

ORIGINAL ARTICLE

Calibration of bioelectrical impedance analysis for body composition assessment in Ethiopian infants using air-displacement plethysmography

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BACKGROUND/OBJECTIVES: Assessment of infant body composition (BC) is crucial to understand the consequences of suboptimal nutritional status and postnatal growth, and the effects of public health interventions. Bioelectrical impedance analysis (BIA) is a feasible, relatively inexpensive and noninvasive method for assessing BC. However, very little research has been conducted in low- and middle-income populations, where efforts to prevent or treat malnutrition in early life are a public health priority. We aimed to develop equations for predicting fat-free mass (FFM) and fat mass (FM) based on BIA in 0- to 6-month-old Ethiopian infants.

SUBJECTS/METHODS: The study comprised a total of 186 BC assessments performed in 101 healthy infants, delivered at Jimma University Specialized Hospital. Infant air-displacement plethysmography (IADP) was the criterion method, whereas weight, length, sex, age and an impedance index (L^2/Z_{50}) were predictors. Prediction equations were developed using stepwise multiple linear regression and the accuracy was evaluated with a 10-fold cross-validation approach.

RESULTS: A linear regression model based on body weight, age and sex predicted FFM, estimated by IADP, with an adjusted R^2 and root mean square error (RMSE) of 0.94 and 200 g, respectively. Adding impedance index to the model resulted in a significantly improved model fit ($R^2 = 0.95$; RMSE = 181 g). For infants below 3 months of age, inclusion of impedance index did not contribute to an improved model fit for predicting FFM compared with a model already comprising weight, sex and age.

CONCLUSIONS: The derived equations predicted FFM with acceptable accuracy and may be used in future field surveys, epidemiological studies and clinical trials conducted in similar sub-Saharan African population groups aged 0–6 months.

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INTRODUCTION

High prevalence of low birth weight and infant malnutrition, combined with increasing rates of obesity, make sub-Saharan African countries prone to nutrition-related diseases.^{1–3} Assessment of body composition (BC), in terms of fat-free mass (FFM) and fat mass (FM), provides valuable information on nutritional status and growth during infancy and childhood, and is becoming increasingly acknowledged as an important tool in paediatric research.^{4–6} There is a particular need for simple techniques that are suitable for application in large clinical trials of nutritional programmes targeting maternal pregnancy nutrition or early infant nutrition in low- and middle-income populations.

Nutritional status and growth in infancy and childhood are most often measured using simple anthropometric indices such as weight-for-height, height-for-age and body mass index.⁷ However, these indicators do not reveal whether the causes or health consequences of suboptimal growth in infancy and early childhood are ascribed to deficits in FM or FFM. Moreover, deficits in FM and FFM are known to have different implications for health status.⁸ Accurate and precise assessment of BC is therefore crucial to improve our understanding of the relation between suboptimal growth in infancy and childhood, and subsequent risk of disease. Infant air-displacement plethysmography (IADP) represents an accurate and precise method for assessment of BC.^{4,9–13} However,

IADP is expensive, non-portable, and thus difficult to implement in many low-resource settings. In contrast to IADP, bioelectrical impedance analysis (BIA) is relatively inexpensive and feasible, and may become a valuable tool for the assessment of infant and childhood BC in low- and middle-income settings.^{14–17} However, the applicability of BIA is limited to populations for whom appropriate age-, sex- and population-specific prediction equations have been developed, based on valid reference methods.^{4,18,19}

BIA was previously shown to be feasible in African infants aged 3–18 months in a small pilot study in the Gambia.²⁰ To the best of our knowledge, however, no equations based on an adequate sample size in sub-Saharan Africa have yet been developed. Thus, the present study aims to develop prediction equations for estimating FFM and FM based on BIA in 0- to 6-month-old Ethiopian infants, using IADP as the criterion method for BC.

