Author’s response to reviews

Title: Lean Body Mass: The Development and Validation of Prediction Equations In Healthy Adults

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Author’s response to reviews: see over
To
The Editor,
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Dear Sir

Re: MS: 9217072930643176: Lean Body Mass: The Development and Validation of Prediction Equations in Healthy Adults

We would like to thank the reviewers for their feedback. We have taken into account this feedback and have attempted to improve the paper by addressing these comments as discussed here.

Thank you.

Yours Sincerely,

Solomon Yu
Corresponding Author
In Response To Reviewer 1 (Heliodoro Aleman-Mateo):

**Issue 1:** ‘Lean body mass: the development and validation of prediction equations
Comments: First of all, the title is simple and nonspecific. Elderly? Adults? Men and women subjects between (age range)? Healthy?’

**Response/Action:** We have added the word Healthy to the title and it now reads: Lean Body Mass: The Development and Validation of Prediction Equations In Healthy Adults

**Introduction Section:**

**Issue 2:**
‘please state the differences and their clinical impacts between fat-free mass, lean body mass, and appendicular lean mass. It is not correct to refer to them as synonyms.’

**Response/Action:** Thank you for pointing this out and we have clarified this on page 4 and paragraph 2 of the introduction:

> Total body weight consists of fat mass and fat free mass. Fat free mass (FFM) consists of bone, muscle, vital organs and extracellular fluid. LBM differs from FFM in that lipid in cellular membranes are included in LBM but not FFM (Janmahasatian *et al.*, 2005). The lipid included in the LBM accounts for only a small fraction of total body weight and has been reported to be up to 3% in men and 5% in women (Janmahasatian *et al.*, 2005). Also, in the literature, LBM has at times included bone mass but at other times not. For example, in a study evaluating chemotherapy toxicity, LBM was assessed using computed tomography (CT) and did not include bone mass(Mourtzakis *et al.*, 2008; Prado *et al.*, 2009).

**Issue 3:**
‘The clinical impact of the loss of lean body mass is related to elderly people but the age range in this study is 18-83 and 22-83 years, therefore should be interesting to talk about the loss and their implication in general population.’

**Response/Actions:** Although we developed the equations in a cohort with a wide age spread, the equations were validated in an older cohort and older people were the focus of this study. In line one of paragraph one of the introduction, we mention that with increasing age, there is a decline in lean body mass and an increase in adiposity.
**Issue 4:**

‘Please clarify the convenience of to use kg of lean body mass instead of bodyweight and please add the most appropriate references (patients cancer).’

**Response/Actions:** We have added the following to page 4, paragraph 1:

In patients with cancer, the use of LBM might be superior to body surface area (Prado et al., 2007). For example, in a prospective study of colon cancer patients treated with 5-fluorouracil (5-FU), the incidence of dose limiting toxicity was examined with respect to conventional dosing of 5-FU/m$^2$ of BSA versus 5-FU/kg of LBM. LBM was a significant predictor of toxicity (p=0.011) but BSA was not (Prado et al., 2007). Similar findings have been reported in other studies (Aslani et al., 2000; Gusella et al., 2002).

**Issue 5:**

‘The limitation of BIA is not only by hydration (this is a basic statement), theremany other issues that limit the use of this technique.’

**Response/Actions:**

We have added to page 5, paragraph 1 of the introduction the following clarification:

Although the bioelectrical impedance analysis method is portable, it still requires the purchase of special equipment and it’s accuracy is also dependent on many other factors such as state of hydration, food intake and exercise (Kyle et al., 2004).

**Issue 6:**

‘What that´s mean: equations by Heitmann and Jamahastian were moreconsistent?’

**Response/Actions:** We have expanded the 1st paragraph, page 5 of the introduction to better clarify this.

In a very recent study of older (>70 years) Australian men, FFM (FFM was calculated by subtracting fat mass from total body mass) as estimated by three PEs where compared to FFM as estimated by DXA (Deurenberg et al., 1991; Heitmann, 1990; Janmahasatian et al., 2005; Mitchell et al.). For two of the PEs, FFM was calculated by subtracting fat mass from total body mass. In this study, the authors proposed that FFM and LBM could be used interchangeably. It was reported that the Deurenberg equation had the smallest mean difference and overestimated $\text{FFM}_{\text{DXA}}$ for overweight men but underestimated $\text{FFM}_{\text{DXA}}$ for all other body mass index (BMI) subgroups. The Heitmann and Janmahasatian equations, on the other hand, overestimated $\text{FFM}_{\text{DXA}}$ across the various BMI categories [7-9].
**Issue 7:**
‘For any validation technique, the reference method is determinant. In this sense, authors compared equations to estimate fat mass, and where normally, fat-free mass is obtained by difference of body weight. First of all, LBM by DXA is different from fat-free mass estimated by the 2C model such densitometry technique. Authors should clarify this issue.

