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ORIGINAL ARTICLE

What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population

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BACKGROUND/OBJECTIVES: The validity of bioelectrical impedance analysis (BIA) for body composition analysis is limited by assumptions relating to body shape. Improvement in BIA technology could overcome these limitations and reduce the population specificity of the BIA algorithm.
SUBJECTS/METHODS: BIA equations for the prediction of fat-free mass (FFM), total body water (TBW) and extracellular water (ECW) were generated from data obtained on 124 Caucasians (body mass index 18.5–35 kg/m^2) using a four-compartment model and dilution techniques as references. The algorithms were validated in an independent multiethnic population ($n = 130$). The validity of BIA results was compared (i) between ethnic groups and (ii) with results from the four-compartment model and two-compartment methods (air-displacement plethysmography, dual-energy X-ray absorptiometry and deuterium dilution).
RESULTS: Indices were developed from segmental *R* and Xc values to represent the relative contribution of trunk and limbs to total body conductivity. The coefficient of determination for all prediction equations was high (R^2: 0.94 for ECW, 0.98 for FFM and 0.98 for TBW) and root mean square error was low (1.9 kg for FFM, 0.8 l for ECW and 1.3 kg for TBW). The bias between BIA results and different reference methods was not statistically different between Afro-American, Hispanic, Asian or Caucasian populations and showed a similar difference (− 0.2–0.2 kg FFM) when compared with the bias between different two-compartment reference methods (− 0.2–0.3 kg FFM).
CONCLUSIONS: An eight-electrode, segmental multifrequency BIA is a valid tool to estimate body composition in healthy euvolemic adults compared with the validity and precision of other two-compartment reference methods. Population specificity is of minor importance when compared with discrepancies between different reference methods.

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Keywords: body composition analysis; bioelectrical impedance; body shape; ethnicity; reference methods; four-compartment model

INTRODUCTION

In recent years, detailed body composition analyses advanced beyond clinical application and now have a specific use in metabolic research and have become an important tool in large-scale studies. It is impractical to obtain complex and expensive information on genetics, metabolomics and lifestyle factors in order to explain a human phenotype that is poorly characterised by body mass index (BMI) or waist circumference.[1,2] There is a high degree of biological diversity in body composition (for example, normal fat mass (FM) or increased adiposity, gynoid or visceral obesity, sarcopenic or athletic nutritional status and normal hydration or excess body fluid) that is inaccurately reflected by simple anthropometric measures. Accordingly, a valid body composition measure is not easy to obtain in epidemiological studies, because the measurement needs to be cost-effective, non-invasive, highly reproducible, most convenient and easy to use.

Bioelectrical impedance analysis (BIA) has been commercially available since the mid-1980s[3] and became an attractive alternative to the more cumbersome conventional reference equipment such as densitometry or dual-energy X-ray absorptiometry (DXA) (see Baracos *et al.*[4] for a recent state-of-the-art position paper on methods of body composition analysis). However, calculating body composition from the impedance to the flow of an electric current through total body fluid requires a number of assumptions (for example, related to body shape and the distribution of current density) that may violate the validity of BIA results. Because of the statistical relationship between impedance and total body water (TBW) or fat-free mass (FFM), many different equations for BIA calibration exist and all of these equations are clearly population specific. The latter implies that the use of a BIA instrument will not necessarily produce a valid result if the equation is not appropriately chosen on the basis of age, gender, level of physical activity, level of body fat and ethnicity.[5] To a large extent, the population specificity of a BIA equation is likely explained by differences in body shape (that is, the length and volume of arms and legs relative to the trunk). However, different levels of agreement between different BIA models and reference methods do not only depend on population characteristics but also on the reference method chosen to generate the equation.

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Recent advances in the technology of multifrequency BIA facilitated the development of new impedance devices that are innovative in design (for example, shape and arrangement of electrodes) and technology (high accuracy of electrical reactance measurement). This new generation of BIA devices claims to provide a very high precision and accuracy because of (i) segmental measurement of arms, legs and the trunk that could reduce the assumptions about body shape,[6] (ii) accurate measurement of both resistance (*R*) and reactance (Xc) at a spectrum of frequencies from 1 to 1000 kHz and (iii) a high precision of posture and contact to electrodes.

The aim of this study was to investigate whether the segmental measurement with the newly developed seca medical Body Composition Analyzer 515/514 (seca gmbh & co. kg, Hamburg, Germany) improves the accuracy of BIA results and reduces the assumptions on body shape that contribute to the population specificity of a BIA algorithm. In a first step, we obtained BIA equations for the prediction of FFM, TBW and extracellular water (ECW) using a four-compartment model, DXA, densitometry and dilution techniques as reference. To improve the algorithm, we developed a new segmental index for the relative contribution of arms, legs and trunk to total body impedance and investigated whether this index relates to body shape and improves the accuracy of the prediction model. In a second step, validation of the equations was performed in an independent multiethnic population.

