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Body Fat Assessment of Young Syrian Adults Using Bioelectrical Impedance Analysis and Deuterium Dilution

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ABSTRACT

Introduction: The purpose of this study was to compare body composition assessed by bioimpedance analysis (BIA) against deuterium dilution (D_2O) techniques. **Methods:** This study was conducted on 215 young Syrian adults. Total body water (TBW), fat free mass (FFM) and fat mass (FM) compartments were calculated from the measurements of resistance made at 50 kHZ using the formula provided by the instrument manufacturer (Bodystat) and D_2O equation. Bland-Altman plot analysis was used to compare methods, and linear regression analysis were performed to detect the relation between the variables (R^2 = 0.81). **Results:** Percentage of body fat derived from BIA (16.2 ±7.3%) and D_2O technique (21.32±6.42%) were significantly different (p<0.001). BIA body composition assessments provided good relative agreement with the selected criterion method of D_2O as indicated by high correlation coefficients (r=0.91). **Conclusion:** When compared with D_2O, BIA overestimated TBW and FFM, and underestimatd FM (P<0.001) in young adults (-5.2%; -3.4 kg).

Key words: Bioelectrical impedance analysis, body composition, deuterium dilution technique

INTRODUCTION

Bioelectrical impedance analysis (BIA) produces a better estimate of fat mass in a wide range of body compositions (Kamimura *et al*., 2003). BIA measurements are determined by the resistance of the body to electrical current flow between points of contact on the body and correlate well with total body water measurements (Erceg *et al*., 2010). Lean tissues contain large amounts of water and electrolysis and is a good conductor of electrical currents. Fat tissue is anhydrous and a poor conductor; therefore, the larger the fat tissue, the higher the resistance to electrical current and the higher the adiposity (Li *et al*., 2013).

The prediction of total body water (TBW), fat free mass (FFM) and possibly the fat mass (FM) from bioelectrical impedance (BI) has been extensively validated in both adult and paediatric population (Kyle *et al*., 2001). Equations for estimating FFM and FM using this tool, with appropriate cross-validation, have been developed in adults, and such equations tend to be specific to the populations for which they were developed and both age and ethnicity may be important predictors (Kyle *et al*., 2001). The existing published BIA

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equations were developed through linear regression analysis by using independent variables such as height, weight, sex, age and impedance (Kyle *et al.*, 2004; Hsieh *et al.*, 2013).

Previous research suggests that BIA models may not be reliable for estimating body composition in a range of populations because the equations are specific to the group on which they are established (Kyle *et al.*, 2004; Stahn, Terblanche & Strobel, 2007). BIA are used in general as an alternative to isotope dilution techniques, which are difficult in field conditions and require expensive instrumentation for isotope enrichment analysis (Battistini *et al.*, 1992).

To date, a systematic work of the measurement properties of BIA method for estimation of TBW, FFM and FM in the young adults group is lacking in Syria. Therefore, our aim was to examine criterion validity, reliability and measurement error of the BIA method in estimating TBW, FFM and FM in young Syrian adults.

METHODS

Subjects

This observational study consisted of a sample of 215 healthy young adults aged 18 to 19-year-old boys, who will serve in the army after a medical examination in the medical army centre in Damascus city, in Syria. Participants were excluded if they were suffering from acute illness that would have produced abnormalities in body composition. Subjects were asked to abstain completely from consuming food and drink in the 12 hours before visiting the laboratory. All anthropometry measurements and sampling were completed during a single visit to the laboratory. The study protocol was approved by the Scientific Research and Ethical Committee of the Atomic Energy Commission of Syria (AECS). Each participant provided informed consent prior to participation after a detailed explanation of the study protocol. This study was performed in accordance with guidelines prescribed by the Helsinki Declaration of the World Medical Association.

Anthropometric measurements

Body weight in light clothing was measured with an electronic scale (Seca, Model: 767; Germany). The accuracy of the scale was confirmed by using weight of known mass (20 kg). Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Model: 225, Germany). Using non-stretchable tapes, waist circumference was measured over the unclothed abdomen at the narrowest point between the costal margin and iliac crest, and hip circumference was measured over light clothing at the level of the widest diameter around the buttocks. All measurement procedures followed the protocol described in the Anthropometric Standardisation Reference Manual (Lohman, Roache & Marto, 1988).

