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# 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

Véronique Josette Essa'a, Henriette Thérèse Dimodi, Patricia Marianne Ntsama and Gabriel Nama Medoua\*

## Abstract

**Background:** Little information is available on the validity of anthropometry or impedance-based equations for prediction of total body water (TBW) in African children. This study was designed to validate and develop equations to predict total body water in Cameroonian children.

**Methods:** TBW was measured by deuterium dilution in 102 children between 24 and 60 months of age and compared with the ones predicted by 5 anthropometric and 7 BIA equations. Multiple linear regression analysis was used to develop prediction equations for TBW from anthropometric parameters.

**Results:** Unacceptable discrepancies in the estimates of TBW at individual level were noted with all the equations tested. The following new anthropometry and BIA equations for the estimation of TBW were respectively developed:  $TBW = 6.488 + 0.434 \times \text{sex} - 0.039 \times \text{age} + 0.670 \times \text{weight} - 0.081 \times \text{MUAC (cm)} - 0.372 \times \text{BMI}$  (adjusted  $R^2 = 0.71$ , RMSE = 3.6), and  $TBW = -6.206 + 0.0037 \times \text{height}^2/Z - 0.041 \times \text{age} + 0.265 \times \text{weight} + 0.1214 \times \text{height}$  (adjusted  $R^2 = 0.68$ , RMSE = 1.4). The cross-validation procedures revealed that the predicted values of TBW compared with measured values are accurate at a group level.

**Conclusion:** The current published anthropometric and BIA equations are invalid for the estimation of TBW in Cameroonian preschool children. The newly developed anthropometry or BIA prediction equations are valid for use in Cameroonian children aged 24–60 months.

**Keywords:** Total body water, Bioelectrical impedance analysis, Anthropometry, Deuterium dilution, Prediction equation, Children, Cameroon

## Background

Nutrition transition, characterized by changes in food consumption habits and lifestyle, which is manifested by increase in consuming energy dense food and decrease of physical activities, has been reported in several developing countries [1]. This situation, known as the double burden malnutrition [2], is associated with increasing prevalence of obesity and non-communicable diseases in people of all age groups, while undernutrition remains a public health

concern [3]. Epidemiological studies found high correlation between obesity in childhood and adult obesity [4, 5]. This implies the need to assess body composition in children.

Body composition which includes fat mass (FM) and fat-free mass (FFM) is a sensitive indicator of current health [6] and nutritional status [7] in children. The best way to assess body composition is by applying multicomponent models, in which measurements of at least two (three-component model) or more (four- or five-component models) constituents of the FFM are made [8, 9]. This approach usually requires measurement of total body water (TBW) (by isotope dilution), body density (usually by hydrodensitometry), and

\* Correspondence: gmedoua@yahoo.fr  
Centre for Food and Nutrition Research, IMPM, P O Box 6163, Yaoundé, Cameroon

mineral content (by energy x-ray absorptiometry [DEXA]) [8, 9]. Although providing accurate information, this model is expensive, technically demanding, and difficult to perform on children.

The two-compartment model in which body weight is divided into FM and FFM is suitable for use in a wide range of settings. In this model, measurement of TBW can be used to predict fat-free mass (FFM), based on the principle that FFM contains a relatively constant proportion of water [10, 11]. TBW can be measured accurately and safely in children by using deuterium dilution technique. Unfortunately, this technique is relatively expensive, not widely available, and difficult to apply to many subjects. Therefore, cheap and simple methods are needed for routine clinical and epidemiological field studies, especially in developing countries. In this regard, anthropometry and bioelectric impedance analysis (BIA) have drawn attention as inexpensive, simple to use, and portable techniques. However, these are indirect methods based on prediction equations developed in a population in which the method was validated against a reference method, and are therefore population-specific [12].

Since the validity of anthropometry and BIA prediction equations in African children has not yet been demonstrated, the aims of the present study were to evaluate if some available prediction equations are applicable for Cameroonian children, and to develop new equations for predicting TBW as measured by deuterium dilution.

## Methods

### Subjects

One hundred two children, girls ( $n = 55$ ) and boys ( $n = 47$ ), between 24 and 60 months of age, were selected from an urban nursery school in Yaoundé, Cameroon. All measurements were performed by trained staff in the morning between 8 am and 1 pm, at a room temperature of  $23 \pm 5$  °C, after being assured that each subject abstained from food and drinks for at least 2 h, did not perform strenuous exercise, and emptying the bladder. The dose of deuterium was administered just after anthropometric and BIA measurements. Thus, the hydration status of a subject was likely to have remained the same throughout the measurement period.

### Anthropometric measurements

Measurements were made using standard procedures [13]. Children were weighed without clothes to the nearest 0.01 kg using an electronic scale (Seca 882, Hamburg, Germany). Height was measured to the nearest millimeter using a portable gauge (Seca 225). Body mass index (BMI) (kilograms per meter squared) was calculated as weight (kilograms) per height (meters) square. Mid-upper arm, waist, and hip circumferences were measured to the nearest 0.1 cm using a non-elastic metric measuring tape (Seca 201).

