

Prediction of fat-free mass and fat mass from bioimpedance spectroscopy and anthropometry: a validation study in 7- to 9-year-old Kuwaiti children

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ETHICS STATEMENT: The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Joint Committee for the Protection of Human Subjects in Research at the Kuwait Institute of Medical Specialization of the Ministry of Health. All children, their parents and teachers were informed about the aims and procedures of the study and a written parent consent form was obtained for each child. The study protocol was approved by the Joint Committee for the Protection of Human Subjects in Research at the Kuwait Institute of Medical Specialization of the Ministry of Health.

ABSTRACT

Objective: Childhood obesity is increasing in many countries, including Kuwait. Currently, adiposity is most commonly assessed from simple anthropometric measurements, e.g. height and weight or combined as body mass index (BMI). This is despite these surrogate measurements being poor indices of adiposity. Bioelectrical impedance analysis (BIA) is a popular method for the assessment of body composition providing a measurement of adiposity as absolute fat mass (FM) or FM expressed as a percentage of body weight (%BF). BIA is, however, an indirect predictive method. This study developed a BIA-based prediction equation for body composition assessment in Kuwaiti children and, additionally, a prediction equation for %BF based on sum of skin-fold (SSF) thickness measurements.

Design: A cross-sectional design was used with primary school recruitment.

Setting: School population in Kuwait City; in-clinic assessments.

Participants: 158 Kuwaiti children aged 7-9 years. Body composition assessed using bioimpedance spectroscopy and skin-folds with prediction equations generate against deuterium dilution measurement of total body water and fat-free mass (FFM) as reference.

Results: The newly developed and cross-validated BIA equation predicted FFM with minimal bias (< 1%) and acceptable 2 standard deviation limits of agreement (± 1.6 kg equivalent to $\pm 10\%$) improving on the predictive performance of comparable published equations. Similarly, SSF predicted %BF with small bias (0.2 %BF) but relatively wide limits of agreement (± 7 %BF).

Conclusions: These new equations are suitable for practical use for nutritional assessment in Kuwaiti children, particularly in epidemiological or public health settings although their applicability in other populations requires further research.

KEY WORDS: Bioelectrical impedance analysis (BIA), sum of skin-folds, percentage body fat, body composition analysis, fat-free mass, total body water, deuterium dilution, children, Kuwait

INTRODUCTION

The prevalence of pediatric obesity is increasing in Kuwait. ⁽¹⁾ The most recent survey (Kuwait Nutrition Surveillance System) in 2018 reports 16.5% of 5-6 year-old boys and girls are obese rising to 32.8% of boys and 27.4% of girls aged 9-10. ⁽²⁾ A further 19.7% of boys and 28.8% of girls were overweight at age 9-10 years in 2018. The most common way to classify pediatric obesity is body mass index (BMI, body weight in kilograms divided by height in meters squared) according to age and sex. Although BMI is efficient in providing a useful indication of body size and shape, it does not provide a precise measure of body composition or spatial information of body components, for example distribution of fat mass or adipose tissue. Furthermore, the relationship between body fat and BMI tends to differ between different ethnic groups. For example, when comparing adult white Europeans, South Asians, Africans and Pacific Islanders, for the same BMI value, Africans have been reported to have the least fat mass, while the South Asians and Pacific Islanders have the highest. ⁽³⁾ Another study indicates that in white children of the same age and sex, fat mass can differ by two-fold while BMI remains the same. ⁽⁴⁾ BMI tends to misclassify children with high bone or muscle mass since it does not differentiate between fat mass (FM) and fat-free mass (FFM). ⁽⁵⁾ Excess body fat is misinterpreted by BMI, accordingly, a more accurate method for assessing body fat is needed.

Obesity is correlated with morbidity and mortality therefore a more reliable and practical method for assessing adiposity is required for a large population. ⁽⁶⁾ Many methods are considered reliable for adiposity assessment, however, not all are appropriate in children or in public health settings. Dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) are inappropriate for a large population because of their high cost ⁽⁷⁾ while the radiation hazard associated with DXA may preclude its use in children. Deuterium dilution can be used to assess total body water (TBW) which following application of age and sex appropriate hydration factors can estimate FFM. The technique is considered a reference method but is generally not routinely usable owing to cost and the need for specialist analytical laboratory facilities. ⁽⁸⁾ Alternatively, studies have shown, that less costly but reliable methods such as bioelectrical impedance analysis (BIA) and skin-fold thickness (SF) are acceptable for use in children. ^(9,10) These methods are, however, indirect relying upon empirically-derived prediction equations to

measure body composition, typically as absolute FFM and FM or %body fat (%BF). Most published equations have been generated for Caucasians and are widely regarded as being ethnicity-specific.⁽¹¹⁾ The Kuwaiti population is relatively homogeneous, being derived from the major population groups of the Arabian Peninsula.⁽¹²⁾ Thus, the aim of this study was to develop new anthropometric and BIA equations, based on deuterium dilution⁽¹³⁾ as the reference method, to assess body composition for Kuwaiti children.

MATERIALS AND METHODS

The present study was a method comparison and validation sub-study as part of a program to establish body composition methodologies at the Kuwait Institute of Scientific Research (KISR). The establishment of the reference deuterium dilution technique has been described previously in the same children as described here.⁽¹³⁾

Participants

A total of 158 7- to 9-year-old children (75 boys and 83 girls) were recruited from primary public schools from Kuwait City, Kuwait. This age group and sample size was chosen to complement a similar study being undertaken under the auspices of the International Atomic Energy Agency (IAEA) in Asian children⁽¹⁴⁾ A non-random purposive sampling approach was used to enrol children encompassing a wide BMI range for each year of age and sex as in⁽¹⁴⁾.

