Title:

Calibration of bioelectrical impedance analysis against deuterium dilution for body composition assessment in stunted Ugandan children

Authors:

Jack Ivor Lewis[1], Henrik Friis[1], Ezekiel Mupere[2], Jonathan C Wells[3], Benedikte Grenov[1]

[1] Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark;

[2] Department of Paediatrics and Child Health, School of Medicine College of Health Sciences, Makerere University, Kampala, Uganda;

[3] Childhood Nutrition Research Centre, Population, Policy and Practice Research and Teaching Department, University College London (UCL) Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

Author disclosures: The authors JIL, HF, EM, JW and BG all declare no conflict of interest

Corresponding author:

Benedikte Grenov, Rolighedsvej 26, DK-1958 Frederiksberg C, Denmark, telephone: +4520456654, email: bgr@nexs.ku.dk

Funding: The research was funded by Dr. Thorvald Madsens Legat

This manuscript contains original data not previously presented elsewhere in any form.

Running title: BIA calibration in stunted Ugandan children

Abbreviations:

^{2}H	Deuterium
BC	Body composition
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CS	Cross-sectional area
FFM	Fat-free mass
FFMI	Fat-free mass index
FM	Fat mass
FMI	Fat mass index
HAZ	Height-for-age z-score
ND	Deuterium dilution space
PA	Phase angle
R	Resistance
RMSE	Root-mean square error
SAM	Severe acute malnutrition
TBW	Total body water
WHO	World Health Organization
WHZ	Weight-for-height z-score
Xc	Reactance
Z	Impedance
Z_{50}	Impedance, measured at 50 kHz

Abstract (250 words):

Background. Bioelectrical impedance analysis (BIA) represents an important tool in body composition (BC) assessment, especially in low-income settings where simple and affordable options are preferred. There is a particular need to measure BC in stunted children, where population-specific BIA estimating equations are lacking.

Objective. We calibrated an equation to estimate body composition from BIA using deuterium dilution (^{2}H) as the criterion method in stunted children.

Methods. We measured BC with ^{2}H and performed BIA in stunted Ugandan children (n = 50). Multiple linear regression models were constructed to predict ^{2}H-derived fat-free mass (FFM) from BIA-derived whole-body impedance (Z_{50}) and other relevant predictors. Model performance was expressed as adjusted R^2 and root-mean square error (RMSE). Prediction errors were also calculated.

Results. Participants were aged 16 to 59 months, 46% were girls and their median [IQR] height-for-age z-score (HAZ) was -2.58 [-2.92, -2.37] according to WHO growth standards. Impedance index (height^2/Z_{50}) alone explained 89.2% variation in FFM and had an RMSE of 583 g (precision error 6.5%). The final model contained age, sex, impedance index and HAZ as predictors, and explained 94.5% variation in FFM with an RMSE of 402 g (precision error 4.5%).

Conclusion. We present a BIA calibration equation for a group of stunted children with relatively low prediction error. This may help evaluate the efficacy of nutritional supplementation in large-scale trials in the same population.

Key words: stunting, body composition, deuterium dilution, bioelectrical impedance analysis

Introduction

Current estimates suggest around 150 million children below 5 years globally suffer from stunting, a form of undernutrition defined as a height-for-age z-score (HAZ) < -2 SD according to the WHO growth standards (1). Childhood stunting increases mortality and morbidity risk, and is associated with poor physical and cognitive development, lower schooling attainment and unmet potential of human capacity (2).

Direct assessment of body composition (BC) is important for monitoring of healthy growth in stunted children, with fat and fat-free tissues contributing to different domains of health status, such as immune function (3) and homeostatic metabolic capacity (4), respectively. For cruder measurements of body mass index (BMI), calculated as weight divided by the square of height, the reduced height of stunted children could result in an inaccurate estimation of childhood adiposity. There are several alternatives for accurately measuring BC in pediatric populations (5,6), including air-displacement plethysmography (7), dual-energy X-ray absorptiometry (8,9) and isotope dilution (10–13). However, the large-scale use of such techniques is complicated by high costs, bulky equipment and/or extensive training requirements.

Bioelectrical impedance analysis (BIA) provides a cheap, portable and relatively easy-to-use alternative for BC estimation, making it well suited to research and monitoring in low-income settings. It has been used extensively to evaluate variability in BC in association with undernutrition and efforts to prevent or treat it (14–18). BIA uses principles of conductivity to measure the electrical impedance of a body, which can then be used to estimate total body water (TBW) and subsequently fat-free mass (FFM) using age- and sex-specific FFM hydration factors (19). In both BIA and isotope dilution methods, the 2-compartment model of BC can be used to derive FM as the difference between weight and FFM. To ensure

accurate conversion of electrical impedance to TBW, a population-specific calibration equation is constructed using a reference method of BC assessment (20). Many examples exist of these equations (11,21–23), which may further include predictive variables such as sex, age and anthropometric measurements. Paradoxically, however, populations with the highest prevalence of stunting, who may stand to gain the most from improved accuracy in BIA assessment, remain the most under-represented in such equations.

From a theoretical perspective, BIA relies on the assumption that the body represents a uniform cylinder, with impedance varying directly in proportion with its length (indexed by height) and inversely with its cross-sectional area (CS) (24). In reality, the limbs (with relatively greater length and smaller CS) contribute substantially more to whole-body impedance than the trunk (25). In stunted children, a reduced limb-to-height ratio is observed (26–28), which likely introduces error to the BIA assessment in these children if equations have been calibrated for non-stunted children. Furthermore, reliable conversion of TBW to FFM relies on accurate tissue hydration constants, which may be perturbed in children suffering from undernutrition (5,29). For this reason, the reporting of TBW in addition to FFM is valuable.

The primary aim of the present study was to calibrate BIA to estimate TBW and FFM in stunted Ugandan children aged 12-59 months, using deuterium dilution as the reference method. As a secondary aim, we compared the performance of two other equations calibrated by deuterium dilution in paediatric populations, developed by Masuda and Komiya (30) (hereon referred to as the "Masuda equation") for children in Japan and Essa'a *et al.* (31) (hereon referred to as the "Essa'a equation") for children in Cameroon. We hypothesized that stunting impacts BC-BIA associations owing to characteristic differences in limb to height ratio, and that our new equation would be more appropriate for our study population.