Anthropometric indices (weight-for-height Z-score (WHZ), weight-for-age Z-score (WAZ), and height-for-age Z-score (HAZ)) were based on the WHO's 2006 Child Growth Standards [14], calculated by using Anthro version 3.2.2 [15].

### Bioelectrical impedance analysis

BIA was performed by a trained staff, using a multi-frequency impedance analyzer (Bodystat QuadScan 4000 with disposable long electrodes, Douglas, Isle of Man, UK) with a tetrapolar placement. Electrodes were placed on the right side of the subject on the hand, wrist, foot, and ankle according to the standard placement stated in the manufacturer's guidelines. Measurements were performed with the subject in supine on a non-conductive surface with their arms and thighs apart, after being assured that the subject did not carry metal objects and that he was in the supine position for at least 15 min. The Bodystat QuadScan 4000 device was calibrated at the beginning of each day using the calibration unit provided by the manufacturer. The values used in the calculations were the mean of duplicate measurements. Only the impedance ( $Z$ ) or resistance ( $R$ ) data at 5, 50, and 100 were used in the calculations of this study. The impedance index was calculated as  $\text{height}^2/Z$  ( $\text{cm}^2/\text{ohms}$ ) or  $\text{height}^2/R$ , depending on the explanatory variable used in the equation tested.  $\text{Height}^2/Z_5$  is assumed to reflect the extracellular water (ECW) and  $\text{height}^2/Z_{100}$  the TBW; their ratio was used as a simple index of the ECW/TBW ratio [16].

### Reference method: deuterium oxide dilution

A dose (0.5 g per kg of body weight) of deuterium oxide (99.8% purity; Cambridge Isotope Laboratories, Inc., Andover, MA, USA) was orally administered to each child and saliva samples (2 mL) were collected before (natural deuterium abundance) and after administering the dose at 3 and 4 h respectively. Saliva collection was carried out after having been assured that the child did not eat or drink in the previous 30 min. Small pieces of cotton wool were used to collect saliva samples, after which saliva was released into sterile tubes by compressing the pieces in a syringe. Samples were stored on ice during transport on the days of fieldwork, brought to the laboratory, and stored at  $-20$  °C until analysis.

The enrichment of deuterium in saliva samples was measured in the range of 2300–2900  $\text{cm}^{-1}$  using a Fourier transformed infrared spectrophotometer (IRAffinity-1, Shimadzu, Kyoto, Japan) with a calcium fluoride cell with a thickness of  $10^{-4}$  m, and a software (Isotope) developed by the Medical Research Council (Human Nutrition Research, Cambridge, UK).

**Table 1** BIA-based prediction equations tested for the prediction of total body water

Equation	Population in whom the equation was developed	Reference
A	The manufacturer's unpublished prediction equation	NS
B	$TBW = 0.700 Ht^2/R - 0.32$	Peru (boys and girls aged 3 months to 9 years)
C	$TBW = 0.593 Ht^2/R + 0.065 Wt + 0.04$	Peru (boys and girls aged 3 months to 9 years)
D	$TBW = -0.50 + 0.60 Ht^2/Z$	United Kingdom (boys and girls aged 5–17 years)
E	$TBW = 0.76 + 0.18 Ht^2/Z + 0.39 Wt$	Peru (Boys and girls aged 3–36 months)
F	$TBW = 0.79 + 0.55 Ht^2/Z$	United Kingdom (boys and girls aged 7–16 years)
G	$TBW = 0.13 + 0.58 Ht^2/Z$	United Kingdom (boys and girls aged 5–17 years)
H	$TBW = 1.84 + 0.45 Ht^2/R + 0.11 Wt$	Illinois (boys and girls aged 5–9 years)

NS without a specified population, *TBW* total body water, *Ht* height, *Wt* weight, *Z* impedance, *R* resistance  
 Bioimpedance electrical analysis measurements of 50 kHz were used for all equations

**Total body water calculations**

**From deuterium oxide dilution**

TBW (kilograms) was calculated from deuterium enrichment, assuming that dilution space of deuterium ( $V_D$ ) or volume of distribution ( $V_D$ ) of a dose of deuterium oxide ( $^2H_2O$ ) is 4.1% higher than TBW due to exchange of hydrogen with non-aqueous hydrogen in the body [17].

$$TBW = \frac{V_D}{1.041}$$

where

$$V_D \text{ (kg)} = \frac{\text{Dose of deuterium (mg)}}{\text{Enrichment of deuterium in saliva (mg/kg)}}$$

**From anthropometry**

The following five anthropometry-based prediction equations were used to estimate the TBW of Cameroonian children:

I Friis-Hansen [18]:

$TBW = k \times \text{weight}^{0.790} \times \text{height}^{0.425}$ , where *k* is 0.195 for subjects aged 2 years to 7 years.

