

Development and cross-validation of predictive equation for estimating total body lean in children

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Abstract

Aim. To develop and cross-validate a predictive equation for estimating lean body mass (LBM) in children, based on bioelectrical impedance analysis (BIA) and anthropometric parameters, and to compare our equation with other predictive methods.

Methods. We evaluated body composition of 155 children (age 5-14 years) by BIA and dual energy X-ray absorptiometry (DXA). Children were divided in two groups: developing set and cross-validation set. Statistical analysis as multiple regression and Bland-Altman methods were performed.

Results. From developing set (105 children) a predictive LBM (kg) equation was created. From the cross-validated set (35 children), our equation was seen to agree with LBM (kg) by DXA. On the contrary, LBM predicted by Schaefer's equation and BIA did not agree with the criterion measure.

Conclusions. This cross-validated equation can be useful in epidemiological studies and also in clinical practice, permitting a better definition and follow up of children's body composition.

Key words

- children
- predictive equation
- lean body mass
- body composition
- dual energy X-ray absorptiometry

INTRODUCTION

The body mass index (BMI) is the most commonly used index for measuring nutritional status in research and epidemiological studies. Also in the pediatric setting, BMI-for-age is used even if an increment of its value is often due to the effect of linear growth or fat-free mass (FFM) rather than fat mass (FM) [1]. BMI is correlated with the main compartments of weight, FM and FFM, but cannot distinguish between them and it may give misleading information on the body composition, especially in children, who have rapidly growing and changing of body conformation. An accurate body composition assessment in children is necessary to discern the effect of nutritional or pharmaceutical treatments in different conditions [2].

Developing accurate body composition-measuring methods for evaluating and monitoring growth and nutritional status in children is an important area of research. Several accurate techniques are available today, such as dual energy X-ray absorptiometry (DXA), air-displacement plethysmography or deuterium dilution, but often they are expensive and generally available only in specialized researcher centers [3].

In order to measure body composition in children, a safe, non-invasive, economical in both cost and time, and valid method is necessary. Bioelectrical impedance analysis (BIA) fulfills many of these requirements and it can be widely used to assess body composition in children as an alternative to the other methods, although when used alone some limitations are evident [4-7].

BIA-based predictive equations, containing several anthropometric parameters, have been developed to improve accuracy in measuring body composition, and have many applications, one of which is to predict FFM and FM values for epidemiologic or clinical purposes. Indeed, several of them have been developed for a variety of childhood populations, some resulting more powerful than others, with interesting but not always conclusive or fully satisfactory results [8-10].

More recently, a BIA-based predictive equation, extrapolated by four compartments (4C) model, has been tested in a widely-aged pediatric population, without been able to achieve satisfactory outcomes in predicting FFM content throughout ages [11].

The aim of our study are: to develop and cross-validate a new predictive equation for estimating lean body mass (LBM) in children, based on BIA and anthropometric parameters, and to compare our equation with other predictive methods.

METHODS

Study population and criteria

As part of the activities related to the “Mensa sana, corpore sano” project, we continuously recruited, from January to December 2013, a total of 155 children (age 5-14 years) through the announcement in elementary and junior high urban schools, where also extracurricular activities took place.

Children were assigned in two groups: developing set and cross-validation set, which is required to cross-validate the predictive equation. Assignment was carried out in random mode (1:1), until the cross-validation set was composed by approximately one third of developing set. Weight limits were not applied to participate to the study. Medical histories were written up with parents or guardians, and the clinical examinations confirmed the healthy status of participants. Written informed consent was obtained from child's parents or guardians in accordance with the institution's ethic policies and procedures. Anthropometric parameters and body composition of all subjects were obtained on the same day.