Inclusion criteria were a healthy Kuwaiti child not on medication or has a medical condition that may affect body composition or metabolic rate. The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Joint Committee for the Protection of Human Subjects in Research at the Kuwait Institute of Medical Specialization of the Ministry of Health. All children, their parents and teachers were informed about the aims and procedures of the study and a completed written parental consent form was obtained for each child.

Anthropometric measurements

Participants were received in the health clinic at KISR. Three trained Ministry of Health professionals were responsible for taking all the anthropometric measurements. Each professional was responsible for one anthropometric measurement for all participants to minimize inter-researcher error. Height was measured, with the children in the Frankfort plane, using a portable stadiometer (Holtain, Crymych, Pembs, UK). Body weight was measured using a digital electronic scale (SECA Robusta 813, Advanced Technology Company K.S.C., Hawali, Kuwait), while the participant was barefoot and wearing light clothes. The average of three measurements was used for the final measured value with a maximum allowable difference of 0.1 cm for height and 0.1 kg for weight, measurement resolution for height and weight respectively. BMI classification and criteria were based on WHO BMI-for-age growth chart ⁽¹⁵⁾, using the calculated BMI ($\text{weight}(\text{kg})/\text{height}^2(\text{m}^2)$). Classification of children as normal weight, overweight or obese based upon BMI-for-age z-score has been described previously. ⁽¹³⁾

Skin-fold thickness was measured with a Holtain skin-fold caliper; measurements were taken while the child was standing and on the right side of the body. An average of three measurement was taken on the four sites (triceps, biceps, subscapular, and suprailiac), with a maximum allowable difference of 0.2 mm.

Waist and hip circumferences were measured using non-stretchable measuring tape; waist circumference (WC) (cm) was measured around the child's bare abdomen at midpoint between lower rib and iliac crest using and hip circumference (HC) (cm) was measured at widest part around hip. All measurements were obtained in triplicate, to 0.1 cm resolution with 0.5 cm as the maximum allowable difference between the three measurements and the mean values used.

Bioimpedance spectroscopy measurement

Protocol: A tetrapolar electrical bioimpedance spectrometer (Imp SFB7, ImpediMed Limited, Pinkenba, Qld, Australia) was used to measure whole body, wrist-to-ankle impedance (Z , ohm) resistance (R , ohm) and reactance (X_c , ohm). Prior to taking the measurements all metal accessories, socks and shoes were removed. Measurements were taken on the right side of the body while lying supine, EKG-style electrodes (single tab, ImpediMed Limited, Pinkenba,

Qld, Australia) were placed 5 cm apart on the hand and foot in the conventional manner as described elsewhere.^(16,17) Measurements were taken after the participant had been in supine for 10 min during the deuterium dilution equilibration phase. Factors known to influence hydration status and BIA measurements including exercise, eating, drinking were supervised and study took place in an air-conditioned room temperature (22°C within a degree) with participants all measured after an overnight fast as described previously.⁽¹³⁾ Measurements were taken in duplicate; each measurement taking approximately 1 second.

Data analysis: Impedance spectrum data were analysed according to the Cole model⁽¹⁸⁾ using Bioimp software (v4.5.0.0, Impedimed Ltd., Brisbane, Australia). The resistance at 50 kHz (R50) was extracted for generation of a single frequency prediction equation and the resistance at zero (R0) and infinite (Rinf) frequencies were obtained from extrapolation of data from a graphical plot of the Cole model⁽¹⁸⁾ and used to predict TBW and FFM according mixture theory⁽¹⁹⁾ using the body composition module of Bioimp. Additional parameters required were the apparent resistivities of intra- and extracellular water (ICW and ECW respectively), a body proportion factor (K_B), body density and an hydration fraction (HF) to convert TBW to FFM (TBW/HF). Published body proportion factors are limited to adults ($K_B \cong 4.3$) but may be computed from anthropometric measurements.⁽²⁰⁾ A K_B value was estimated using anthropometric data obtained in the present study and from the literature.⁽²¹⁾ Age and sex appropriate hydration factors were taken from Wells et al.⁽²²⁾ In preliminary analyses the older published factors of Lohman were tested and yielded slightly larger (3%) TBW values.⁽²³⁾ Resistivity coefficients for children have not been published so those used by Moon et al.⁽²⁴⁾ and Ward et al.⁽²⁵⁾ were tested. In addition, Moissl's modification of mixture theory incorporating BMI was assessed.⁽²⁶⁾

Total body water measurement by deuterium dilution

Total body water was measured by the deuterium dilution technique following the protocol of the IAEA⁽⁸⁾ as described in detail previously.⁽¹³⁾ Briefly, a baseline urine sample was collected after an overnight fast, followed by administration of a 10% D₂O dose based on body weight. Post-dose urine samples were collected 4 h after the intake of the dose. Urine samples were analysed to determine deuterium enrichment using isotope ratio mass spectrometry (IRMS, Nu

Instruments, UK). A 4% correction for the difference between isotope dilution space and TBW was applied. FFM was calculated from TBW using the same hydration fraction as used for the impedance data, i.e., age and sex appropriate hydration factors according to Wells et al. ⁽²²⁾ .

Data analysis

Anthropometric predictions of body composition

Multiple regression was used to predict %BF from skin-fold measurements. To be consistent with published prediction equations the sum of skin-folds (SSF) was log transformed.⁽²⁷⁾ The dependent variable was %BF determined from deuterium dilution, independent (predictor) variables were SSF and sex (coded female=0, male=1). Assumptions of normality and constant variance made in multiple regression were checked and met. Multi-collinearity between independent variables was assessed by determining the variance inflation factor (VIF); a value less than 10 being deemed acceptable. The standard error of the estimate error, calculated as the square root of the mean square error, coefficient of determination (R^2) values, Akaike information criterion (AIC), Mallows' C_p and the predicted residual error sum of squares (PRESS) statistic were used to determine goodness of fit of the regression model.