SUBJECTS AND METHODS

Subjects and setting

Subjects were recruited from Jimma University Specialized Hospital (JUSH) in Jimma Town, Ethiopia between January and July 2012. All 0- to 6-month-old infants born at term (gestation ≥ 37 weeks) at JUSH maternity ward, living in Jimma town, with a birth weight ≥ 1500 g, without congenital malformations were eligible for the study. The study was nested in a larger

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longitudinal study, entitled the Infant Anthropometry and Body Composition Study (iABC-1), that has been described in detail elsewhere.^{13,21} A total of 236 times, eligible infants, participating in iABC-1, were invited to participate in the present study. In 196 instances, participation was accepted, and BC assessments were performed. Before analysis, eight assessments were excluded because of incomplete data and further two assessments were excluded because of a fat percentage < 1. Thus, 186 BC assessments (97 in girls and 89 in boys) were performed in 101 infants. A total of 57 infants were assessed once and 18, 14, 9 and 3 infants were assessed 2, 3, 4 and 5 times, respectively. The median (interquartile range) interval between assessments of infants included in the study multiple times was 28.5 (28–40.5) days.

For all study subjects, written informed consent was obtained from one of the parents or a caregiver. The study was granted ethical permission from the Ethical Review Board of JUSH (Ref. no. RPGE/312/2011). All the measurements were collected by trained Ethiopian research nurses and were of no harm to the study subjects.

Data collection

Data on infant length were obtained in duplicates to the nearest 0.1 cm using an SECA 416 Infantometer (SECA, Hamburg, Germany). Weight, FFM and FM were measured to the nearest 0.001 kg using the PEA POD—an IADP (COSMED, Rome, Italy). The theory and the standard operating procedures used in the present study has been described elsewhere.^{21,22} In brief, IADP relies on densitometry and uses a two-component model of BC to derive FFM and FM based on the relationship between the assessed body density, and the known density of FM and the age- and sex-specific reference values for the density of FFM.^{11,23} A constant density of FM of 0.9007 g/cm³ and the age-specific densities of FFM as suggested by Fomon *et al.*²⁴ and validated in a study of infants from Jimma by Andersen *et al.*¹³ were applied. All the calculations were performed by the PEA POD's in-built computer (software version 3.3.0). During the 2-min body volume measurement, the infants were lying in a supine position on a plastic tray in a closable transparent test chamber, and did not wear any clothes besides a tight-fitted wig cap. A complete PEA POD measurement lasted ~ 10 min.

Whole-body impedance at 50 kHz (Z_{50}) was measured using the QuadScan 4000 (multifrequency bioelectrical impedance analyser from Bodystat (Bodystat, Douglas, Isle of Man)). The conventional theoretical BIA model treats the body as a single cylinder. Adjustment of impedance for body length (l), known as the 'impedance index' (L^2/Z_{50} , expressed in cm²/Ω at 50 kHz), then allows estimation of cylinder volume, a proxy for total body water and FFM. Numerous studies have shown impedance index to be a strong predictor of total body water and FFM among children and adults.^{5,25–28}

The infants were measured while sleeping or being very calm, placing them in a supine position on a non-conductive surface with insulating material between their limbs. A tetrapolar configuration was used, attaching the current electrodes to the right dorsal surface of the third metacarpal of the hand and the right dorsal surface of the third metatarsal of the foot. The sensing electrodes were attached to the right dorsum of the wrists and ankles midway between the styloid processes and malleoli, respectively. In case of inadequate separation of a minimum of 3 cm between the current drive and voltage sensing electrode, due to a small hand and foot, the current drive electrode was moved to the ventral side of the hand and foot to ensure a larger electrode separation, as previously recommended for neonates.²⁹ All impedance measurements were carried out in duplicate and the mean was calculated.