Materials and Methods

This study was designed as an amendment to The Role of Milk Protein and Whey Permeate in Lipid-based Nutrient Supplements (LNS) on the Growth and Development of Stunted Children in Uganda (MAGNUS) intervention trial (ISRCTN13093195) (32). The primary outcomes of the MAGNUS study were change in knee-heel and total length in stunted Ugandan children aged 12-59 mo over a 12-week intervention with one of four LNS formulations in a 2x2 factorial design (*n = 4 x 150*) or non-supplemented control (*n = 150*). Data collection for the MAGNUS study took place between February 2020 and December 2020 at two sites within the Jinja District of Eastern Uganda. Inclusion criteria for the original MAGNUS study were children living within the study catchment area, aged 12-59 mo, a HAZ <-2 according to the WHO growth standards (1), and the primary caregiver willing to return for follow-up visits. Children with severe acute malnutrition (SAM), defined as a mid-upper arm circumference (MUAC) <115 mm or a weight-for-height z-score (WHZ) <-3 or bipedal pitting oedema were excluded from the study and referred to a local hospital for treatment. Children with medical complications, disabilities effecting eating or anthropometric measurement, or an allergy to peanuts or milk were further excluded. The current study took place in October – November 2021 at one of the MAGNUS study sites, Buwenge health center IV. Potential participants who were still aged <59 mo were identified from the original MAGNUS register. From this list, a sample of eligible participants were selected to cover a wide range of size and nutritional status across ages and sex. This strategy ensures a calibration equation relevant across the full range of the specific population (33). Relevant caregivers were contacted by telephone and invited for screening. Due to aging of original MAGNUS participants, some children from the local health center aged 12-24 mo were identified and invited for screening. To be eligible for inclusion, participants had to be

aged 12-59 mo with supporting documentation, still stunted with a HAZ <-2 (1), with their primary caregiver having provided written informed consent to participate. Participants were excluded if they presented with clinical signs of sickness or if they were SAM according to the above criteria.

Ethical approval and Informed consent:

The amended study was conducted in accordance with the ethical principles stated in the current version of the Declaration of Helsinki and all applicable local regulatory requirements. The study amendment was approved by the School of Medicine Research Ethics Committee at Makerere University (Ref. 2019-013) and The Ugandan National Council of Science and Technology (Ref. SS 4927). Study information was delivered to caregivers in the local languages of Lusoga, Luganda or English, as appropriate. Written informed consent was obtained from willing caregivers on behalf of their child according to local regulations. Study staff were all involved in the original MAGNUS study and completed a five-day training program before data collection. All participants were tested for malaria infection, and additionally for HIV if they had not been tested in the MAGNUS study.

Bioelectrical impedance analysis

Participants arrived on the morning of the one-day examination fasted since the night before. Directly after enrollment, all participants underwent BIA assessment by a single study nurse. Whole-body impedance was measured at 50 KHz using Bodystat 500 instrumentation (Bodystat, Isle of Man, UK), calibrated daily according the manufacturers' instructions. Measurements took place with the child in a supine position on an insulated matt wearing

only dry loose-fitting clothes. A position score was assigned by the nurse, ideally requiring straight arms and legs resting on the ground without limbs touching. Disposable small-sized gel-adhesive electrodes were placed in tetrapolar ipsilateral formation, on the right-hand side of the body. Specifically, two electrodes of one cable were attached behind the knuckle of the middle finger and beside the ulna head, and two electrodes of the other cable were attached behind the second toe and between the medial and lateral malleoli. To avoid electrical interference, a minimum distance of 3 cm between electrodes was maintained. For particularly small infants, the proximal electrode could be moved up the arm or leg to maintain this distance. Impedance (Z), resistance (R), reactance (Xc) and phase angle (PA) were recorded; Z and R to the nearest whole ohm (Ω), Xc to the nearest 0.1 Ω and PA to the nearest 0.1°. A duplicate measurement was taken approximately three minutes after the first. Accepted ranges for individual measurements were; 300 – 1600 Ω for R, 20.0 – 90.0 Ω for Xc and 2.0 – 7.0° for PA. Measures had to be within these accepted ranges, plus duplicate R and Xc measures had to be within 20.0 Ω and 10.0 Ω of each other, respectively. A third measure was taken if any range requirement was violated and a mean average of two appropriate readings used in analysis. The within-subject coefficient of variation for Z was 0.05%.

Anthropometric measurements

Participant weight was measured to the nearest 100 g in light clothing using digital scales (SECA 874, Hamburg, Germany) which were calibrated daily. Length (<24 mo) or height (≥24 mo) was measured to the nearest 1 mm using a wooden measuring board (Shorrboards®, Maryland, USA). MUAC was measured at the midpoint between the acromion and olecranon processes using a non-stretchable coloured UNICEF tape to the

nearest 1 mm. All measurements were performed in triplicate and the median used for analysis.

Deuterium dilution technique

Deuterium doses were prepared at the Department of Biochemistry & Sports Science, Makerere University, Uganda. Two dose sizes were prepared, appropriate for smaller and larger children, respectively. For small doses, 99.8% deuterium oxide (Cambridge Isotope Laboratories Inc., MA, USA) was passed through a 0.2-µm syringe filter into a borosilicate stock bottle and diluted to a target concentration of 7.4% (w/w) with bottled water (Rwenzori, Uganda). The solution was divided into individual 11-ml dose bottles to deliver ~0.74 g of D_2O. For large doses, the same process was followed but to a target concentration of 5.0% (w/w). The solution was divided into individual 21-ml dose bottles to deliver ~1 g of D_2O. Both dose sizes contained a D_2O dose of between 0.05 and 0.10 g per kg body weight according to previous recommendations (34). Dose bottles were sealed, frozen at -20ºC, and transferred to the field site freezer. These bottles stayed frozen until required for use each morning, unused doses were returned to the freezer for a maximum of three days before being discarded. The three stages of the deuterium dilution technique; pre-dose saliva sampling, dosing, and post-dose saliva sampling took place at separate workstations to avoid contamination. New dry gloves were worn at all times when processing saliva samples.