SUBJECTS AND METHODS

Phase 1 of the study was designed to develop BIA equations for prediction of FFM, ECW and TBW based on a four-compartment model, sodium bromide (NaBr) and deuterium dilution (D_2O) as reference methods. A total of 124 Caucasian men and women (BMI 18.5–35 kg/m^2) aged 18–65 years were recruited by notice board posting and writing to people who participated in former studies at the Institute of Human Nutrition and Food Science in Kiel, Germany. Phase 2 of the study was to validate the developed equations in an independent multiethnic sample of 130 men and women (BMI 19.8–33.7 kg/m^2) aged 18–65 years (32 Caucasians, 36 Asians, 31 Afro-Americans and 31 Hispanics) recruited at the New York Obesity Nutrition Research Center, USA. The following exclusion criteria were applied: acute and chronic diseases (especially hypertension, renal and cardiac insufficiency), regular intake of medications (except for contraceptives), amputation of limbs, electrical implants as cardiac pacemaker, metallic implants (except tooth implants), pregnancy or breastfeeding period, current alcohol abuse and extensive tattoos on the arms or legs. Oedema of ankles was excluded by inspection (and manual compression if appropriate). The study was approved by the medical ethics committee of the Christian-Albrechts-Universität zu Liel, Kiel, Germany, and St Luke's-Roosevelt Hospital, New York, NY, USA. All subjects provided their fully informed and written consent before participation. Blood pressure measurements were obtained while the subjects were in a seated position, using a standard manual sphygmomanometer.

The subjects were asked to report to the study centres between 0700 and 0730 hours and 10 h after the last food and liquid intake. After taking a blood sample of 10 ml whole blood, D_2O and NaBr were orally administered (in Kiel: 400 mg D_2O per kg body weight and 50 mg NaBr per kg body weight; in New York: 1.5 g NaBr and 11 g D_2O per person). Body composition was then measured using air-displacement plethysmography (ADP), DXA and BIA. In addition, anthropometric data were recorded. Four hours after the baseline blood sampling and ingestion of D_2O and NaBr tracers, a second blood sample of 10 ml of whole blood was collected.

Anthropometrics

Body height and weight were obtained on the measuring station seca 285 (combination of a scale and a stadiometer) to the nearest g and 0.5 cm with an accuracy of ± 50 g up to 100 kg for the scale and ± 2 mm for the stadiometer. The length of the right arm was measured from acromion scapulae to articulatio radioulnaris distalis and the length of the right leg from crista iliaca to malleolus lateralis by means of a non-stretchable measurement tape (circumference measuring tape seca 201). Circumferences of the upper arm, hip and waist were measured. Hip circumference was measured at the level of the symphysis and waist circumference was determined midway between the lowest rib and the uppermost border of the iliac crest at the end of normal expiration.

Bioelectrical impedance analysis

The seca medical Body Composition Analyzer 515/514 consists of a platform with an integrated scale, a handrail system and a display and operation unit. The device uses four pairs of electrodes that are positioned at each hand and foot, with one electrode in each pair through which the electrical current enters the limb and the other electrode detects the voltage drop. The eight-electrode technique enables segmental impedance measurement of the right arm, the left arm, the trunk, the right leg, the left leg and the right and the left body side. Impedance is measured with a current of 100 μA at frequencies of 1, 1.5, 2, 3, 5, 7.5, 10, 15, 20, 30, 50, 75, 100, 150, 200, 300, 500, 750 and 1000 kHz. The feet are placed on top of the electrodes so that the heel touches the smaller posterior and the forefoot touches the bigger anterior electrode. Each side of the ascending handrail carries six electrodes, of which two were chosen depending on the person's height. According to the manufacturer's instructions, the angle between the arms and the body should be 30 degrees. To get the right choice of grip position, the subject has to stand upright with outstretched arms. The hands touch the electrodes so that the electrode separator is positioned between the middle and ring finger. The electronics automatically starts a measurement when the person contacts all electrodes correctly.

Participants were asked not to exercise within 12 h and drink alcohol within 24 h before the impedance measurement. Before measurement, subjects were standing for at least 10 min. The duration of measurement was 75 s. Resistance (*R*) and reactance (Xc) values were recorded to the nearest 0.1 Ω. BIA values obtained at the 5- and 50-kHz frequencies were used for the generation of the prediction equations. As a measure of the volume of the conductor (that is, the body), the impedance index was calculated by scaling for height squared (height^2/*R*). All measurements in each study centre (Kiel and New York) were recorded by the same investigator.

The reproducibility of measurement was obtained from two replicate measurements of 15 participants (11 women and 4 men, age 25–64 years, BMI 22.0 ± 2.3 kg/m^2). The differences among duplicates were calculated as absolute values. The technical error of the measurement ($\sqrt{}\Sigma$(intra-observer difference)^2/2 × number of duplicates) was 0.221 kg FM, and the percentage of reliability (technical error × 100/overall mean of the measurements) was 1.276%.

Four-compartment model

FM was calculated using a four-compartment model that includes body volume (by ADP), TBW (by D_2O) and bone mineral content (BMC by DXA) using the equation of:[7]

$$\mathrm{FM\,(kg)} = 2.7474{*}\mathrm{body\ volume(l)} - 0.7145{*}\mathrm{TBW(l)} + 1.4599{*}\mathrm{BMC\,(kg)} - 2.0503{*}\mathrm{weight\,(kg)}$$

A detailed description of each method and its precision has previously been reported.[8] FFM was calculated as the difference between body mass and FM.

ADP was performed using the BOD-POD device (Life Measurement Instruments, Concord, CA, USA). Before each measurement, a two-step calibration was carried out. Two repeated body volume measurements were performed, averaged and corrected for predicted body surface area and measured thoracic gas volume using the BOD POD software (version 1.69; Life Measurement Instruments). The coefficient of variation for repeated body volume measurements was 0.2%. FM was calculated from body density using Siri's equation.[9] FFM_{ADP} was calculated as weight—FM_{ADP}.

