

BMJ Open Development of bioelectrical impedance-based equations for the prediction of body composition of Malawian adolescents aged 10–18 years: a cross-sectional study

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To cite: Divala OH, Mwakhwawa Q, Phiri MM, *et al.* Development of bioelectrical impedance-based equations for the prediction of body composition of Malawian adolescents aged 10–18 years: a cross-sectional study. *BMJ Open* 2022;**12**:e058551. doi:10.1136/bmjopen-2021-058551

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-058551>).

Received 23 October 2021
Accepted 17 February 2022



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ABSTRACT

Objectives The accuracy of bioelectrical impedance analysis (BIA) depends on population-specific prediction equations and there is no population-specific equation for predicting fat-free mass (FFM) in Malawian adolescents. This study aimed at determining the agreement between FFM measured by deuterium oxide dilution technique (the reference) and FFM by BIA; and propose BIA-based prediction equations to estimate FFM for Malawian adolescents.

Design This was a cross-sectional study.

Setting The study was conducted in Blantyre, Malawi

Participants 186 Malawian adolescents aged between 10 and 18 years were included in this study. Body composition was estimated by both the BodyStat BIA analyser and the deuterium oxide dilution method.

Results BIA inbuilt equation underestimated FFM compared with deuterium oxide dilution ($p=0.039$). The new prediction equation for FFM (kg) = $-4.316 + 0.425 \times \text{height}^2(\text{cm})/\text{resistance}(\Omega) + 1.287 \times \text{sex}(\text{male}=1, \text{female}=0) + 0.307 \times \text{age}(\text{years}) + 0.344 \times \text{weight}(\text{kg}) + 0.019 \times \text{reactance}(\Omega)$ yielded an R² of 0.926. The equation for total body water (TBW) (kg) = $-2.152 + 0.328 \times \text{height}^2(\text{cm})/\text{resistance}(\Omega) + 0.910 \times \text{sex}(\text{male}=1, \text{female}=0) + 0.307 \times \text{age}(\text{years}) + 0.249 \times \text{weight}(\text{kg}) + 0.015 \times \text{reactance}(\Omega)$ yielded an R² of 0.922. The Bland-Altman plot illustrated a good level of concordance between the FFM and TBW predicted by the new equations and the values derived using deuterium dilution method.

Conclusions The new BIA prediction equations for estimating FFM and TBW could be used to assess with very good accuracy and precision the body composition of Malawian and adolescents with similar characteristics.

INTRODUCTION

The double burden of malnutrition is becoming a great public health concern in low-income and middle-income countries (LMICs). In Malawi as in most LMICs, stunting and wasting have shown a modest decline while overweight and obesity are

Strengths and limitations of this study

- This is one of the few studies from low-income and middle-income countries (LMICs) to develop and validate bioelectrical impedance analysis-based equations for use in adolescents and young people from LMIC.
- The study uses robust methodology by employing development and validation groups with deuterium oxide dilution technique as the reference.
- The prediction equations are based on a sample that included adolescents with a history of previous malnutrition which may have affected their pubertal development and body composition
- The developed prediction equations did not take into consideration the impact of the pubertal developmental stage which may affect body composition.
- The developed prediction equations are only generalisable to Malawian adolescents or those with similar characteristics and further research should be conducted to test the accuracy of the new equations in other settings.

increasing rapidly. Evidence suggests a high correlation between obesity in childhood and adult obesity.¹ Therefore, accurate and reliable indicators are required for the early detection of excess adiposity and trends of childhood obesity.

In clinical and/or epidemiological studies, anthropometric measurements such as body mass index (BMI), waist circumference, and waist-hip ratio are widely used to define and assess overweight and obesity. Although these techniques are simple to measure and remain valuable tools in monitoring the size and shape of a child in relation to standards, they do not precisely distinguish between increased fat mass (FM) or fat-free mass (FFM)^{2–6} leading to misclassification in large-framed and/or muscular children.

Additionally, anthropometric measurements cannot predict the change in body fatness, which is a widely used approach to evaluate the effect of intervention programmes on malnutrition. It is therefore important that the ideal monitoring tools should directly assess adiposity.