II Morgenstern et al. [19]:

Boys from 3 months to 13 years:  $TBW = 0.0846 \times (\text{weight} \times \text{height})^{0.65}$

Girls from 3 months to 13 years:  $TBW = 0.08037 \times (\text{weight} \times \text{height})^{0.65}$

III Mellits and Cheek [20]:

Boys  $\leq 132.7$  cm:  $TBW = -1.927 + 0.465 \times \text{weight} + 0.045 \times \text{height}$

Boys  $\geq 132.7$  cm:  $TBW = -21.993 + 0.406 \times \text{weight} + 0.209 \times \text{height}$

Girls  $\leq 110.8$  cm:  $TBW = 0.076 + 0.507 \times \text{weight} + 0.013 \times \text{height}$

Girls  $\geq 110.8$  cm:  $TBW = -10.313 + 0.252 \times \text{weight} + 0.154 \times \text{height}$

IV Wickramasinghe et al. [21]:

$TBW = 0.13 \times \text{height} + 0.27 \times \text{weight} + 1.82 \times \text{sex} - 10.35$ ; boy = 1 and girl = 0

V Morgenstern et al. [22]:

**Table 2** General characteristics of our sample of Cameroonian children (*n* = 102)

		Minimum	Maximum	95% CI
Female [ <i>n</i> (%)]	55 (53.9)			
Age (month)	50.4 ± 7.0	24.0	60.0	48.8 to 51.8
Body weight (kg)	16.7 ± 2.0	12.6	22.7	16.3 to 17.1
Height (cm)	107.0 ± 6.3	90.9	118.8	105.7 to 108.2
Mid-upper arm circumference (cm)	16.2 ± 0.9	13.4	18.9	16.0 to 16.4
Waist circumference (cm)	51.0 ± 2.6	43.7	58.7	50.5 to 51.6
Hip circumference (cm)	54.2 ± 3.2	47.0	68.2	53.5 to 54.8
Waist/hip ratio	0.9 ± 0.1	0.8	1.1	0.9 to 1.0
Weight-for-height Z-score	-0.60 ± 0.86	-2.46	1.28	-0.78 to -0.45
Height-for-age Z-score	0.66 ± 1.35	-3.42	3.31	0.37 to 0.91
Weight-for-age Z-score	0.02 ± 0.91	-2.67	2.53	-0.16 to 0.20
ECW/TBW ratio index ( $Z_5/Z_{100}$ )	0.89 ± 0.03	0.63	0.92	0.88 to 0.90

Mean ± SD; *ECW* extracellular water, *TBW* total body water,  $Z_{100}$  impedance at 100 kHz,  $Z_5$  impedance at 5 kHz  
 Measured by deuterium dilution

**Table 3** TBW derived from deuterium dilution, anthropometric, and BIA measurements of Cameroonian children ( $n = 102$ )

	Total body water (kg)	95% CI	TBW/weight (%)	95% CI
Standard				
Isotope dilution	9.6 ± 1.7 <sup>b</sup>	9.3 to 9.9	57.0 ± 7.2 <sup>c</sup>	55.7 to 58.4
Anthropometry				
I	13.3 ± 1.6 <sup>f</sup>	13.0 to 13.6	78.7 ± 1.2 <sup>g</sup>	78.5 to 78.9
II	10.8 ± 1.3 <sup>e</sup>	10.6 to 11.1	64.1 ± 2.1 <sup>f</sup>	63.7 to 64.5
III	10.5 ± 1.4 <sup>de</sup>	10.3 to 10.8	62.4 ± 2.5 <sup>ef</sup>	61.9 to 62.8
IV	9.0 ± 1.7 <sup>a</sup>	8.7 to 9.3	53.2 ± 6.2 <sup>b</sup>	52.0 to 54.4
V	10.7 ± 1.3 <sup>de</sup>	10.5 to 11.0	63.7 ± 4.0 <sup>ef</sup>	62.9 to 64.4
BIA				
A	10.0 ± 1.8 <sup>bc</sup>	9.7 to 10.4	59.5 ± 7.5 <sup>d</sup>	58.4 to 60.9
B	10.2 ± 1.8 <sup>bcd</sup>	9.8 to 10.5	60.2 ± 5.8 <sup>d</sup>	59.0 to 61.4
C	10.0 ± 1.6 <sup>bc</sup>	9.7 to 10.3	59.4 ± 4.9 <sup>d</sup>	58.4 to 60.4
D	8.5 ± 1.5 <sup>a</sup>	8.2 to 8.8	50.2 ± 5.0 <sup>a</sup>	49.2 to 51.2
E	10.0 ± 1.2 <sup>bc</sup>	9.8 to 10.3	59.6 ± 1.5 <sup>d</sup>	59.3 to 59.9
F	9.0 ± 1.4 <sup>a</sup>	8.8 to 9.3	53.6 ± 4.5 <sup>b</sup>	52.7 to 54.5
G	8.8 ± 1.5 <sup>a</sup>	8.5 to 9.1	52.5 ± 5.4 <sup>b</sup>	51.5 to 53.6
H	10.4 ± 1.3 <sup>cde</sup>	10.2 to 10.7	62.0 ± 3.8 <sup>e</sup>	61.3 to 62.8

BIA bioimpedance electrical analysis, I to H prediction equations

Means in the same column not sharing a common superscript letter are significantly different ( $P < 0.05$ ) according to Tukey's *B* test

**Table 4** Cross-validation of anthropometric and BIA-based equations with isotope dilution for prediction of TBW in Cameroonian children ( $n = 102$ )