A whole-body DXA scan was performed to measure BMC and FM using a Hologic Discovery A densitometer and the whole-body-software 12.6.1:3 (Hologic, Inc., Bedford, MA, USA) in the Kiel study centre and an iDXA (GE Lunar, Madison, WI, USA) software version 11.4 in New York. FFM_{DXA} was calculated as weight − FM_{DXA}.

D_2O was used to estimate TBW. After obtaining 10 ml of venous blood samples, each participant received an oral dose of 0.4 g of deuterium oxide (D_2O, 99.8%; Carl Roth GmbH, Karlsthal, Germany) per kg body weight with an amount of 100 ml of tap water. Four hours later, a second blood sample was taken. $^2H/^1H$ enrichment of the serum samples was measured by isotope ratio mass spectrometry. Plasma samples were analysed for their ^2H_2O content using an isotope ratio mass spectrometer (Element analysator EA3000, Eurovector, Milan, Italy; CF-IRMS Isoprime Elementar Analysensysteme, Hanau, Germany; Agroisolab GmbH, Jülich, Germany).

The following equation was used to calculate TBW:

$$\text{TBW (kg)} = ((\text{dose} \times 99.9)/20 \times (18.02/\text{atom percent excess}) \times 10^{-3})/1.04$$

where dose is the dose of ^2H_2O in g, 99.9 is the AP of ^2H_2O, 20 is the molecular weight of ^2H_2O, 18.02 is the molecular weight of unlabeled water, atom percent excess is $AP_{plateau} - AP_{baseline}$ and 1.04 is the correction for hydrogen dilution space.

Intraindividual coefficient of variation for plasma deuterium AP was $0.18 \pm 0.09\%$. FFM_{D_2O} was calculated as TBW/0.732, where 0.732 resembles the hydration constant of FFM.[10]

Extracellular water

An oral dose of NaBr providing 50 mg of bromide per kilogram of body weight was administered simultaneously with deuterium-enriched water. Bromide was quantified in plasma samples using a non-destructive liquid X-ray fluorescence technique, with reproducibility of $\pm 0.8\%$. Corrected bromide space was used as the proxy for ECW and calculated using the following formula: corrected bromide space = Br dose/Br elevation in plasma $\times 0.90 \times 0.95 \times 0.94$, where 0.90 is the correction factor for non-extracellular distribution, 0.95 is the Donnan equilibrium factor and 0.94 is the correction for water content in plasma.[11]

Statistics

Data analyses were performed with the SPSS software, version 15.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics are presented as means ± s.d. All data were normally distributed. Differences between independent samples (for example, men and women) were analysed using unpaired *t*-test. Differences between parameters of body composition assessed by different methods were tested using paired *t*-test. Repeated measures analysis of variance was used to compare biases between different methods. Comparisons between ethnic groups were performed by analysis of variance with Bonferroni *post-hoc* test. Pearson's correlation coefficient was calculated for relationships between variables. A *P*-value <0.05 was considered significant.

Development of BIA algorithms. Regression equations for prediction of FFM (measured by the four-compartment model), TBW and ECW (measured by deuterium and NaBr dilution) from impedance were derived in the study population from Kiel. Stepwise multiple regression analysis was used to determine the optimal combination of prediction parameters to fit the models. Coefficient of determination (R^2, proportion of the total variance in the dependent variable that is explained by the independent variables) and the root mean square error (RMSE) were calculated, and predictor variables were included in the model only if their addition resulted in a significant increase in the explained variance of the dependent variable and a substantial change ($>10\%$) in β-coefficients of independent variables. The impedance index at 50 kHz (or 5 kHz in case of ECW) was used as the primary independent variable, and then secondary variables were tested as additional predictors (Xc at 50 kHz (or 5 kHz), weight, age, sex and the newly developed indices for segmental impedance). Optimal combination of predictor variables was selected by consideration of the correlation between predicted and observed data (which should be maximised) and by the RMSE statistic. The RMSE statistic is defined as the square root of the sum of the squared deviations between prediction and observation, divided by the number of observations minus the number of parameters used for prediction, which should be minimised.[12]

$$\text{RMSE} = \sqrt{\frac{\sum(\text{Predicted value} - \text{Observed value})^2}{(\text{Number of observations} - \text{Number of parameters} - 1)}}$$

Validation of BIA equations in an independent sample. To determine the relative contribution of (i) population specificity and (ii) the reference method on the validity of the BIA result, we tested the new algorithms in an independent multiethnic population and compared (i) the validity of BIA results between different ethnic groups and (ii) the results from BIA versus the results from different criterion methods (ADP, DXA, D_2O and the four-compartment model). Linear regression analysis was used to determine the relative agreement between FFM predicted from impedance measurement and FFM_{4C} or FFM_{ADP}, FFM_{DXA} and FFM_{D_2O} as a reference. Similarly, TBW and ECW predicted by BIA were compared with respective results from dilution methods. Analysis according to Bland and Altman was used to determine absolute agreement between the body composition assessed by criterion methods (four-compartment model, densitometry, DXA or dilution method) and BIA. According to this approach, the bias is the difference between measured and predicted values of FFM, TBW or ECW, and the error is the s.d. of the bias.[13] The dependency of the bias on the mean of measured and predicted values was tested using correlation analysis. The limits of agreement, calculated as bias ± 2 s.d. error (that is, 95% confidence interval of the individual difference), were used to test agreement between the two methods (measured and predicted FFM values).

