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ORIGINAL ARTICLE

Comparing single-frequency bioelectrical impedance analysis against deuterium dilution to assess total body water

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BACKGROUND/OBJECTIVES: In this study, we aimed to validate the accuracy of single-frequency bioelectrical impedance analysis (SF-BIA) at 50 kHz to assess total body water (TBW) against the reference technique deuterium dilution (D_2O) and to explore if the simple clinical parameters extracellular fluid (ECF) composition and body shape explain individual differences between D_2O and SF-BIA ($Diff_{BIA-D_2O}$).

SUBJECTS/METHODS: We assessed TBW with D_2O and SF-BIA in 26 women and 26 men without known disease or anomalous body shapes. In addition, we measured body shape with anthropometry and ECF composition (osmolality, albumin, glucose, urea, creatinine, sodium and potassium).

RESULTS: On group average, SF-BIA to predict TBW agreed well with D_2O (SF-BIA, 39.8 ± 10.1 l; D_2O, 40.4 ± 10.2 l; and $Diff_{BIA-D_2O}$ − 0.7 l). In four individuals ('outliers'; 15% of the study population), $Diff_{BIA-D_2O}$ was high (− 6.8 to + 3.8 l). $Diff_{BIA-D_2O}$ was associated with individual variations in body shape rather than ECF composition. Using gender-specific analysis, we found that individual variability of waist circumference in men and arm length in women significantly contributed to $Diff_{BIA-D_2O}$. When removing the four 'outliers', these associations were lost.

CONCLUSIONS: In the majority of our sample, BIA agreed well with D_2O. Adjusting for individual variability in body shape by anthropometrical assessment could possibly improve the accuracy of SF-BIA for individuals who deviate from mean values with respect to body shape. However, further studies with higher subject numbers are needed to confirm our findings.

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Keywords: body composition; bioelectrical impedance analysis; body shape; total body water

INTRODUCTION

Water is the origin of all things (Thales, 624-546 BC). Apart from acting as a universal solvent for virtually all biochemical reactions, water has direct influences on many physiological processes, including energy metabolism.[1] To further explore the role of body water, a suitable method for its accurate measurement is required. The reference method to assess total body water (TBW) is deuterium dilution (D_2O). However, this method is expensive and time consuming. TBW is commonly assessed with bioelectrical impedance analysis (BIA). According to the guidelines of the European Society of Clinical Nutrition and Metabolism, this technique works well in healthy subjects with a suitable prediction equation.[2] When compared with D_2O, the mean differences to BIA were only − 0.5 l in men and − 0.3 l in women in a study of 1474 normal adults.[3] However, in a few individuals, BIA produced a large and unexplained bias of up to 10 l in men and 9 l in women,[3] clearly limiting its utility. A better understanding of the underlying mechanisms leading to these large discrepancies is required. To this end, we should recall the basic principles of BIA. According to Kyle *et al.*[2], an empirical relationship can be established between the impedance quotient ($length^2$/resistance (R)) and the volume of water. BIA prediction equations account for the fact that the body is not a uniform cylinder, and its conductivity is not constant, by introducing a coefficient. This coefficient depends on various factors, and errors occur when there are alterations either in resistivity of the conductive material or in variations of body shape.[2] In this pilot study, we aimed to validate the accuracy of single-frequency BIA (SF-BIA) at 50 kHz to assess TBW against the reference technique D_2O and to explore if the simple clinical parameters extracellular fluid (ECF) composition and body shape explain large individual differences between D_2O and SF-BIA. In this case, these parameters could possibly be used to improve the accuracy of SF-BIA in the future.

PATIENTS AND METHODS

The Institutional Review Board of the Charité approved the study and we obtained written informed consent from all participants before study entry. We recruited 52 adult volunteers (26 women and 26 men) with a wide range of body mass index (19.0–38.3 kg/m^2) and without known disease or apparent anomalous body shapes, by advertisement among university staff. All participants underwent assessment of body composition and body shape as described below.