**Response/Actions:** We agree with the reviewer and this has been amended accordingly. Page 5, line 18, in reference to the existing prediction equation, we have clarified this and accordingly changed LBM to FFM. We have also in the introduction clarified how these terms are defined.

**Issue 8:**
‘Please give more information about why the biochemical variables should improve the precision and accurate of the new predictive equation. In otherwords, what is the background that supports this related hypothesis? Please review the following paper: Visser M, Kritchevsky SB, Newman AB, Goodpaster BH, Tylavsky FA, Nevitt MC, Harris TB. Lower serum albumin concentration and change in muscle mass: the Health, Aging and Body Composition Study. Am J Clin Nutr. 2005 Sep;82(3):531-7.’

**Response/Actions:** We have amended the first paragraph on page 6 to better clarify the justification for evaluating the potential of biochemical variables in improving the performance of the prediction equations.

The addition of biochemistry variables might improve the performance of prediction equations despite the increased costs and this should be explored. Creatine Kinase (CK) is found predominantly in the skeletal muscle and serum levels might be related to lean muscle mass (Norton et al., 1985). There has only been one study evaluating the relationship between LBM and plasma creatine kinase activity (CK) and a weak and partial correlation ($r < 0.262$) between log CK and LBM was reported (Swaminathan et al., 1988). Serum albumin is said to reflect protein reserve and lower albumin levels are associated with loss of lean mass (Visser et al., 2005).
Methodology Sections:

**Issue 9:**
*Were the new equations validated against DXA baselinemeasurements? Is this a cross-sectional data analysis? Ethnicity? Please state about the criterion to defined the designed and validation sample. Please clarify.*

**Response/Actions:**
We have clarified that the DXA were undertaken at follow up for NWAHS group and baseline for the FAMAS group. This is cross sectional data analysis. Accordingly this has been clarified as below:

- Page 6, last line: Participants with complete anthropometric and DXA measurements at follow up (2004-06) aged ≥50 were included in this analysis.
- Page 7, line 6-7: DXA measurements at baseline (2002-2005) were obtained on 700 participants aged 50 years and over.

We discussed ethnicity as a limitation and clarified in the discussion: Furthermore, only Caucasians were studied and therefore generalizing these results to other ethnic communities is not possible and ethnic specific PEs will need to be developed.

The inclusion and exclusion criteria are clarified on page 7, last paragraph for the CASA population. It reads: The inclusion criteria were being aged 18 and above, able to comply with study protocol and weight stable over the last 3 months. The exclusion criteria were those with a serious medical illness, an acute illness in the pass 3 months or in the 2 weeks following blood sampling, an inability to stop medications for 3 days prior to blood sampling, being in receipt of vaccinations and pregnancy.

For the Validation Cohort (VC): This was a convenience sample of 52 healthy subjects (age 22 – 83 years) recruited through advertisement for another study (Tai et al., 2009). Subjects with known medical illness including gastrointestinal disease or symptoms, significant respiratory, renal or cardiac disease and who were pregnant were excluded from this study.

**Issue 10:**
*‘Respect to the anthropometric predictive variables: what about of some related muscle circumference such as calf and arm (basic anthropometric measurements).’*

**Response/Actions:** We agree that this will be something important to be explored in future studies but these measurements were not part of this research study. We have accordingly discussed this in the limitation section in the discussion (page 15, line 10).

The use of other anthropometry measurements such as calf or arm circumference may improve the performance of prediction equations and needs to be explored in future studies.
**Issue 11:**

‘Please clarify which of the multi-regression analysis was used for the selection of the predictive models: All possible regression or Stepwise, or ….?’

**Response/Actions:** We investigated all possible regressions. This was discussed in the statistical analysis section (page 10, line 2):

The best PEs (as assessed by adjusted $R^2$: the proportion of the variance of the dependent variable accounted for by the independent variables, and adjusted for the number of independent variables) were developed considering up to 6 equations with n predictors. For each n, the PE for validation was selected by considering the adjusted $R^2$ value and likely clinical utility.

**Issue 12:**

‘How many models do the authors found in this sample?’