The pure error (accuracy) statistic was used for cross-validation of BIA results (that is, testing the predictive power of the BIA equation for data not used in the equation's development). The pure error was calculated as the root mean square of the differences between predicted and measured data (the smaller the pure error, the greater the accuracy of the tested equation).

$$\text{Pure error} = \sqrt{\frac{\sum(\text{Predicted value} - \text{Observed value})^2}{\text{Number of observations}}}$$

Results for pure error are compared between different ethnic groups and between different methods used as a reference (ADP, DXA and D_2O). The pure error should be similar to the RMSE (precision) obtained using the data from which the predictive equation was derived (see above).

RESULTS

Basic characteristics and results from different methods of body composition analysis for the Caucasian study population (Kiel study centre only) are given in Table 1 stratified by gender. Age and BMI ranges of participants were 18–65 years and 20.0–34.7 kg/m^2. In the data set from Kiel study centre, invalid data occurred three times for the measurement of ECW by NaBr dilution, four times for the measurement of TBW by D_2O and once for the measurement of total body volume by ADP. This led to invalid results of the four-compartment model in five cases. Invalidity of data was judged by checking the plausibility of data (implausible results were identified by between-method comparisons and in the case of ECW from ECW/ICW ratio >1 or <0.6). Invalid data were substituted as follows: ECW was calculated from the relationship between ECW/ICW and % FM (ECW/ICW = 0.0046 × % FM according to DXA + 0.642; $r = 0.37$, $P < 0.01$) and TBW

Table 1. Descriptive characteristics of the Caucasian study population in phase 1 (Kiel) and results for FFM derived from different methods of body composition analysis (MW ± s.d.)

	Females (n = 62)	*Males (n = 62)*	*All (n = 124)*
Age, years	40.6 ± 12.7	40.2 ± 11.7	40.4 ± 12.2
Weight, kg	67.8 ± 13.1	83.6 ± 11.4	75.7 ± 14.6
Height, cm	167 ± 7	179 ± 6	173 ± 9
BMI, kg/m^2	24.1 ± 3.7	25.9 ± 3.3	25.0 ± 3.6
Waist circumference, cm	83.0 ± 11.3	92.0 ± 10.4	87.5 ± 11.7
Arm circumference, cm	29.1 ± 3.1	32.1 ± 3.1	30.6 ± 3.4
Arm length, cm	55.1 ± 3.0	59.7 ± 3.1	57.4 ± 3.8
Leg length, cm	90.5 ± 7.0	95.0 ± 5.3	92.8 ± 6.6
FFM_{ADP}, kg	44.6 ± 5.4	64.1 ± 7.4	54.3 ± 11.7
FFM_{DXA}, kg	45.5 ± 6.4	64.8 ± 7.4	55.1 ± 11.9
FFM_{D_2O}, kg	45.9 ± 6.0	65.0 ± 7.4	55.6 ± 11.7
FFM_{4C}, kg	45.4 ± 5.8	65.2 ± 7.4	55.4 ± 12.0
FFM_{BIA}, kg	45.4 ± 6.0	65.4 ± 6.6	55.4 ± 11.8

Abbreviations: ADP, air-displacement plethysmography; BIA, bioelectrical impedance analysis; BMI, body mass index; D_2O, deuterium dilution; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; MW, molecular weight; 4C, four-compartment model.

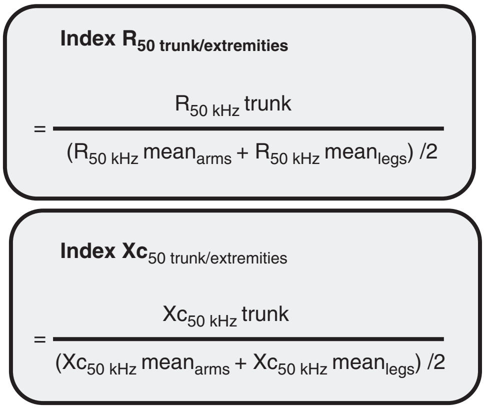

Figure 1. Two different indices were developed from measured *R* and Xc values (mean of left and right body side) to represent the relative contribution of trunk and extremities to total body conductivity.

(TBW = ECW + ICW; ECW/ICW = ECW/(TBW − ECW)). TBW was calculated as $((FFM_{ADP} + FFM_{DXA})/2) \times 0.732$ (assuming 73.2% water content of FFM). FFM_{4C} was substituted as the mean of FFM from the remaining two valid methods (ADP, DXA or D_2O). In a first step, the analyses were carried out excluding invalid data (case wise). To confirm the results, analyses were subsequently repeated with invalid data substituted as described above.

Indices for body shape from segmental BIA

Two different indices, Index $R_{50\ \text{trunk/extremities}}$ and Index $Xc_{50\ \text{trunk/extremities}}$, were developed from segmental *R* and Xc values (means of left and right body side) to represent the relative contribution of the trunk and extremities to total body conductivity (Figure 1). The new indices correlated with trunk length and waist and arm circumference (index from segmental *R*-values: $r = 0.43$, $P < 0.001$; $r = 0.25$, $P < 0.01$ and $r = 0.25$, $P < 0.01$; index from segmental Xc-values: $r = 0.46$, $P < 0.001$; $r = 0.19$, $P < 0.05$ and $r = 0.40$, $P < 0.001$). The index from segmental *R*-values correlated with the ratio of trunk length to mean extremity length ((length of arm + leg)/2) ($r = 0.35$, $P < 0.001$), whereas the index from segmental Xc-values correlated with arm and leg length ($r = 0.22$, $P < 0.05$ and $r = 0.38$, $P < 0.001$, respectively). In all, 40% of the variance in Index $R_{50\ \text{trunk/extremities}}$ was explained by gender and the ratio of trunk length to mean extremity length; 63% of the variance in Index $Xc_{50\ \text{trunk/extremities}}$ was explained by gender and arm length. Other variables (age, leg length, arm or hip circumference) were not independent predictors of either indices.