After a 20-minute nil-by-mouth period was observed, pre-dose saliva samples were collected using a cotton stick with a closed mouth. The cotton was removed from the stick and saliva expressed using a 10-ml syringe into a labeled cryotube. For small doses, 10 ml 7.4% deuterium solution was drawn into a syringe which was wiped dry and weighed in a ziplock bag along with five pieces of gauze to the nearest 0.01 g using calibrated laboratory scales.

169 The remaining 1 ml of solution was pipetted into a cryotube for later analysis. For large
170 doses, 1 ml 5.0% deuterium solution was pipetted from the dose bottle into a cryotube for
171 later analysis. The bottle and remaining 20 ml of solution was weighed in a ziplock bag along
172 with a drinking straw and five pieces of gauze as above.

173 Small doses were administered into the corner of the mouth using the syringe and large doses
174 were consumed through the straw provided. In both cases, any spillage was collected on the
175 pre-weighed gauze and finally all items were returned to the same pre-weighed bag to
176 calculate the exact dose received. Participants fasted for 15 minutes post-dose and were
177 monitored in case of regurgitation. Participants then ate one banana to standardize food
178 consumption during the equilibration period. They also had access to bottled water
179 (Rwenzori, Uganda), but consumption was measured by study staff and limited to <200 ml.
180 After a 3-hour equilibration period under observation, a post-dose saliva sample was
181 collected using the same method as for the pre-dose sample. Pre-dose saliva, dose samples
182 and post-dose saliva were collected into separate ziplock bags, and stored in separate freezer-
183 boxes for up to 6 hours on site before being transferred to a freezer at -20^{o}C. The complete
184 sample set was transferred to Makerere biorepository for freezing at -80^{o}C, before being sent
185 to Iso-Analytical Ltd., United Kingdom, for analysis.

186

187 Deuterium analysis

188 Deuterium enrichment of saliva was analysed by isotope-ratio mass spectrometry. Deuterium
189 dilution space (N_D) was calculated by the plateau method using the following equation (34):

190 $$N_D\,(kg) \quad = \quad ((T * A)/a).\ *.\ ((E_D - E_T)/(E_S - E_P))$$

191 where T is the mass of dose consumed by the child, a is the portion of dose diluted for
192 analysis in grams, A is the mass of tapwater for the dilution, and E is isotopic enrichment in

delta per mille (‰) where D, T, S and P refer to the samples of the dose, tap water, post-dose saliva and pre-dose saliva respectively. TBW was calculated as $N_D/1.044$ to account for non-aqueous proton exchange (35). TBW was converted to FFM using previously published age- and sex-specific lean tissue hydration factors best suited to the age range of our sample (19) and as recommended by the International Atomic Energy Agency (36). FM was the difference between weight and FFM.

Statistical analyses

All statistical analyses and plots were performed using R version 4.2.1 (37), including the *anthro*, *lmtest* and *ggplot* packages. Fat-free mass index (FFMI) and fat mass index (FMI) were generated using the formulae:

$$\text{FFMI (kg/m}^2\text{)} = \text{FFM / height}^2$$

$$\text{FMI (kg/m}^2\text{)} = \text{FM / height}^2$$

Impedance index was calculated as H^2/Z_{50}, where H was height or length in cm and Z_{50} was whole-body impedance in Ω measured at 50 kHz.

Descriptive statistics are presented as mean (SD) for continuous variables except age, weight and height, which are presented as mean (range). Stunting categories were defined using cutoffs of below -2 and below -3 z-scores for HAZ according to the WHO growth standards (1) for moderate and severe stunting, respectively. Sex differences at baseline were tested using linear model ANOVA and Pearson's Chi-squared tests as appropriate.

The calibration equation was developed iteratively using multiple linear regression models using the full (n = 50) sample. FFM measured by deuterium dilution, the dependent variable, was modelled by independent variables added into the model in a stepwise fashion.

Independent variables of interest were identified *a priori*, as was the decision to omit participant weight from model selection. Likelihood ratio tests for nested models were used to compare model performance and assess benefit of added terms. The stepAIC function within the *MASS* package confirmed the final model with a forward and backward stepwise approach. The independent variables of the full model were used to prepare an additional model predicting TBW and a simple calibration equation, containing only H^2/Z_{50}, was also generated for FFM and TBW. Model assumptions of linearity, and normality and homoscedasticity of residuals were confirmed using *autoplot*. Multicollinearity between independent variables was further ruled out. Adjusted R^2 (%), root mean square error (RMSE) (kg), and percentage of prediction error (RMSE / mean tissue weight by ^2H (kg) x 100) were calculated for FFM to assess prediction error of the calibration equation.

The Masuda and Essa'a equations were identified following a literature search for age-appropriate BIA calibrations, further restricted to those in which deuterium dilution was used as the reference technique. They were applied to our sample to generate FFM and FM. Bland-Altman plots were constructed to compare their performance against body composition as measured by ^2H dilution. Simple linear regression models were constructed to assess correlation between the average value and difference between methods. A *P*-value of <0.05 was considered significant.

Results

A description of the study population is presented in **Table 1.** Of the 50 enrolled participants, 29 (58%) had taken part in the MAGNUS study and the remainder were newly recruited. The MAGNUS study population in Buwenge was predominantly rural-dwelling, with few

mothers having received formal education and the local diet was low in animal-source foods. The HAZ and WHZ of the current sample are comparable to the MAGNUS sample (unpublished data, Grenov B & Friis H, University of Copenhagen, 2022). Most participants were moderately stunted and one boy was moderately acutely malnourished.

The mean (sd) dose of deuterium was 1.22 (0.32) g/kg bodyweight. During the ^{2}H equilibration period, participants consumed a median [IQR] of 65 [53, 118] ml of water. Boys had 1.02 [95%CI: 0.31, 2.00] kg higher FFM and 0.87 [95%CI: 0.45, 1.28] kg/m^2 higher FFMI than girls.