Bioelectrical impedance analysis

Resistance (R) and reactance (X) were measured at 50 kHz using a bioimpedance analyser (Quadscan 4000, Bodystat Limited, UK). These measurements were carried out with the adult lying supine on the bed, the arms near but not touching the body and the legs abducted. The skin of the right hand and foot was swabbed with alcohol before the electrodes were placed.

Source electrodes were placed on the dorsal surface of the foot over the distal portion of the second metacarpal. Sensing electrodes were placed at the anterior ankle between the tibial and fibular malleoli and at the posterior wrist between the styloid processes of the radius and ulna. The adolescent laid still for at least 5 min before the measurements were made. The average of repeated measurements of R and X agreeing to within 2 ohm of each other was used in subsequent analyses.

During the measurement, the instrument recorded whole body impedance from the hands to the feet by applying an electric alternating current flux of 0.2 mA at an operating frequency of 50 kHz. Finally, percentage of body fat (BF%) was calculated from the whole impedance value and the pre-entered personal data (weight, height, physical activity level, waist and hip circumference) of the corresponding subject. TBW, FFM, FM, or BF% were calculated from the measurements of resistance made at 50 kHz using the formula provided by the instrument manufacturer (BIA-man). This formula included "height^2/impedance (cm^2/ohm)". The equation used in Quadscan 4000 unit is especially suited for the age group of 6 – 17 years. Therefore, the TBW values were taken from the instrument and the FM calculated using the same hydration factor used in the D_2O method.

Body composition by total body water

Hydrometry was considered as the reference method in this study. Total body water (TBW) was determined by the isotope dilution technique using deuterium oxide according to the plateau method (Coward, 1990). TBW was assessed by deuterium dilution measured with mass spectroscopy (Valencia *et al.*, 2003). An accurately weighed dose equivalent to 0.05 g D_2O kg^{-1} body weight (99.8 % atom present excess; Cambridge Isotope (D_2O) laboratories, (Inc, United Kingdom) was given orally to subjects. The dose flask was rinsed with 30 ml of water and this rinsed water was also ingested to ensure completeness. Saliva samples were taken before the administration of the dose to each subject after a 6- to 12-h fast and either 3 or 4 h after the dose was administered. Absorbent salivates (Watterolle Swab Tampon, Sarstedt, Germany) were used to collect the saliva. Saliva samples were analysed by using Isotope Ratio Mass Spectrometer (Isoprime, GV Instruments Limited, UK). The values obtained were expressed relative to secondary standards (low-enrichment and high-enrichment standard water was similarly prepared to normalise data against V-SMOW-SLAP-GISP (Vienna Standard Mean Ocean Water/Standard Light Antarctic Precipitation. Greenland Ice Sheet Preciptation). All samples were prepared and analysed in triplicate. The equation used for the calculation of deuterium dilution space (N) was as follows (Halliday & Miler, 1977):

$$N = (TA/a)*((Ea - Et)/(Es - Ep)).$$

where A is the amount of isotope given in grams, a is the portion of the dose in grams retained for mass spectrometer analysis, T the amount of tap water in which the portion of a is diluted before analysis, and Ea, Et, Ep, and Es are the isotopic enrichments in delta units of the portion of dose, the tap water used, the pre-dose saliva sample, and the post-dose saliva sample, respectively. The deuterium dilution space was assumed to over-estimate TBW by a factor of 1.04 (Forbes, 1987). Fat free mass (FFM) was calculated from TBW, assuming that FFM has a hydration constant of 0.738 (Lohman, 1992). Fat mass was calculated as scale weight minus FFM.