**Response/Actions:** For the purpose of this study, the best 6 models with n predictors were considered where $n \geq 6$, otherwise the best n models.

**Issue 13:**

‘Please state the criteria for the selection of the predictive model or how the precision was assessed ($R^2$, SEE and don’t forget to include the values of Mallow (Cp)).’

**Response/Actions:** We have described the statistical method. Adjusted $R^2$ was used. The use of Mallows’ $C_p$ is inappropriate here since its use is intended for pre-specified models (see eg. Harrell FE (2001) Regression Modeling Strategies, Springer)

**Issue 14:**

‘Also, add the term assumption of the lineal regression before VIF and CN, add other parameter of assumption of lineal regression analysis.’

**Response/Actions:** We have clarified our statistical method as discussed above.

**Issue 15:**

‘Is it valid to consider the clinical utility? So what is the importance of statistic to test precision, accuracy and bias? Please clarify.’

**Response/Actions:** We feel that it is valid to consider clinical utility. Using insights gained from knowledge of the subject matter will usually improve predictive models. In the present case, two
models may have very similar adjusted $R^2$ values, but one may be preferred because some of the variables involved are much simpler to measure and more practical for implementation.

**Issue 16:**
‘My major concern is about the validation procedure used for the new anthropometric equations. Why two different validation procedures were used? All this section is confusing, and there is not a serious criterion to test the accuracy of the new anthropometric equations.’

**Response/Actions:** The reference method in this study was the DEXA. This was the criterion used to test against. Before embarking on a more costly process of analyzing biochemistry in the larger cohort (NWAHS and FAMAS), all PEs were initially validated in a convenience sample where biochemistry was already available. From this investigation, it was noted that the addition of blood biochemistry to the PE provided only marginal benefits and could not justify the increased costs and so, only the PEs containing anthropometric were validated in the larger cohort.

**Issue 17**
‘In the statistical analysis section, please to clarify how accuracy was tested. Please clarify concordance correlation, agreement and bias and precision?’

**Response/Actions:** We have already discussed that the predictive performance of the PE was tested using the method of Bland-Altman analysis (page 10, line 8). Furthermore, we also clarified that precision and bias were calculated according to the method of Sheiner and Neal. Both of these methods give a reflection of how close or far the measured/predicted LBM was compared to the reference LBM (as measured by DXA). Both of this method has been widely used to compare the performance of predicted value vs. the reference value. Concordance correlation was also calculated to provide information on how well the 2 methods (reference vs. measured) correlated. Concordance correlation was also calculated to provide information on how well the 2 methods (reference vs. measured) correlated. The concordance correlation coefficient (Lin, 1989 & 2000) evaluates the degree to which pairs of observations fall on the 45° line through the origin. It contains a measurement of precision (Pearson correlation coefficient), which measures how far each observation deviates from the best-fit line, and is a measure of precision, and a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy.
Results Section:

**Issue 18:**
‘Why the results are presented by sex and age group?’

**Response/Actions:** It has been previously reported that LBM PE are affected by gender and age. Also, it is important to be aware how the equations perform in different age and gender categories just as it is important to determine how they perform in various weight groups.

Discussion Section:

**Issue 19:**
*Do the authors test the accuracy of the Janmahasatian, Deur and Hietequation. What that’s mean: that appears to perform better???*

**Response/Actions:** The discussion section has been modified accordingly. This can be found in page 14, second paragraph.

A significant finding from this study is the development of a new anthropometric PE for LBM: $LBM = 22.932326 + 0.684668 \text{ (weight)} - 1.137156 \text{ (BMI)} - 0.009213 \text{ (age)} + 9.940015 \text{ (if male)}$. The close approximation of LBM was reflected by its small bias (ME=0.74kg) and precision (RMSE=3.74kg). It overestimated $LBM_{DXA}$ across gender, age and BMI groups. This PE may be useful in care settings where access to DXA may be limited, providing clinicians a practical alternative to assess LBM. Furthermore, it also provides a bedside option in hospitals for ill and frail patients where transport for DXA assessment may be difficult. Whilst BIA may be simple technique to be used at the beside, reliable BIA measurement requires factors such as hydration status, food intake and exercise to be controlled (Kyle *et al.*, 2004). Skin fold measurement may be a cheap option but the accuracy of the measurement relies on the skill of the operator and the loss of subcutaneous tissue in older people may affect accuracy (Omran *et al.*, 2000). Therefore, this anthropometric PE offers a practical and relatively reliable means of LBM assessment. Interestingly, the Deurenberg equation although said to be estimating FFM, appeared to have less bias with a ME of 0.02 kg but similar precision with a RMSE of 3.95 when compared to our newly developed PE. Also, across gender, age and BMI groups, it at times over-estimated and at other times under-estimated the $LBM_{DXA}$ (Mitchell *et al.*). The newly developed PE appeared to have better precision (smaller RMSE) and less bias (lower ME) than the Deurenberg equation only in women and in obese older individuals.