Equation	Correlation coefficient <sup>a</sup>	Bias (kg) <sup>b</sup> [95% CI]	Error (kg) <sup>c</sup>	95% Limits of agreement (kg) <sup>d</sup> [95% CI]
Anthropometry				
I	0.72	3.7 <sup>a</sup> [3.4, 3.9]	3.8	1.2 [0.8, 1.6] to 6.1 [5.7, 6.5]
II	0.73	1.2 <sup>a</sup> [1.0, 1.4]	1.7	-1.2 [-1.5, -0.8] to 3.5 [3.1, 4.0]
III	0.72	0.9 <sup>a</sup> [0.7, 1.1]	1.5	-1.5 [-1.9, -1.1] to 3.3 [2.9, 3.7]
IV	0.67	-0.6 <sup>a</sup> [-0.9, -0.4]	1.5	-3.4 [-3.8, -2.9] to 2.1 [1.7, 2.6]
V	0.66	1.1 <sup>a</sup> [0.8, 1.3]	1.7	-1.5 [-1.9, -1.0] to 3.7 [3.2, 4.1]
BIA				
A	0.61	0.4 [0.1, 0.7]	2.0	-2.7 [-3.2, -2.2] to 3.5 [3.0, 4.0]
B	0.66	0.6 <sup>a</sup> [0.3, 0.9]	1.6	-2.2 [-2.7, -1.7] to 3.4 [2.9, 3.9]
C	0.67	0.5 [0.2, 0.7]	1.4	-2.2 [-2.7, -1.8] to 3.1 [2.7, 3.6]
D	0.66	-1.1 <sup>a</sup> [-1.3, -0.8]	1.7	-3.7 [-4.2, -3.3] to 1.6 [1.1, 2.0]
E	0.71	0.5 [0.2, 0.7]	1.3	-2.0 [-2.4, -1.5] to 2.9 [2.5, 3.3]
F	0.66	-0.5 <sup>a</sup> [-0.8, -0.3]	1.4	-3.1 [-3.6, -2.7] to 2.1 [1.6, 2.5]
G	0.66	-0.7 <sup>a</sup> [-1.0, -0.5]	1.5	-3.4 [-3.8, -2.9] to 1.9 [1.4, 2.4]
H	0.68	0.9 <sup>a</sup> [0.6, 1.1]	1.5	-1.7 [-2.1, -1.2] to 3.4 [3.0, 3.8]

BIA bioimpedance electrical analysis, TBW total body water

<sup>a</sup>Significant at  $P < 0.001$

<sup>b</sup>Predicted minus measured value (a positive value of the bias reflects an overestimation by the prediction equation)

<sup>c</sup>Pure error; prediction error of 0.0–1.8 kg is considered ideal [25]

<sup>d</sup>The smaller the 95% limits of agreement, the greater the agreement of the equation with the deuterium dilution technique

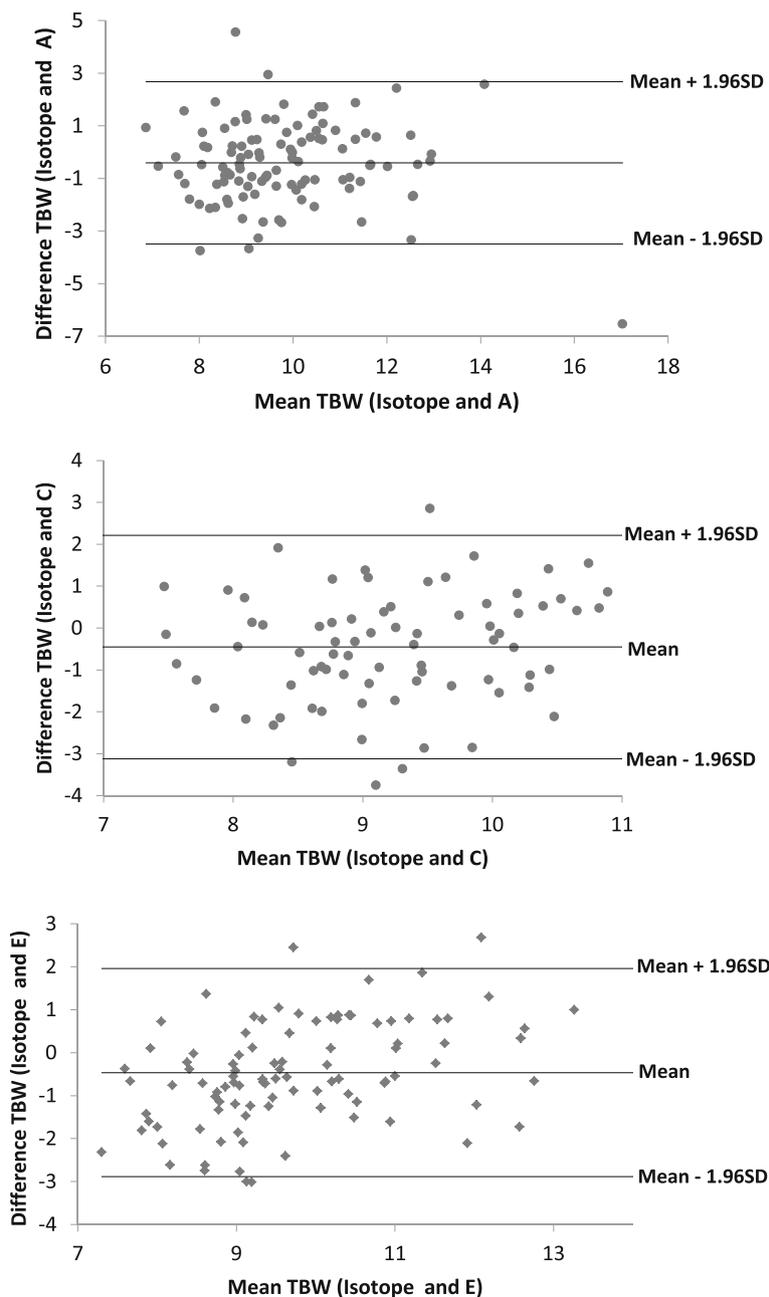
Boys:  $TBW = 0.10 \times (\text{height} \times \text{weight})^{0.68} - 0.37 \times \text{weight}$   
 Girls:  $TBW = 0.14 (\text{height} \times \text{weight})^{0.64} - 0.35 \times \text{weight}$

**Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics 21. The Shapiro-Wilk test was used to test normality of data distribution. Results were expressed as mean ± standard deviation. Type I error risk was set at 0.05 for all analyses. Comparisons between dependent variables were determined using analysis of variance and Tukey's *B* test was used for post-hoc comparisons of means when appropriate. Pearson's

**From BIA**

Table 1 displays the seven impedance-based prediction equations used to estimate the TBW of Cameroonian children.



**Fig. 1** The Bland-Altman plots illustrating the difference between TBW values predicted by the equations A, C and E, and TBW measured by isotope dilution

correlations were used to study the relationship between the measured and predicted (obtained by BIA and anthropometric prediction equation) values. The difference between the measured and predicted values (bias) was tested against 0 (paired Student's *t* test). Pure error was calculated using the following equation [13]:

$$\text{Pure error} = \sqrt{\sum (y-y')^2/n}$$

where *y* is the measured value, *y'* is the predicted value, and *n* is the number of subjects in the sample.

The test of Bland and Altman [23] was used to determine bias and limits of agreement between values predicted by equations and those measured by deuterium dilution. Limits of agreement were defined as upper and lower 95% confidence intervals and were determined by the mean differences ±1.96 × standard deviation [24].

In order to propose a new equation, the database was split in two subsamples and children were randomly allocated assigned to the equation development set (51 subjects) or the equation validation set (51 subjects). In the equation development set, multiple linear regression analysis was used to develop equations for predicting TBW as measured by deuterium, from anthropometric parameters such as sex, age, weight, height, and BMI or from BIA parameters. The resulted prediction equations were cross-validated by

its application to the equation validation set and by using the Bland and Altman procedure to assess the agreement between the predicted and measured values.

**Results**

Table 2 displays the descriptive characteristics of the study population. Forty-six percent of the children included in the study were males and 54% were females. The mean age was 50.4 months, the mean weight was 16.7 kg, and the mean height was 107 cm. The children's mean Z-scores (WHZ, HAZ, and WAZ) were within the normal range, suggesting that they had a good growth and nutritional status. However, 3.9% of the children were classified as wasted (WHZ < -2), 2.0% as stunted (HAZ < -2), 2.0% as underweight (WAZ < -2), and 3.9% as overweight (WAZ > 2). The analysis of variance showed that TBW of subjects was significantly affected (*P* < 0.05) by the methods used for its determination (Table 3). Most prediction equations (I, II, III, IV, V, D, E, G, and H) showed significant difference with the isotope dilution while some others such as A, B, C, and E showed non-significant differences according to Turkey's *B* test, but this test does not indicate agreement between methods.

Comparisons between values of TBW measured by isotope dilution and predicted by each of the anthropometric and BIA equations are presented in Table 4. Significant correlations (*P* < 0.001) were noted between all predicted values of TBW and values measured by

**Table 5** Correlation matrix between some anthropometric, impedance, and hydration characteristics of Cameroonian children with bias of TBW

Bias	Anthropometry						Impedance Z	Hydration ECW/TBW
	Sex	Age	Height	Weight	BMI	MUAC		
Anthropometry								
I	NS	NS	-0.287**	NS	-0.278**	-0.304**	NS	NS
II	NS	NS	NS	NS	NS	NS	NS	NS
III	NS	NS	NS	NS	-0.217*	NS	NS	NS
IV	-0.502**	NS	NS	NS	NS	NS	NS	NS
V	0.459**	NS	NS	NS	NS	NS	NS	NS
BIA								
A	NS	NS	0.597**	0.700**	NS	0.425**	0.861**	NS
B	NS	NS	NS	NS	NS	NS	-0.395**	NS
C	NS	NS	NS	NS	NS	NS	-0.309**	NS
D	NS	NS	NS	NS	NS	NS	-0.244*	NS
E	NS	NS	NS	NS	-0.266**	NS	NS	NS
F	NS	NS	NS	NS	NS	NS	NS	NS
G	NS	NS	NS	NS	NS	NS	-0.210*	NS
H	NS	NS	NS	NS	NS	NS	NS	NS