Statistical methods

The statistical analyses were performed using Stata 12 (StataCorp, College Station, TX, USA). Statistical significance was considered at a P -value of < 0.05. Descriptive statistics are presented as means and s.d. Stepwise multiple linear regression analysis was used to develop the prediction equations, using FFM and FM estimated by the PEA POD as dependent variables, respectively, and the impedance index and selected covariates as predictor variables. Likelihood ratio tests were applied to test whether extension of the prediction model resulted in a significantly improved model fit. Regression diagnostics was applied to all linear regression analyses (i.e. assumptions about linearity of the model, normally distributed residuals and homoscedasticity). To evaluate the predictability of the developed models on data not used in the modelling and potential overfitting, a k -fold cross-validation was applied.³⁰ The present k -fold cross-

validation was chosen to be 10-fold. Thus, observations were randomly distributed in 10 equally sized subsets of data. Subsequently, a total of 10 prediction models were generated. Each prediction model was developed based on nine alternating subsets of data, using the remaining one subset, not used in the modelling, to validate the prediction model. Thus, a total of 10 prediction errors (root mean square error (RMSE)) were obtained from the 10 validations. A combined error, expressed as the average RMSE from all 10 validations, was calculated and used to assess the predictive performance of the developed models for FFM and FM.

Bland–Altman plots with 95% limits of agreement were used to assess the level of agreement between the values assessed with the PEA POD and the predicted values estimated with the new equations based on impedance index and selected covariates.³¹

RESULTS

A description of the study subjects stratified on sex and age groups is presented in Table 1. Of the 186 BC assessments, 21, 46, 39, 40, 24 and 16 were conducted at birth, 1, 2, 3, 4 and 5–6 months, respectively.

Prediction of FFM and FM

In the total study population, FFM estimated by the PEA POD was plotted against impedance index (L^2/Z_{50}) showing a positive linear correlation, Pearson's $r=0.89$ (Figure 1). Weight was the single most significant independent predictor of FFM, accounting for 93.5% of the total variability in FFM. However, using likelihood ratio tests, we found that adding impedance index, age and sex as predictor variables in addition to body weight resulted in an improved model fit (P -value for all likelihood ratio tests in the stepwise forward model search < 0.01). The final model accounted for 95.4% (adjusted R^2) of the total variability in FFM estimated by the PEA POD with an RMSE of 181 g (Table 2). The RMSE of the final model corresponded to a prediction error of 4.4% (RMSE/mean FFM (PEA POD) × 100%). Prediction of FM used a similar approach. The final model predicting FM included impedance index, age and sex as predictor variables and accounted for 92.4% of the total variability in FM estimated by the PEA POD with an RMSE of 180 g (Table 2). The RMSE of the final model corresponded to a prediction error of 13.1% (RMSE/mean FM (PEA POD) × 100%).

Additionally, subanalysis was conducted stratifying the study population into above and below 3 months of age. In the age group below 3 months, impedance index did not contribute to an improved model fit for predicting FFM, compared with a model already comprising weight, sex and age. However, in the above 3 months of age group, inclusion of impedance index resulted in an improved model fit for predicting FFM. Compared with a simple anthropometric model including weight, sex and age, the model comprising impedance index reduced the RMSE for FFM by 16% (Table 3).

Validation of the prediction models

Using a 10-fold cross-validation on the models developed in the total study population, we found an average RMSE of predicting FFM and FM of 184 and 183 g, respectively. A Bland–Altman plot revealed a mean difference between the predicted and the measured FFM of 0 g with 95% limits of agreement of –351; 351 g (Figure 2) and FM of 0 g with 95% limits of agreement of –350; 350 g (Figure 3). No significant difference in bias across the different levels of average FFM was observed ($P=0.14$). The magnitude of bias across different levels of average FM was borderline significant ($P=0.06$).

DISCUSSION

In the present study, prediction equations for FFM and FM were developed and cross-validated based on a large sample of

