisfactory weight gain.

We would suggest to express also those values in term of Z-score at birth

Table 1 was modified to include infants’ weight-for-age and length-for-age z scores.

Concerning the discussion:

Line 245 In reference to Bandana equation specifically developed for infants of developing countries, how to explain that Fat Mass is always underestimated in infants of developing countries. Is it due to the protein consumption in those population?

The Bandana equation was developed on Indian infants. They recommended it for infants in developing countries. However, we think that the underestimation probably is due to genetic variations rather than high protein consumption because populations in developing countries usually consume low protein diets due to poor economical backgrounds. It is also possible that infants in developing countries might have more total body fat but less subcutaneous fat thus leading to the consistent underestimation when using skinfold equations. More sensitive instrumentation is needed to distinguish subcutaneous fat from internal body fat which is beyond the scope of this paper. Therefore, the manuscript has not been revised in response to this comment.
Reviewer's report 2

Reviewer: Jean-Philippe Godin

Responses to Reviewer's report:
The major compulsory revision is about Table 4, and its description in the “results” and “discussion” sections. The reviewer would strongly suggest working on the reporting of the statistical analyses supporting the results, discussions. It means better describe the bias (systematic bias, yes or no, is it significantly different from 0) and to clarify the interpretation (see next comments).

Kindly refer to our responses in the following comments.

Minor essential revisions:
Page 2: Line 40; Please could you replace “accuracy” by “interchangeability of methods or agreements” or rewrite the sentence using “interchangeable methods”. The accuracy should be reserved for method validation when know isotopic materials are measured and compared to their reference isotopic values.

The word “accuracy” has been replaced with “agreement” throughout the text wherever applicable.

Page 4: Would it possible for the authors to add more information about the protocol regarding the fluids intake of the infants during the equilibration period of the isotope (from the injection to the first urine collection), did the authors have recorded the volume of fluid intake and if yes, how did they take into account this volume? Or did they weight the infants during this period? Is there any precaution due to the climate that may affect the administration, fluids losses (as sweet), that would need to be taking into account?

A new sentence has been added at the end of the Calculations section as follows: The back extrapolation method rather than the equilibration method was used in this study because it normalized the effects of fluid intake, metabolic water, or fluid losses through urination, respiration and sweet on the body water compartment.

Page 5: For infants, there is a significant variability in peer-reviewed papers about the timing for collecting post-doses urine sample (from 3h to few days). Could the authors add a comment about their choice?

We agree with the reviewer that researchers have collected urine at 3, 4 or 5 h post dose. We collected 2 post dose samples (5h and on day 3) and used the back extrapolation method as it is considered more accurate for infants and young children. Please see our response above.

Page 5: Line 107; could the author add the name of the reference materials used and at which levels they studied the accuracy and reproducibility?
The following sentences and a new reference have been added at the end of the paragraph under *Analysis of the 18O enrichment* section of the revised manuscript: These reference materials included the Vienna-Standard Mean Ocean Water, the Vienna-Standard Light Antarctic Precipitation, and the reference water samples enriched with $^{18}$O (IAEA 304A and 304B) (Wong WW, Klein PD, Parr RM, Clements SA. Interlaboratory analysis of reference water samples enriched with deuterium and oxygen-18. Appl Radiat Isot 1993, 44: 561-566.). The $^{18}$O content of these reference materials ranged from natural abundance to 500 ‰.

Page 6, line 125: the hydration factor of lean tissue is effectively well documented in infants based on the papers mentioned by the authors. Did the authors assess the “hydration status” of the infants using clinical observations? Or are they some specific recommendations for future studies in infants?

Unfortunately, the study was not designed to evaluate the hydration status of the infants. Since the percentage of total body water relative to body weight as shown in Table 2 among the Sri Lankan infants was well within the normal ranges reported among well-nourished infants in industrialized countries, we were not concerned about the hydration status of these infants. Since the study was not designed specially to determine hydration status, we are not in the position to provide any specific recommendations for future studies in infants.

Page 7, line 42: Replace “annexure” by “annexe”

Suggested correction done.

Page 7: The reviewer would recommend adding a sentence mentioning that the first check was to assess that there is no systematic bias. This was performed by checking the plot of differences and visually checked that they were well distributed around zero. If this is not the case, there is a bias (a systematic or not) and this was assessed by studying the regression (with the slope and intercept).