Body composition can be measured using accurate and precise techniques such as hydro densitometry, deuterium oxide dilution technique, dual-energy X-ray absorptiometry, quantitative MRI and ultrasound. However, these techniques are expensive and technically demanding. Bioelectrical impedance analysis (BIA) provides a desirable option as it is a relatively cheap, non-invasive and simple method for assessing body composition.⁷ BIA determines the electrical impedance of body tissues, which provides an estimate of total body water (TBW) that is converted to an estimate of FFM, with assumed constant values for the hydration of lean tissue.⁸ However, the estimation of body composition by BIA is based on predictive equations that were developed in populations with specific characteristics. Due to differences in ethnicity and body composition, these equations are valid only for a population in which they were developed.⁹⁻¹³

It is, therefore, important that there is a valid equation for measuring body composition in Malawian adolescents. Once validated, BIA has the potential to be scaled up for use in larger population surveys as it is low-tech and is affordable in line with recent suggestions.¹⁴ The deuterium oxide dilution technique is a safe, non-invasive method that can be used in all population groups, including pregnant women and children.^{15 16} Therefore, this study aimed to determine the accuracy of the BIA inbuilt equations in assessing the body composition of Malawian adolescents and, if necessary, to develop and validate prediction equations using the deuterium oxide dilution as a reference technique.

MATERIALS AND METHODS

Participants

A convenient sample of 186 Malawian adolescents aged between 10 and 18 years was included. Data used in this study were collected between September 2018 and February 2019, from healthy Malawian adolescents. The subjects included clinically healthy adolescents with a history of treatment for acute malnutrition and their age-matched sibling and age/sex-matched community controls without a history of severe malnutrition.

Anthropometric measurements

Weight was measured to the nearest 0.1 kg using a SECA electronic scale (SECA, Hamburg, Germany) with the participant barefoot and wearing light clothes. Height was measured to the nearest 0.1 cm using a SECA stadiometer (SECA, Hamburg, Germany) with the participant barefoot in a standing position. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m). Mid upper arm circumference (MUAC)

was measured to 1 mm using non-stretchable plastic insertion tapes (TALC, St. Albans, UK)

Bioelectrical impedance analysis

The BIA was performed by trained personnel, using a dual-frequency Bodystat 1500 MDD system (Bodystat, Douglas, UK). Adolescents were measured while lying in a supine position with arms and legs slightly abducted from the trunk. The BIA electrodes were placed on the dorsal surfaces of the ankle and wrist. For this analysis impedance, resistance (R), percentage of fat mass (%FM), FM, FFM and TBW at 50 kHz were used. The resistance index (height^2/R) was calculated as the square of the height (in cm) divided by the resistance (in ohms).

Deuterium oxide dilution

The plateau protocol was used for body composition measurement through the isotope dilution of deuterium oxide. Briefly, a gravimetrically weighed amount of deuterium oxide 99.8% (0.1mg/kg of body weight) was orally administered to participants after collecting a baseline saliva sample. Then two saliva samples were collected at 3.5 hours and 4 hours postdose administration. The enrichment of deuterium oxide in the three samples was assessed Fourier Transform Infrared Spectrometer (Agilent 4500 Series FTIR) and TBW was subsequently calculated using the mean of 3.5 and 4 hours samples.

Statistical analysis

Data were collected by electronic data collection forms using the Open Data Kit (ODK) software and data collected using tablets. For the enrichment data obtained from measuring deuterium from the saliva samples: samples were run in duplicate, if the difference between duplicate runs was more than 3ppm, samples were rerun and if samples were not enough, only enrichment at 3.5 hours was used. The descriptive statistics are presented as means (SD) for normally distributed data or median (IQR) for skewed distribution.

The accuracy of the inbuilt BIA equations was assessed by the Bland and Altman approach which determined bias and limits of agreement between the reference method with estimates from inbuilt BIA equations. To develop the equations, the subjects were randomly split into two subgroups: one group for the development of the equation and the other for the cross-validation. The randomly split dataset consisted of 93 subjects in the development group and 93 subjects in the validation group. FFM and TBW derived from the deuterium dilution method (DDM) were separately used as a dependent variable for the development of their prediction equations. The independent variables were age, sex, height, weight, reactance and resistance index. Multicollinearity was assessed by a variance inflation factor (VIF), a VIF of less than 10 was acceptable.