Anthropometric measurements

Body height (ht) was measured standing without shoes using a stadiometer (SECA instruments, UK) and recorded to the nearest 0.1 cm. Body weight (BW) was measured by using a scale (SECA instruments, UK), and recorded to the nearest 0.1 kg, the subjects wearing only the underwear. BMI was calculated by the standard formula: body weight (kg)/height² (m)². Waist circumference (WC) was measured with the subject standing, midway between the last rib and the upper edge of the iliac crest, and hip circumference (HC) was measured at the greater gluteal curve. Both measurements were taken with inelastic centimeter to the closest 0.1 cm. The waist-to-hip ratio (WHR) was calculated using the above-taken measures. Also the waist-to-height ratio (WHtR) was calculated for evaluating the possible cardiovascular and metabolic risk in this pediatric population [12, 13].

Bioelectrical impedance analysis

Total body resistance (R) and reactance (Xc), and the resultant impedance ($Z = \sqrt{R^2 + Xc^2}$), all expressed in ohm, were directly measured with a single-frequency 50 kHz analyzer BIA-101 (Akern Srl, Italy). For a more accurate evaluation of body composition, BIA-derived impedance index (ZI) which has been demonstrated to better predict FFM than the other BIA parameters alone, and has been preferentially used in the literature to elaborate BIA-based predictive equations, was used. ZI was calculated according to the standard formula: body height² (cm)²/total body impedance (ohm) [14, 15].

All children were measured in an assumed normally hydrated state and had not eaten or participated in physical activities at least two hours prior the measurement. BIA analysis occurred following standard procedures, delivering the absolute and percentages values of FM, FFM, and total body water (TBW).

Dual energy X-ray absorptiometry

Body composition analysis was assessed by DXA, the reference method in this study (i-DXA, GE Medical Systems, Milwaukee, WI, USA), according to the previously described procedure for the evaluation of FM and LBM [16-18]. Radiation exposure was equivalent to 0.01 millisievert. The output from DXA scan was analyzed through a dedicated pediatric DXA computer software in order to get measure of absolute and percent values of FM, LBM, and also bone mineral content (BMC).

The coefficient of variation (CV% = 100*standard deviation/mean) intra- and inter-subjects ranged from 1% to 5%. The coefficient of variation for BMC measurements is ≤ 1%; the coefficient of variation on this instrument for five subjects scanned 6 times over a 9-month period were 2.2% for FM and 1.1% for LBM measurements.

Statistical analysis

Since a skew distribution was observed for several continuous variables in this study, median, first and third quartile (Q1, Q3) were preferred as summary statistics. Categorical variables were described by absolute and relative frequencies. Continuous covariates were compared by t-test, or Mann-Whitney test when a significant shift from normality was found.

To develop a predictive model for measuring LBM (kg), a general linear model was fitted to the observed data. Age, height, weight, sex, WC, HC, WHR and the BIA-derived ZI, were considered all as potential significant predictors. Final predictors of LBM were identified by a non-automate backward selection, taking clinical interpretation and correlation structure among covariates into account. Plots of smoothed *studentized* residuals vs continuous covariates were used to assess linearity. Influence measures were computed to assess if the final model was unduly influenced by few observations. Poorly predicted observations were identified by studentized residuals.

Correlation analysis (Pearson or Spearman) were performed for analyzing the associations among the predicted LBM (kg) value obtained using our proposed

equation with the LBM (kg) measured by the reference method DXA. FFM from BIA and also a reference BIA-based equation already validated in children, namely the Schaefer's equation [19], were associated with LBM (kg) from DXA.

It was evaluated the possible influence of tissue distribution in different body compartments when determining body composition by BIA. Indeed, from previous studies using BIA, measurements of the forearm and lower leg accounted for the majority of total body impedance but contributed little to total body weight, whilst the trunk accounted for around 40% of total body weight but only for around 10% of total body impedance. Moreover, the variability in the ratio of limbs-to-trunk impedance may confound the relationship between total body impedance (Z) and FFM values obtained by BIA [20,21]. For such reasons, covariance analysis was performed in the whole study sample by assessing the relationship between the BIA-derived ZI and LBM (kg) measured by DXA, through the use of an arbitrary cut-off for the percentage of LBM at the trunk in respect to the total LBM, in order to highlight differences in this population.

Finally, the new predictive equation was cross-validated against an external group according to Bland & Altman [22].