Furthermore, we have avoided the use of the phrase of “performed better” in the results section and clarified the use of ME and RMSE. Please see page 12, last paragraph:

Table 4 compares the performance of the various PEs across various BMI groups. Once again, PE1 has the smallest ME and RMSE than the Janmahasatian and Heitmann equations across all the BMI groups analyzed. Heitmann, Janmahasatian and PE1 consistently over-estimated $LBM_{DXA}$ across the various BMI groups. PE1, in comparison with the Deurenberg equation has a lower ME and RMSE in the obese BMI ($>30 \text{ kg/m}^2$) and underweight BMI ($<22 \text{ kg/m}^2$) groups. Interestingly, the Deurenberg equation has less bias and better precision than PE1.
in predicting LBM_{DXA} in the 22-27kg/m^2 BMI group. The Deurenberg equation overestimated LBM_{DXA} except in the underweight and obese categories.

**Conclusions Section:**

*Considering all my major comments the conclusion should be rewritten.*

Based on the major comments from the reviewer, we have accordingly rewritten the discussion and conclusion sections taking into account the feedback provided in the earlier sections.
In Response To Reviewer 2 (Aldo Scafoglieri):

Abstract:

**Issue 1:** Lean body mass is abbreviated three times please correct

**Response/Actions:** This is corrected accordingly in the abstract (page 3 – line 4 and line 12).

Introduction:

**Issue 1:** page 4: Please provide reference year for Hilmer et al. ...

**Response/Actions:** This has been amended

**Issue 2:** Please correct Heitmann and Jamahastian to Heitmann and Janmahasatian

**Response/Actions:** The spelling error has been corrected on page 5, line 5

Methods:

**Issue 1:** Please try to avoid too many abbreviations. Page 7: What does DC stand for? or do you mean VC instead?

**Response/Actions:** The DC has been removed (page 7 line 19) as it does not add any value.

Results:

**Issue 1:** page 10: What does DC stand for?

**Response/Actions:** It was an error and has been corrected to “VC” which stand for validation cohort (page 11 line 1).

**Issue 2:** page 11: please correct Durenberg to Deurenberg

**Response/Actions:** This has been corrected in page 12 line 15.
Discussion:

**Issue 1:** page 13 line 1: omit the abbreviation '(PEs)'

**Response/Actions:** This has been corrected in page 14 line 1

**Issue 2:** page 13 2nd paragraph: The authors state that the loss of subcutaneous tissue in older people may affect accuracy of skinfold measurement. How does the accumulation of adipose tissue under the fascia generalis affect accuracy? Please explain. Please elaborate on why the majority of PEs overestimated LBM by DXA.

**Response/Actions:**
As the discussion has been significantly modified based on comments from reviewer 1, then this issue has not been discussed in detail.

References:

**Issue 1:** Please provide year of publication for reference 1,2,5,7

**Response/Actions:** This has been corrected accordingly

Table 1:

**Issue 1:** Please omit abbreviation '(VC)' from title

**Response/Actions:** This has been removed from the title (page 20 line 1)

**Issue 2:** Provide statistical significance level for Concordance R

**Response/Actions:** The significance level for concordance R is expressed as 95% CI and this has been added in page 21.

Table 2,3,4:

**Issue 1:** Please add CI, SD, R to table captions

**Response/Actions:** CI, SD and R has been added to the table captions (page 21-23)

**Issue 2:** Please omit VC from caption

**Response/Actions:** This has been removed from the captions (page 21-23)
Issue 3: Please correct DEXA to DXA

Response/Actions: This has been corrected in the captions (page 21-23)

Issue 4: Please write DXA in underscript when used in conjunction with LBM. Please also do the same for PE1, PE2, PE3, PE4

Response/Actions: On reviewing of this suggestion, to make it easier for reader to understand the table, it was decided to change the shorthand of PE1, PE2 and PE3 to Heitmann equation, Janmahasation equation and Deurenberg equation respectively (page 21-23).