The equation containing impedance index only took the following form:

$$FFM\ (kg) = 1.669 + 0.836*(H^2/Z_{50})$$

where height is measured in cm. This equation had an adjusted R^2 of 89.2% and an RMSE of 583 g, corresponding to a prediction error of 6.4% (**Table 2**). **Figure 1a** and **Figure 1b** show the association between H^2/Z_{50} and FFM measured by ^{2}H. Following model selection, the best performing model took the form:

$$FFM\ (kg) = 3.796 + 0.214*(HAZ) + 0.488*(Sex) + 0.068*(Age) + 0.400*(H^2/Z_{50})$$

where age was in months and sex was coded as female = 0 and male = 1. This full model explained 94.5% variation in FFM, and had an RMSE of 402 g corresponding to a prediction error of 4.6%. Each term in the model; LAZ, sex, age and impedance index, were independent predictors of FFM and the addition of each term improved model performance as tested by likelihood-ratio tests ($P < 0.001$). **Figure 1b** shows the association between FFM

measured by ^{2}H and estimated by the full BIA model. Accordingly, the same RMSE for FM resulted in a prediction error of 16.7%. Equivalent model specifications for TBW are presented in **Table 2**.

We tested for systematic differences between the methods with Bland-Altman plots. The Masuda equation showed a small, non-significant underestimation of FFM by 119 g with 95% limits of agreement of -903; 665 g, compared to ^{2}H (**Figure 2a**). The reverse bias was evident for FM (**Figure 2b**). Regression models did not detect an association between the mean and difference of the Masuda equation- and ^{2}H-derived FFM ($P = 0.903$) or FM ($P = 0.152$). Bland-Altman plots for the Essa'a equation showed considerable underestimation of FFM by 1708 g with 95% limits of agreement from -2969 to 447 g, compared to ^{2}H (**Figure 2c**). The reverse bias was evident for FM (**Figure 2d**). Regression models detected an inverse association between the mean and difference of the two methods for FFM ($\beta = -256$ g, $P < 0.001$), and a borderline positive association for FM ($\beta = 249$ g, $P = 0.056$). Histograms of residuals for impedance index-only and full models predicting FFM and TBW are presented in **Supplementary figure 1**.

Discussion

In this study, we calibrated an equation to predict FFM from bioelectrical impedance assessment in stunted Ugandan children using ^{2}H dilution as the criterion method. The simple equation containing only impedance index as an independent variable performed well, accounting for 89% of the variation in FFM. Similar equations calibrating BIA with ^{2}H in children, relying on impedance index as a sole predictor, have R^2 ranging from 42% to 91% (11,21,31,33,38–43) , placing our equation well. The spread of data points from the regression in **Figure 1a** and **Figure 1b** supports a strong performance across the full range of

FFM present in our sample. The addition of age, sex and LAZ into our model increased its predictive performance to account for 94.5% variation in FFM, which remains strong compared to existing fully-specified models (68% - 96%) (11,21,33,38–45). It should be noted, however, that the majority of the previously calibrated equations are generated within children over five years of age, often an easier age to achieve accurate BIA readings. This improvement over our simple impedance index model is visualized by the residual reduction in **Figure 1b** and **Supplementary figure 1**. The 402 g RMSE (4.6% prediction error) for FFM in our full model is acceptable, but indicates some variation in BIA assessment at the individual level. This is a well-established reality for BIA and supports its application in group-level investigations and monitoring (46), moreover it should be noted that some of the disagreement between techniques must relate to error in TBW assessment. Though the ideal reference method from the perspective of BIA theory, as water is the primary conducting substance in the body, deuterium dilution estimates TBW with an accuracy of 1-2% (47) for reasons such as dosing inaccuracies and laboratory instrument error (48). Regardless of methodology, such prediction error in TBW and hence FFM is propagated to FM estimation with increased relative magnitude, owing to the fact that it is the minority component of body weight. In the present case the prediction error for FM is 16.7% using the full equation, although the magnitude of this prediction error could partly be explained by the relative thinness of our stunted children.

Weight is often included in BIA calibration equations, offering a small improvement in R^2. However, our decision to omit weight from model selection was based on fundamental principles of the technique. By including weight in the equation predicting FFM, the calculation of FM from FFM and weight in the second step can result in an unwanted autocorrelation across the two steps of FM prediction. The chosen approach allows more independent assessment of the two components of body composition. The addition of HAZ

317 into our model increased adjusted R^2 by a further ~1% (**Table 2**), which we interpret as
318 suggesting the degree of stunting influences on the performance of BIA even within a wholly
319 stunted population, albeit to a small degree. The inclusion of a HAZ term into future
320 prediction equations would therefore be important in a population where the HAZ range
321 extended into non-stunted categories.

322 This is the first BIA calibration equation developed specifically for use in stunted children.
323 Being affordable and relatively simple to implement in low-income and field settings, BIA is
324 well-suited to populations with a greater stunting prevalence (46). The accuracy of BIA is
325 reliant on population-specific calibrations against a reference technique. With the presented
326 equation, we provide an opportunity for wider application of BIA in populations currently
327 under-represented by calibration equations and within whom body composition assessment is
328 increasingly recognized as valuable (5,27). As described by the nutrition transition (49), low-
329 income populations classically associated with nutritional deficits and higher rates of stunting
330 are increasingly exposed to energy-dense and nutrient-poor food. Accordingly, increasing
331 rates of overweight and obesity manifesting in the same individuals have particularly adverse
332 effects on the risk of non-communicable disease, termed a double-burden of malnutrition (3).
333 In these settings, body composition assessment appears to hold the key to understanding the
334 long-term consequences of catch-up growth (10,50). There has been a hesitancy in
335 recommending nutritional supplementation to combat stunting for fear of preferential accrual
336 of FM over FFM (51,52), and inadvertently contributing to the double burden[3]. Support for
337 this concern is mixed (53), and we anticipate this equation can be applied in further
338 investigations involving larger population samples to provide more concrete evidence of the
339 effects of nutritional supplementation in stunted children.