We instructed participants to restrain from exercise and alcohol or excessive fluid consumption on the day before testing. On the evening before the tests, they provided a baseline urine sample of at least 50 ml at

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home at 2230 hours. Immediately afterwards they drank the deuterium dose, rinsed the deuterium bottle with 50 ml of tap water and drank the rinsing water. We advised the participants to fast and not to consume any fluids after the deuterium ingestion, and to void the next morning. The remaining tests were carried out at our Clinical Research Center, where the participants arrived at 0830 hours to provide the post-dose urine sample, assuring a standardized time interval of 10 h between the two samples. Urine samples were kept at $-20\,^{\circ}C$ till mass spectrometric analysis, which was done at the University of Maastricht, according to a previously described protocol.[4]

After voiding, the participants rested for 10 min in a supine position. All BIA measurements were carried out using BIACorpus RX 4000 (Medi-Cal Healthcare GmbH, Karlsruhe, Germany), after the subjects were carefully placed into a position suitable for BIA measurements, assuring separate placement of legs in an angle of about 30 degrees. After cleaning the skin with disinfectant, we placed two single-use electrodes (BIA Classic Tabs; Medi-Cal Healthcare) on the dorsal surface of both hands and feet, according to the manufacturer's instructions. Whole-body impedance was measured at 50 kHz analysis and TBW was calculated from the dominant side of the body according to a standard equation.[3] We assessed body height with a Laser Stadiometer (Soehnle Leifheit AG, Nassau, Germany) and body weight with electronic scales attached to the BodPod system (Life Measurement Inc., Concord, CA, USA). We assessed body shape by measuring circumferences with a non-stretchable measuring tape at the standardized reference points as follows:

Waist circumference	half way between lower rib and iliac crest
Hip circumference	at the level of trochanter major
Mid-upper arm circumference	on the dominant side midway between acromion and olecranon while the participants held their arm flexed at 90 degrees at the elbow
Mid thigh circumference	midway between trochanter major and proximal border of patella
Shoulder circumference	at the site defined by marking the intercept of the vertical line from the acromion towards the olecranon and horizontal line between the tips of the shoulder blades on each side of the body

We measured body lengths on the dominant side while the person was supine for BIA testing as follows:

Arm length	from the acromion to the proximal border of the proximal electrode on the surface of the hand
Upper body length	from the acromion to the iliac crest
Leg length	from the iliac crest to the proximal border of the proximal electrode on the surface of the foot

We obtained a fasting blood sample by venipuncture for analysis in a commercial laboratory on the same day. The sample was analyzed for serum albumin (turbidimetric assessment), plasma glucose (hexokinase/G6P-DH), urea (kinetic ultraviolet test), creatinine (Jaffé analysis), sodium (indirect ion-specific electrode), potassium (indirect ion-specific electrode), using commercially available assays (Roche, Mannheim, Germany). Plasma osmolality was assessed by freezing point depression (Roebling, Berlin, Germany). We excluded potassium and osmolality data of five participants from analysis because sample preparation was not carried out according to the standard procedures.

We applied SPSS (version 18.0; SPSS Inc., Cary, NC, USA) for statistical analysis, presented data as mean ± s.d. and carried out between-group comparison with one-way analysis of variance followed by two-tailed *post-hoc* Dunnett's *t*-tests. For intra-individual comparisons, we applied the paired *t*-test with $P<0.05$ considered as statistically significant, and for the method comparison, we used Kendall's Tau correlation coefficient and Bland–Altman analysis of agreement.[5] We considered subjects as 'outliers' if the measurement difference between BIA and D_2O was above or below the group mean ± 2 s.d. To identify parameters that explain the measurement difference between BIA and D_2O ($Diff_{BIA-D_2O}$), we carried out a stepwise regression analysis with $Diff_{BIA-D_2O}$ as dependent and further possible predictors shown in the results section as independent variables.