A double cross-validation was performed in which a randomized, sex-stratified 50:50 split of the total sample was carried out ($n = 79$ per group). Equations developed in each group were cross-validated in the opposite group. Covariance analysis and comparison of the slopes and intercepts was used to compare the regression models between the two groups and if not significant, a single equation from the whole sample was also generated with predicted performance assessed using the LOOCV procedure (Solverstat 2019 R0; software available at <https://solverstat.wordpress.com/>, last accessed 29th November 2022). The performance of the new prediction equation was compared to published prediction equations for similar populations of children (Supplementary Table 1).^(9,28–33) For those equations which predict body density,^(28,31–33) %BF was calculated using the modified Siri equation of Weststrate and Deurenberg⁽³⁴⁾ as recommended by Reilly et al.⁽²⁷⁾

Bioelectrical impedance predictions of body composition

The measured resistance at 50 kHz (R50) was used to develop prediction equations using stepwise multiple regression analysis. FFM was the dependent variable and the predictor variables examined were weight, age, sex (male = 1, female = 0), and resistance index (RI) based on height ($\text{height}^2/\text{resistance}$). Assumptions of normality and constant variance and multicollinearity were assessed as used for anthropometric prediction. Cross-validation of the best performing predictor was undertaken using the same split-group procedure as for anthropometric predictions. Single-frequency predictions of FFM were compared with both published prediction equations for similar populations and those determined using mixture theory modelling as described above.

Statistical analysis

Statistical analyses were performed using MedCalc v19.8 for Windows (MedCalc Software, Broekstraat 52, B-9030 Mariakerke, Belgium). Results are expressed as mean and standard deviation except where otherwise indicated. Differences in participant characteristics between sexes were examined by independent sample t-test for continuous variables and z-test for proportional variables. Statistical significance was defined as $P < 0.05$. Data were assessed for normality using Kolmogorov-Smirnov test and for outliers using generalized extreme studentized deviate (ESD) procedure at an alpha level of 0.05.

Predictive performance of equations, either anthropometric or impedance-derived, was assessed using Pearson correlation, paired sample t-test, Lin's concordance correlation, Cohen's D for paired samples, residual standard deviation for random differences (Passing-Bablok regression) and Bland-Altman limits of agreement analysis. Relative ranking of prediction equations was based upon median absolute percentage error (MAPE), two-one-sided t-test equivalence analysis for paired data and Gower similarity index. The TOST procedure measures the minimum % difference at which the mean values are considered equivalent and were calculated using the Excel spreadsheet provided by Lakens (available at <https://files.osf.io/v1/resources/q253c/providers/osfstorage/58455f749ad5a100475fe734?action=download&direct&version=7>, last accessed 29th November 2022). The Gower index varies between 0 and 1 where 1 represents identity between measurements. Gower indices were

calculated using Gower (v1.1) (available at <https://www.pbarrett.net/Gower/Gower.html>, last accessed 29th November 2022) Statistical significance was set at $P < 0.05$.

RESULTS

Participant characteristics

Physical and reference body composition data of the participants are presented in Table 1. Total participant number was 158 (75 boys and 83 girls) aged 7- to 9-years-old Kuwaiti children. There were no significant differences in any body composition parameter with the exception of %BF between the sexes. A wide range in %BF was observed, varying from 14.2% to 57.5% with %body fat being significantly larger ($P < 0.01$) in females than males (means 38.7% versus 35.1%). BMI classification, based on the WHO growth reference charts⁽¹⁵⁾, showed a bimodal distribution with similar proportions of boys and girls in the normal and obese classes (40-50.7%) and few (8-16.9%) classed as overweight. General characteristics of the two sub-groups of participants (prediction generation and validation) are presented in Table 2. There were no significant differences and each group was representative of the whole population.

Anthropometric prediction of body composition

Prediction of %BF fat from log-transformed sum of skin-folds is presented in Table 3. Prediction models were developed for each sub-group for both males and females separately and by including sex as a predictor variable. Relationships between predictor variables and reference %body fat were highly significant ($P < 0.0001$) with strong coefficients of determination (R^2), > 0.83 except for females alone, $R^2 = 0.76$. Prediction error (RMSE) was similar across all models, approximately 3.5 kg.

Cross-validation of the generated prediction equation (including sex as a predictor variable) was excellent with minimal bias ($\pm < 0.3$ kg) as indicated by high correlation (> 0.91), high % similarity (TOST $< 2.5\%$ similarity), high Gower index and low MAPE ($< 6.5\%$) (top panel, Table 4). Pearson and concordance correlations between predicted and reference %BF values were virtually identical indicating no systematic differences. Overall prediction error was small (2.5 kg) with 1.96SD limits of agreement between methods of ± 7.1 kg.

Comparison of predictive performance of the new equation with comparable and commonly used published equations is present in the lower panel, Table 4. Data are presented for all participants stratified by sex. Generally, published equations, although providing highly correlated data, performed poorly at predicting body composition both at the population level, biases ranging from 4.4 to 22.3 kg, and in individuals with limits of agreement up to ± 21.9 kg (Table 4, Supplementary data Figure 1). The best performing published equation was that of Wendel et al. (29)

Prediction of total body water from bioelectrical impedance measurements

Four different models were explored for predicting TBW from impedance measurements using: resistance index (RI, height^2/R at 50 kHz); weight alone; RI and weight and RI, weight and sex. Each model was developed separately in cross-validation groups 1 and 2 (Table 5). Generally, all potential predictor variables were highly significant ($P < 0.001$) with high coefficients of determination (R^2) being observed (> 0.87). Sex was the weakest predictor variable being not significant in Group 2 but significant in group 1. Prediction error was around 1 kg for all models. Overall, the final prediction model included all three variables with an R^2 of 0.934 and prediction error of 0.84 kg.