Statistical analysis

All anthropometric data were analysed using statistical package for social science (SPSS Version 17.0.1, 2001, SPSS Inc., Chicago, USA). Descriptive statistics of anthropometric variables of the subjects were computed as mean±standard deviation (SD). Bland and Altman was used to compare the agreement between measured body FM by the reference method (deuterium dilution) and FM estimated from the prediction equations based on BIA (Bland & Altman, 1986). Linear regression analysis was performed to detect the relation between the variables, and the coefficients of determination (R^2) for each regression model. The level of

Table 1. Anthropometric characteristics of young Syrian adults (18 – 19 years) (N= 215)

Variable	*(Mean ±SD)*	*(95%CI)*
Height (cm)	172.0 ± 7.0	(171.2 - 172.8)
Weight (kg)	68.9 ± 12.4	(67.2 - 70.57)
BMI (kg/m2)	23.2 ± 3.8	(22.7 - 23.8)
Hip circumference (cm) (HC)	95.3 ± 7.8	(94.2 - 96.3)
Waist circumference (cm) (WC)	76.4 ± 9.2	(75.2 - 77.6)
Impedance at 50 kHz (Ω)	499.3 ± 52.6	(492.3 - 506.4)

Table 2. Body composition of subjects determined by bioelectrical impedance analysis (BIA) and isotope dilution technique using deuterium oxide (N= 215)

Variable	*(Mean ±SD)*	*(95%CI)*
TBW-BIA (L)	42.1 ± 4.5	(41.5 - 42. 7)
TBW-D2O (L)	39.6 ± 4.8	(38.9 - 40. 2)
P-value	<0.00	<0.00
TBW-BIA (%)	61. 9 ± 5.4	(61.2 - 62.6)
TBW-D2O (%)	58.2 ± 4.8	(57.5 - 58.8)
P-value	<0.00	<0.00
TFM- BIA (kg)	11.9 ± 7.5	(10.9 - 12.9)
TFM- D2O (%)	15.3 ± 7.4	(14.3 - 16.3)
P-value	<0.00	<0.00
TFM- BIA (%)	16.2 ± 7.3	(15.2 - 17.2)
TFM- D2O (kg)	21.3 ± 6.4	(20.5 - 22.2)
P-value	<0.00	<0.00

significance was determined at a P value of <0.05.

The intra-class correlation coefficient (ICC) was used to test the validity of BF%, FM and FFM measured by BIA-man compared to D_2O (Bartko & Capenter, 1976). Values of ICC below 0.4 were considered as an indication of poor reproducibility, values between 0.4 and 0.7, medium-term reproducibility and values above 0.7 as having good reproducibility. The ICC of BIA *vs* D_2O for TBW(%), TBW(L), FFM(%), FFM(kg), BF(%) and BF(kg) were 0.937, 0.975, 0.943, 0.975, 0.943 and 0.982, respectively.

RESULTS

A total of 215 young adults were investigated in this study. Table 1 shows the (Mean ± SD), max and min values of general characteristics of the young adults included in this study. The average of height, weight, BMI, hip circumference waist circumference, and impedance at 50 kHz of the young adults were 172.0 cm, 68.9 kg, 23.2 kg/m^2, 95.3 cm, 76.4 cm and 499.3 ohm, respectively.

Body composition

Table 2 presents the TBW, FFM and FM data (means ± SD), max and min values

Table 3. Comparison of BIA method of body composition with DD

Comparison	*Regression analysis*		*Bland and Altman*		*SEE*
	R2	*p-Value*	*Mean bias ± SD of bias*	*95% limits of agreement*	
BIA-man vs. DD					
Total body water (%)	0.790	< 0.00	3.7 ± 2.5	-1.13 to 8.60	2.41
Total body water (L)	0.911	< 0.00	2.5 ± 1.4	-0.34 to 5.32	1.34
Free fat mass (%)	0.808	< 0.00	5.1 ± 3.2	-1.13 to 11.42	3.21
Free fat mass (kg)	0.911	< 0.00	3.4 ± 1.95	-0.46 to 1.21	1.82
Body fat (%)	0.797	< 0.00	- 5.2 ± 3.2	-11.43 to 1.13	3.38
Fat mass (kg)	0.932	< 0.00	- 3.4 ± 1.95	-7.22 to 0.45	1.96

