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Original article

Validation of total body water analysis by bioelectrical impedance analysis with deuterium dilution in (pre)school children[☆]Marieke de Beer^{a,b,c,*}, Tessa Timmers^a, Peter J.M. Weijs^{d,e}, Reinoud J.B.J. Gemke^{a,b}^a Department of Pediatrics, VU University Medical Center, Amsterdam, The Netherlands^b EMGO+ Institute for Health and Care research, University Medical Center, Amsterdam, The Netherlands^c Department of Epidemiology, Documentation and Health Promotion, Municipal Health Service, Amsterdam, The Netherlands^d Department of Nutrition & Dietetics, Internal Medicine, VU University Medical Centre, Amsterdam, The Netherlands^e Department of Nutrition & Dietetics, School of Sports and Nutrition, Hogeschool van Amsterdam, The Netherlands

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SUMMARY

Background & aims: Accurate field methods to assess body composition in young children are scarce. The aim of this study was to validate bioelectrical impedance analysis with deuterium based total body water analysis in (pre)school children.

Methods: We examined fifty-six 4–7 year old children, 36% boys. Total body water estimated with standardized single frequency bioelectrical impedance analysis was compared with deuterium dilution technique. An established equation (Kushner) was tested, recalibrated and subsequently cross-validated in an independent sample.

Results: Compared to deuterium dilution (mean total body water 12.66 kg, $n = 56$), bioelectrical impedance analysis underestimated total body water by 0.51 kg ($p = 0.002$) and this varied across the range of total body water ($r = 0.41$; $p = 0.002$). Recalibration of the equation yielded: total body water (kg) = $0.439 \text{ Height}^2 \text{ (cm}^2\text{)}/\text{Resistance (}\Omega\text{)} + 0.027 \text{ Weight (kg)} + 4.014$ ($R^2 = 0.74$, $\text{SEE} = 0.96 \text{ kg}$). Application of this equation in the independent sample ($n = 20$) showed a non-significant bias in total body water (mean bias -0.30 kg , $p = 0.17$).

Conclusions: These data indicate that BIA underestimates total body water by 0.51 kg in (pre)school children, when using the Kushner equation. Recalibration and cross-validation enables enhanced assessment of body composition in (pre)school children for use in epidemiological field studies.

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1. Introduction

Field methods that accurately assess body composition in children are scarce. Currently, body mass index ($\text{BMI} = \text{weight}/\text{height}^2$) is the most commonly used routine measure that gives an indication of relative weight. International BMI-for-age cut offs have been proposed to classify overweight as well as undernutrition.^{1,2} However, BMI levels among children should be interpreted with caution. Although high BMI-for-age is a good indicator of excess fat

mass (FM), BMI differences among thinner children can be largely due to fat-free mass (FFM).³ Following this, to identify the need for intervention in case of suspected overweight or undernutrition, more information is needed about whole body FM and whole body FFM which is reflected in body composition.

Body composition is best addressed by applying a multi-component model, in which measurements of different body properties are combined.⁴ The three-component (3C) model for instance is based on the measure of weight (Wt), total body water (TBW) and body volume (BV). It distinguishes fat mass, water and fat-free dry mass. Although highly accurate, these methods are relatively expensive, sometimes rather invasive and therefore not suitable in routine (field) applications.

Bioelectrical Impedance Analysis (BIA) is potentially more suitable for survey and outpatient clinical use, particularly in pre-school and school aged children. BIA estimates FFM (and indirectly FM) by measuring the resistance (R) of body tissue to a weak imperceptible current. The measured resistance is first of all used to

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; DEU, deuterium dilution; FFM, fat free mass; FFM_{H} , hydration percentage fat free mass; $^2\text{H}_2\text{O}$, deuterium water; Ht, height; TBW, total body water; Wt, weight.

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estimate total body water (TBW).⁵ For the estimation of TBW in children, a large number of BIA equations are available. An important practical concern is that the prediction equations are population specific. Therefore, it is recommended to validate equations in the population or sample in which they are used, by comparing BIA to a reference method, such as hydrometry.⁶

In the next step, FFM is calculated from TBW using the percentage of hydration of FFM (hydration constant). Because chemical composition of FFM changes during childhood and is different in boys and girls age- and sex-specific constants for hydration of FFM are recommended.^{7–9} Finally, FM can be calculated by subtracting FFM from total weight (Wt).