All data were analyzed by using SAS (version 9.3, SAS Institute, Cary, NC) and R (version 2.15.1) software programs. Statistical significance was set for *p*-values less or equal to 0.05.

RESULTS

The developing set was composed by 120 children, 65 girls and 55 boys (54% vs 46%). Few participants (12-13%) could not perform all the study testing and were excluded from the statistical analyses when required (13 children did not perform the BIA, and 1 child the DXA due to technical problems with the instruments, while 1 child did not had the HC measure). The cross-validation set group was composed by 35 children, 18 girls and 17 boys (51% vs 49%). Main body composition parameters of both groups are presented, divided by gender, in Table 1.

Developing set median age was 8.51 years for girls and 9.03 for boys (*p* > 0.05). At comparative analysis, height, weight, BMI, WC and HC were similar in both genders (*p* > 0.05). Moreover, WHR (*p* = 0.00) and WHtR (*p* = 0.03) resulted significantly greater in boys. Data from DXA proved a significantly higher LBM (kg) in boys (*p* = 0.01), who also showed a greater, but not significant, BMC (*p* = 0.07). No difference was found between genders in FM values. Data from BIA evidenced that R and Xc were significantly higher (*p* = 0.00 and *p* = 0.02, respectively) in girls. In the cross-validation set, median age was years 8.00 for girls and 9.00 for boys (*p* > 0.05). Boys and girls presented homogeneous body composition, except for R, which was higher in girls (*p* = 0.04), and FFM (%), which was higher in boys (*p* = 0.04) (Table 1).

Body composition of overall developing set was assessed by DXA and revealed a higher FM (2.50 kg, equal to 23.84%) and a lower LBM (-2.56 kg, equal to

11.5%) when compared to measurements obtained by BIA, from which significantly differed in absolute and percentage values (*p* < 0.0001).

With the purpose to derive a predictive model for evaluating LBM (kg) in children, we combined BIA and anthropometric parameters as its potential predictors. Multivariable regression analysis identified five significant predictors of LBM (kg) in our pediatric population: ZI and four anthropometric parameters, such as HC, WHR, height, and age, which showed a significant effect in predicting LBM (kg) in the overall sample. The *R*² of the final model was equal to 0.962, with a mean root square errors of 1.90 kg (Table 2).

To improve our model, we searched for poorly predicted and influential observations. Inspection of residuals led to identify only four poorly predicted observations (3.8%), in all but one of them the predicted LBM was greater than the observed value as measured by DXA. A single observation was found to be highly influential on the fitting of the final model. In order to test model robustness, this observation was excluded from the analysis, and the model was refitted.

Based on this final model, the following predictive equation for evaluating LBM (kg) in children is then proposed:

$$\text{Predicted LBM (kg)} = -27.597 + 0.337 \times (\text{ZI}) + 0.094 \times \text{HC (cm)} + 9.593 \times \text{WHR} + 0.360 \times \text{age} + 0.164 \times \text{height (cm)}$$

Predicted LBM (kg) obtained by applying the proposed equation to our study cohort did not significantly diverge to the ones obtained by DXA (*p* > 0.05). Our equation underestimated LBM in 0.66 kg (3.06%), when compared to LBM measured by DXA. Extrapolating data for estimating FM, through the difference between total body weight and LBM, our equation found an overestimation of 0.68 kg (6.54%), which was not significantly different comparing to FM assessed by DXA.

At Pearson's correlation analysis, the predicted values of LBM (kg) by applying our equation were highly correlated with measurements of LBM (kg) obtained by DXA (*r* = 0.98). Values of FFM (kg) attained by using BIA or a validated BIA-based predictive equation for children as reference method, namely the Schaefer's equation, showed both a lower correlation with the corresponding values of LBM (kg) assessed by DXA in this pediatric population (*r* = 0.957 and *r* = 0.966, respectively).

The association between total LBM (kg) measured by DXA and BIA-derived ZI was further investigated by taking into account differences of tissue distribution in the vary body compartments, specifically the relative amount of trunk LBM evaluated by DXA. A cutoff value of 45% was chosen for the percentage of trunk LBM, corresponding to the median value observed in our cohort and very similar to previously used cut-off. At covariance analysis, subjects with trunk LBM below 45% of the total LBM (kg) showed a significantly different ZI slope when compared with subjects with trunk LBM above 45% (*R*² = 0.917).