Table 1. Description of the study subjects

	Birth (n=21) Mean $\pm$ s.d.	1 Month (n=46) Mean $\pm$ s.d.	2 Months (n=39) Mean $\pm$ s.d.	3 Months (n=40) Mean $\pm$ s.d.	4 Months (n=24) Mean $\pm$ s.d.	5–6 Months (n=16) Mean $\pm$ s.d.
Age (days)						
Girls	1 $\pm$ 1	46 $\pm$ 8	74 $\pm$ 6	102 $\pm$ 6	132 $\pm$ 4	180 $\pm$ 5
Boys	1 $\pm$ 0	48 $\pm$ 4	76 $\pm$ 6	104 $\pm$ 6	132 $\pm$ 6	178 $\pm$ 8
Gestational age (weeks)^{a,b}						
Girls	39 (38–41)	39 (37–40)	39 (37–41)	39 (38–40)	39 (37–40)	38 (37–39)
Boys	39 (37–40)	39 (37–40)	39 (37–41)	39 (37–41)	39 (37–41)	39 (37–39)
Weight (kg)						
Girls	2.99 $\pm$ 0.24	4.25 $\pm$ 0.75	5.45 $\pm$ 0.76	5.77 $\pm$ 0.88	6.32 $\pm$ 0.66	6.65 $\pm$ 0.86
Boys	3.15 $\pm$ 0.34	5.13 $\pm$ 0.52	5.89 $\pm$ 0.69	6.69 $\pm$ 0.68	7.09 $\pm$ 0.93	7.49 $\pm$ 1.32
Length (cm)						
Girls	49.0 $\pm$ 1.8	53.9 $\pm$ 2.8	57.6 $\pm$ 2.4	59.4 $\pm$ 3.2	61.8 $\pm$ 2.2	63.0 $\pm$ 2.1
Boys	49.7 $\pm$ 1.2	56.6 $\pm$ 2.1	59.4 $\pm$ 2.1	61.9 $\pm$ 2.4	63.0 $\pm$ 3.0	65.6 $\pm$ 2.4
BMI (kg/m²)						
Girls	12.4 $\pm$ 0.7	14.5 $\pm$ 1.5	16.3 $\pm$ 1.2	16.3 $\pm$ 1.5	16.5 $\pm$ 1.0	16.7 $\pm$ 1.6
Boys	12.7 $\pm$ 1.1	16.0 $\pm$ 1.2	16.6 $\pm$ 1.3	17.4 $\pm$ 1.3	17.8 $\pm$ 1.8	17.3 $\pm$ 2.1
Ponderal index (kg/m³)						
Girls	25.4 $\pm$ 1.9	26.8 $\pm$ 2.2	28.3 $\pm$ 1.9	27.5 $\pm$ 2.7	26.7 $\pm$ 1.6	26.5 $\pm$ 2.6
Boys	25.6 $\pm$ 2.2	28.3 $\pm$ 2.4	28.0 $\pm$ 2.3	28.2 $\pm$ 2.6	28.3 $\pm$ 3.3	26.3 $\pm$ 2.2
FM (kg)						
Girls	0.17 $\pm$ 0.08	0.94 $\pm$ 0.35	1.49 $\pm$ 0.40	1.62 $\pm$ 0.46	1.79 $\pm$ 0.45	1.78 $\pm$ 0.54
Boys	0.27 $\pm$ 0.17	1.15 $\pm$ 0.29	1.51 $\pm$ 0.31	1.85 $\pm$ 0.40	1.97 $\pm$ 0.52	2.00 $\pm$ 0.63
FFM (kg)						
Girls	2.82 $\pm$ 0.23	3.32 $\pm$ 0.48	3.97 $\pm$ 0.41	4.17 $\pm$ 0.52	4.53 $\pm$ 0.44	4.87 $\pm$ 0.47
Boys	2.88 $\pm$ 0.24	3.97 $\pm$ 0.30	4.38 $\pm$ 0.43	4.85 $\pm$ 0.41	5.12 $\pm$ 0.50	5.49 $\pm$ 0.75
Body fat (%)						
Girls	5.5 $\pm$ 2.5	21.4 $\pm$ 5.7	26.9 $\pm$ 4.5	27.6 $\pm$ 4.9	28.1 $\pm$ 4.8	26.3 $\pm$ 5.7
Boys	8.4 $\pm$ 4.6	22.3 $\pm$ 3.9	25.5 $\pm$ 2.9	27.4 $\pm$ 4.1	27.3 $\pm$ 4.7	26.2 $\pm$ 4.3
Impedance, Z₅₀ (Ω)						
Girls	741 $\pm$ 89	853 $\pm$ 94	832 $\pm$ 93	841 $\pm$ 86	855 $\pm$ 77	916 $\pm$ 38
Boys	763 $\pm$ 102	757 $\pm$ 47	784 $\pm$ 89	770 $\pm$ 60	785 $\pm$ 61	840 $\pm$ 131
Impedance index, L²/Z₅₀ (cm²/Ω)						
Girls	3.3 $\pm$ 0.4	3.5 $\pm$ 0.6	4.1 $\pm$ 0.7	4.3 $\pm$ 0.8	4.5 $\pm$ 0.5	4.3 $\pm$ 0.3
Boys	3.3 $\pm$ 0.4	4.2 $\pm$ 0.4	4.6 $\pm$ 0.7	5.0 $\pm$ 0.6	5.1 $\pm$ 0.6	5.3 $\pm$ 1.2