The aim of the present study was to assess the validity and reliability of an established BIA equation for 4–7 year old children. Furthermore, to derive a recalibrated equation when this BIA equation differed significantly from hydrometry.

2. Materials and methods

For the recalibration sample, a total of thirty-six healthy children were recruited from four local primary schools in Amsterdam, the Netherlands. The measurements were performed in September and October of 2009. Participants were visited at school in the morning for baseline urine collection and the administration of deuterium water (²H₂O). In the afternoon they visited the Nutritional Assessment Laboratory of the Hogeschool van Amsterdam, University of Applied Sciences, accompanied by a parent for a second urine collection and a 1 h measurement session. A second independent sample of 20 children, referred as the external cross-validation sample, was recruited. They were participants of a large prospective cohort study in Amsterdam and were of the same age as the recalibration sample children (www.abcd-study.nl).¹⁰ Permission was granted by the institutional review board of the VU University Medical Centre. All parents gave written informed consent.

2.1. Anthropometrics

Height (Ht) was measured to the nearest millimeter using a Leicester portable height measure (Seca, Hamburg, Germany) and weight (Wt) to the nearest 100 g using the electronic weighing scale that is part of the Bod Pod® (validation group) or to the nearest 100 g using a Marsden weighing scale, model MS-4102 (Marsden weighing group, Oxfordshire, UK) (cross-validation group).

2.2. BIA

After the child had emptied his/her bladder, arm-to-leg BIA was measured using the Bodystat 1500MDD system (Bodystat Inc., Douglas, UK). The BIA measurement was performed after 2 min in supine position with the legs abducted to 45°. A pair of electrodes was attached at the right wrist/hand and the right ankle/foot and a 50 kHz alternating current (800 µA) was passed through the body. The voltage drop was measured and the resistance (R) calculated. The procedure was repeated and the average of the 2 measurements was used for further calculations. We chose to use the Kushner et al.⁵ formula for the calculation of TBW, because it was based on population based samples of an age range comparable to our sample and because it is incorporated into the bodystat software.

$$\bullet \text{ TBW (kg)} = 0.59 \text{ Ht}^2 (\text{cm}^2)/R (\Omega) + 0.065 \text{ Wt (kg)} + 0.04$$

2.3. Total body water, measured by deuterium dilution

Total body water (TBW) was determined by ²H₂O dilution with a dose equivalent to 0.05 g per kg body weight. Doses were sweetened by lemonade. After drinking the ²H₂O dose the dose container was rinsed with 20 mL of tap water and given to the participants to drink, in order to ensure complete ingestion of the total dose. Urine samples were collected pre-dose and 4 h post-dose using a urine collection system. These were stored in special glass vials at –20 °C, and then analyzed using isotope-ratio mass spectrometry (IRMS).¹¹

For calculating TBW, it was assumed that ²H₂O dilution space overestimated TBW by a factor of 1.04, therefore TBW was divided by 1.04.¹²

2.4. Statistics

2.4.1. Validation of the Kushner equation

Paired sample *t*-tests were used to compare TBW calculated with BIA and obtained from ²H₂O. For assessment of TBW agreement between BIA and ²H₂O the statistical graphic approach proposed by Bland and Altman was used.¹³ Pearson's correlation analysis was performed of the variables generated on the *x* and *y* axes of the Bland–Altman plot. This was used to report whether the bias between techniques varied in relation to the amount of TBW.

2.4.2. Recalibration of the prediction equation for TBW and cross-validation

Linear regression analysis was used to recalibrate the relationship between Ht²/R incorporating weight in the regression model and TBW from ²H₂O. The standard error of the estimate (SEE) was calculated, in order to indicate accuracy of body composition estimates in individuals. The regression equation was then used to predict TBW in the cross-validation group. The same analyses were applied as described for the validation of the existing equation. All analyses were conducted using SPSS statistical software (version 16.0).