Table 1

Comparative analysis among anthropometric, DXA and BIA parameters (median, first and third quartiles)

variables	Developing set			Cross-validation set		
	Girls (n 65)	Boys (n 55)	p-value	Girls (n 18)	Boys (n 17)	p-value
	Median (Q ₁ -Q ₃)	Median (Q ₁ -Q ₃)		Median (Q ₁ -Q ₃)	Median (Q ₁ -Q ₃)	
	Anthropometric data (1 missing data)			Anthropometric data		
Age (year)	8.51 (8.00-11.00)	9.03 (8.00-11.00)	0.27	8.00 (7.00-10.25)	9.00 (7.00-11.00)	0.68
Height (m)	1,35 (1,20-1,48)	1,36 (1,28-1,46)	0.70	1.33 (1.24-1.40)	1.35 (1.22-1.44)	0.68
Weight (kg)	31.80 (25.00-46.10)	36.40 (27.8-48.0)	0.23	31.95 (24.27-45.90)	34.10 (26.55-42.90)	0.96
WC (cm)	60.00 (54.00-68.00)	64.0 (57.0-73.0)	0.05	62.00 (55.75-78.25)	62.00 (55.00-72.00)	0.96
HC (cm)	72.00 (64.00-87.00)	73.50 (67.00-85.00)	0.74	73.50 (66.25-84.00)	71.00 (64.00-82.00)	0.57
WHR	0.83 (0.79-0.87)	0.87 (0.84-0.90)	0.00*	0.85 (0.81-0.89)	0.87 (0.84-0.88)	0.37
WHtR	0.44 (0.41-0.49)	0.46 (0.43-0.51)	0.03*	0.47 (0.42-0.52)	0.46 (0.43-0.50)	0.96
BMI (kg/m²)	17.41 (14.9-22.09)	19.90 (16.20-23.19)	0.10	17.96 (16.22-23.45)	18.58 (15.79-22.13)	0.68
	Data from DXA (1 missing data)			Data from DXA		
LBM (kg)	20.06 (17.08-26.96)	23.96 (20.23-29.00)	0.01*	20.48 (15.52-24.67)	22.19 (18.43-27.80)	0.35
FM (kg)	10.34 (6.46-17.41)	10.56 (5.95-18.43)	0.90	11.97 (7.45-19.24)	8.33 (5.60-16.18)	0.30
LBM (%)	64.49 (57.10-71.20)	69.74 (57.87-74.06)	0.12	65.30 (56.40-71.52)	74.70 (64.45-77.60)	0.08
FM (%)	32.16 (25.00-39.44)	36.30 (31.00-42.65)	0.10	34.70 (28.47-43.60)	25.30 (22.40-35.55)	0.08
BMC (kg)	1.07 (0.92-1.47)	1.28 (1.03-1.60)	0.08	1.12 (0.96-1.31)	1.18 (0.95-1.37)	0.63
	Data from BIA (13 missing data)			Data from BIA		
R (ohm)	693.50 (633.00-755.00)	620.00 (577.00-696.00)	0.00*	679.00 (635.25-738.25)	625.00 (579.50-699.00)	0.04*
Xc (ohm)	66.50 (62.00-79.00)	61.00 (56.00-71.00)	0.02*	63.50 (59.00-66.00)	60.00 (56.00-67.00)	0.50
ZI (cm²/ohm)	25.31 (20.54-37.14)	29.77 (25.18 - 34.84)	0.06	25.14 (19.51-30.04)	29.64 (22.84-33.25)	0.22
FFM (kg)	22.05 (18.50-34.70)	27.10 (21.90-33.70)	0.08	21.70 (18.50-31.00)	25.30 (20.10-30.80)	0.39
FM (kg)	8.70 (4.80-14.90)	7.20 (5.0-14.70)	0.78	9.95 (6.20-14.90)	7.30 (6.00-1.10)	0.48
FFM (%)	75.55 (67.80-80.70)	77.00 (69.30-82.60)	0.31	68.80 (67.60-75.90)	76.50 (71.70-81.20)	0.04*
FM (%)	24.45 (19.30-32.20)	23.00 (17.4-30.70)	0.31	31.20 (24.10-32.40)	23.50 (21.30-30.00)	0.09
TBW (lt)	17.90 (15.4-27.10)	21.20 (17.10-28.50)	0.06	17.40 (15.40-24.50)	19.90 (15.60-26.80)	0.33