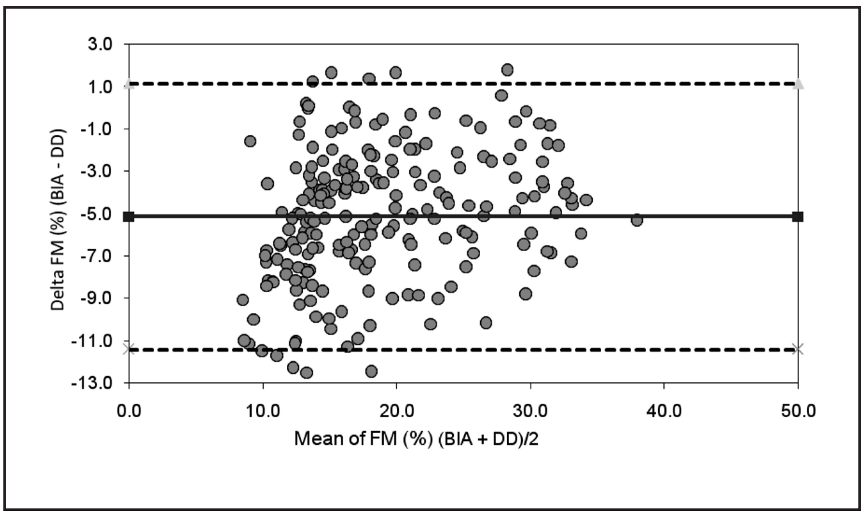

Figure 1. The difference (bias) in the predicted FM (%) measured by BIA and FM (%) measured by DD

as measured by bioelectrical impedance analysis (BIA-man) and deuterium dilution (D_2O) techniques. When compared with D_2O, BIA overestimated TBW and FFM, and underestimated FM (P<0.001) in young adults. Significant difference (*p*<0.001) was found between the methods: TBW was 61.9 ± 5.4% *vs* 58.2 ± 4.8%, and the %BF was 16.2 ± 7.3% *vs* 21.3 ± 6.4%, for BIA and D_2O equations, respectively. Thus, the equation of BIA underestimated, the %BF in young Syrian adults (-5.2%; -3.4 kg), when compared to the D_2O equation. As shown in Table 3, the standard error of the estimate (SEE) results were indicative of good reproducibility between BIA-man and D_2O for TBW, and FM (R^2 = 0.80 and SEE = 3.38).

Agreement between BIA and D_2O **equations**

The Bland-Altman plot of difference between the FM measured by BIA versus the FM from D_2O is illustrated in Figure 1. Bland and Altman analysis showed that the bias expressed as the mean of

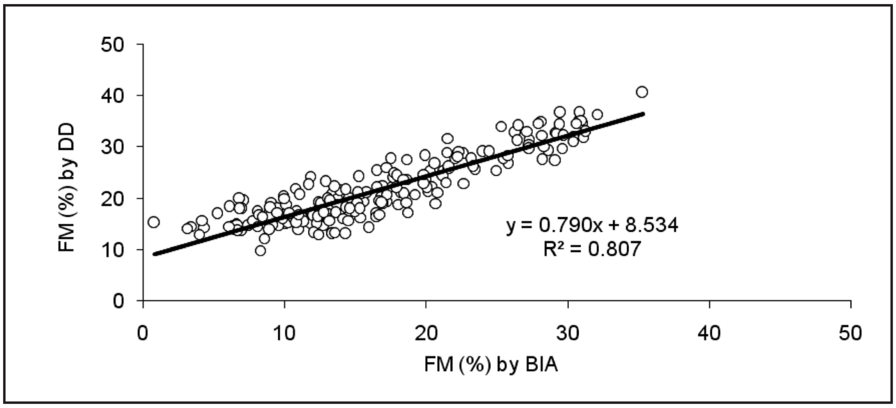

Figure 2. Line regression between FM% by DD and FM% by BIA for Syrian adolescents (18-19 years)