The results of cross validation are presented in the upper panel, Table 6. Bias was minimal $< \pm 0.5$ kg with small limits of agreement (± 1.6 kg).

Comparison with published equations are presented in the lower panel of Table 6 and Supplementary figure 2. Comparison was performed against bioimpedance spectroscopic (BIS) prediction approaches (methods of Moissl (26), Moon (24) and Ward (21,25)) and empirically-derived single-frequency (50 kHz) prediction equations (Clasey (35), Rush (36), Jemmaa (37), De Lorenzo (38), El Harchaoui (39) and Horlick (40)). The prediction equation generated in this study performed the best closely followed by the BIS methods of Ward using personalised K_B values and Moissl. The best performing single frequency predictors were those of El Harchaoui and Horlick. Limits of agreement for these predictions were broadly similar (ranging from ± 2.0 to ± 2.2 kg).

Prediction of body composition (FM and FFM) from bioelectrical impedance and skin-fold measurements

Total body water volumes for all participants obtained using the best performing BIA-based predictor equation (Model 4, Table 5) were transformed to FFM values using age-appropriate hydration fractions. Fat mass was determined by difference with body weight and %BF calculated. Similarly FFM and FM were calculated from the derived skin-fold equation for %body fat (Table 4). Predicted FFM, FM and %BF values were compared to reference values calculated from deuterium dilution-determined TBW using limits of agreement analysis and are presented in Fig 1.

Mean differences in FFM were close to zero (MAPE approximately 3%) for both the BIA-based predictions and the SSF-based predictions with limits of agreement similar ($\pm 9.9\%$ for BIA and 11% for SSF) to those seen in the split-group analysis, Tables 4 and 6 for BIA and SSF predictions respectively, (Fig1A). A slight but significant ($P < 0.05$) proportional bias was also observed. Impedance-based prediction was slightly better than SSF-based prediction, MAPE = 2.8% compared to 3.2% respectively. Figs 1B and 1C present similar analyses for FM and %BF. Conversely, SSF predicted FM and %BF slightly better than BIA with limits of agreement of approximately $\pm 17.5\%$ compared to $\pm 21\%$ although MAPE was slightly smaller for BIA (5%) than for SSF (6.1%).

DISCUSSION

Childhood obesity in Kuwait is currently estimated to have a prevalence of around 30% ⁽²⁾ and is a recognised risk factor for cardiovascular disease and metabolic syndrome ⁽⁴¹⁾ in this population. However, most estimates of adiposity and body composition in general are based on either simple height and weight measures or BMI ^(42,43) despite the acknowledged inadequacies of these measurements for this purpose. ⁽⁴⁴⁾ The present study has provided a validated BIA prediction equation for body composition for use in Kuwaiti children.

BIA technology is popular for estimating body composition as a practical alternative to expensive and technically complex methods such as DXA suitable for use in epidemiological and public health settings. The BIA method is based upon a two-compartment model of the body

(body mass = fat mass + fat-free mass) and is an indirect method since it does not measure body composition *per se* but predicts this using transformation algorithms from the measured electrical resistance of the body. Since transmission of an electrical current through the body occurs via the conductive water containing tissues, electrical resistance is quantitatively related to body water volume by

$$TBW = \rho \frac{L^2}{R} \quad \dots [1]$$

where TBW = total body water (L), L = the conductive length (cm), (typically stature is used as a proportional surrogate since true conductive length is unknown), R (ohm) is the measured resistance and ρ is the specific resistivity (ohm.cm). Transformation algorithms are typically empirically derived by calibration against a reference body composition method and developed by regression of the resistance index (L^2/R) against reference TBW volume. Additional potential predictor variables may be included in a multiple regression. In the present study, the accepted reference method of deuterium dilution was used to measure TBW. Multiple regression was used to develop the best-fitting prediction algorithm with the final equation including RI, sex and weight. Although included as a predictor in the final model, the effect size of sex was relatively small and, in the cross-validation, was only a significant predictor in one of the two validation groups. This is not entirely surprising since sex-differences in body composition of children are generally considered to occur in both FFM and, primarily, in FM in post-pubertal children. ⁽¹⁰⁾ Sex as a predictor variable did, however, improve albeit slightly prediction performance supporting its retention in the final model. Furthermore, the ratio of intra-to extracellular water in TBW is generally higher in females again supporting inclusion of sex as a covariate. ⁽⁴⁵⁾

Although BIA is most appropriately calibrated against TBW, of more practical interest to nutritionists is body composition in terms of FM and FFM. TBW may be converted to estimates of FFM by assuming an hydration fraction for FFM. Body water volume and as a proportion of FFM is generally held within a tight physiological range with 0.732 being the accepted assumed hydration fraction of FFM in healthy adults. ⁽⁴⁶⁾ Hydration fraction is higher in newborns and infants and gradually approaches adult values around puberty. ⁽⁴⁶⁾ In the present study, age-appropriate hydration fractions were used to convert TBW volumes to FFM and, in accord with the 2-compartment model, provide estimates of FM and %BF. The newly-developed prediction

equation provided excellent prediction of FFM at the population level (<1% bias), the limits of agreement were relatively wide, $\pm 9.9\%$ but comparable to or smaller than those observed in other studies and for alternative prediction equations tested in this population. There is no consensus as to what is a clinically acceptable agreement limit, although 10% is often considered acceptable. (47)

Although BIA is most appropriately used to predict FFM, in clinical practice it is more commonly used to predict FM and %BF. Since these are calculated by difference of FFM and body weight, this becomes a doubly-indirect prediction with the likelihood of increased error due to error propagation. Error may be further compounded as a result of this calculation involving subtracting FFM that is typically proportionally larger than FM from body weight to calculate the much smaller FM. In the present study, for example, mean FFM was approximately 2-fold larger than FM but was up to 6-fold larger for the participant with the lowest %BF (14.2%). Notably however, SSF which more directly assesses body fat as subcutaneous adipose tissue exhibited smaller limits of agreement than BIA. These observations suggest that, while the differences are small, BIA may be more appropriate to assess FFM and SSF adiposity in this age group.