Abbreviations: BMI, body mass index; FFM, fat-free mass; FM, fat mass. ^aGestational age is presented as median (range). ^bFour missing.

Ethiopian infants. The results suggest that the accuracy of predicting FFM and FM was significantly improved when impedance index was included in models already comprising weight, sex and age. Inclusion of impedance index reduced the RMSE by 10% for both FFM and FM, and therefore brings an important additional benefit to the models. The 10-fold cross-validation yielded almost similar prediction errors as the developed prediction models of FFM and FM based on the full sample, indicating that the final models on average performed well on data not used in the modelling. However, the cross-validation was applied on the same overall data sample and it is therefore likely that the RMSE estimates outside the present sample will be slightly inflated.

At population level, the average agreement between predicted and measured FFM and FM, respectively, was excellent. However, the relatively wide 95% limits of agreement for both FFM and FM suggest that the predictive accuracy at an individual level may be limited, and thus more appropriate for predictions at a population level.

Despite almost similar RMSE, the prediction error for FM was considerably higher than for FFM (13.1% vs 4.4%). This was expected, as FM compared with FFM constitutes a much smaller proportion of the total body weight. Hence, the prediction error of FM can be very high in infants with little FM. For example, the average FFM and FM in newborns from Jimma has previously been found to be 2804 and 232 g, respectively,²¹ yielding

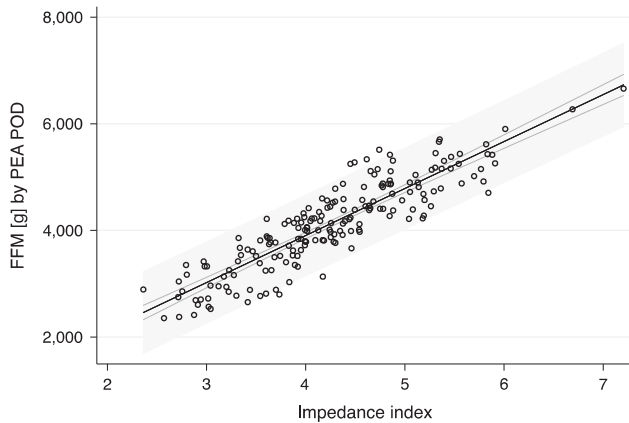


Figure 1. Impedance index (L^2/Z_{50}) plotted against FFM estimated by IADP. The narrow curved lines shows the 95% confidence intervals of the regression line and the grey area represents the prediction interval (i.e. confidence interval for an individual forecast) ($n = 186$).

Table 2. Multiple regression analyses of weight (kg), length (cm), sex, age (days) and impedance index (L^2/Z_{50}) for predicting fat-free mass (kg) and fat mass (kg) measured by the PEA POD ($n = 186$)