In the development group, several linear regression models were run. The initial model included a single independent variable with further models adding

independent variables one by one. Potential interaction between predictors was assessed by including an interaction term in distinct regression and applying the Wald test. A model with the highest adjusted R^2 , the lowest SE of estimate (SEE) value, and the lowest p-value was selected as the best fit to predict FFM.

The new FFM and TBW equations were validated in the validation group. The performance of the new equations was assessed using pure error which was calculated as the square root of the sum of squared differences between the observed and the predicted values divided by the number of subjects in the cross-validation group; a smaller pure error indicates greater accuracy of the equation.¹⁷ Paired t-test was used to test the difference between the measured and predicted values (bias) of FFM and TBW. The Bland and Altman plots were used to test the agreement between the reference method with estimates from the new prediction equation. A $p \leq 0.05$ was considered statistically significant. Data were analysed using Stata V.14.0 (StataCorp).

Patient and public involvement

No patient involved.

RESULTS

We included 186 participants with an average age of 14.1 years and 53.7% were female. The mean (\pm SD)

body weight and height were 41.1 kg (9.65) and 149.2 cm (9.83). The anthropometry and body composition measurements (both by BIA and deuterium oxide dilution technique) that were taken from subjects in both the development and validation groups were not significantly different at a significance level of $p > 0.05$ (table 1). Age and sex distribution of anthropometric and body composition indices were not significantly different between the development and validation groups (online supplemental tables 1 and 2).

Table 2 shows a comparison between body composition estimates measured by deuterium oxide dilution and BIA. BIA significantly underestimates FFM, mean difference of 0.93 (95% CI 0.22 to 1.64). Similarly, the Bland-Altman analysis in figure 1 shows a significant difference in FFM obtained from the deuterium oxide dilution technique and the BIA system plotted against the average FFM of both methods. The bias was 0.93 with a 95% CI (0.21 to 1.65), and the 95% limits of agreement between the methods were -8.89 to 10.75 . This reflected an inaccurate estimation of the BIA in-built prediction equation compared with the deuterium oxide dilution technique.

The regression models for the prediction equations are presented in table 3. The FFM (kg) was the dependent variable, resistance index (Ht^2/R), sex weight, age were the significant predictor variables included in the equation. In the final model, resistance index (Ht^2/R),

Table 1 Characteristics of participants and comparison of development and cross-validation group

Variable	All subjects (n=186)	Development group (n=93)	Validation group (n=93)	P value*
Age (years), mean \pm SD	14.0 \pm 1.91	13.9 \pm 1.76	14.1 \pm 2.05	0.549
Female, n (%)	107 (57.5)	54 (58.1)	53 (57.0)	0.882†
Anthropometry				
Weight (kg), mean \pm SD	41.1 \pm 9.65	41.7 \pm 10.24	40.4 \pm 9.03	0.359
Height (cm), mean \pm SD	149.2 \pm 9.83	149.5 \pm 10.60	149.0 \pm 9.04	0.682
BIA				
R (ohms), mean \pm SD	644.4 \pm 86.9	645.4 \pm 90.35	643.3 \pm 83.71	0.873
Ht^2/R ($cm^2/ohms$) mean \pm SD	35.6 \pm 8.04	35.4 \pm 7.75	35.8 \pm 8.35	0.721
Xc (ohms), mean \pm SD	59.8 \pm 8.06	59.0 \pm 8.27	60.6 \pm 7.80	0.176
% FM, mean \pm SD	18.5 \pm 6.29	17.7 \pm 5.93	19.2 \pm 6.57	0.093
TBW, mean \pm SD	25.4 \pm 5.86	25.4 \pm 5.67	25.3 \pm 6.07	0.949
FFM, (kg), mean \pm SD	32.6 \pm 7.85	32.4 \pm 7.77	32.9 \pm 7.96	0.694
FM, (kg), mean \pm SD	7.7 \pm 3.84	7.2 \pm 3.55	8.2 \pm 4.07	0.080
DDM				
%FM, mean \pm SD	17.7 \pm 7.49	17.3 \pm 7.03	18.2 \pm 7.93	0.411
TBW (kg), mean \pm SD	25.3 \pm 5.65	25.0 \pm 5.14	25.5 \pm 6.14	0.544
FFM (kg), mean \pm SD	33.6 \pm 7.64	33.2 \pm 6.96	33.9 \pm 8.28	0.694
FM (kg), mean \pm SD	7.6 \pm 4.44	7.4 \pm 4.39	7.8 \pm 4.50	0.508

*A t-test development versus validation group.