The newly developed SSF and impedance predictors were compared to a number of published equations. These equations were selected since they are in widespread use or were developed in comparable populations of children. In general published SSF equations markedly underestimated %BF compared to the reference values. The notable exception being predictions obtained with the equation of Wendel et al. which although still underestimating were closer to reference values. Wendel et al. and others, e.g., (48) also observed underestimation of %BF when predicted by these same published equations. The reasons for this underestimation are unclear. It is possible that equations such as those of Durnin and Rahman (28) or Brook (33) are now over five decades old and were developed in populations of children with body compositions that are not commensurate with those of contemporary children. A further possibility is that SSF-based equations typically predict body density which is then transformed to %BF. The original transformation method, and still in common use, was the Siri equation. (49) In the present study, we used the modification recommended by Weststrate and Deurenberg. (34) This produces lower estimates of %BF than Siri; for example, mean %BF from Durnin and Rahman using this

approach was 17.4% but was increased to 24.0% when transformed using the Siri equation. The newly developed prediction equation did not require conversion of body density to %BF since %BF was calculated directly from FM calculated from deuterium dilution-derived FFM. Similarly the best performing Wendel et al. equation also did not invoke body density since this equation was derived again from directly measured %Bf using DXA.

Prediction of FFM from impedance measurements was generally acceptable at a population level, the worst performing published equation (Rush et al.) overestimated mean FFM by 15% with most equations providing estimates within 10% of reference values. Limits of agreement were typically around ± 2 kg or $\pm 12\%$. The newly developed equation had minimal bias on cross-validation (0.05 kg) and improved limits of agreement ± 1.6 kg or $\pm 10\%$. Although there is no acknowledged performance standard for predicting body composition, limits of agreement of this magnitude are generally considered as clinically acceptable for clinical use in individuals.⁽⁴⁷⁾ The generalizability of the new equation is unknown. Empirically-derived prediction equations frequently show population specificity and are not readily transferable between populations. Conversely, bioimpedance spectroscopy approaches for assessing body composition use a biophysical modelling approach to estimating TBW and FFM and are considered to be less susceptible to population specificity since the models use directly values for resistivity (Equation 1).⁽¹⁹⁾ However, it should be recognized that these resistivity values still require empirical determination and, to date, this has only been undertaken in adults.⁽²⁵⁾ Nonetheless, BIS prediction of TBW performed creditably when appropriate corrections for difference in body proportions of children compared to adults was applied.⁽²¹⁾

The study has strengths and limitations. A strength of the study was acknowledgement that although BIA assesses directly FFM, %BF is often of greater interest to dietitians and public health nutritionists. Analysis of data was extended to consider predictive performance for FM and %BF. In addition to BIA, the independent method of skin-fold measurements was assessed and shown to provide acceptable estimates of %BF comparable with those from BIA. The current study used a BIS device, which is typically more expensive and technologically more complex than single frequency bioimpedance analysis (SF BIA) devices. The new equation was, however, developed using impedance data collected at 50 kHz, the frequency used by SF BIA devices extending usability to lower resource settings. A limitation of the study is the relatively

narrow age range of the participants, 7-9 years. The newly developed equation should be tested for predictive performance across a wider range of ages although good predictive performance of BIS indicates broader applicability of this approach.

CONCLUSIONS

Both a sum of skin-folds prediction equation for %BF and a single frequency BIA prediction equation for estimation of TBW and FFM in Kuwaiti children aged 7-9 years that showed good predictive performances were developed. The minimal mean biases indicate that the equations should be particularly useful in epidemiological research and for providing body composition data when developing public health strategies to combat increasing childhood obesity. The acceptable limits of agreement suggest that the equations should also prove useful when assessing body composition in an individual in a clinical setting.

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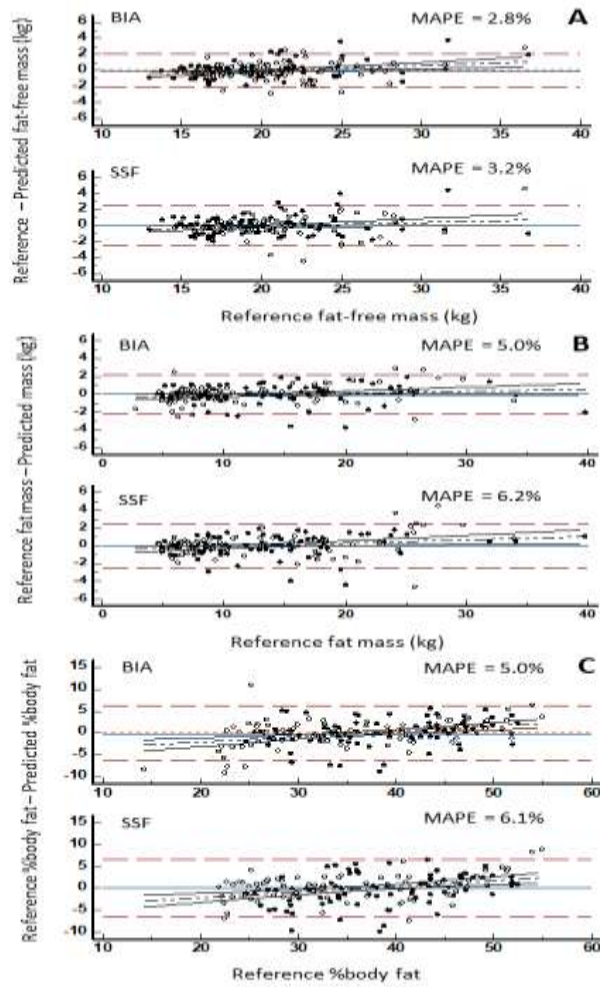