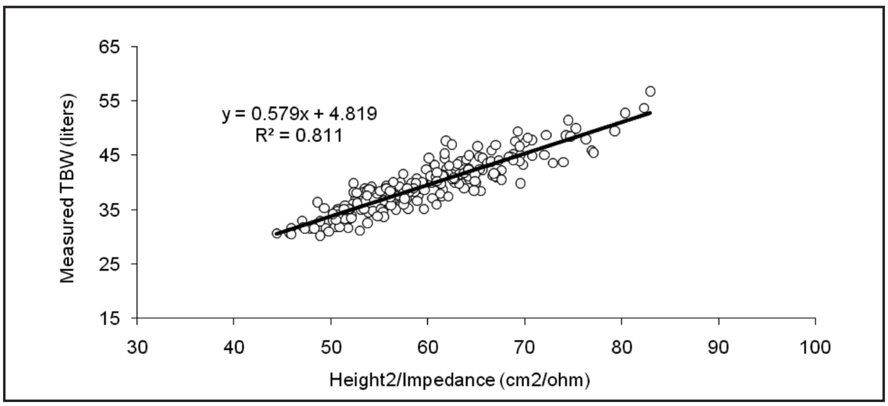

Figure 3. Linear regression between TBW (L) by DD and height ^{2}/impedance (cm^2/ohm) for Syrian adolescents (18-19 years)

difference in FM measured by BIA *vs* FM measured by D_2O is -5.15 ± 3.20 %. The limits of agreement for estimated FM BIA *vs* FM by D_2O at 2 SD was ± 6.28 %. The Bland-Altman plot revealed the mean difference to be small and the limits of agreement narrow because 95% of the determined data was within the 2 SD (Figure 1).

There was significant inter-method agreement between the bioelectric impedance measurement (BIA-man) and deuterium oxide dilution (D_2O) (p<0.001).

Linearity of the BIA-BF% relationship

Figure 2 illustrates the simple linear regression analysis performed on the total sample with FM determined by D_2O as a dependent variable (y-axis) and FM determined by BIA as the independent variable (x-axis). Visual inspection of the scatter plot also shows a positive relationship between the BF% determined by D_2O and BIA ($R^2 = 0.81$; $p< 0.001$) (Figure 2). Also, a positive relationship between the TBW (L) determined by D_2O and height^2/impedance (cm^2/ohm) ($R^2 = 0.81$; $p< 0.001$) is shown (Figure 3). This regression is linear. The regression equations for predicting from BIA are as follows:

Body fat (%) = 0.7907 X BF% determined by BIA + 8.5345 (s.e.e. 2.82%, $R^2 = 0.8077$).

Total Body Water (L) = 0.5792 X Height^2/ Imp (cm^2/ohm) + 4.8197 (s.e.e. 2.10%, R^2 = 0.8114).

DISCUSSION

The BIA is a non-invasive safe method which is portable, and widely used to estimate body composition (Camarneiro *et al.,* 2013). BIA is used for estimating TBW, FM and FFM on the basis of mathematical formulae using measurements of resistance, reactance, and impedance (Kyle *et al*., 2001).

This is the first study examining the relationship between BIA and body composition in a nationally representative sample of a healthy young adult group in Syria. Therefore, the present study provides the most recent body composition estimates by BIA of young healthy Syrian adults. A primary finding of this investigation was that the BIA significantly underestimated mean %BF (mean deference; -5.15%) while it overestimated mean TBW (mean deference; +3.74%) or FFM (mean deference; +5.14%) when compared to D_2O. Similar results were reported by Martinoli *et al*. (2003) who concluded that the TBW estimates obtained with BIA represented significant over-estimates with respect to the reference values obtained using D_2O dilution. A review of previous BIA studies with different cohorts found considerable variability in inter-method agreement (Jack, Gray & Eric, 2011; Erceg *et al*., 2010; Kyle *et al*., 2004). BIA overestimated BF by more than 3% in men when BF was higher than 15% (Sun *et al.*, 2005). Kyle, *et al*. (2004) suggest that BIA works well in healthy subjects with stable water and electrolytes balance with a BIA equation appropriate to age, sex and race. On the other hand, Dehghan & Merchant (2008) have cautioned against the use of BIA for large epidemiological populations where race, ethnic group, and conditions vary.