ECW extracellular water, NS not significant, TBW total body water, Z impedance index  
\**P* < 0.05; \*\**P* < 0.01

isotope dilution. However, correlation measures the strength of a relation between two variables not the agreement between them. Paired comparisons with the equations A, C, and E showed non-significant biases, while significant ( $P < 0.05$ ) biases were noted with the other prediction equations. Equations IV, D, F, and G respectively underestimated TBW by 0.6, 1.1, 0.5, and 0.7 kg, while equations I, II, III, V, B, and H overestimated TBW by 3.7, 1.2, 0.9, 1.1, 0.6, and 0.9 kg. The Bland-Altman approach [23, 24] was used to better assess the agreement of each prediction equation with the deuterium dilution technique. In all cases, large 95% limits of agreement with large confidence interval (CI) were noted, demonstrating a lack of agreement between the anthropometry- and BIA-based prediction equations tested and the isotope dilution, with unacceptable discrepancies at individual level. This is illustrated in Fig. 1 with prediction equations that gave non-significant biases according to paired Student's  $t$  test and where residuals of some children exceeded the 95% confidence limits of TBW. Despite the fact that there is no absolute criterion value for pure error that marks successful validation, most prediction equations gave pure error values that were within the limit recommended by Houtkouper et al. [25], except the equations I and A.

The relationship between the bias and sex, and some anthropometric parameters (age, height, weight, BMI, and MUAC), impedance index, and hydration (ECW/TBW ratio) characteristics of children were tested to determine any systematic error related to these characteristics (Table 5). Significant negative and positive correlation of the bias with sex were found for anthropometry-based equations IV and V, respectively indicating gradual underestimation and overestimation of TBW by these equations when the child is a boy or a girl. No significant correlation was found between bias and age suggesting no effect of TBW by increasing age. Significant negative and positive correlations of the bias with height were respectively found for equations I and A, respectively indicating gradual underestimation and overestimation of TBW by these equations in children with increase in height. Weight and bias were positively correlated for equation A only, indicating gradual overestimation of TBW by this equation with increase of weight. BMI and bias were negatively correlated for three equations (I, III, and E) indicating gradual underestimation of TBW by these equations with increase of fatness. Mid-upper arm circumference (MUAC) and bias were negatively and positively correlated respectively for equations of I and A, indicating gradual underestimation and overestimation of TBW by these equations respectively with increase of MUAC.

Impedance index and bias were positively correlated for equation A, indicating a gradual overestimation of TBW by this equation with increase impedance index

in children. Furthermore, this index and bias were negatively correlated for four BIA equations (B, C, D, and G) indicating a gradual underestimation of TBW by these equations with increase impedance index in children. No significant correlation was found between bias and ECW/TBW ratio suggesting no effect of TBW by extracellular fluid.

Prediction equations for TBW were developed in the equation development set with sex, age, weight, height, BMI, and MUAC as possible predictor variables for anthropometry-based equation (Table 6) and sex, age, weight, height, and  $\text{height}^2/Z$  as possible variable for BIA-based equation (Table 7). The following equations which

**Table 6** Anthropometry-based prediction equations for TBW in the equation development set

Predictor variables	Adjusted $R^2$	RMSE	$P$
Sex, age, weight, height, BMI, MUAC	0.69	3.30	0.000191
Sex, age, weight, BMI, MUAC	0.71	3.61	0.000073
Sex, age, height, BMI, MUAC	0.43	3.01	0.004752
Sex, age, MUAC, BMI	0.30	2.91	0.016322
Sex, BMI, MUAC	0.34	3.35	0.006719
Age, MUAC, BMI	0.25	3.00	0.022761
MUAC, BMI	0.28	3.66	0.008530
MUAC	0.17	4.03	0.017568
BMI	-0.04	0.12	0.946799
Sex, age, weight, MUAC	0.64	3.47	0.000219
Sex, age, height, MUAC	0.44	3.29	0.002812
Weight, MUAC	0.67	5.30	0.000012
Sex, weight, MUAC	0.67	4.42	0.000030
Age, weight, MUAC	0.66	4.39	0.000037
Sex, age, MUAC	0.22	2.91	0.030647
Sex, height, MUAC	0.46	3.78	0.001066
Age, height, MUAC	0.39	3.56	0.002919
Sex, MUAC	0.24	3.47	0.014472
Age, MUAC	0.16	3.01	0.043851
Sex, age, BMI	-0.05	1.35	0.615107
Sex, age, height, BMI	0.35	3.06	0.008451
Sex, age, weight, height	0.64	3.81	0.000120
Sex, age, weight	0.66	4.37	0.000041
Sex, age, height	0.38	3.52	0.003466
Sex, weight	0.67	5.33	0.000011
Age, weight	0.63	5.16	0.000026
Weight	0.65	7.26	0.000004
Height	0.39	5.72	0.000516
Weight, height	0.63	5.16	0.000026
Weight, height, MUAC	0.64	4.33	0.000055
Sex, weight, height, MUAC	0.65	3.83	0.000107