Figure 1: Comparison of body composition predicted by skin-fold-based anthropometric and impedance-based algorithms compared to the reference method of deuterium dilution

Panel A Fat-free mass (kg)
 Panel B Fat mass (kg)
 Panel C Percentage body fat (%BF)

Key ○ Female participants
 ● Male participants
 - - - - - Limits of agreement (± 1.96 SD)
 - - - Regression line
 Bias

Table 1 Physical and body composition characteristics of participants

Variable	Males (n= 75)	Females (n=83)	P-value ^a
Age (years)	8.2 ± 0.6 (7.0 to 9.4)	8.1 ± 0.7 (7.0 to 9.4)	0.79
Weight (kg)	33.9 ± 10.8 (19.1 to 65.5)	33.6 ± 11.1 (18.2 to 76.53)	0.88
Height (cm)	129.8 ± 6.4 (113.9 to 148.8)	129.0 ± 7.4 (112.2 to 149.5)	0.49
Triceps skinfold (mm)	14.1 ± 6.7 (5.0 to 31.0)	14.5 ± 5.2 (5.1 to 25.3)	0.66
Biceps skinfold (mm)	8.50 ± 5.2 (2.5 to 22.0)	8.7 ± 4.2 (3.0 to 20.0)	0.83
Supscapular skinfold (mm)	12.5 ± 9.1 (3.4 to 35.0)	12.5 ± 7.0 (5.0 to 32.0)	1.00
Suprailiac skinfold (mm)	13.0 ± 9.4 (2.9 to 34.0)	12.7 ± 7.5 (2.8 to 34.07)	0.83
Waist circumference (cm)	63.6 ± 12.9 (48.0 to 99.0)	62.9 ± 12.2 (44.7 to 102.0)	0.72
Hip circumference (cm)	74.8 ± 10.9 (59.0 to 102.0)	75.2 ± 10.7 (58.0 to 112.0)	0.84
Waist-hip ratio	0.84 ± 0.06 (0.75 to 0.99)	0.83 ± 0.06 (0.7 to 1.0)	0.68
BMI (kg/m ²)	19.8 ± 5.1 (13.6 to 35.2)	19.8 ± 4.7 (13.5 to 35.7)	0.98
BMI classification Normal (%):Overweight (%):Obese (%)	38 (50.7) : 6 (8) : 31 (41.3)	36 (43.4) : 14 (16.9) : 33 (39.8)	
BMI z-score ^b	1.60 ± 2.0 (-1.9 to 6.8)	1.4 ± 1.5 (-1.3 to 5.3)	0.39
TBW (kg)	16.3 ± 3.0 (11.2 to 28.1)	15.4 ± 3.4 (10.0 to 28.3)	0.39
Fat-free mass (kg)	21.1 ± 3.9 (14.5 to 36.5)	19.9 ± 4.4 (13.0 to 36.8)	0.39
Fat mass (kg)	12.7 ± 7.2 (2.8 to 39.7)	13.8 ± 7.4 (4.9 to 34.1)	0.34
%BF	35.1 ± 9.8 (14.2 to 55.0)	38.7 ± 7.8 (24.7 to 57.5)	0.01
FFMI (kg/m ²)	12.5 ± 1.5 (9.9 to 17.5)	11.8 ± 1.5 (9.4 to 17.1)	0.44
FMI (kg/m ²)	7.4 ± 3.9 (2.1 to 18.2)	8.0 ± 3.4 (3.3 to 18.5)	0.44

Abbreviations: BMI = body mass index; TBW = total body water; %BF = percentage body fat. Values are means ± S.D., range in parenthesis. ^a Differences were analyzed with a two-sample t-test. ^b WHO growth reference standards.⁽¹⁵⁾

Table 2 General characteristics of participants for the split samples

Variable	Group 1 (n=79)	Group 2 (n=79)	P-value ^a
Female, %(n)	51.9 (41)	53.2 (42)	0.73 ^b
Age (years)	8.1 ± 0.7	8.2 ± 0.7	0.18
Weight (kg)	33.7 ± 10.6	33.8 ± 11.3	0.97
Height (cm)	129.0 ± 6.8	129.8 ± 7.1	0.51
BMI (kg/m ²)	19.7 ± 4.9	20.0 ± 4.9	0.77
Impedance (Z, Ω)	738.3 ± 92.5	734.7 ± 80.6	0.79
Resistance (R, Ω)	735.4 ± 92.4	731.7 ± 80.7	0.79
Reactance (Xc, Ω)	64.8 ± 7.1	64.8 ± 7.7	0.95
Height ² / Resistance (RI, cm ² / Ω)	23.2 ± 4.5	23.5 ± 4.4	0.67
TBW (kg)	15.8 ± 3.2	15.8 ± 3.3	0.89
Fat-free mass (kg)	20.5 ± 4.1	20.6 ± 4.3	0.87
Fat mass (kg)	13.3 ± 6.9	13.3 ± 7.7	0.97
%BF (%)	37.2 ± 8.7	36.8 ± 9.3	0.75

Abbreviations: BMI = body mass index. Values are means ± S.D. ^aDifferences were analyzed with a two-sample t-test; ^bz-test for proportions.