Table 1. Demographic characteristics, body composition and body shape of the study participants

	Men (n = 26)	*Women* (n = 26)
Age (years)	36 ± 10 (23–62)	37 ± 11 (21–57)
BMI (kg/m^2)	25.8 ± 4.8 (19.0–38.3)	24.1 ± 3.5 (18.7–31.6)
TBW_{D_2O} (l)	48.9 ± 7.0 (39.1–61.4)***	32.0 ± 3.8 (24.8–42.0)
TBW_{BIA} (l)	48.2 ± 7.3 (37.3–66.2)***	31.4 ± 3.1 (24.8–38.2)
Shoulder circumference (cm)	119 ± 12 (98–146)***	100 ± 7 (90–114)
Mid-upper arm circumference (cm)	34 ± 5 (27–45)***	29 ± 3 (24–35)
Waist circumference (cm)	93 ± 15 (67–130)**	81 ± 11 (67–106)
Hip circumference (cm)	100 ± 11 (80–129)	98 ± 8 (83–115)
Thigh circumference (cm)	57 ± 7 (50–75)	56 ± 4 (48–62)
Arm length (cm)	61 ± 3 (55–67)***	53 ± 4 (46–63)
Leg length (cm)	99 ± 6 (89–114)***	89 ± 6 (75–97)

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; D_2O, deuterium dilution; TBW, total body water. Data are presented as mean ± s.d. (range). $^{**}P<0.01$ and $^{***}P<0.001$ men vs women.

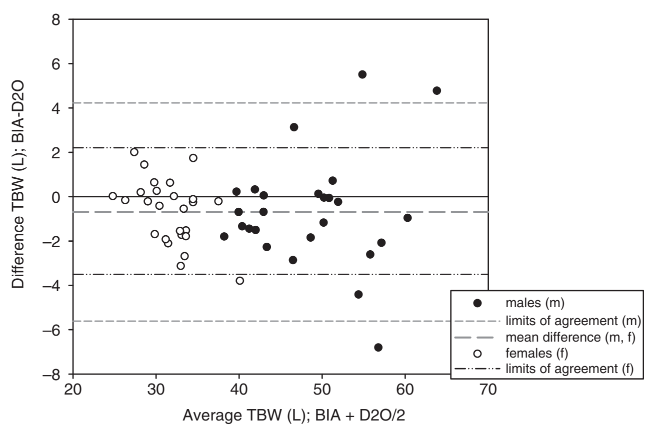

Figure 1. Bland–Altman analysis of agreement between methods.

RESULTS

Demographic characteristics, body composition and body shape of the study participants are presented in Table 1. ECF composition and osmolality of the study participants were within or very close to reference values (data not shown). We found no significant mean measurement difference between TBW_{BIA} and TBW_{D_2O} (entire study group -0.7 ± 2.0 l; men: -0.7 ± 2.5 l and women -0.6 ± 1.5 l). TBW_{BIA} and TBW_{D_2O} were highly correlated both in men and women ($r^2=0.90$ and 0.76, respectively), with root mean square errors of 2.5 and 1.2 l, respectively. As simple correlation coefficients can be misleading when judging on agreement between two methods, a more detailed method comparison of BIA and isotope dilution (D_2O) with Bland–Altman analysis of agreement is shown in Figure 1. Although mean group $Diff_{BIA-D_2O}$ was similar for men and women, maximum individual $Diff_{BIA-D_2O}$ was larger in men than in women (-6.8 and $+5.5$ vs -3.8 and $+2.0$ l, respectively). Concomitantly Bland–Altman analysis showed wider limits of agreement (mean ± 1.96 s.d.) in men vs women (-5.9 to 4.2 vs -3.5 to 2.3 l).