The paragraph has been modified as follows: The Bland and Altman pair-wise comparison method [24] was used to evaluate the agreement between the % FM predicted using the anthropometric equations and the % FM measured using the $^{18}$O dilution method as the reference by plotting the differences between the two measurements against the averages of the two measurements to make sure that the differences were well distributed around zero. If not, regression analysis was used to test the differences between the two methods and their average % FM values.
Lines 150-152: Could the authors add/complete their sentences by mentioning the “not significant from 1” for the slope. In addition, the authors did not mention the analysis of the intercept? Could you please justify or explain?

Since we did not compare or plot the %FM predicted using the skinfold equations directly with the %FM measured by the isotope dilution method, we were not testing whether the slope or the intercept of the regression line between these two measurements were significantly different from 1 or zero, respectively. The simple regression analysis between the two measurements would produce a single bias but would not be able to tell whether the differences between the two measurements were constant or changed depending on the size of the measurements. Therefore, the Bland and Altman pair-wise comparison method rather than the simple regression analysis was used to analyze our data. Since we are not using the simple regression analysis to compare the %FM obtained using the two methods, we did not discuss the slope or the intercept of the regression method or test whether the slope or the intercept were significantly different from 1 or zero, respectively. Therefore, we did not make any changes in the revised manuscript to address this comment.

Page 8, statistical analysis:
Lines 148, 151, please could you remove “accuracy” and replace it by “interchangeable methods” as mentioned previously.

Suggested correction done.

Lines 167-168: The differences reported are so huge and un-physiological, that one hypothesis is that the equations reported in the papers cited were wrong! Could the authors check again in other papers that the equations were correct? If there are well reported, could the authors add a warning?

Equations were checked and they are correct. We have removed the unphysiological values from both Table 3 and Table 4 but included them in the text as per suggestion.

Lines 175-179: The authors wrote “For equations with no relationship between…”. The reviewer would strongly suggest clarifying this sentence by mentioning the type of bias (i.e. no bias, or a systematic proportional bias).

The paragraph has been modified as follows: For equations with no relationship (P>0.05) between the differences and the average values, the lower and upper limits of agreement or 95% confidence interval between the two methods (skinfold equations vs. $^{18}$O dilution) are shown as bias – 2SD and bias + 2SD, respectively. For equations showing significant relationship between the differences and the average values, the bias as well as the lower and upper limits of agreement varied depending on the % FM values. Since the minimum and maximum values for % FM among the Sri Lankan infants ranged between 20% and 45%, the limits of agreements for these equations were calculated at 20% and 45% FM for illustrational purposes.
Line 186: In Table 5 and Annexe, the equation and results for boys using the Durnin & Wormsley are not reported. Is it because this equation was only developed for girls? (as far as the reviewer noticed, the reviewer did not find this info in the manuscript, could you please add a sentence about it? 

Yes, the Durnin & Wormsley equation was developed for girls only. The sentence has been modified as follows: The equation by Durnin and Wormsley [22], which was developed specially for girls, also showed no significant relationship between the differences and average values among the female infants.

Lines 192: The reviewer strongly advised to describe the rest of the results reported in Table 4 by adding a paragraph on other comparisons by mentioning their bias.

The footnote of the table has been greatly expanded to clarify the importance of headings for each column. A new paragraph has been added in the Results section to address the results of the other equations as follows: Unfortunately, all the other prediction equations showed significant relationship (P < 0.05) between the differences and the average values indicating that the difference would not remain constant as we have observed with the equations proposed by Bandana, Goran or Durnin & Wormesley but would change depending on the value of %FM. For example, with the Shaikh’s equation for baby boys, the equation would underestimate %FM by 5.49% (bias). However, the regression analysis between the differences and the average values revealed a significant slope (P=0.000). Therefore, at 20% FM, the Shaikh’s equation could either underestimate %FM by 10.11% (LL at 20%) or overestimate %FM value by 16.61% (UL at 20%). At 45% FM, the Shaikh’s equation would underestimate %FM by 58.32% (LL at 45%) or by 31.60% (UL at 45%). Similar interpretations would apply to the other prediction equations showing significant relationship between the differences and the average values.