Equation	Adjusted R^2 (%)	RMSE (kg)
Prediction of fat-free mass		
$0.379 + 0.881 \times (L^2/Z_{50})$	79.5	0.383
$1.000 + 0.569 \times \text{weight}$	93.5	0.215
$1.169 + 0.552 \times \text{weight} + 0.145 \times \text{sex}^a$	94.2	0.204
$1.303 + 0.508 \times \text{weight} - 0.171 \times \text{sex} + 0.002 \times \text{age}$	94.4	0.200
$-0.195 + 0.439 \times \text{weight} - 0.151 \times \text{sex} + 0.034 \times \text{length}$	94.6	0.196
$1.015 + 0.358 \times \text{weight} - 0.132 \times \text{sex} + 0.003 \times \text{age} + 0.233 \times (L^2/Z_{50})$	95.4	0.181
Prediction of fat mass		
$-1.120 + 0.586 \times (L^2/Z_{50})$	58.4	0.422
$-0.995 + 0.431 \times \text{weight}$	89.2	0.215
$-1.168 + 0.448 \times \text{weight} + 0.148 \times \text{sex}$	90.3	0.204
$-1.300 + 0.492 \times \text{weight} - 0.174 \times \text{sex} - 0.002 \times \text{age}$	90.7	0.200
$0.206 + 0.562 \times \text{weight} + 0.155 \times \text{sex} - 0.035 \times \text{length}$	91.1	0.196
$-1.010 + 0.643 \times \text{weight} - 0.134 \times \text{sex} - 0.003 \times \text{age} - 0.234 \times (L^2/Z_{50})$	92.4	0.180

Abbreviation: RMSE, root mean square error. ^aMale = 0; female = 1.

prediction errors of 6.5% for FFM, but as much as 77.6% for FM, indicating limited use of predictions of FM at younger ages.

A total of 44 infants were assessed multiple times on different occasions throughout the study, but each of the assessments were treated as independent entities in the analysis. Thus, the observations could be regarded as non-independent. The median interval between measurements of infants included multiple times were 28.5 days and as infants change considerably in their size and shape in the first few months, it is unlikely that multiple assessments within infants have affected our analysis.

We found only one study developing prediction equations using BIA in an infant population from sub-Saharan Africa. Wells *et al.*²⁰ studied a small sample ($n = 20$) of 3- to 18-month-old Gambian infants, using deuterium dilution to estimate FFM. The study concluded that despite BIA having poorer accuracy than direct BC methods like deuterium dilution, the developed equations including BIA could be used to compare BC between groups at the population level with an acceptable accuracy.

Table 3. Multiple regression analyses of weight (kg), length (cm), sex, age (days) and impedance index (L^2/Z_{50}) for predicting fat-free mass (kg) measured by the PEA POD in children below ($n = 106$) and above ($n = 80$) 3 months of age

Equation	Adjusted R^2 (%)	RMSE (kg)
Prediction of fat-free mass (< 3 months)		
$0.625 + 0.780 \times (L^2/Z_{50})$	75.4	0.327
$1.118 + 0.542 \times \text{weight}$	93.1	0.174
$1.296 + 0.521 \times \text{weight} - 0.145 \times \text{sex}^a$	94.1	0.160
$1.173 + 0.570 \times \text{weight} - 0.125 \times \text{sex} - 0.002 \times \text{age}^b$	94.4	0.156
$1.102 + 0.462 \times \text{weight} - 0.120 \times \text{sex} + 0.117 \times (L^2/Z_{50})$	94.5	0.155
Prediction of fat-free mass (≥ 3 months)		
$1.340 + 0.720 \times (L^2/Z_{50})$	73.2	0.333
$0.896 + 0.588 \times \text{weight}$	84.0	0.258
$1.215 + 0.552 \times \text{weight} - 0.168 \times \text{sex}$	85.2	0.248
$1.099 + 0.508 \times \text{weight} - 0.202 \times \text{sex} + 0.003 \times \text{age}$	87.0	0.232
$-0.897 + 0.408 \times \text{weight} - 0.191 \times \text{sex} + 0.002 \times \text{age} + 0.045 \times \text{length}$	88.4	0.220
$0.731 + 0.313 \times \text{weight} - 0.140 \times \text{sex} + 0.005 \times \text{age} + 0.308 \times (L^2/Z_{50})$	90.7	0.196

Abbreviation: RMSE, root mean square error. ^aMale = 0, female = 1. ^bLength was not significant and thus excluded from the model.