3. Results

3.1. Characteristics of subjects

Fig. 1 shows the flowchart of the study procedure. Two girls from the recalibration group showed higher than expected baseline measurement levels of deuterium. We imputed these values with average values of baseline measurement. Furthermore, one child of the cross-validation group was excluded because we administered deuterium dilution twice (with an interval of 1 week) because she did not show up at the first measurement session. Therefore the second baseline measurement showed too high a level of

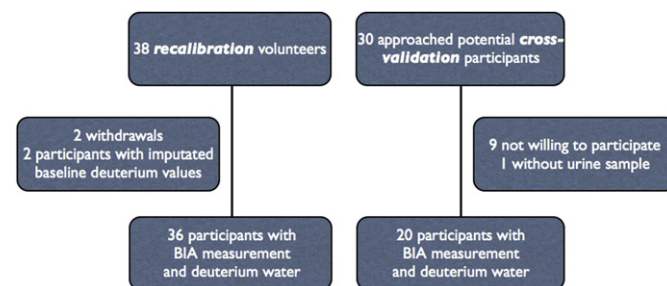


Fig. 1. Flowchart of participants in the study.

deuterium. Table 1 shows a description of the characteristics of the recalibration and the cross-validation sample, stratified by gender.

3.2. Validation of Kushner BIA equation

We found an underestimation in TBW of 0.51 kg ($p = 0.002$) using the Kushner equation in BIA when compared to deuterium water. This bias varied across the range of TBW with a correlation of 0.41 ($p = 0.002$) with a smaller difference between the techniques at a higher amount of TBW (Fig. 2).

3.3. Recalibration of the prediction equation and cross validation

The correlation between Ht^2/R and TBW_{DEU} was 0.84 ($p < 0.001$). We recalibrated the existing equation: $TBW = 0.439 (Ht^2/R) + 0.027 Wt + 4.014$, with R^2 of 0.74, standard error of the estimate (SEE) of 0.96 kg.

Using the recalibrated formula we found a non-significant underestimation of TBW in the cross-validation sample of -0.30 kg ($p = 0.17$). This bias did not vary across the range of TBW with a correlation of -0.11 ($p = 0.65$) (Fig. 3).

4. Discussion

Analysis of body composition in children becomes increasingly important in light of the expanding prevalence of overweight and obesity and is also suitable for use in children with undernutrition. BIA represents one of the few potentially applicable methods for this purpose in field settings. There are several available BIA-equations for the prediction of TBW in children. The Kushner equation incorporated in the Bodystat system is based on a sample of 24 children (4.8–9.8 years). The equation was cross validated with 13 children (6.4–9.9 years).⁵ These reference groups are comparable with the current study group of 4.4–7.5 years respectively 5.1–5.9 years. Previous BIA validation studies in adults and children have reported that equations generated in one population do not always transfer successfully to others.^{14–16} The present study shows that the Kushner BIA equation for school age children, when compared to the Deuterium dilution method underestimated TBW and therefore FFM. This underestimation is non-systematic which means values cannot simply be converted to get an accurate value of TBW. Recalibration of the equation yielded: $TBW = 0.439 (Height^2/Resistance) + 0.027 Weight + 4.014$. Application of this equation showed a small non-significant bias. Importantly this bias was systematic, thereby allowing more robust assessment of body composition in (pre)school aged children for use in epidemiological field studies.

This study has several strengths. First, we used a larger recalibration and cross validation group with a more homogenous age

Table 1
Description of the sample.

	Recalibration sample (n = 36)				Cross-validation sample (n = 20)			
	Males (n = 14)		Females (n = 22)		Males (n = 6)		Females (n = 14)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Age (years)	5.9	0.9	5.6	1.0	5.5	0.2	5.6	0.2
Weight (kg)	21.0	2.8	23.48	7.9	22.7	3.3	20.5	2.4
Height (cm)	113.6	5.84	114.66	8.7	118.0	4.3	113.9	5.8
BMI (kg/m ²)	16.3	1.68	17.44	3.3	16.2	1.5	15.8	1.0
R_{BIA} (Ω)	712.8	54.71	741.02	72.0	694.6	80.0	775.3	68.4
TBW_{DEU} (kg)	12.8	1.30	12.44	2.1	13.7	1.6	12.4	1.5

BMI, body mass index; R_{BIA} , resistance; TBW_{DEU} , total body water.