† χ^2 test.

BIA, bioelectrical impedance analysis; DDM, deuterium dilution method; FFM, fat-free mass; FM, fat mass; Ht^2/R , height²/resistance (Resistance Index); R, resistance; TBW, total body water; Xc, Reactance.

Table 2 Comparison between deuterium oxide dilution technique and BIA in measuring body composition in Malawian adolescents aged 10–18 years

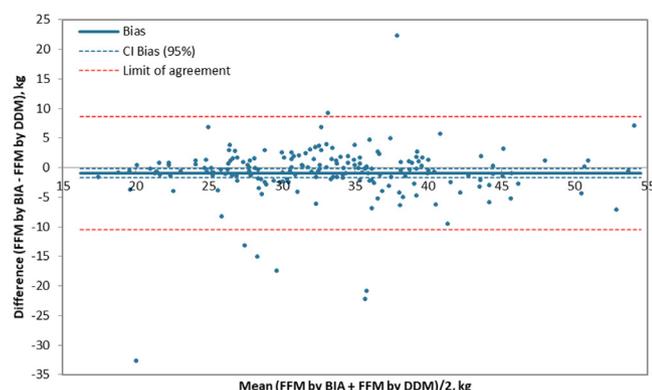
Body composition	BIA, mean±SD	DDM, mean±SD	Mean difference (95% CI)	P value*
Overall (n=186)				
FFM (kg)	32.6±7.85	33.6±7.64	−0.93 (−1.64 to −0.22)	0.011
TBW (kg)	25.4±5.86	25.3±5.65	0.07 (−0.53 to 0.67)	0.827
FM (kg)	7.7±3.84	7.6±4.44	0.13 (−0.32 to 0.57)	0.568
%FM	18.5±6.29	17.7±7.49	0.70 (−0.24 to 1.65)	0.145
Female (n=107)				
FFM (kg)	32.2±7.35	32.5±6.49	−0.32 (−1.28 to 0.65)	0.516
TBW (kg)	25.1±5.54	24.5±4.82	0.59 (−0.25 to 1.42)	0.165
FM (kg)	8.9±4.17	9.2±4.65	−0.26 (−0.93 to 0.04)	0.440
%FM	20.8±6.11	20.9±6.78	−0.12 (−1.49 to 1.25)	0.867
Male (n=79)				
FFM (kg)	33.3±8.49	35.0±8.80	−1.76 (−2.80 to −0.72)	0.001
TBW (kg)	25.7±6.28	26.4±6.51	−0.64 (−1.49 to 0.21)	0.136
FM (kg)	6.1±2.62	5.5±3.02	0.66 (0.14 to 1.17)	0.013
%FM	15.3±5.04	13.5±6.20	1.82 (0.61 to 3.04)	0.004

*t-test.

DDM, deuterium dilution method; FFM, fat-free mass; FM, fat mass; %FM, fat mass percentage; TBW, total body water.

sex weight, age, and reactance accounted for the largest variability (R^2 ; 92.6%) and lowest SEE of 1.95 kg for FFM. The best prediction equation for TBW accounted for 92.2% of the variation in TBW with a SEE of 1.47. The final prediction equation of BIA for estimation of FFM and TBW that had the highest adjusted R and the lowest SEE value were as follows:

$$\text{FFM (kg)} = -4.316 + 0.425 * \text{height}^2(\text{cm}) / \text{resistance}(\Omega) + 1.287 * \text{sex} (\text{male}=1, \text{female}=0) + 0.307 * \text{age}(\text{years}) + 0.344 * \text{weight}(\text{kg}) + 0.019 * \text{reactance}(\Omega).$$

$$\text{TBW (kg)} = -2.152 + 0.328 * \text{height}^2(\text{cm}) / \text{resistance}(\Omega) + 0.910 * \text{sex} (\text{male}=1, \text{female}=0) + 0.307 * \text{age}(\text{years}) + 0.249 * \text{weight}(\text{kg}) + 0.015 * \text{reactance}(\Omega)$$
**Figure 1** Bland and Altman plot comparing (fat-free mass (FFM) FFM determined by bioelectrical impedance analysis (BIA) and FFM determined by deuterium dilution method (DDM).