340 As a secondary aim, we compared the performance of two other calibration equations
341 generated in children using ^2H. The Masuda equation was developed in healthy Japanese

children aged 3 to 6 years and contained resistance index, weight, age and sex as independent variables (30). The equation performed excellently in their own sample, accounting for 96% of variation in TBW (our equation achieved 94% for TBW). The performance of the Masuda equation for predicting FFM and FM in our children was excellent, as shown by Bland-Altman comparison in **Figure 2a and Figure 2b**, respectively. The limits of agreement were comparable to our own for FFM (-903; 665 g vs. -801; 792 g) and showed no bias across the range of FFM. The Masuda children were overall older, taller and heavier (mean: ~5 years; ~109 cm; ~20 kg) and based on a crude assessment of means were within expected growth norms according to WHO standards. To interpret the performance, we believe it relevant to consider the potential for similarities in body proportions between our stunted Ugandan children and healthy Japanese children. A recent population-based study has shown Japanese and Ugandan adolescents to have similar height deficits compared to the WHO reference (54). This suggests that while the stunting prevalence of Japanese children may be low, other sources of height variability may be sufficiently powerful to create similarities in limb development compared to stunted children elsewhere. Supporting this notion, recently published reference values have shown that Japanese children have relatively shorter legs compared to British children from birth to 12 years, irrespective of height (55).

The Essa'a equation was developed in Cameroonian children aged 2 to 5 years, with a mean (sd) weight of 16.7 (2.0) kg and height of 107.0 (6.3) cm, i.e. heavier and taller than our population, and contained resistance index, weight, age and height as independent variables predicting TBW (31). Although one might expect this demographic to compare more similarly to our own, the equation performed poorly in our sample, substantially underestimating FFM with a bias towards underestimating further at greater levels of FFM (**Figure 2c, Figure 2d**) making it unsuitable for use. The equation also did not perform well in the sample from which it was generated, accounting for 68% of variation in TBW, which

could begin to explain its performance in ours. The mean (sd) HAZ of the Essa'a sample was 0.66 (1.35) z-scores above the WHO median and therefore much greater than our children. We originally hypothesized that altered limb to trunk proportions in stunted children could be an important consideration in a calibration equation, which is perhaps evidenced here and is further supported by the significant HAZ term in our own equation.

A strength of our study was the use of ^{2}H dilution as the criterion method, with proven agreement with the gold-standard four-component model (56). The quality of our BIA measurements was also high, with all position scores achieving an "ideal" rating, inter-observer variability removed due to a single trained nurse performing all assessments, and intra-observer variation minimal. This is an important consideration for future studies utilizing the equation, in which the accuracy and standardization of BIA measurements should be a priority. Factors critical to BIA methodology in children have been discussed in a recent review and include electrode placement, voiding, skin preparation and body position (57). However, with adequate standardization and training with these factors in mind, inter-measurer CVs of 2.6% and 0.2% for R have been achieved in neonates and children, respectively (58,59). The careful sample selection ensured that we had good coverage across wide ranges of age, weight and height in each sex.

Regarding limitations of the study, many BIA calibration studies perform a form of cross-validation to assess the performance of the final equation on a different sub-sample of the population. We took the decision to avoid such a validation technique due to our sample size, instead opting to allocate all data points (n = 50) to equation generation. We also carefully selected the sample across a wide range of nutritional status, in order to ensure high accuracy of the equation in thinner and heavier children. Although this could be seen as a weakness of the study, the equation would likely have been similar, but due to the smaller sample size the RMSE would have been greater, and confidence in each coefficient would have been lower.

Nevertheless, one may consider the lack of cross-validation as a preclusion to comparing this equation's performance with others as we have done. The predictive error between BIA devices has been discussed in various reviews (57,60), and should be considered when comparing BC studies. However, the devices used in our study and the compared studies have important commonalities, namely a single (50 kHz)-frequency whole-body assessment with hand-hand and foot-foot electrode placement, which provides a good foundation for comparison. We intended to generate an equation relevant for ages one to five years but were unable to recruit children below 16 months. The performance of the equation along the full range of FFM without bias, though, encourages us to believe it remains relevant for application in children from 12 months. Although this study was designed to maximize the predictive ability of the generated equation among the stunted population of the MAGNUS intervention trial, the inclusion of non-stunted children from the same population would have strengthened our investigation and may have provided further evidence that stunted linear growth influences BIA-BC assessment. It may be further argued that the applicability of our equation may falter longitudinally as children recover from stunting. Further investigations to test our hypothesis are therefore encouraged.

In conclusion, we have developed a prediction equation for estimating body composition from bioelectrical impedance, which may perform well in stunted Ugandan children. The study supports the use of BIA as a reliable technique in a low-income setting where children may have higher rates of stunting and that stunting may affect the prediction of TBW from BIA assessment.

Acknowledgements

All authors were involved in study design; JIL, BG and EM implemented and/or supervised the study; JIL and JW analyzed the data; JIL, HF, JW and BG interpreted the data; JIL drafted the manuscript, which has been critically reviewed and approved by all authors.

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

447

448

References

1. WHO Mulitcentre Growth Reference Study Group. Child Growth Standards: Length/height-for-age, weight-forage, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva, Switzerland: WHO; 2006.

2. Prendergast AJ, Humphrey JH. The stunting syndrome in developing countries. Paediatr Int Child Health 2014;34(4):250-65.

3. Wells JCK, Sawaya AL, Wibaek R, Mwangome M, Poullas MS, Yajnik C, et al. The double burden of malnutrition: etiological pathways and consequences for health. Lancet 2020;395(10217):75-88.

4. Wells JCK. The capacity-load model of non-communicable disease risk: Understanding the effects of child malnutrition, ethnicity and the social determinants of health. Eur J Clin Nutr 2018;72(5):688-97.

5. Wells JCK. Body composition of children with moderate and severe undernutrition and after treatment: A narrative review. BMC Med 2019;17(215):1-9.

6. Wells JCK. Body composition in infants: Evidence for developmental programming and techniques for measurement. Rev Endocr Metab Disord 2012;13(2):93–101.