All results were expressed as median (first quartile-third quartile). Statistical significance attributed to results with * $p < 0.05$ between girls and boys. BIA: bioelectrical impedance analysis; DXA: dual energy x-ray absorptiometry; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; BMI: body mass index; FM: fat mass; FFM: fat-free mass; BMC: bone mineral content; R: resistance; Xc: reactance; ZI: BIA-derived impedance index; TBW: total body water.

Table 2

Multivariable regression analysis with final model for predicting LBM (kg) (n = 105, missing data = 15)

Predictor	Regression coefficients			R ²	RMSE	p-model
	β	SE	p-value			
Intercept	-27.597	4.136	< 0.0001*			
ZI (cm ² /ohm)	0.337	0.035	< 0.0001*			
HC (cm)	0.094	0.020	< 0.0001*			
WHR	9.593	2.734	0.0007*			
Age (year)	0.360	0.121	0.0037*			
Height (cm)	0.164	0.031	< 0.0001*			
Overall model				0.962	1.90	< 0.0001*

β : Unstandardized regression coefficient; SE: standard error; RMSE: root mean square error; R²: r-squared value; p-model, significance level for model; FFM: fat-free mass; BIA: bioelectrical impedance analysis; ZI: BIA-derived impedance index; HC: hip circumference; WHR: waist-to-hip ratio.

Our new predictive equation was validated against an independent group of 35 children (17 boys and 18 girls) (Table 1). The mean difference between predicted LBM (kg) and measured LBM (kg) in the overall

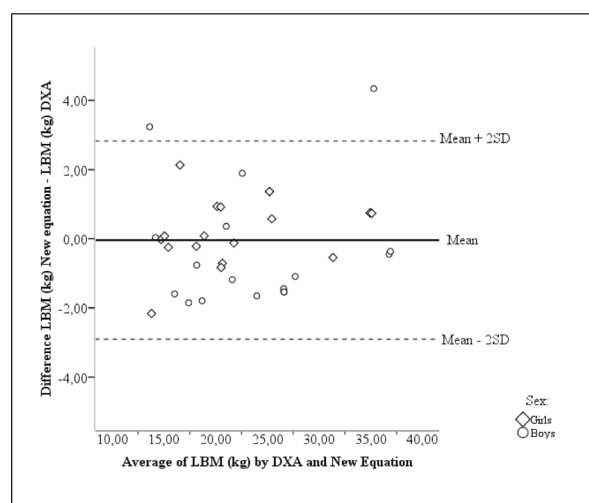
sample was -0.04 kg and limits of agreement of +2.82 to -2.90 kg. The differences and agreement between measured LBM (kg) and LBM (kg) predicted by our equation, Schaefer's equation and BIA method are de-

Table 3

Comparison of LBM/FFM estimated by our equation and other predictive methods with LBM measured by DXA in the cross-validation set

Methods	Sample	Cross-validation set		
		Predicted LBM/FFM (kg) Median (Q ₁ ;Q ₃)	Difference (kg) Median (Q ₁ ;Q ₃)	Agreement p-value
Our new equation	Girls	20.44 (17.03;25.88)	0.08 (-0.32;0.92)	0.879
	Boys	23.15 (17.12;26.49)	-1.09 (-1.56;0.19)	
Schaefer's equation	Girls	21.93 (18.75;26.30)	1.75 (1.52;2.58)	<0.0001
	Boys	25.93 (19.42;29.16)	1.02 (0.76-2.65)	
BIA	Girls	21.70 (18.22;31.00)	2.12 (1.34-5.43)	<0.0001
	Boys	25.30 (20.05;30.80)	2.50 (1.50-3.99)	

Results were expressed as median (first quartile-third quartile). Difference (kg) corresponds to LBM/FFM estimated by predictive methods minus LBM measured by DXA. Agreement p-value was performed by one sample t-test for total sample. LBM: lean body mass; FFM: fat-free mass; ZI: BIA-derived impedance index.