The developed equations were applied to the cross-validation group to evaluate their accuracy. **Table 4** presents the comparison of FFM and TBW estimated by the new equation and the isotope dilution technique. There was no significant difference between the FFM measured by isotope dilution technique (33.9 ± 8.28) and that estimated by the new BIA equation (34.0 ± 7.5), p -value=0.743. Similarly, there was no significant difference between the TBW measured by isotope dilution technique (25.5 ± 6.14) and that estimated by the new BIA equation (25.6 ± 5.52), p -value=0.773. The FFM equation had an accuracy (pure error) of 2.68 kg while the TBW prediction equation yielded an accuracy of 2.01 kg. Furthermore, in the whole sample of this study, the FFM and TBW values estimated with the new validated BIA equation among girls and boys did not differ from what was measured by the deuterium dilution method (DDM) (**table 5**).

The Bland and Altman approach showed a non-significant bias for TBW (0.06; 95% CI −0.355 to 0.476) and FFM (0.092; 95% CI −0.463 to 0.647). The limits of agreements ranged from −5.19 to 5.37 for FFM and −3.90 to 4.02 for TBW (**figure 2**).

DISCUSSION

This study assessed the accuracy of an inbuilt BIA equation in determining body composition in comparison to deuterium oxide dilution. The study also developed and cross-validated a new bioelectrical impedance-based equation for the prediction of body composition in adolescents aged 10–18 years in Malawi using deuterium oxide dilution as a reference technique. We have demonstrated

Table 3 Regression models developed for the prediction of total body water in Malawian adolescents aged 10–18 years

	Predictors	β (SE)	P value	R ²	SEE	Collinearity statistics	
						Tolerance	VIF
FFM	Intercept	-4.316 (2.54)	0.093	0.926	1.95		
	Ht ² /R	0.425 (0.067)	0.001			0.152	6.56
	Weight	0.344 (0.052)	0.001			0.184	5.43
	Age	0.500 (0.134)	0.001			0.740	1.35
	Sex	1.287 (0.500)	0.012			0.672	1.49
	Xc	0.019 (0.029)	0.523			0.716	1.40
TBW	Intercept	-2.152 (1.92)	0.264	0.922	1.47		
	Ht ² /R	0.328 (0.051)	<0.001			0.152	6.561
	Weight	0.249 (0.040)	<0.001			0.184	5.428
	Age	0.307 (0.101)	0.003			0.740	1.351
	Sex	0.910 (0.377)	0.018			0.672	1.489
	Xc	0.015 (0.022)	0.503			0.716	1.397

FFM, fat-free mass; Ht²/R, Resistance Index (height²/resistance); SEE, SE of the estimate; Sex, male=1, female=0; TBW, total body water; VIF, variance inflation factor; Xc, reactance.

that FFM estimates from inbuilt BIA equations are significantly biased when compared with the DDM technique in Malawian adolescents. We have subsequently developed a model to predict FFM and TBW among Malawian adolescents using BIA which is a widely used technique for estimating body composition and it is particularly useful in large, population-based studies because it is quick, portable, inexpensive and non-invasive.