7. Admassu B, Wells JCK, Girma T, Belachew T, Ritz C, Owino V, et al. Body composition during early infancy and its relation with body composition at 4 years of age in Jimma, an Ethiopian prospective cohort study. Nutr Diabetes 2018;8(1)1-9.

8. van Beijsterveldt IALP, van der Steen M, de Fluiter KS, Spaans SAMJ, Hokken-Koelega ACS. Body composition and bone mineral density by Dual Energy X-ray Absorptiometry: Reference values for young children. Clin Nutr 2022;41(1):71–9.

9. de Fluiter KS, van Beijsterveldt IALP, Goedegebuure WJ, Breij LM, Spaans AMJ, Acton D, et al. Longitudinal body composition assessment in healthy term-born infants until 2 years of age using ADP and DXA with vacuum cushion. Eur J Clin Nutr 2020;74(4):642–50.

10. Fabiansen C, Yaméogo CW, Iuel-Brockdorf AS, Cichon B, Rytter MJH, Kurpad A, et al. Effectiveness of food supplements in increasing fat-free tissue accretion in children with moderate acute malnutrition: A randomised 2 × 2 × 3 factorial trial in Burkina Faso. PLoS Med 2017;14(9):1-18.

11. Wells JCK, Hawton K, Darch T, Lunn PG. Body composition by 2H dilution in Gambian infants: Comparison with UK infants and evaluation of simple prediction methods. Br J Nutr 2009;102(12):1776-82.

12. Jain V, Kumar B, Devi S, Jain A, Jana M, Kurpad AV. Body composition from birth to 2 years in term healthy Indian infants measured by deuterium dilution: Effect of being born small for gestational age and early catch-up growth. Eur J Clin Nutr 2022;76(1):1165-71.

13. Skau JKH, Touch B, Chhoun C, Chea M, Unni US, Makurat J, et al. Effects of animal source food and micronutrient fortification in complementary food products on body composition, iron

status, and linear growth: A randomized trial in Cambodia. Am J Clin Nutr 2015;101(4):742-51.

14. Kangas ST, Kaestel P, Salpéteur C, Nikièma V, Talley L, Briend A, et al. Body composition during outpatient treatment of severe acute malnutrition: Results from a randomised trial testing different doses of ready-to-use therapeutic foods. Clin Nutr 2020;39(11):3426-33.

15. Bourdon C, Bartels RH, Chimwezi E, Kool J, Chidzalo K, Perot L, et al. The clinical use of longitudinal bio-electrical impedance vector analysis in assessing stabilization of children with severe acute malnutrition. Clin Nutr 2021;40(4):2078-90.

16. Kasonka L, Munthali G, Rehman AM, Chisenga M, Wells S, Wells JCK, et al. Anthropometry, body composition and chronic disease risk factors among Zambian school-Aged children who experienced severe malnutrition in early childhood. Br J Nutr 2021;128(1):453-60.

17. Bahwere P, Balaluka B, Wells JCK, Mbiribindi CN, Sadler K, Akomo P, et al. Cereals and pulse-based ready-to-use therapeutic food as an alternative to the standard milk- and peanut paste-based formulation for treating severe acute malnutrition: A noninferiority, individually randomized controlled efficacy clinical trial. Am J Clin Nutr 2016;103(4):1145-61.

18. Lelijveld N, Seal A, Wells JCK, Kirkby J, Opondo C, Chimwezi E, et al. Chronic disease outcomes after severe acute malnutrition in Malawian children (ChroSAM): a cohort study. Lancet Glob Health 2016;4(9):e654–62.

19. Lohman TG. Assessment of Body Composition in Children. Pediatr Exerc Sci 1989;1:19-30.

20. Sun SS, Chumlea C, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. Am J Clin Nutr 2003;77(1):331-71.

21. Divala OH, Mwakhwawa Q, Phiri MM, Owino V, el Kari K, Maleta KM. 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(4):1-8.

22. Wibæk R, Kæstel P, Skov SR, Christensen DL, Girma T, Wells JCK, et al. Calibration of bioelectrical impedance analysis for body composition assessment in Ethiopian infants using air-displacement plethysmography. Eur J Clin Nutr 2015;69(10):1099-104.

23. Hegelund MH, Wells JCK, Girma T, Faurholt-Jepsen D, Zerfu D, Christensen DL, et al. Validation of bioelectrical impedance analysis in Ethiopian adults with HIV. J Nutr Sci 2017;6(e62):1-7.

24. Kyle UG, Bosaeus I, de Lorenzo AD, Deurenberg P, Elia M, Gómez JM, et al. Bioelectrical impedance analysis - Part I: Review of principles and methods. Clin Nutr 2004;23(5):1226-43.

25. Fuller NJ, Elia M. Potential use of bioelectrical impedance of the 'whole body' and of body segments for the assessment of body composition: comparison with densitometry and anthropometry. Eur J Clin Nutr 1989;43(11):779-91.

26. Pomeroy E, Stock JT, Stanojevic S, Miranda JJ, Cole TJ, Wells JCK. Trade-Offs in Relative Limb Length among Peruvian Children: Extending the Thrifty Phenotype Hypothesis to Limb Proportions. PLoS One 2012;7(12):1-10.

27. Wells JCK, Devakumar D, Manandhar DS, Saville N, Chaube SS, Costello A, et al. Associations of stunting at 2 years with body composition and blood pressure at 8 years of age: longitudinal cohort analysis from lowland Nepal. Eur J Clin Nutr 2019;73(2):302-10.

28. Gunnell DJ, Smith GD, Frankel SJ, Kemp M, Peters TJ. Socio-economic and dietary influences on leg length and trunk length in childhood: a reanalysis of the Carnegie (Boyd Orr) survey of diet and health in prewar Britain (1937-39). Paediatr Perinat Epidemiol 1998;12(1):96-113.

29. Hoffman DJ, Sawaya AL, Martins PA, McCrory MA, Roberts SB. Comparison of techniques to evaluate adiposity in stunted and nonstunted children. Pediatrics 2006;117(4):e725-32.