Development of BIA prediction equations for FFM, TBW and ECW

Results of the stepwise regression analyses are given in Table 2. The β-coefficients for the prediction equations are proprietary to the system manufacturer seca. In all, 98% of the variance in FFM_{4C} was explained by the predictors Ht^2/R_{50}, Xc_{50}, Index $R_{50\ \text{trunk/extremities}}$, weight,gender and age; 94% of the variance in ECW_{NaBr} was explained by Ht^2/R_{50}, weight and Index $R_{50\ \text{trunk/extremities}}$; and 98% of the variance in TBW_{D_2O} was explained by the predictors Ht^2/R_{50}, Xc_{50}, weight, Index $R_{50\ \text{trunk/extremities}}$, Index $Xc_{50\ \text{trunk/extremities}}$, age and gender.

The s.e. of the estimates (= RMSE) were 1.91 kg FFM, 0.79 l ECW and 1.34 kg TBW. Agreement between measured and predicted values was analysed by regression and Bland–Altman analyses and are shown in Figures 2–4. Correlations between values measured by gold-standard methods and the BIA models were high. The observed limits of agreement (s.d. of the difference between reference method and BIA result multiplied by 2) were low, and no systematic error was found. Bias and limits of agreement between BIA and the four-compartment model were narrow (-0.05 ± 3.71 kg FFM, Figure 2) and in the same range when compared with biases and limits of agreement among reference methods (FFM_{ADP-D_2O}: 1.10 ± 4.71 kg; $FFM_{ADP-DXA}$: -0.70 ± 5.23 kg and FFM_{D_2O-DXA}: 0.36 ± 3.57 kg).

Table 2. Results of three stepwise regression analyses in phase 1 (Kiel) with FFM_{4C} (kg), TBW_{D_2O} (l) and ECW_{NaBr} (l) as the dependent variables

Predictors of FFM_{4C} (kg)	R^2	*P-value*	*RMSE, kg*
Ht^2/R_{50} (Ω)	0.93	<0.001	3.24
Xc_{50} (Ω)	0.95	<0.001	2.64
Index $R_{50\ \text{trunk/extremities}}$ (Ω)	0.96	<0.001	2.39
Weight, kg	0.97	<0.001	2.16
Gender	0.97	<0.001	2.05
Age, year	0.98	<0.001	1.91
Intercept		0.092	
Predictors of ECW_{NaBr} (l)			
Ht^2/R_{50} (Ω)	0.89	<0.001	1.07
Weight, kg	0.92	<0.001	0.89
Index $R_{50\ \text{trunk/extremities}}$ (Ω)	0.94	<0.001	0.79
Intercept		<0.001	
Predictors of TBW_{D_2O} (kg)			
Ht^2/R_{50}(Ω)	0.93	<0.001	2.26
Xc_{50} (Ω)	0.95	<0.001	1.92
Weight, kg	0.96	<0.001	1.65
Index $R_{50\ \text{trunk/extremities}}$ (Ω)	0.97	<0.001	1.44
Index $Xc_{50\ \text{trunk/extremities}}$ (Ω)	0.97	0.046	1.40
Age, year	0.98	0.002	1.36
Gender	0.98	0.027	1.34
Intercept		0.488	

Abbreviations: ECW, extracellular water; FFM, fat-free mass; RMSE, root mean square error; TBW, total body water.

Validation of BIA equations in an independent sample

The study population for phase 2 (New York study centre only) is characterised in Table 3 stratified by ethnic group and gender. Age and BMI ranges were 19–65 years and 18.7–34.4 kg/m^2 for Caucasians, 21–64 years and 20.2–33.7 kg/m^2 for Afro-Americans, 19–64 years and 18.9–31.7 for Asians, and 18–64 years and 20.5–33.3 kg/m^2 for Hispanics, respectively. In the total data set, invalid data occurred two times for the measurement of ECW by NaBr dilution, six times for the measurement of TBW by D_2O and six times for the measurement of total body volume by ADP. This led to invalid four-compartment model results in 12 cases. These data were substituted as described above.

Impact of ethnicity on the validity of BIA equations

Comparison of the validity of the results from BIA equations between different ethnic groups is shown in Table 4. The mean bias for prediction of FFM, ECW and TBW was low in all ethnic groups and did not significantly differ between Caucasians, Asians, Afro-Americans and Hispanics, respectively. Significant underestimation of FFM by BIA was observed in Asians and Afro-Americans, whereas significant overestimation of ECW by BIA occurred in Caucasians and Asians, respectively. Pure error of the prediction did not exceed RMSE of the equations (see Table 2) in Asians and Hispanics but was slightly higher in Caucasians and Afro-Americans (for prediction of ECW, FFM and TBW). Bias for the prediction of FFM, TBW and ECW (reference method—BIA result) did not differ between ethnicities (all $P > 0.05$).