This observed bias from the in-built prediction equation is similar to what was observed in Bangladeshi children¹⁸ South African preadolescents,¹⁹ Moroccan children²⁰ and Tunisian children.²¹ This could be explained by differences in the body structures of our population vs the population that was used to derive the inbuilt predictive equation. However, a most recent study which also used the DDM technique as a reference reported that the inbuilt BIA equation agrees well with reference methods in European children and adolescents.²²

It is well established that body composition differs between men and women, with men having more lean mass than women, and women having more fat mass.^{23–25} This is likely the case in our sample where there was a significant difference found between the FFM values measured with BIA and DDM. Additionally, in the case where the dual-frequency Bodystat 1500 MDD system is

used as in this population, the integrated equation underestimates TBW for men due to the propriety of their body composition where the components of lean mass are higher.^{25 26}

When developing the equation for the prediction of FFM and TBW in this population, resistance index and body weight explained 90.5% and 90.7% of the variability in FFM and TBW, respectively. This has also been observed in previous studies where resistance index and weight were the strongest predictors of body composition.^{22 27 28} The inclusion of age, sex and reactance improved the predictability of the equation by further increasing the R² to 0.926 and 0.922 for FFM and TBW, respectively.^{29 30} The new equations provided an excellent agreement with direct measures of FFM and TBW with a high R² and low SEE as measures of fit. These findings are comparable with those reported from other studies with comparable participant characteristics and using the deuterium oxide dilution technique as the reference method where R² ranged from 0.65 to 0.99 with an SEE ranging from 0.41 to 3.81.^{20 21 31–34}

As prediction equations are being applied to other samples, their accuracy tends to decrease. Therefore, it has to be cross-validated in another sample to confirm both its validity, and applicability. In our cross-validation,

Table 4 Comparison between FFM and TBW measured by isotope dilution using FTIR and by new BIA equation in the cross-validation sample

Body composition	New BIA equation, mean±SD	DDM, mean±SD	Bias (95% CI)	P value*	Pure error
FFM (kg)	34.0±7.51	33.9±8.28	0.09 (-0.46 to 0.65)	0.743	2.68
TBW (kg)	25.6±5.52	25.5±6.14	0.06 (-0.36 to 0.48)	0.773	2.01

**t-test

BIA, bioelectrical impedance analysis; DDM, deuterium dilution method; FFM, fat-free mass; FTIR, Fourier Transform Infrared Spectrometer; TBW, total body water.

Table 5 Comparison of TBW and FFM from the reference method and prediction equations and stratified by sex

Body composition	New BIA equation, mean±SD	DDM, mean±SD	Mean difference (95% CI)	P value*
Overall (n=186)				
FFM (kg)	33.6±7.11	33.6±7.64	0.05 (−0.28 to 0.39)	0.761
TBW (kg)	25.3±5.22	25.3±5.65	0.04 (−0.21 to 0.29)	0.761
Female (n=107)				
FFM (kg)	32.7±6.35	32.5±6.49	0.15 (−1.28 to 0.59)	0.488
TBW (kg)	24.6±4.66	24.5±4.82	0.11 (−0.22 to 0.43)	0.512
Male (n=79)				
FFM (kg)	34.9±7.87	35.0±8.80	−0.08 (−0.62 to 0.45)	0.755
TBW (kg)	26.3±5.80	26.4±6.51	−0.05 (−0.46 to 0.35)	0.791

SD, standard deviation

*t-test.

.BIA, bioelectrical impedance analysis; DDM, deuterium dilution method; FFM, fat-free mass; %FM, fat mass percentage; FM, fat mass; TBW, total body water.

the Bland and Altman analysis showed a good agreement between the predicted and the measured values of FFM and TBW. The limits of agreement assessed by the Bland and Altman approach were very small and comparable to previous studies.^{31 35 36} The FFM equation yielded a pure error of 2.68 and for TBW the pure error was 2.01. Nonetheless, the new prediction equations derived in the current study are only applicable within the same or similar populations and age range.