RMSE root mean square error

**Table 7** BIA-based prediction equations for TBW in the equation development set

Predictor variables	Adjusted $R^2$	RMSE	$P$
Sex, age, weight, height, $Ht^2/Z$	0.68	1.40	0.000112
Age, weight, height, $Ht^2/Z$	0.68	1.40	0.000056
Weight, height, $Ht^2/Z$	0.67	1.40	0.000032
Sex, age, weight, $Ht^2/Z$	0.63	1.43	0.000144
Age, weight, $Ht^2/Z$	0.60	1.44	0.000103
Weight, $Ht^2/Z$	0.62	1.43	0.000027
Sex, age, $Ht^2/Z$	0.37	1.55	0.004143
Sex, $Ht^2/Z$	0.40	1.54	0.001302
Age, $Ht^2/Z$	0.39	1.55	0.001516
$Ht^2/Z$	0.42	1.53	0.000306
Height, $Ht^2/Z$	0.63	1.42	0.000024
Sex, age, height, $Ht^2/Z$	0.64	1.42	0.000114
Sex, height, $Ht^2/Z$	0.61	1.43	0.000090
Age, height, $Ht^2/Z$	0.66	1.41	0.000037

RMSE root mean square error, Z is the impedance at 50 kHz, Ht height

presented the highest adjusted  $R^2$ , the lowest root mean square error (RMSE) value, and the lowest  $P$  value were selected:

$$\begin{aligned} \text{Anthropometry : TBW (kg)} &= 6.488 + 0.4339 \\ &\times \text{sex (male} = 1, \text{female} = 0) \\ &- 0.039 \times \text{age (month)} \\ &+ 0.6696 \times \text{weight (kg)} - 0.081 \\ &\times \text{MUAC (cm)} - 0.372 \times \text{BMI} \end{aligned}$$

$$\begin{aligned} \text{BIA : TBW (kg)} &= -6.206 + 0.0037 \\ &\times \text{height}^2/Z - 0.0410 \times \text{age} \\ &+ 0.2652 \times \text{weight} + 0.1214 \\ &\times \text{height} \end{aligned}$$

The new equations were applied in the equation validation set. Good agreement was obtained with the isotope dilution with a pure error value within the ideal limit recommended by Houtkooper et al. [25] (Table 8). The Bland-Altman plots illustrated a good level of concordance between the TBW predicted by the new equations and TBW measured by isotope dilution (Fig. 2).

## Discussion

A lack of agreement was noted in the present study between the isotope dilution and all the anthropometry- and BIA-based equations tested, with unacceptable discrepancies at individual level for clinical purposes, as residuals of some children exceeded the 95% confidence limits of TBW. This result agrees with previous studies showing that anthropometry and BIA prediction equations used for TBW and body composition have the tendency to be population-specific and that inappropriate use of published prediction equations can lead to systematic errors and inaccurate estimations [26, 27]. In this regard, Tzamaloukas et al. [28] showed that each anthropometric measurement included in anthropometric equations for the prediction of body composition estimate a unique body composition which is for the average subject of the population in whom the equations were developed, while Haroun et al. [29] showed for the BIA model that variation of the relative body geometry between ethnic groups confound the relative distribution between weight and impedance among limb and trunk. However, apart from biological variations, errors in the measurements could also contribute to the bias between measured and predicted TBW by BIA. Despite the standardized approach used for BIA measurement, an improper control of some factors including hydration status, body position, skin temperature, electrode placement, previous exercise, and dietary intake may affect BIA measurement and prediction of TBW in children [30, 31].

To the best of our knowledge, there is no prediction equation for Cameroonian or African children that was developed or cross-validated to estimate body composition or TBW. In this study, one anthropometry and one BIA prediction equations for the estimation of TBW in Cameroonian children aged 24–60 months were developed. An adjusted  $R^2$  of 0.71 and RMSE of 3.6 were obtained for the anthropometry-based prediction equation while adjusted  $R^2$  of 0.68 and RMSE of 1.4 were obtained for the BIA-based prediction equation. This result agrees with previous studies displaying the method criterion ( $R^2 = 0.65–0.99$ ,  $\text{RMSE} = 0.41–3.81$ ) for TBW equations [32, 33]. Moreover, our prediction equations showed ideal prediction accuracy according to Houtkooper et al.'s [25] classification system for prediction errors.