Table 3 Anthropometric predictive models for percentage body fat* in Kuwaiti children aged 6.5 – 9.6 years

Participant group	Model Predictors	Regression coefficient	P value	R ²	RMSE (kg)	Mallow's C(p)	PRESS	Akaike information criterion	VIF
Group 1	LogSSF +	32.360	0.0001	0.834	3.697	3.0	1064	204.4	1.0
	Sex+	-2.862	0.0001						
	Intercept	-14.070							
Group 2	LogSSF +	35.005	0.0001	0.846	3.578	3.0	1104	209.7	1.0
	Sex+	-1.903	0.0001						
	Intercept	-18.696							
All participants	LogSSF +	33.578	0.0001	0.839	3.624	3.0	2114	409.8	1.0
	Sex+	-2.437	0.0001						
	Intercept	-16.203							
All females	LogSSF +	32.5320	0.0001	0.757	3.857	2.0	na	226.1	na
	Intercept	-14.4916	0.0001						
All males	LogSSF +	34.2637	0.0001	0.885	3.360	2.0	na	183.8	na
	Intercept	-19.7375	0.0001						

Abbreviations: SSF = sum of 4 skin-folds (biceps, triceps, sub-scapular, suprailiac); RMSE = root mean square error, PRESS = predicted error sum of squares; VIF = variance inflation factor; na = not applicable to single variable regression;* reference method, deuterium dilution

Table 4 Anthropometric prediction of percentage body fat* in Kuwait children aged 6.5 – 9.6 years

Prediction method	Validation group	Sex	Mean \pm SD (%BF \pm SD)	Bias* (%BF \pm SD)	Limits of agreement (%)	r_p	r_c	Cohen's d effect size for paired data	RSD (%BF)	MAPE (%)	TOST (%)	Gower index
Split-group cross-validation												
Reference %BFD ₂ O	Group 2	Both	36.8 \pm 9.3									
Prediction Group 1 (Table 3)	Group 2	Both	36.7 \pm 8.1	0.1 \pm 0.8	-7.2 – 7.4	0.918	0.909	0.03	2.5	6.2	2.2	0.943
Reference %BFD ₂ O	Group 1	Both	37.2 \pm 8.7									
Prediction Group 2 (Table 3)	Group 1	Both	37.4 \pm 8.5	-0.2 \pm 0.7	-7.2 – 6.9	0.911	0.911	0.05	2.6	6.5	2.4	0.932
Comparison with published predictors (all participants)												
Reference %BFD ₂ O	All	M	35.1 \pm 9.8									
	All	F	38.7 \pm 7.8									
This study (LOOCV)	All	M	35.1 \pm 9.0 ^a	0.0 \pm 3.3	-7.5 to 7.5	0.941	0.938	0.30	2.3	5.1	1.8	0.927
	All	F	38.7 \pm 7.0 ^a	0.0 \pm 3.8	-7.5 to 7.5	0.870	0.865	0.92	2.7	6.4	1.8	0.901
	All	M	14.5 \pm 8.5 ^a	20.6 \pm 3.4	13.9 to 27.3	0.941	0.262	6.54	2.9	61.3	60.7	0.588
Durnin and Rahman ⁽²⁸⁾	All	F	20.0 \pm 6.3 ^a	18.8 \pm 3.8	11.6 to 25.6	0.870	0.186	6.36	3.1	48.1	50.1	0.625
	All	M	22.1 \pm 6.5 ^a	12.1 \pm 3.8	4.6 to 19.7	0.934	0.546	4.30	3.9	40.9	39.5	0.757
Slaughter et al. ⁽⁹⁾	All	F	22.9 \pm 10.8 ^a	16.6 \pm 4.0	9.1 to 23.7	0.856	0.226	3.12	3.3	43.2	43.7	0.668

a

Johnston et al. (32)	All	M	12.8 ± 9.2 ^a	22.3 ± 3.3	15.8 to 28.8	0.941	0.248	6.83	3.1	68.7	65.4	0.554
	All	F	16.6 ± 6.2 ^a	22.1 ± 3.9	14.9 to 28.9	0.870	0.142	6.15	3.1	56.9	60.0	0.558
Wendel et al. (29)	All	M	30.3 ± 7.5 ^a	4.8 ± 4.0	-3.1 to 12.6	0.928	0.778	1.45	2.8	13.6	15.9	0.791
	All	F	34.3 ± 6.1 ^a	4.4 ± 4.1	-3.0 to 11.6	0.849	0.687	1.14	3.3	11.7	13.3	0.898
Brook ⁽³³⁾	All	M	18.2 ± 10.5 ^a	16.9 ± 3.5	9.9 to 23.4	0.941	0.394	4.84	3.6	50.8	50.1	0.663
	All	F	18.2 ± 10.3 ^a	20.5 ± 5.2	10.3 to 30.4	0.870	0.236	4.40	5.1	53.8	55.3	0.589
Deurenberg et al. (31)	All	M	20.8 ± 7.6 ^a	14.2 ± 3.7	6.9 - 21.6	0.940	0.389	4.75	3.3	41.5	42.8	0.715
	All	F	24.0 ± 6.5 ^a	14.7 ± 3.8	7.7 to 21.4	0.869	0.273	4.01	3.2	37.3	39.9	0.705
Alkutbe et al.	All	M	25.6 7.5	9.5 3.5	1.1 to 18.3	0.896	0.530	2.39	2.7	27.0	30.5	0.707
	All	F	29.1 7.4	9.4 4.4	2.5 to 16.3	0.889	0.493	2.68	2.5	25.0	26.4	0.660

^aReference (from deuterium dilution) – predicted. Abbreviations: RSD = residual standard deviation; MAPE = median absolute percentage error; TOST = two one-sided t tests.