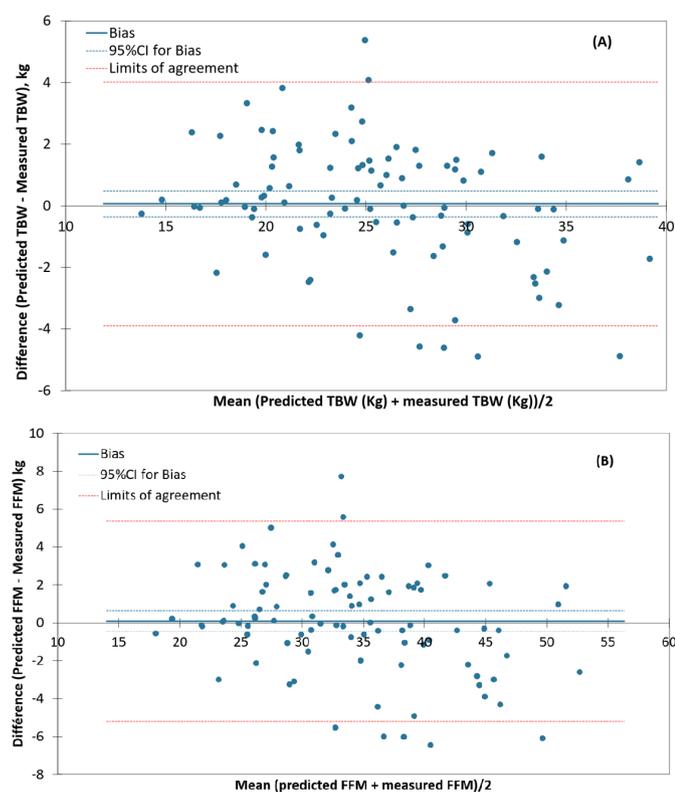


Figure 2 Bland and Altman plot between (A) total body water (TBW) and (B) fat-free mass (FFM) determined by deuterium dilution method (DDM) and those determined by the new prediction equation.

Some limitations need to be considered when interpreting and using the new equations. This study included a sample of adolescents that were previously malnourished who may have delayed puberty and differ in body composition from adolescents without a history of acute malnutrition. However, emerging evidence suggests that exposure to and treatment for acute malnutrition using therapeutic milks in early life may not necessarily be associated with modified body composition later in life.³⁷ So, while this is a concern, we think that it may only have had minimal effect. Another limitation is that the study included a wide age range; despite adjusting for sex, age and anthropometry, this study did not adjust for pubertal development stages which affect body composition. However, age and sex distribution of anthropometric and body composition indices were not significantly different between the development and validation groups. It should be noted that the new equations are only useful for Malawian adolescents or those with similar characteristics and further research should be conducted to test the accuracy of the new model in different LMIC contexts. Despite these limitations, our sample size is comparable to previously published validation studies on methods of assessing body composition and the use of a development and validation group improved the development of the new prediction equations.^{18 20 28 38}

CONCLUSION

This study has demonstrated that the measurement of FFM in Malawian adolescents aged 10–18 years, using the inbuilt BIA equation, is not accurate, thus questioning the usefulness of the inbuilt equation for the assessment of the important disease risk factors. As an alternative, we have developed a novel prediction equation for estimating FFM and TBW based on weight, resistance index, age and sex. The developed equations showed a good agreement with the deuterium oxide dilution technique

and could be used for Malawian adolescents or those with similar characteristics. Therefore, these equations can be used in both field and clinical settings, depending on the availability of the BIA.

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Acknowledgements The authors thank the guardians and children for their participation and commitment to the study. Gratitude is also extended to the study team who assisted in the data collection and conducting body composition assessments.

Contributors Conceptualisation: QM, MMP and KMM; Data curation: OHD, QM and MMP; Formal analysis: OHD and KEK; Methodology, QM, MMP and KMM; Project administration, KMM; Writing—original draft: OHD; Writing—review and editing, QM, MMP, VO, KEK and KMM; Guarantor, KMM. All authors have read and agreed to the published version of the manuscript.

Funding The study was implemented with support from the International Atomic Energy Agency under a regional project RAF6052 ‘Using Nuclear Techniques to Assess Body Composition in Children Previously Treated for Moderate and Severe Acute Malnutrition and Their Medium-Term Benefits and Risks in Six Countries’. The Department of Nutrition, HIV and AIDS, Ministry of Health, Malawi supported QM with personal stipend. The School of Public Health and Family Medicine, College of Medicine, University of Malawi supported data collection activities.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained from parent(s)/guardian(s)

Ethics approval This study involves human participants and was approved by College of Medicine Research and Ethics Committee (COMREC) Reference number: P.09/17/2281. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data for this article will be shared on reasonable request from the corresponding author.

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