**Figure 1**

Bland-Altman representation of individual differences between predicted LBM by the new equation and measured LBM by DXA, plotted against the average of measured LBM values and predicted LBM values. LBM: lean body mass; DXA: dual energy x-ray absorptiometry.

scribed in Table 3. According to Bland-Altman method (Figure 1), our new LBM predictive equation for children was the only one in agreement to DXA-measured LBM ($p = 0.88$) and besides, there was no potential bias found ($p = 0.42$).

DISCUSSION

Measuring accurately body composition is still a challenge in the adult setting but even more in the pediatric one, where a developing physique represent a difficult situation to precisely evaluate lean and fat mass. The easily-obtainable BMI, despite widely used for epidemiological and clinical purposes, also in reason of its correlation with the cardiovascular and metabolic risk, has several limitations, especially in differentiating the real amount and body compartments distribution of lean and fat mass. For these reasons, BMI could be inadequate, especially in the pediatric setting, for accurately monitoring body composition and the interventions on it, or to implement risk prevention strategies. Other anthropometric parameters, such as WC, HC and WHR, or WHtR in pediatrics, are also used to characterize and

follow up the nutritional status and related predisposition to diseases [23-26].

More sophisticated and accurate techniques to measure body composition, such as air-displacement plethysmography or deuterium dilution, are difficult to perform, expensive, often available only in specialized centers, with results valuable merely in the research setting, and have a limited application in children, for safety reasons [27]. In particular, DXA is able of directly measuring, with high precision, the amount of LBM, FM and BMC in the different body compartments, although its amount of X-radiation [28].

Among other techniques, BIA instead is simple, non-invasive, inexpensive, and appropriate for routine use in the monitoring of nutritional status, even in children. Despite some limitations, such as the tendency of overestimating FFM while underestimating FM, the inability of measuring BMC and of assessing precisely body composition compartments, it can indirectly estimate the amount of FFM, FM and body fluids, thus allowing the implementation and follow up of dietetic and pharmacological interventions in clinical practice [29]. To improve the ability of BIA for estimating body composition, several predictive equations have been developed by combining BIA and anthropometric parameters. However, their reliability and application in the clinical setting is limited throughout different ages and conditions. Indeed, developing BIA-based predictive equations in children have proved difficult, especially to assess the degree of fatness, due to extreme variability of their body composition by ages, sex and pubertal development.

From this standpoint, our study aimed to design an original and dependable predictive equation to estimate LBM in children, derived by BIA and anthropometric parameters against DXA as the criterion measure. Besides, we aimed cross-validate our equation and confront LBM (kg) by DXA with other reference predictive equations and methods.

When analyzing the data from our pediatric population, normal ranges for age and sex as *per* reference charts and proposed cut-offs were found, thus characterizing our children as a normal weight population with apparently low cardiovascular and metabolic risk [30-32].

Children's age did not differ in both genders and it

was relatively below the average for expecting the pubertal development. For this reason the study group could be considered as composed mainly by pre-pubertal children, although the puberty state has not been recorded. BW and BMI were uniform in boys and girls, with WHR significantly greater in the former, underlining a tendency to an android distribution of FM. Also, WHtR was significantly greater in boys although within the normal limit (< 0.5) [13].

The additional more detailed analysis of body composition performed by DXA evidenced a significantly higher amount of absolute LBM (kg) in boys than in girls, which could be explained as *per* usual sex differences. From BIA, R and Xc were significantly higher in girls than in boys, outlining again the difference in body composition of the two genders. As expected, DXA and BIA disclosed in evaluating body composition, the latter overestimating FFM while underestimating FM, supporting our approach of using the more accurate DXA as validation method to develop any predictive equation.