30. Masuda T, Komiya S. A Prediction Equation for Total Body Water from Bioelectrical Impedance in Japanese Children. J Physiol Anthropol Appl Human Sci 2004;23(1):35-9.

31. Essa'a VJ, Dimodi HT, Ntsama PM, Medoua GN. Validation of anthropometric and bioelectrical impedance analysis (BIA) equations to predict total body water in a group of Cameroonian preschool children using deuterium dilution method. Nutrire 2017;42(1):1-10.

32. Pesu H, Mutumba R, Mbabazi J, Olsen MF, Mølgaard C, Michaelsen KF, et al. The Role of Milk Protein and Whey Permeate in Lipid-based Nutrient Supplements on the Growth and Development of Stunted Children in Uganda: A Randomized Trial Protocol (MAGNUS). Curr Dev Nutr 2021; 5(5):1-9.

33. Devakumar D, Grijalva-Eternod CS, Roberts S, Chaube SS, Saville NM, Manandhar DS, et al. Body composition in Nepalese children using isotope dilution: The production of ethnic-specific calibration equations and an exploration of methodological issues. PeerJ 2015;3(e785):1-12.

34. Davies P, Wells JCK. Calculation of total body water in infancy. Eur J Clin Nutr 1994;48(7):490-5.

35. Racette S, Schoeller D, Luke A, Shay K, Hnilicka J, Kushner R. Relative dilution spaces of 2H- and 18O-labeled water in humans. Am J Physiol Endocrinol Metab 1994;267(1):585-90.

36. International Atomic Energy Agency. Introduction to body composition assessment using the deuterium dilution technique with analysis of urine samples by isotope ratio mass spectrometry [Internet]. Vienna, Austria: IAEA; 2010. Available from: http://www.iaea.org/Publications/index.html

37. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2022 [cited 2022 Sep 7]. Available from: https://www.R-project.org/.

38. el Harchaoui I, el Hamdouchi A, Baddou I, el Menchawy I, Benjeddou K, Saeid N, et al. Development and validation of bioelectrical impedance analysis equations for prediction total body water and fat-free mass using D 2 O technique in Moroccan children aged between 8 and 11 years old. Eur J Clin Nutr 2018;72(12):1663-72.

39. Nightingale CM, Rudnicka AR, Owen CG, Donin AS, Newton SL, Furness CA, et al. Are Ethnic and Gender Specific Equations Needed to Derive Fat Free Mass from Bioelectrical Impedance in Children of South Asian, Black African-Caribbean and White European Origin? Results of the Assessment of Body Composition in Children Study. PLoS One 2013;8(10):1-11.

40. Liu A, Byrne NM, Ma G, Nasreddine L, Trinidad TP, Kijboonchoo K, et al. Validation of bioelectrical impedance analysis for total body water assessment against the deuterium dilution technique in Asian children. Eur J Clin Nutr 2011;65(12):1321-7.

41. Prins M, Hawkesworth S, Wright A, Fulford AJC, Jarjou LMA, Prentice AM, et al. Use of bioelectrical impedance analysis to assess body composition in rural Gambian children. Eur J Clin Nutr 2008;62(9):1065-74.

42. Rush EC, Puniani K, Valencia ME, Davies PSW, Plank LD. Estimation of body fatness from body mass index and bioelectrical impedance: Comparison of New Zealand European, Maori and Pacific Island children. Eur J Clin Nutr 2003;57(11):1394-401.

43. Houtkooper LB, Going SB, Lohman TG, Roche AF, Van Loan M. Bioelectrical impedance estimation of fat-free body mass in children and youth: a cross-validation study. J Appl Physiol 1985;72(1):366-73.

44. Kourkoumelis N, Grujic VR, Grabez M, Vidic A, Siksna I, Lazda I, et al. New bioelectrical impedance analysis equations for children and adolescents based on the deuterium dilution technique. Clin Nutr ESPEN. 2021;44(1):402-9.

45. Ndagire CT, Muyonga JH, Odur B, Nakimbugwe D. Prediction equations for body composition of children and adolescents aged 8–19 years in Uganda using deuterium dilution as the reference technique. Clin Nutr ESPEN. 2018;28(1):103-9.

46. Wells JCK, Fewtrell MS. Measuring body composition. Arch Dis Child 2006;91(7):612-7.

47. Scholeller DA. Hydrometry. In: Roche A, Heymsfield S, Lohman T, editors. Human body composition. 1st ed. Champaign, IL: Human Kinetics; 1996. p. 25-43.

48. Kuriyan R. Body composition techniques. Indian J Med Res 2018;148(1):648-58.

49. Popkin BM. The Nutrition Transition: An Overview of World Patterns of Change. Nutr Rev 2004;62(7):140-3.

50. Wibaek R, Vistisen D, Girma T, Admassu B, Abera M, Abdissa A, et al. Associations of fat mass and fat-free mass accretion in infancy with body composition and cardiometabolic risk markers at 5 years: The Ethiopian iABC birth cohort study. PLoS Med 2019;16(8):1-22.

51. Hoffman DJ, Sawaya AL, Verreschi I, Tucker KL, Roberts SB. Why are nutritionally stunted children at increased risk of obesity? Studies of metabolic rate and fat oxidation in shantytown children from São Paulo, Brazil. Am J Clin Nutr 2000;72(1):702-7.

52. Martins PA, Hoffman DJ, Fernandes MTB, Nascimento CR, Roberts SB, Sesso R, et al. Stunted children gain less lean body mass and more fat mass than their non-stunted counterparts: a prospective study. Br J Nutr 2004;92(5):819-25.

53. Fabiansen C, Phelan KPQ, Cichon B, Yaméogo CW, Iuel-Brockdorff AS, Kurpad A, et al. Short Malnourished Children and Fat Accumulation With Food Supplementation. Pediatrics 2018;142(3):1-8.

54. Rodriguez-Martinez A, Zhou B, Sophiea MK, Bentham J, Paciorek CJ, Iurilli ML, et al. Height and body-mass index trajectories of school-aged children and adolescents from 1985 to 2019 in 200 countries and territories: a pooled analysis of 2181 population-based studies with 65 million participants. Lancet 2020;396(10261):1511-24.