With respect to $Diff_{BIA-D_2O}$, we identified three men and one woman as 'outliers'; the 3 men showing a $Diff_{BIA-D_2O}$ of -6.8, 4.8

Table 2. Correlation coefficients between anthropometrical parameters and bioimpedance raw data, as well as the measurement difference between BIA and D_2O

	Men (n = *26)*			*Women* (n = *26)*		
	R	*Xc*	$Diff_{BIA-D_2O}$	*R*	*Xc*	$Diff_{BIA-D_2O}$
Age	0.052	− 0.340	0.305	− 0.219	− 0.397*	0.505**
Height (cm)	− 0.247	− 0.488*	− 0.176	0.203	0.378	− 0.530**
BMI (kg/m^2)	− 0.575**	− 0.548**	0.585**	− 0.378	− 0.121	0.289
Shoulder circumference (cm)	− 0.716***	− 0.527**	0.216	− 0.319	− 0.027	0.102
Mid-upper arm circumference (cm)	− 0.725***	− 0.441*	0.357	− 0.337	0.129	0.127
Waist circumference (cm)	− 0.415*	− 0.597**	0.642***	− 0.398*	− 0.158	0.330
Hip circumference (cm)	− 0.451*	− 0.598**	0.461*	− 0.046	0.133	0.095
Mid-upper thigh circumference (cm)	− 0.375	− 0.252	0.372	− 0.052	0.270	0.154
Arm length (cm)	0.041	− 0.352	0.122	0.316	0.549**	− 0.637***
Leg length (cm)	0.054	− 0.157	− 0.117	0.400*	0.601**	− 0.616**

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; D_2O, deuterium dilution; R, resistance; Xc, reactance. Data are displayed as Pearson's correlation coefficient (*r*). *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

Table 3. Stepwise regression analysis with the measurement difference as dependent variable

Independent variable	*ß/ln ß*	P	*Adjusted R^2*
Men (n = *26)*			0.388
Variables entered			
Waist	0.642	0.000	
Excluded variables			
BMI	0.067	0.848	
Hip	− 0.054	0.826	
Women (n = *26)*			0.382
Variables entered			
Arm length	− 0.637	0.000	
Excluded variables			
Height	− 0.164	0.467	
Leg length	− 0.288	0.295	

Abbreviations: BMI, body mass index; R, resistance. Waist, waist circumference (cm); Hip, hip circumference (cm) Independent variables were BMI, waist and hip circumference for males and height and leg and arm length for females.

and 5.5 and the woman of − 3.8 l. R and reactance (Xc) showed considerable associations with anthropometrical parameters, whereas the relation with ECF composition and osmolality was negligible (data not shown). Correlations of anthropometrical parameters with impedance raw data and the measurement difference between BIA and D_2O for men and women are shown in Table 2. Shoulder and arm circumference in the men was significantly related with R and Xc, but not with $Diff_{BIA-D_2O}$. In the women, none of the measured circumferences, but limb lengths, were related to $Diff_{BIA-D_2O}$. We next carried out stepwise linear regression analyses to identify and evaluate parameters explaining $Diff_{BIA-D_2O}$ and selected the independent body shape-related variables if there was a significant correlation with the measurement difference according to Table 2. The results of the regression analyses are presented in Table 3.

We found a significant contribution to $Diff_{BIA-D_2O}$ from waist in men and from the length of arms in women. Two of the three men that were detected as 'outliers' (5.5 and 4.8 l difference) also had the highest waist circumferences in the male group (120 and 130 cm when compared with the group average of 93 ± 15 cm). The woman detected as 'outlier' (− 3.8 l) had the longest arms in the female group (62.5 cm when compared with the group mean of 53.0 ± 4 cm). When repeating the stepwise regression after excluding the outliers, none of the independent variables entered the prediction model in the male group, whereas in the female group, leg instead of arm length entered the model ($P=0.003$; adjusted $r^2=0.292$).

DISCUSSION

When compared with D_2O, SF-BIA at 50 kHz worked well and underestimated group mean TBW by only 0.7 l. Even though our study was of small sample size, accuracy of SF-BIA to predict group mean TBW was good both in healthy men and women and equal to the accuracy in a previous large-scale investigation on 1474 subjects.[3] However, although SF-BIA accurately predicted group mean TBW, maximum individual measurement differences ($Diff_{BIA-D_2O}$) came up to − 6.8 l in our study. The magnitude of the observed $Diff_{BIA-D_2O}$ was higher in men when compared with women (Figure 1). Sun *et al.*[3] also reported higher deviations of TBW_{BIA} from TBW assessed with dilution techniques in men when compared with women.[3] This state-of-affairs might be explained by gender-specific effects of body composition and shape and/or higher absolute amount of TBW present in men, emphasizing the need for a gender-specific approach in our investigations.