Our extensive and deepen statistical analysis, when considering all these results, was able to provide a predictive equation for evaluating LBM (kg) in children. BIA contributed through the derived ZI, that confirmed its relevance in predicting body composition even in the pediatric setting, whilst, among the anthropometric parameters, age, height, WHR and HC appeared to be significant predictive factors for estimating LBM (kg). In particular, the presence of HC and WHR in our equation together with height and age, was important to obtain a more adherent estimation of body composition in a dynamically changing physique, in regards of linear growth and especially lean and fat mass development, thus accounting also for their distribution and related risk.

When using our predictive equation to estimating LBM and FM content in the developing set, the values obtained practically coincided with those measured by using DXA. Using our equation to extrapolation FM (kg) values could be possible, although FM might be slightly overestimated since BMC is not separately quantified. As BMC generally represents a small parcel of body weight and resulted less than 5% of the total body weight in this study, its factual interference in estimating body composition and its compartments with our predictive equation could be considered negligible.

When analyzing the associations between our predictive equation for LBM and the effective value measured by using DXA, a significant correlation was found. Moreover, our model resulted significantly superior in evaluating LBM when compared to values obtained by using BIA or another predictive equation validated in the pediatric setting, such as the Schaefer's equation. Such outcomes could be explained by the incapacity of BIA or other BIA-based equation of discriminating exactly lean, fat and bone mass and also to account for the precise tissue distribution in the different body compartments when estimating body composition.

BIA considers the body as a unique cylinder instead of a grouping of cylinders, and this could be a confounding factor for distinguishing limbs and trunk contribution on total body impedance and body weight.

For such reason, we analyzed the influence of tissue distribution in different compartments on estimating body composition by evaluating the associations between the BIA-derived ZI and the total LBM measured by DXA, using a cut-off of 45% for LBM content at the trunk, for dissecting the study sample. Indeed, a significantly different regression slope was found in children having a percentage of trunk LBM below or above the chosen cut-off, thus confirming the relevance of tissue distribution, especially the abdominal region, when assessing body composition and its compartments. Therefore, taking into account body circumferences when developing predictive equations for children, as we have done in this study, allows an improvement of body composition estimation.

An independent set was used to cross-validate our predictive equation and verify the agreement of other predictive methods. The lowest difference between predicted and measured LBM (kg) was seen in the girls, when our new equation was used. In the overall sample, according to Bland-Altman plot and one-sample t-test, our equation was seen to agree with DXA criterion measured without bias interference. LBM (kg) predicted by BIA and Schaefer's equation, instead, did not agree with LBM (kg) measured by DXA in Italian children population.

Despite the good results upraised by this study, several limitations could be mentioned as the lack of pubertal status data, even if pre-puberty in the vast majority of the study population could be assumed because of its prevalent young age. The absence of body skinfolds measurement as further evaluation of tissue distribution; the impracticality of disengaging the bone mass from FM in the final valuation of body composition, in spite of its small contribution on total body weight.

CONCLUSION

In conclusion, our study was able to develop and cross-validate a predictive equation for estimating body composition, mainly LBM, in the pediatric population by using impedance index by BIA and few easily-obtainable anthropometric parameters. The inclusion of HC and WHR, together with age and height, has showed to better assess the dynamic changes of the physique and nutritional status in children and to overmatch our previously proposed equation for evaluating the FFM and FM compartments. This equation can be useful in epidemiological studies, in the research setting and also in clinical practice, permitting a better definition and follow up of children's body composition. Further studies in larger populations are necessary as well as the development of direct FM predictive equation to help even more the diagnosing and managing obesity in childhood and the related risk.

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Author's Contributions

The authors' responsibilities were as follows: CC and LDR drafted the manuscript (equal contribution); LDR designed the study; PG and LR performed the

protocol and collected the data; RCM analyzed the data; all authors contributed to the interpretation of data and revision of the manuscript; ADL and IP had primary responsibility for the final content of the manuscript.

Conflict of interest statement

All authors declare no conflict of interest.

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