In the total study population, the bias between BIA results and reference methods showed modest correlations with body height

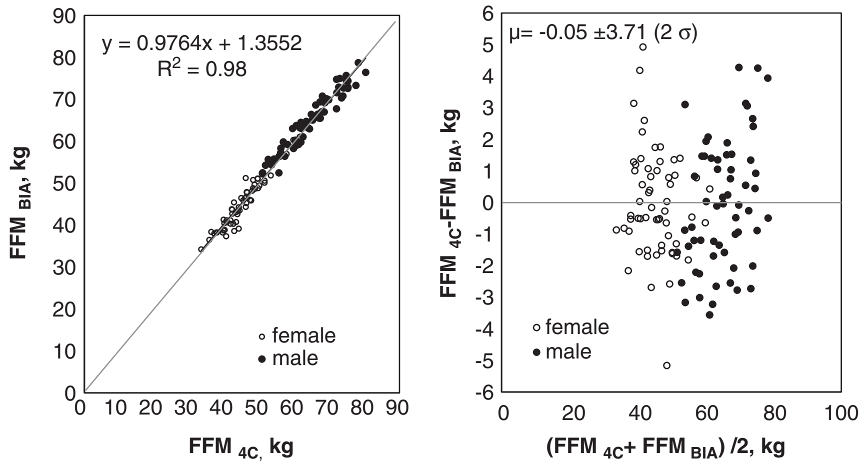

Figure 2. Regression analysis (left figure) and Bland–Altman plot of limits of agreement in FFM between four-compartment model (4C) and BIA (right figure). Open symbols for females; closed symbols for males.

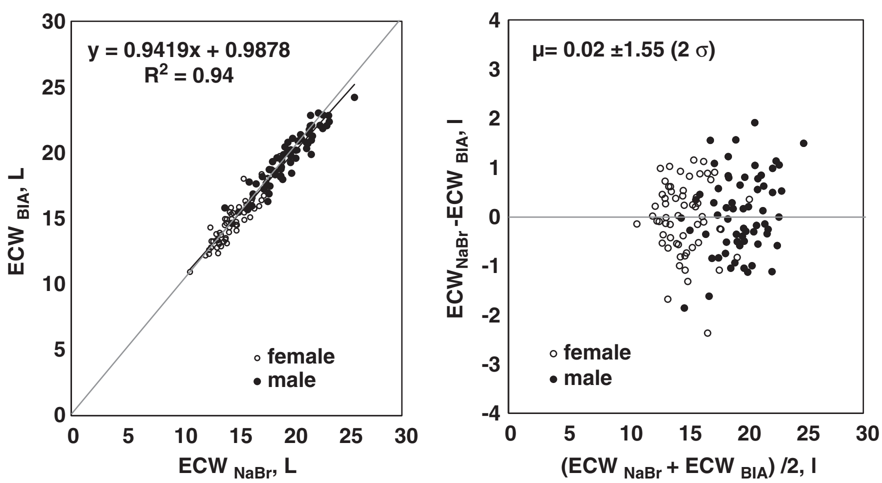

Figure 3. Regression analysis (left figure) and Bland–Altman plot of limits of agreement in ECW between NaBr dilution and BIA (right figure). Open symbols for females; closed symbols for males; $n = 130$.

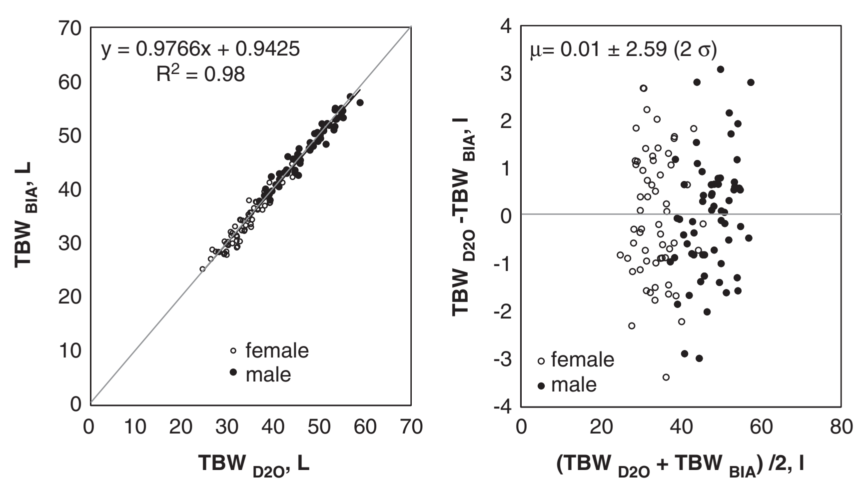

Figure 4. Regression analysis (left figure) and Bland–Altman plot of limits of agreement in TBW between D_2O and BIA (right figure). Open symbols for females; closed symbols for males; $n = 130$.

and arm length (ΔFFM_{4C-BIA} $r=-0.26$ and $r=-0.22$; ΔTBW_{D_2O-BIA} $r=-0.19$ and $r=-0.19$; $\Delta ECW_{NaBr-BIA}$ $r=-0.29$ and $r=-0.25$; all $P<0.05$). No other relationships were observed between biases and anthropometric variables, age or % FM $trunk_{DXA}$.