Influence of ECF composition on the measurement difference between SF-BIA and D_2O

Large perturbations of plasma sodium concentrations caused by saline infusion and thirsting had a significant effect on impedance measurements and the subsequent calculation of TBW.[6] Therefore, one of our hypotheses was that individual physiological variability in ECF composition might at least in part explain the large measurement inaccuracy of BIA seen in some individuals. Instead, we found that the prediction of TBW from impedance measurements at 50 kHz seemed relatively unaffected by physiological variations in ECF composition and osmolality in our healthy volunteers. Extracellular electrolyte composition in healthy people seemed to be held constant to an extent that impedance at 50 kHz remained unaffected. Other techniques, such as multiple frequency BIA or bioimpedance spectroskopy might be more sensitive to picking up changes in ECF composition.

Influence of body shape on the measurement differences between SF-BIA and D_2O

Body shape variations seen in our group of healthy volunteers significantly affected SF impedance and at least partly explained $Diff_{BIA-D_2O}$. Two of the three men showing exceptionally high $Diff_{BIA-D_2O}$ also had a high waist circumference, and the female with the high $Diff_{BIA-D_2O}$ had relatively long arms. When removing these few outliers from the analysis, the former prediction power of waist circumference and arm length for $Diff_{BIA-D_2O}$ disappeared. The large measurement difference seen in some individuals might thus be caused by the fact that these persons do not fit well into the general geometric principles derived from the average of a healthy population. Concomitantly, we have previously shown that truncal circumferences explain a small but significant part of the measurement difference of body fat between BIA and air-displacement plethysmography in a group of overweight volunteers.[7] Because of a more systematic approach to characterize body shape in the current study, we are able to expand on our previous findings. However, to generate and cross-validate prediction equations that include body shape parameters, a very large sample size would be needed.

On one hand, our findings can be debated as based on a few outliers. On the other hand, they are in line with earlier work, suggesting a noteworthy influence of body shape on the accuracy of BIA. First, Lukaski[8] proposed that the bioimpedance method may be prone to errors in individuals with excessively long limbs relative to the torso length back in 1997. Second, differences in circumferences of various body segments are likely to explain conflicting results in previous studies, showing that leg impedance contributed between 12 and 44% to whole-body impedance, and the trunk between 10 and 46%.[9,10] Concomitantly, in hemodialysis patients, the body shape-derived Kb factor used to adjust impedance measurements for specific segmental resistivity as part of the bioimpedance spectroskopy technique improved estimation of body fluid volume.[11]

Segmental BIA has been developed to overcome inconsistencies between R and mass of the trunk, yet this technique requires further evaluation.[2] Although the body composition data presented in the results section of our study were derived from tetrapolar impedance measurements between the hand and foot of the dominant body side, we had applied a tetrapolar system that also measures segmental impedance by placing two electrodes each on both hands and feet. When we included the resulting segmental data as independent parameters to predict TBW_{D_2O}, Xc measured from the right to the left leg was selected as an independent variable. This maneuver increased r^2 from 0.92 to 0.95 in men. In women, R from the left arm to the left leg was selected as an independent variable. The adjusted r^2 increased from 0.86 to 0.92. We thus suggest that segmental SF-BIA might have advantages in the healthy and normal population. However, at the present time, additional research is needed to examine the benefit, standardization and accuracy of segmental SF-BIA measurements.

In conclusion, agreement between BIA and D_2O was high on average. Individual variations of body shape, but not of ECF composition, explained the measurement differences between SF-BIA at 50 kHz and D_2O. Our pilot data suggest that the observed large measurement differences in some individuals might be avoided by adjusting impedance measurements for individual variability in truncal circumferences and limb lengths. However, to confirm our findings, a study with a larger sample size is needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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