As in the present study, D_2O dilution technique has often been used as a reference method to assess body composition because the D_2O is a capable non-invasive method to measure body composition based on body water content (Mohrmann *et al*., 2006). D_2O as a reference method can be a good option in developing regions that do not have access to the gold standard methodology such as the four-compartment models (Valencia *et al*., 2003). The average body fat found in our study through the method of dilution oxide was 21.32% for Syrian adolescents. Camarneiro *et al*. (2013) found similar results for children and healthy 11-14 year- old adolescents with 22.75% body fat for boys.

The main finding of this study is that in a group of healthy Syrian adolescents, BIA body composition assessments provided a good relative agreement with the selected criterion method, D_2O, as indicated by high correlation coefficients (R^2 = 0.91). Bland-Altman plots revealed no systematic error in the estimation of percentage body fat with the BIA method from the D_2O. Current research indicates that there is a consensus on the usability and accuracy of BIA devices in Syrian adolescents. For the BIA method examined in the present study using the equation of BIA-man, percent correlation with D_2O for BF, FFM, and TBW was generally good.

Our results support previous studies which indicate that segmental BIA measures correlate well with a criterion method but under or overestimate the individual %BF measures (Chouinard *et al*., 2007; LaForgia, Gunn & Withers, 2008). Other studies suggest that segmental BIA provides a relatively accurate estimate of percentage body fat in specific populations such as high school aged children (Kriemler *et al.,* 2009). Sixteen different BIA devices were applied in the literature; for the majority of BIA devices, adhesive electrodes were used in a tetrapolar position (electrodes placed on hand, foot, wrist and ankle). Other BIA devices used pressure contacts in tetrapolar (i.e. foot-to-foot and hand-to-hand devices) and octopolar positions (hand-to-foot devices) (Talma *et al.,* 2013).

The validity of BIA is not without criticism. According to Pimentel *et al.* (2010), these differences may be associated with error in several instances: when the device measures impedance,when choosing the equation to be used, the lack of specificity of the equation used for the sample or to a combination of one or more of these reasons. In general, the typical prediction error of the traditional BIA method has been reported to range from 3.0 - 4.0% (Heyward & Wagner 2004).

Studies on the validation of equations have been developed in several bipolar and tetrapolar BIA devices. The disagreement between the studies may be due to the variance in the equations and equipment used, the adoption of different protocols, different ethnic groups and body composition.

Good results were obtained from a total of 24 studies for reliability for %BF, FFM and FM: ICCs were ≥ 0.96 and correlations were ≥ 0.82, except for one study (r = 0.27 for boys and r = 0.38 for girls (Lubans *et al.*, 2011; Talma *et al.*, 2013). They, therefore, concluded that there is strong evidence for good reliability of BIA. In agreement with those studies, our results are indicative of good reproducibility between BIA man and D_2O for TBW, FFM and FM (r = 0.94)
In the present study, an equation relating FM (derived from FFM) has been developed, based on data from deuterium dilution measurements of total body water, to weight, height and BIA resistance that is applicable to Syrian adolescents in the 18-19 years age range. Height and BIA resistance were entered into the equation as impedance index, height squared/ resistance. Equations were developed in this study using FFM as the criterion variable rather than FM because of the functional relation between bioelectrical impedance and the hydrated lean tissue of the body. These equations can be used to calculate total body fat and body fat as a percentage of body weight for each individual.

More longitudinal studies and on the BIA devices is needed before recommendations for their use as a clinical tool can be made. Future research should assess the validity of bioelectrical impedance analysis among more culturally diverse samples, as well as older adults and obese individuals in Syria.

CONCLUSIONS

Our results indicate that the BIA-Bodystat cannot accurately assess %BF, FM, TBW or FFM in young Syrian adults. Therefore, it needs validation prior to use on young Syrian adults. Our data confirm that body impedance could be considered a valid alternative to deuterium oxide dilution for assessment of TBW in young adult populations.

Conflict of interest
The authors declare that there are no conflicts of interest.

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