Impact of different reference methods on the validity of BIA equations

Table 5 summarises the validity of BIA results for FFM using four-compartment model (4C) and different two-compartment methods as a reference. Biases between BIA and all two-compartment methods (DXA, ADP and D_2O) were significantly lower when compared with the bias between BIA and the four-compartment model (all $P<0.01$). In contrast, the prediction of FFM by the BIA equation agreed equally well with all two-compartment methods of body composition analysis. Pure error of the prediction was 2 kg FFM when judged against the four-compartment model, DXA or D_2O. The pure error of FFM prediction compared with FFM measured by ADP was slightly higher (2.4 kg). The bias between BIA results and different two-compartment reference methods showed a similar dimension when compared with the bias between different two-compartment reference methods ($\Delta FFM_{ADP-DXA}$: 0.04 ± 1.96 kg; ΔFFM_{ADP-D_2O}: −0.25 ± 2.16 kg; ΔFFM_{D_2O-DXA}: 0.29 ± 1.54 kg).

DISCUSSION

This study reports on an eight-electrode, segmental multi-frequency BIA device to estimate body composition in healthy and euvolemic adults with a validity and precision that matches that of other two-compartment reference methods, including ADP, D_2O and DXA.

Table 3. Descriptive characteristics of the study population from phase 2 (New York) stratified by ethnicity

	Females	*Males*	*All*
Caucasians	(n = 16)	(n = 16)	(n = 32)
Age, years	42.7 ± 13.7	43.1 ± 15.7	42.9 ± 14.5
Weight, kg	68.0 ± 12.0	81.9 ± 15.0	74.9 ± 15.1
Height, cm	164 ± 5	175 ± 7	170 ± 8
BMI, kg/m^2	25.1 ± 34.1	26.8 ± 4.6	26.0 ± 4.4
Asians	(n = 18)	(n = 18)	(n = 36)
Age, years	40.7 ± 13.0	41.3 ± 14.4	41.0 ± 13.5
Weight, kg	58.1 ± 6.4	69.3 ± 11.1	63.7 ± 10.6
Height, cm	160 ± 4	172 ± 6	166 ± 8
BMI, kg/m^2	22.6 ± 1.9	23.3 ± 3.5	23.0 ± 2.8
Afro-Americans	(n = 15)	(n = 16)	(n = 31)
Age, years	37.1 ± 10.5	40.9 ± 11.7	38.7 ± 11.1
Weight, kg	67.8 ± 10.1	81.4 ± 16.7	75.2 ± 15.3
Height, cm	166 ± 6	176 ± 8	172 ± 8
BMI, kg/m^2	24.6 ± 3.7	26.0 ± 3.8	25.4 ± 3.8
Hispanics	(n = 16)	(n = 15)	(n = 31)
Age, years	40.5 ± 13.4	39.7 ± 11.7	40.1 ± 12.4
Weight, kg	69.3 ± 4.1	80.3 ± 12.1	74.6 ± 10.4
Height, cm	158 ± 7	174 ± 5	165 ± 10
BMI, kg/m^2	27.9 ± 2.9	26.7 ± 4.2	27.3 ± 3.6

Abbreviation: BMI, body mass index.

Table 5. Validity of BIA results for FFM compared with different two-compartment reference methods in the total study population (phase 2, New York)

	Multiethnic study population
FFM_{BIA}, kg	51.0 ± 10.5
FFM_{4C}, kg	51.8 ± 10.3
Bias (FFM_{4C-BIA}), kg	0.8 ± 1.9
pure error, kg	2.0
FFM_{ADP}, kg	50.8 ± 10.1
Bias ($FFM_{ADP-BIA}$), kg	−0.2 ± 2.4
Pure error, kg	2.4
FFM_{DXA}, kg	50.8 ± 10.1
Bias ($FFM_{DXA-BIA}$), kg	0.2 ± 2.0
Pure error, kg	2.0
FFM_{D_2O}, kg	51.1 ± 10.4
Bias (FFM_{D_2O-BIA}), kg	0.1 ± 2.7
Pure error, kg	2.0

Abbreviation: ADP, air-displacement plethysmography; BIA, bioelectrical impedance analysis; D_2O, deuterium dilution; DXA, dual-energy X-ray absorptiometry; ECW, extracellular water; FFM, fat-free mass.

Table 4. Comparison of the validity of the results from BIA equations between different ethnic groups in phase 2 (New York)

	Caucasians	*Asians*	*Afro-Americans*	*Hispanics*
FFM_{4C}, kg	53.1 ± 10.8	47.8 ± 8.7	57.2 ± 11.0	50.0 ± 8.5
FFM_{BIA}, kg	52.4 ± 10.3	47.1 ± 9.9	55.7 ± 11.4	49.6 ± 8.8
Bias (FFM_{4C-BIA}), kg	0.7 ± 2.1	0.7 ± 1.8*	1.5 ± 1.7***	0.4 ± 1.8
Pure error, kg	2.1	1.9	2.2	1.9
ECW_{NaBr}, l	15.9 ± 2.9	14.3 ± 2.3	17.1 ± 2.6	15.4 ± 2.2
ECW_{BIA}, l	16.4 ± 2.9	14.6 ± 2.5	17.2 ± 3.0	15.5 ± 2.3
Bias ($ECW_{NaBr-BIA}$), l	−0.5 ± 1.0**	−0.4 ± 0.6**	−0.1 ± 0.8	−0.2 ± 0.7
Pure error, l	1.1	0.7	0.8	0.7
TBW_{D_2O}, kg	38.6 ± 7.9	34.2 ± 6.7	41.3 ± 8.1	36.0 ± 6.1
TBW_{BIA}, kg	38.3 ± 7.5	34.1 ± 7.1	40.9 ± 8.3	36.3 ± 6.3
Bias (TBW_{D_2O-BIA}), kg	0.3 ± 1.7	0.2 ± 1.3	0.4 ± 1.4	−0.3 ± 1.3
Pure error, kg	1.7	1.3	1.5	1.3

Abbreviations: ADP, air-displacement plethysmography; BIA, bioelectrical impedance analysis; D_2O, deuterium dilution; DXA, dual-energy X-ray absorptiometry; ECW, extracellular water; FFM, fat-free mass; TBW, total body water; 4C, four-compartment model. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ for difference between results from BIA and reference method.