Table 5 Predictive models for total body water in Kuwaiti children aged 6.5 – 9.6 years

Model	Predictors	Regression coefficient	P value	R ²	RMSE (kg)	Mallow's C(p)	PRESS	Akaike information criterion	VIF
Group 1 (n = 79; 41 F, 38M)									
Model 1	RI +	0.6593	0.0001	0.886	1.082	2.0	95.2	14.5	na
	Intercept	0.4915							
Model 2	Weight +	0.2821	0.0001	0.872	1.148	2.0	110.7	23.8	na
	Intercept	6.2631							
Model 3	RI +	0.3715	0.0001	0.939	0.796	3.0	54.5	33.0	4.2
	Weight +	0.1422							
	Intercept	2.3668							
Model 4	RI +	0.3417	0.0001	0.943	0.777	4.0	52.7	36.0	4.7
	Weight +	0.1521							
	Sex +	0.4110							
	Intercept	2.5269							
Group 2 (n = 79; 42 F, 37M)									
Model 1	RI +	0.6912	0.0001	0.872	1.186	2.0	117.0	28.9	na
	Intercept	-0.3894							
Model 2	Weight +	0.2694	0.0001	0.855	1.261	2.0	128.5	36.1	na
	Intercept	6.4724							
Model 3	RI +	0.3934	0.0001	0.923	0.922	3.0	71.6	9.84	4.1

	Weight +	0.1346	0.0001						4.1
	Intercept	2.0565							
Model 4	RI +	0.3700	0.0001						4.5
	Weight +	0.1433	0.0001						4.4
	Sex +	0.3648	0.0926	0.926	0.909	4.0	71.1	10.8	1.1
	Intercept	2.1431							

All participants (n = 158; 83 F, 75 M)

Model 4	RI +	0.3549	0.0001						4.5
	Weight +	0.1479	0.0001						4.4
	Sex +	0.3873	0.0063	0.934	0.836	4.0	115.3	52.5	1.1
	Intercept	2.3525							

RI = resistance index (height^2/R , cm^2/Ω); Weight (kg); Sex (M= 1, F = 0); Height (cm), X_c (reactance, Ω). R^2 = coefficient of determination. Abbreviations: RMSE = root mean square error; PRESS = predicted residual error sum of squares; VIF = variance inflation factor; na = not applicable to single variable regression.

Table 6 Comparison of predictive impedance-based equations for total body water in Kuwaiti children aged 6.5 – 9.6 years

Prediction	Validation group	Mean \pm SD (kg)	Bias* (kg \pm SD)	Limits of agreement (kg)	Cohen's d effect size for paired data	r_p	r_c	RSD (kg)	MAPE (%)	TOST (%)	Gower index
Split-group cross-validation											
Reference IRMS-TBW (kg)	Group 2	15.8 \pm 3.3									
Prediction model 4 Group 1 (Table 5)	Group 2	15.9 \pm 3.1	-0.04 \pm 0.9	-1.8 to 1.7	0.012	0.962	0.961	0.63	2.5	1.4	0.971
Reference IRMS-TBW (kg)	Group 1	15.8 \pm 3.2									
Prediction model 4 Group 2 (Table 5)	Group 1	15.7 \pm 3.1	0.05 \pm 0.6	-1.4 to 1.5	0.015	0.971	0.971	0.54	2.8	1.6	0.968
Comparison with published predictors (all participants)											
TBWD ₂ O (kg)	All	15.8 \pm 3.2									
Prediction model 4, Table 5 (LOOCV)	All	15.8 \pm 3.1	0.0 \pm 0.8	-1.6 to 1.6	0.00	0.966	0.966	0.58	2.8	0.7	0.967
Moissl et al. ⁽²⁶⁾	All	16.2 \pm 3.0 ^a	-0.38 \pm 1.0	-2.3 to 1.6	0.40	0.952	0.943	0.70	4.3	3.3	0.960

Moon et al. ⁽²⁴⁾ ($K_B = 4.3$)	All	16.6 ± 3.8 a	$-0.79 \pm$ 1.2	-3.0 to 1.4	1.02	0.963	0.926	0.67	5.5	6.6	0.947
Ward et al. ⁽²⁵⁾ $K_B = 4.3$	All	16.1 ± 3.7 a	-0.3 ± 1.0	-2.3 to 1.6	1.30	0.966	0.951	0.64	4.3	8.3	0.932
Ward et al. ^(21,25) $K_B = \text{Personal}$	All	15.2 ± 3.8	0.61 ± 1.1	-1.4 to 2.8	0.14	0.965	0.939	0.65	6.0	1.9	0.947
Clasey et al. ⁽³⁵⁾	All	17.1 ± 3.2 a	$-1.31 \pm$ 0.9	-3.2 to 0.5	1.37	0.956	0.881	0.69	9.0	9.0	0.928
Rush et al. ⁽³⁶⁾	All	18.1 ± 4.0 a	$-2.35 \pm$ 1.2	-4.7 to 0.0	2.68	0.964	0.779	0.67	14.8	14.8	0.897
Jemaa et al. ⁽³⁷⁾	All	17.4 ± 3.8^a	$-1.57 \pm$ 1.2	-4.0 to 0.9	1.57	0.949	0.851	0.79	9.7	9.7	0.918
De Lorenzo et al. ⁽³⁸⁾	All	17.8 ± 3.6 a	$-2.01 \pm$ 1.0	-4.0 to -0.0	2.33	0.964	0.817	0.65	12.8	12.8	0.898
El Harchaoui et al. ⁽³⁹⁾	All	14.9 ± 3.7 a	0.88 ± 1.0	-1.1 to -2.9	1.05	0.964	0.926	0.66	7.0	7.0	0.945
Horlick et al. ⁽⁴⁰⁾	All	16.5 ± 3.5^a	$-0.72 \pm$ 1.0	-2.6 to 1.1	0.81	0.964	0.939	0.64	5.2	5.2	0.952

*Reference-predicted. Abbreviations: r_p = Pearson correlation coefficient; r_c = concordance correlation coefficient; RSD = residual standard deviation MAPE = median absolute percentage error; TOST = two one-sided t tests. ^aP<0.001 (paired t test)