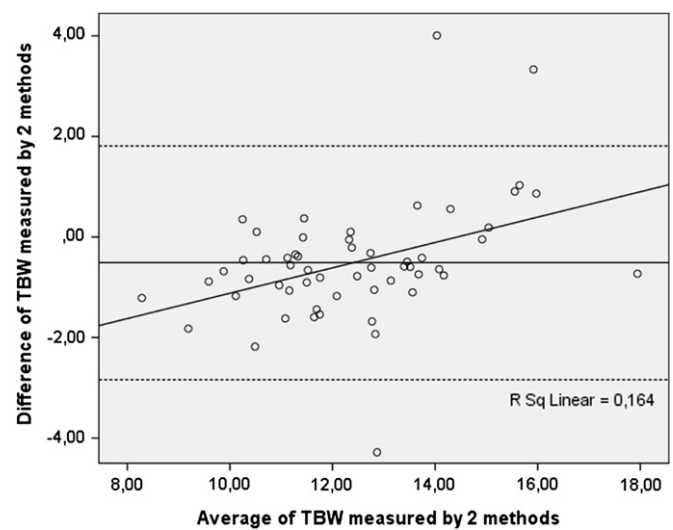


Fig. 2. Bland–Altman plot showing the agreement between the differences (BIA existing equation–Deuterium dilution) and mean (BIA existing equations + Deuterium dilution) of TBW ($r = 0.41$; $p = 0.002$) (recalibration and cross-validation sample, $n = 56$).

range than was previously used. Second, because we cross-validated the recalibrated equation in a different sample, we were not only able to assess a potential bias, but we could also examine whether this bias was systematic and therefore whether it is possible to correct for a potential bias.

There are some limitations of the present study. First, children were allowed to eat and drink during the 4 h equilibration period. We did not adjust for dilution of the dose by water intake during the 4 h equilibration period. This could have resulted in an overestimation of TBW. Secondly, although the cross-validation is a strength, it is possible that the bias did not reach significance due to the smaller sample size. However the small underestimation seems to be systematic which means values can be converted to get an accurate value of TBW.

In this study we were not able to address the issue whether BIA is also an accurate method for children with a body composition in the more extreme ranges, like obesity. In this group limitations

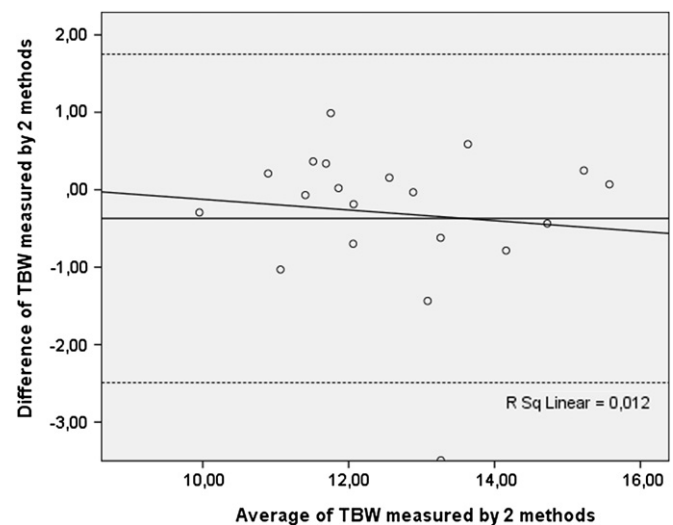


Fig. 3. Bland–Altman plot showing the agreement between the differences (BIA recalibrated equation – Deuterium dilution) and mean (BIA recalibrated equation + Deuterium dilution) of TBW ($r = -0.11$; $p = 0.65$) (cross-validation sample, $n = 20$).

could be due to a difference in hydration constants. According to Wells et al.⁹ hydration is higher in higher-BMI SDS individuals. Therefore they conclude that, when high levels of accuracy are required, 2-component techniques like BIA are inherently limited in this group because of their assumption of constant values for lean tissue composition.⁹ Whether BIA is suitable for clinical use for the follow up of body composition in individual children, particularly in the more extreme ranges of BMI, therefore remains to be established and should be further addressed in future research.

In conclusion bio-impedance analysis appears a feasible, reasonably accurate and affordable tool for epidemiologic studies of body composition of school-aged children.

Conflict of interest statement

None declared.

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