The coefficient determination for all generated prediction equations is high (values between 0.94 for ECW and 0.98 for FFM and TBW) and the RMSE is low. These data confirm the results from previous studies that have shown the high potential of BIA to accurately predict body composition.[14–16]

However, previous studies have also shown that BIA is most appropriate for estimating body composition of groups in epidemiological and field studies but has limited accuracy in individuals.[17,18] In contrast to these studies, the present findings clearly show that the agreement between BIA and reference methods was as good as the agreement among different reference methods. The application of the generated prediction equations to the independent population for validation purposes revealed that the pure error of the prediction was in the range of the RMSE. The following reasons may contribute to the high accuracy of the generated prediction equations:

- Highly reproducible positioning of the participant for the BIA measurement that is facilitated by variable handrail positions depending on the height of the person.
- Eight electrodes are used and therefore the upper and lower body, left and right side of the body are assessed.
- The new indices that were developed from measured *R* and Xc values to represent the relative contribution of trunk and extremities to total body conductivity could adjust for differences in body shape that contribute to inaccuracies in previous BIA measurements.

Because BIA measures the impedance to the flow of an electric current through total body fluid, the conductive volume (V, which represents TBW or FFM) is proportional to the square length of the conductor (Ht^2) and inversely correlated to resistance (*R*) of the cross-sectional area, ($V = p \times Ht^2/R$, where *p* is the specific resistance of the conductor). Montagnese *et al.*[19] recently reported that no single BIA equation applies across the age range of 4–24 years, because at certain ages or pubertal stages, the slope and intercept of the equation relating lean mass to $height^2/Z$ change (*Z*, impedance $= (R^2 + Xc^2)^{1/2}$). Regression equations relating FFM_{4C} to $height^2/R$ also differed between ethnic groups in the present study (Caucasians: slope 0.94, intercept 7.02; Hispanics: slope 0.90, intercept 9.47; African Americans: slope: 0.97, intercept 7.19; Asians: slope 0.86, intercept 8.62). However, prediction equations were generated by multiple regression analysis, including Xc_{50} and Index $R_{50\ \mathrm{trunk/extremities}}$ as independent variables (Table 2), which may have compensated for population differences because ΔFFM_{4C-BIA} did not differ between ethnic groups.

From the principles of BIA, it can also be deduced that whole-body impedance is mainly based on the impedance of the distal parts of the limbs near the electrodes.[20,21] The population specificity of BIA equations due to the differences among racial groups (for example, black populations have longer limbs than white populations) may therefore mostly relate to differences in the proportion of limb lengths.[5,22] In the current study, the indices (Index $R_{50\ \mathrm{trunk/extremities}}$ and Index $Xc_{50\ \mathrm{trunk/extremities}}$) that were developed from segmental *R* and Xc values may partly compensate for differences in body shape because these indices correlated with trunk length, arm length and waist and arm circumferences. In line with this argument, the bias between BIA result and reference method showed no correlation with waist circumference, and only a weak association with arm length. A previous study found that waist circumference was the only significant predictor of systematic error in % FM between BIA and DXA ($r = 0.60$, $P < 0.0001$) and concluded that eight-electrode, segmental multifrequency BIA is a valid method to estimate % FM in adults with BMI classified as normal weight and overweight, but not as obese.[23] The segmental BIA indices used in the present study for the purpose of compensating for differences in body fat distribution need to be investigated in populations with a higher BMI range and greater levels of obesity.

Comparison of bias (ΔBIA result − reference method) between ethnicities (Table 4) with bias between methods (Table 5) revealed that the contribution of population specificity is of minor importance for the accuracy of the generated prediction equation when compared with the impact of the reference method on the BIA result.

In conclusion, modern BIA technique is a valid tool to estimate body composition in healthy and euvolemic adults, which can compare with the validity and precision of other two-compartment reference methods, such as ADP, D_2O or DXA. Population specificity is of minor importance when compared with results from different reference methods. Future studies should include individuals with a wider BMI and age range, as well as the validation of TBW and ECW in patients with fluid overload. Finally, to enhance the validity of BIA for body composition assessment, a cut-off should be generated based on the ECW-to-TBW ratio or bioimpedance vector analysis in order to limit the prediction of FFM and FM to healthy euvolemic individuals and to avoid the generation of inaccurate results in patients with dehydration or fluid overload.

CONFLICT OF INTEREST

ABW and MJM serve as consultants for seca Gmbh & Co. KG, Hamburg, Germany. ABW has also received lecture fees from Medicom, seca and Unilever. DG has received lecture fees from seca. JJK serves as a consultant to Abbott Nutrition, Ohio, USA. JJK has also received grant support from Unilever and seca. The remaining authors declare no conflict of interest.

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