Lines 250-252: The reviewer would strongly advise the authors to clarify the sentence. Is it a systematic bias, if yes, could the authors add if the bias is significantly or not different from 0. The authors only discussed Bandana et al. equation and what about Goran et al; and Durnin & Wormsley results and the others. Then between Bandana, Durnin and Goran, which one would they advise to use? If the authors cannot conclude, could they add some further recommendation for further studies based on their experiences?

The Results section has been modified and a new paragraph has been added at the end of the section as mentioned above.

Page 10; about the TBW of boys and girls. The reviewer likes the comparison with Fomons and Butte et al. Could the authors add a sentence about these comparisons. It seems that the delta
(TBW between boys and girls are a bit higher in this paper as compared to other published data). Could the authors add a comment (or hypothesis) explaining this offset?

The TBW values of the 4.5-month-old Sri Lankan infants (56.7±2.9% for girls and 61.3±5.9% for boys) were well within the values reported by Fomons (59.6% for girls at 4 months of age and 60.1% for boys at 4 months of age) as we have shown in the manuscript. Our values were higher when compared to the values reported by Butte on 3-month-old USA infants (55.4% for girls and 55.9% for boys). The difference could simply be due to the difference in hydration status between the Sri Lankan infants and the USA infants. The Discussion section has been modified to address this comment as follows: The TBW values of the 4.5-month-old Sri Lankan infants were well within the values reported by Fomons. Our values were higher when compared to the values reported by Butte on the 3-month-old USA infants. The difference could simply be due to the difference in hydration status between the Sri Lankan infants and the USA infants.

Page 11, line 242: when the authors described “mean and SD”, could the authors removed one “%”

   The suggested change done.

Lines 258 and 269: Replace “Third world countries” by “Developing countries”

   The suggested change done.

In Table 2; could you please add more results about 18O isotopic enrichment calculated for the equilibration period and the isotopic enrichments measured at 5hrs and at 3 days.

   The 18O content of the urine samples collected from the infants at baseline and at 5 hours and 3 days post-dose have been added in Table 2.

In Table 3; there are two similar typing errors in page 23: “Prepubertral”

   This was corrected.

In this table, could you please explain and report how did you calculate the BD (body density) used in Durnin & Rahaman and in Brook equations?

   As summarized in the annexe, majority of the published work used skinfold measurements to predict %FM. However, with Durnin & Rahaman and Brook, they used the skinfold measurements to predict body density and then converted body density into %FM. These equations, including the equations to predict body density, are all listed in the annexe.

In Table 4, the reviewer would strongly advise to:
   o Add CI (confidence interval) at 95% of the bias after the “Bias”;

   For the Bland-Altman pair-wise comparison, the 95% confidence interval of the Bias is represented by the Bias-2SD and the Bias+2SD.
o Add CI (confidence interval) at 95% of the slope after the “slope”,

  Since we did not use the regression analysis to compare the %FM between the two methods, we did not analyze the slope and therefore would not be able to provide the CI of the slope as suggested.

o Remove the SD of the bias

  The SD of the bias generated by the Bland Altman pair-wise comparison is needed to calculate the Bias-2SD and Bias+2SD as well as the lower limits (LL) and upper limits (UL) for equations showing significant relationship between the differences and the average %FM values. Therefore, it is essential that we keep it in the Table.

o Rename “constant” by “intercept” and add the CI as well to see rapidly if the CI includes 0 or not.

  The “constant” has been changed to “intercept” in the revised Table. As stated in our earlier response, the CI is represented by the Bias-2SD and Bias+2SD.

o Remove P because we don’t know to which variable P is referring to.

  The footnote of the Table has been greatly expanded to clarify the P represented the p value of the regression analysis performed between the differences and the average %FM values.

o The LL and UL limits (at 20 and 45%) are not reported or discussed in the manuscript, so it is worthwhile to keep them in Table 4. What are you conclusions for these values?

  These limits are included in the Table to illustrate how bad the predicted %FM value could deviate from the measured %FM value. The second sentence of the Conclusions indicated that these equations are not appropriate for predicting %FM in Sri Lankan infants.

Typing error in “Deurenberg”; “Deurenberge” between Table 4 and Table in Annexe

This was corrected.

I hope we have sufficiently addressed the comments of the reviewer and that our revised manuscript will be accepted for publication in BMC Pediatrics.

I look forward to hearing your favorable decision.

Sincerely yours,

Thushari Bandara