**Table 8** Cross-validation of the new equations with isotope dilution for prediction of TBW ( $n = 51$ )

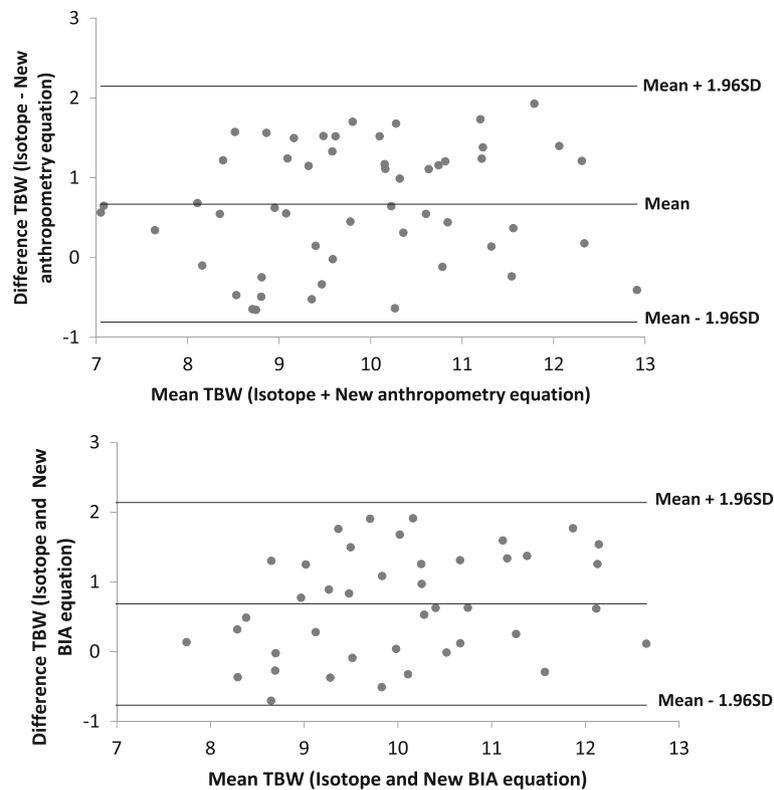
Equation	Correlation coefficient <sup>a</sup>	Bias (kg) <sup>b</sup> [95% CI]	Error (kg) <sup>c</sup>	95% Limits of agreement (kg) <sup>d</sup> [95% CI]
Anthropometry	0.86	-0.7 [-0.9, -0.5]	1.0	-2.1 [-2.5, -1.8] to 0.8 [0.5, 1.2]
BIA	0.87	-0.7 [-1.3, -0.1]	0.9	-2.1 [-2.4, -1.9] to 0.8 [0.6, 1.0]

<sup>a</sup>Significant at  $P < 0.001$

<sup>b</sup>Predicted minus measured value (a positive value of the bias reflects an overestimation by the prediction equation)

<sup>c</sup>Pure error; prediction error of 0.0–1.8 kg is considered ideal [25]

<sup>d</sup>The smaller the 95% limits of agreement, the greater the agreement of the equation with the deuterium oxide dilution technique



**Fig. 2** The Bland-Altman plot illustrating the difference between TBW predicted by the new equations and TBW measured by isotope dilution

In this study, the BIA equation included the impedance index, body weight, height, and age. This result was consistent with previous studies showing that the prediction of TBW from impedance index could be improved by the inclusion of anthropometric data such as body weight, age, and sex, which could correct differences across groups [34, 35]. The equation based on anthropometric measurements included body weight, BMI, MUAC, age, and sex. The precision of this model was comparable to that of the impedance equation. The cross-validation procedures revealed that the predicted values of TBW compared with measured values are accurate at a group level. The precision assessed with the pure error and the limits of agreement assessed by Bland-Altman approach facilitates generalization from the sample to the population. Both BIA and anthropometric new prediction equations had precise estimates of TBW, and therefore could be used to assess TBW in Cameroonian preschool children.

Although the use of deuterium oxide dilution technique for cross-validation analysis constitute a strength in the present study, the relatively small sample size used could be a limitation as participants were not fully representative of the Cameroonian preschool children population. Therefore, findings should be construed with caution when attempting to generalize prediction equations.

## Conclusions

In conclusion, the available published anthropometric and BIA equations provide unacceptable discrepancies in the estimates of TBW at individual for Cameroonian preschool children. The present study presented two new gender-dependent equations to estimate TBW in Cameroonian children aged 24–60 months.

## Abbreviations

BIA: Bioelectrical impedance analysis; BMI: Body mass index; DEXA: Energy x-ray absorptiometry; ECW: Extracellular water; FFM: Fat-free mass; FM: Fat mass; HAZ: Height-for-age Z-score; MUAC: Mid-upper arm circumference; RMSE: Root mean square error; TBW: Total body water; WAZ: Weight-for-age Z-score; WHO: World Health Organization; WHZ: Weight-for-height Z-score

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## Availability of data and materials

Important data for this study are contained in the manuscript. Subjects' individual data are not shared in this work for ethical reasons. Authors did not include data sharing clearance during ethical approval.

**Authors' contributions**

GN M initiated the study, drafted the research protocol, coordinated and led the research implementation in the field, analyzed the data, and drafted the manuscript. VJE, HTD, and PMN helped with subjects' enrolment, sample collection and analysis as well as preparation of the manuscript. All authors edited the manuscript and approved its final contents.

**Ethics approval and consent to participate**

The protocol was approved by the ethics committee of the Institute of Medical Research and Medicinal Plants Studies (IMPM). Informed written consent was obtained from each caregiver of children participating in the study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors confirm that they have no competing interest in any company or organization sponsoring the research currently and at the time the research was done.

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