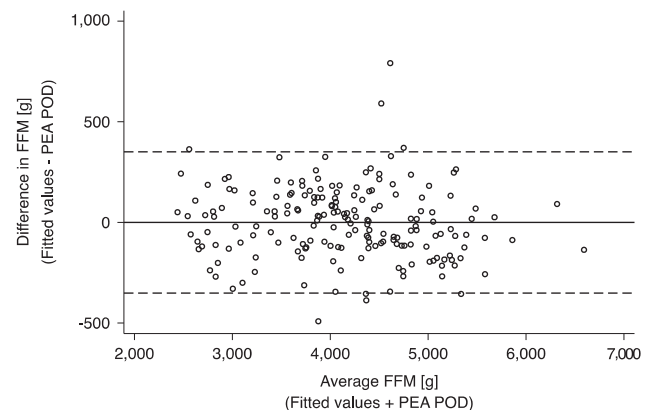


Figure 2. A Bland-Altman plot of the difference between FFM estimated by IADP and predicted FFM using BIA ($n = 186$).

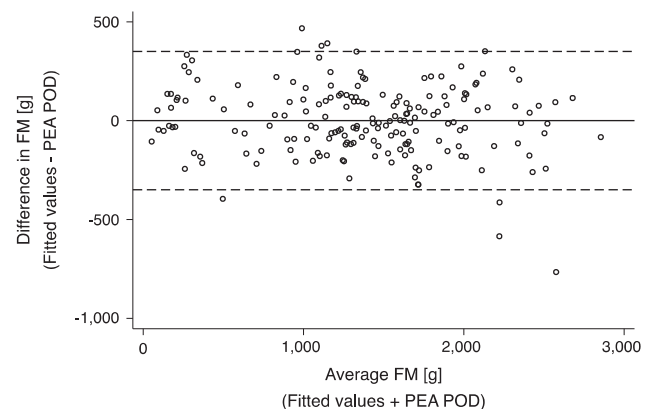


Figure 3. A Bland-Altman plot of the difference between FM estimated by IADP and predicted FM using BIA ($n = 186$).

In the literature, it is disputed whether BIA in children offers a significant advantage over simpler anthropometric prediction models including age, sex, weight and length/height. In the present study, compared with a simple model including weight,

length and sex, a model further incorporating impedance index performed better at predicting FFM and FM, with ~8% smaller RMSE (Table 2). However, our findings from the stratified subanalysis suggest that impedance index measured using BIA contributes to an improved model fit in the age group above 3 months, but not in the age group below 3 months for predicting FFM (Table 3). This finding is in agreement with a recent study on Australian infants by Lingwood *et al.*³² This study predicted FFM using IADP and concluded that BIA did not provide additional improvements in comparison with simple anthropometric measures in infants younger than 3 months. However, at 3 and 4.5 months of age the prediction of FFM improved slightly when BIA was included. In the present study, compared with the final prediction model based on the total study population, the R^2 value for the final model based on infants above 3 months has decreased. This is most likely due to the smaller group size and greater variability in BC as the infants becomes older. For the infants below 3 months, there is less variability in BC, and the relationship with weight, age and sex is stronger. This might have strengthened the predictive ability of the prediction model based on the total study population. Dung *et al.*³³ measured BC using dual energy X-ray absorptiometry (DXA) and BIA in preterm infants (mean gestational age at study 38.6 weeks) and suggested that BIA provides little improvements over anthropometric measures in the prediction of FFM during the first months of life. The specific cutoff point of when BIA improves the prediction of FFM compared with a simple anthropometric model has not been established from current research, and future studies on BIA in infancy should aim to establish such cutoff age. However, our study is one of the first to show that BIA is valuable for estimating BC in early infancy.

Studies in older children found that BIA performed well in predicting FFM. Ejlerskov *et al.*³⁴ developed prediction equations for FFM in 99 healthy Caucasian children aged 3 years using DXA as the criterion method. The authors found that predicted FFM from the developed equations was in good agreement with the estimates of FFM using DXA scans. They found a relatively wide distribution of the individual predictions and suggested that the equations could be used in studies at a population level. Other studies conducted in children have concluded that impedance index is a strong predictor of FFM, estimated using DXA scans, and that BIA measurements can be used to estimate FFM at the population level.^{5,15,16,26}