55. Inokuchi M, Matsuo N, Takayama JI, Hasegawa T. Reference values of inside leg length and inside leg length to stature ratio for Japanese children, 0–12 years of age. Ann Hum Biol 2022;49(1):1-9.

56. Fields DA, Goran MI. Body composition techniques and the four-compartment model in children. J Appl Physiol 2000;89(1):613-20.

57. Brantlov S, Ward LC, Jødal L, Rittig S, Lange A. Critical factors and their impact on bioelectrical impedance analysis in children: a review. J Med Eng Technol 2017;41(1):22-35.

58. de Palo T, Messina G, Edefonti A, Perfumo F, Pisanello L, Peruzzi L, et al. Normal Values of the Bioelectrical Impedance Vector in Childhood and Puberty. Nutrition 2000;16(1):417-24.

59. Piccoli A, Fanos V, Peruzzi L, Schena S, Pizzini C, Borgione S, et al. Reference Values of the Bioelectrical Impedance Vector in Neonates in the First Week After Birth. Nutrition 2002;18(1):383-7.

60. Talma H, Chinapaw MJM, Bakker B, Hirasing RA, Terwee CB, Altenburg TM. Bioelectrical impedance analysis to estimate body composition in children and adolescents: A systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. Obes Rev 2013;14(11):895-905.

Tables and figures

Table 1. Characteristics of 50 stunted Ugandan children aged 16-59 months[1]

	Male (*n = 27*)	Female (*n = 23*)	*P*-value
Age (months)	37 (17 - 59)	37 (16 - 56)	0.940
Anthropometrics			
Weight (kg)	11.8 (7.3 - 15.0)	10.9 (6.7 - 13.8)	0.243
Height (cm)	86.0 (72.0 - 98.2)	84.3 (66.7 - 96.5)	0.508
Mid-upper arm circumference (cm)	14.9 (1.0)	14.5 (1.2)	0.248
Body mass index (kg/m^2)	15.9 (1.0)	15.3 (0.9)	0.062
Height for age	-2.71 (0.55)	-2.81 (0.89)	0.853
Weight for height	-0.24 (0.82)	-0.39 (0.69)	0.500
Body mass index for age	0.15 (0.80)	-0.09 (0.68)	0.262
Height-for-age Z category			
Moderate stunting	22 (82%)	18 (78%)	0.777
Severe	5 (19%)	5 (22%)	
Weight-for-height Z category			
≥0	11 (41%)	8 (35%)	0.560
-2 to <0	15 (56%)	15 (65%)	
-3 to <-2	1 (4%)	0 (0%)	
Body composition (^{2}H)			
Fat-free mass (kg)	9.5 (1.9)	8.5 (1.6)	0.046
Fat mass (kg)	2.3 (0.7)	2.5 (0.7)	0.298
Fat-free mass index (kg/m^2)	12.7 (0.7)	11.9 (0.7)	<0.001
Fat mass index (kg/m^2)	3.2 (0.8)	3.5 (0.8)	0.143
Body fat (%)	19.7 (4.5)	22.6 (4.4)	0.023
Bioelectrical impedance			
Impedance, Z_{50} (Ω)	803 (70)	895 (93)	0.001
Impedance index (H^2/Z_{50})	9.4 (2.0)	8.1 (2.0)	0.033

[1]Presented as mean (range), mean (sd) or n (%), as appropriate; Age, Weight and Height presented as mean (range)

Table 2. Multiple regression analyses of sex, age (months), impedance index (H^2/Z_{50}) and height-for-age as predictors of total body water and fat-free mass (kg) as measured by ^{2}H-dilution in 50 stunted Ugandan children aged 16-59 months

Equation	Adjusted R^2	RMSE (g)	Prediction error (%)
Predicting FFM			
$1.669 + 0.836*(H^2/Z_{50})$	0.892	583	6.5
$5.065 + 0.984*(sex) + 0.119*(age)$	0.868	638	7.1
$2.756 + 0.406*(sex) + 0.059*(age) + 0.485*(H^2/Z_{50})$	0.941	421	4.7
$3.796 + 0.488*(sex) + 0.068*(age) + 0.400*(H^2/Z_{50}) + 0.214*(HAZ)$	0.950	402	4.5
Predicting TBW			
$1.486 + 0.628*(H^2/Z_{50})$	0.893	435	6.2
$4.042 + 0.720*(sex) + 0.089*(age)$	0.858	496	7.1
$2.239 + 0.269*(sex) + 0.042*(age) + 0.379*(H^2/Z_{50})$	0.937	326	4.7
$3.050 + 0.333*(sex) + 0.049*(age) + 0.312*(H^2/Z_{50}) + 0.167*(HAZ)$	0.942	311	4.4

Sex coded as male = 1, female = 0.

FFM Fat-free mass, H^2 height squared, HAZ height-for-age z-score, RMSE root mean square error, TBW total body water, Z_{50} impedance at 50 KHz

Figure 1

A) Fat-free mass measured by ^{2}H dilution plotted against impedance index (H^2/Z_{50}) among 50 stunted Ugandan children aged 16-59 months. Grey shading represents 95% confidence error of estimate. **B)** Fat-free mass estimated by newly generated bioelectrical impedance equation plotted against fat-free mass measured by ^{2}H dilution among 50 stunted Ugandan children aged 16-59 months. Grey shading represents 95% confidence error of estimate. BIA bioelectrical impedance analysis.

Figure 2

Bland-Altman plots of 50 stunted Ugandan children aged 16-59 months comparing **A**) fat-free mass estimated by the Masuda bioelectrical impedance equation and measured by ^{2}H dilution, **B**) fat mass estimated by the Masuda bioelectrical impedance equation and measured by ^{2}H dilution, **C**) fat-free mass estimated by the Essa'a bioelectrical impedance equation and measured by ^{2}H dilution, **D**) fat mass estimated by the Essa'a bioelectrical impedance equation and measured by ^{2}H dilution. Dotted lines represent ±1.96 SD limits of agreement. Regression line presented with 95% confidence error of estimate.