Major strengths of the collected data are the large sample size and the use of a previously validated method to estimate FFM in infants and children.^{9,10,13,22} Furthermore, the PEA POD's underlying assumptions, regarding the age- and sex-specific reference values of the density of FFM, as proposed by Fomon *et al.*,²⁴ have recently been confirmed in the present study population by Andersen *et al.*¹³ The present study is one of the first to develop prediction equations in a healthy infant population. When validating previously developed equations by Lingwood *et al.*³² on the present sample, we found that FFM was significantly overestimated. Compared with FFM estimated by the PEA POD, the predicted FFM was on average overestimated by 250–500 g, with limits of agreement of up to 1 kg. These findings accentuates that BIA equations are not universally applicable across population groups. Ethnic groups are well established to differ in their physique and limb lengths. Studies of children and adolescents have demonstrated that ethnic groups require different BIA equations, hence we expect the same scenario in infants.^{35,36} Thus, while the study on Ethiopian infants by Andersen *et al.*¹³ suggests that the composition of FFM may be the same across population groups, our study suggest that the relationship between FFM and impedance index is specific to each population group.

According to Heyward and Wagner,³⁷ one of the limitations when conducting studies using BIA is the intraindividual variation

in whole-body resistance because of factors that alter the hydration state, for example, diarrhoea or timing of breastfeeding. Only healthy infants were included and timing of breastfeeding was standardised to the extent possible; thus, intraindividual variation in hydration state is unlikely to have affected the accuracy of the BIA measurements. It is recommended that the subject is placed in a supine position for several minutes before measurement, to obtain a more even distribution of the body water.³⁸ Predominantly babies were measured while sleeping, after they had been in a supine position for ~10 min. Thus, it is unlikely that uneven water distributions or agitated study subjects have affected the accuracy of the measurements. Another potential source of error is that insufficient separation between electrodes in newborns with small hands and feet can cause electrical interference between the electrodes and artificially inflate the body impedance. Sesmero *et al.*³⁹ found that separation between the current and the sensing electrode affected the measurement. In a few infants with small hands and feet, ideal separation to avoid interference between electrodes was not possible. The current electrodes was therefore moved to the ventral rather than the dorsal surfaces of the hand and foot as recommended by Lingwood *et al.*²⁹ The study by Lingwood *et al.*²⁹ concluded that rearrangement of electrodes did not affect the impedance measurement significantly.

The prediction models were developed in a relatively homogeneous sample of 0- to 6-month-old Ethiopian infants and the measurements were performed in a standardised manner in a controlled environment. This should improve the accuracy of predicting FFM and FM in the current population, but might impair the validity of the equations outside the present population. Future studies should aim to cross-validate the developed equations in other population groups, in rural areas and in other countries in sub-Saharan Africa, as the developed equations may prove as a feasible tool to obtain accurate predictions of FFM. As the composition of FFM (e.g. decreasing hydration) changes significantly during the first several months of life,⁴⁰ it is dubious that the prediction equations can be used beyond this specific age group.

The main significance of our work is that BIA improved the accuracy of estimating BC in early life in a sub-Saharan African population. There is increasing interest in testing the efficacy of public health nutrition programmes using randomized trials. Although BIA has limits to accuracy in individuals and does not improve the predictive accuracy in infants below 3 months of age, the technique is nevertheless ideal for application across groups, and may therefore provide a new opportunity for the assessment of BC in large clinical trials, where more specialized techniques are not feasible.

We conclude that the developed prediction equations estimated FFM and FM, in a population of 0- to 6-month-old Ethiopian infants with sufficient accuracy to be applied in future studies predicting BC at a population level. The prediction models based on the total study population were significantly improved when impedance index was included. Subanalysis showed that inclusion of impedance index did not contribute to an improved model fit for predicting FFM in infants below 3 months of age. To the best of our knowledge, this study is the first to validate BIA, using accurate BC methods in a large sample in sub-Saharan Africa. Future studies should aim at validating the developed equations in similar sub-Saharan African populations and other population groups. The technique may be particularly valuable in randomized trials evaluating public health nutrition programmes for pregnant mothers and infants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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