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A critical analysis of whole body bioimpedance spectroscopy (BIS) for the estimation of body compartments in health and disease

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ABSTRACT

Aim of the study was to assess the accuracy and precision of a BIS device and the relative contribution of BIS beyond the anthropometric parameters. The output of the Impedimed device (SFB7) and the relative contribution of height, weight, age, sex and resistance values at zero and infinite frequency (R_{zero} and R_{inf} respectively) to the prediction of total body water (TBW_d, deuterium space), of extracellular fluid (ECF_{br}, sodium bromide space) and of fat mass (FM_{DXA}) were assessed in 116 subjects (32 healthy subjects and 84 patients with disorders of body composition). Using a repeated randomization procedure, new equations for TBW, ECF and FM were derived.

The SFB7 gave measures of determination similar to those obtained with equations that included only anthropometric data. The SFB7, but not the newly derived regression equations, underestimated TBW and ECF by 3.82 ± 3.37 (mean $\pm$ SD) and by 0.93 ± 2.62 l and overestimated FM by 6.55 ± 3.86 kg. Nine of 16 patients with ECF overload as detected by ECF_{br} were also detected by BIS. BIS measurements contribute marginally but not significantly beyond anthropometric data to the prediction of TBW, ECF and FM, either in healthy subjects or in patients with disturbed body composition.

1. Introduction

Whole body impedance spectroscopy has been advocated since 1992 as a simple method to assess TBW, ECF and FM [1–4]. This methodology is mainly used in epidemiological studies to assess body composition and nutritional status [5–7]; however, it is also used to assess hydration in disease states [8–11]. Anthropometric data, namely height, weight, sex and age, are mandatory inputs [12]. It has been shown for single frequency whole body impedance measurements that anthropometric inputs represent the mayor contributors to the prediction whereas the impedance measurements themselves are of the least importance [13–15]. We are not aware that the relative contributions of whole body BIS and of anthropometric measurements for the estimation of TBW, ECF and FM have ever been evaluated. We assessed the validity of a currently used BIS device in health and disease in comparison with anthropometric calculations alone. Whole body DXA, and deuterium and sodium bromide dilution studies were used as gold standard methods. The hypothesis was that R_{zero} and R_{inf} contribute significantly to the prediction of body compartments in health and disease. The inclusion of disease states extends the range of observed values of body compartments enormously and therefore should help to assess the contribution of whole body BIS to prediction equations. Furthermore, the transition of health to disease is smooth and often arbitrary.

2. Subjects and methods

The study included 116 subjects: 32 healthy controls were students at the Medical University Graz and healthy individuals from a gymnastic club. Consecutive patients referred to the Department of Internal Medicine were asked to volunteer for the study. Eighty-four of them who agreed to participate were included in the study. The diagnoses were quite representative for admittances to a general internal ward. They included e.g. congestive heart failure, coronary heart disease, essential hypertension, atherosclerosis,

Abbreviations: Anthro, anthropometry (height, weight, sex and age); BIS, bioimpedance spectroscopy using the expression $Ht^2/Rzero$ and $Ht^2/Rinf$; %BWt, percent of body weight; ECFbr, extra-cellular fluid assessed by sodium bromide dilution; FMDXA, fatmass assessed by Dual-energy X-ray absorptiometry; Rzero, resistance at zero frequency; Rinf, resistance at infinite frequency; SFB7, impedimed SFB7 device; TBWd, total body water by deuterium dilution; TBW, total body water; ECF, extra-cellular fluid; FM, fatmass.

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kidney disease, chronic renal failure, gastrointestinal diseases, type II diabetes, morbid obesity, osteoporosis, cancer, chronic polyarthritis and anorexia nervosa. The patients already on treatment were studied without changing their home medication. The study complies with the Declaration of Helsinki. It was approved by the institutional Ethics Committee and all patients gave written informed consent. Anthropometric data and body compartments as assessed by gold-standard methods and by the SFB7 device in healthy subjects and patients are given in Table 1.

3. Study protocol

All measurements were performed on the same day following a 12-hour fast. A blood sample was taken to determine background deuterium and sodium bromide concentrations. One hundred ml of tap water containing 312.48 mg deuterium (D_2O) and 676 mg sodium bromide were administered orally. The doses were not normalized to body weight in order not to add additional sources of error. Four hours later a second blood sample was taken from the brachial vein. We measured height to the nearest centimeter with a caliper while the subjects were standing and weight with an electronic scale to the nearest 100 grams (Soehnle No. 7347). BIS measurements (SFB7 by Impedimed Inc., San Diego) were performed in supine position following 15 min supine rest as recommended by the manufacturer. The equations used in the SFB7 are not provided by the manufacturer. However, we have calculated intracellular fluid (ICF) and ECF according to the Hanai equations [7] and have found correlation coefficients of 0.999 for both with no deviations to the lines of identities. This ascertains that the Hanai formulas are implemented in the SFB7 device. Whole body DXA (Hologic QDR4500A software version 12.6) was performed between 8 am and 12 am as recommended by the manufacturer. In order to calculate TBW from DXA measurements (which, besides FM measurements, are only used for assessing the quality of our deuterium measurements [see Fig. 1]) lean body masses as derived by DXA were multiplied by a factor of 0.73 [16].

4. Analytics

The blood samples were centrifuged at 4000 RPM for 12 min; the supernatant serum was then ultra-filtrated (Amicon Ultra-15, PLTK Ultracel-PL Membrane, 30 kDa, Millipore®) at a speed of 4000 RPM for 25 min. Between 4 and 6 ml of ultra-filtrate were recovered. The samples were stored at −28 °C for further analysis.

4.1. Deuterium measurements

Deuterium was measured in the continuous flow mode by chromium reduction using a ceramic reactor [17]. The method of Morrison et al. was modified as follows: a high temperature oven (HEKAtech, Germany) was fitted with a EuroAS 300 liquid auto sampler (EuroVector, Italy). The oven was configured with a Cr packed reactor held isothermally at 1050 °C. Serum samples obtained by ultracentrifugation of human plasma contained in 2-ml septa-sealed vials were placed onto the carousel of the liquid auto sampler, which was fitted with a 10 μl injection syringe (SGE Europe). A sequence of one wash cycle of 3.5 μl volume was carried out for each sample prior to injection into the Cr reactor. Serum samples of 1.8 ml were then injected in quintuples into the septa-sealed injector port. The resulting vapor was flushed into the reactor by the carrier helium gas via a 1-mm interior diameter stainless steel probe extending into the ceramic reactor tube (Al_2O_3). In the reactor, water was reduced by the Cr, resulting in

Table 1
Test results in healthy subjects and patients.

	Healthy Subjects									Patients								
	M (n = 18)					F (n = 14)				M (n = 55)					F (n = 28)			
	Mean	SD	Min	Max	% of Gold standard	Mean	SD	Min	Max	% of Gold standard	Mean	SD	Min	Max	% of Gold standard	Mean	SD	Min	Max	% of Gold standard
Age (y)	51.93	19.41	24.99	77.00		32.55	12.56	23.94	63.19		69.53	14.43	28.05	94.19		73.28	12.27	34.66	92.47	
Height (cm)	178.00	5.62	169.00	188.00		166.54	5.47	153.00	174.50		173.76	7.45	158.00	192.00		159.18	6.73	141.00	172.00	
Weight (kg)	80.09	8.92	66.70	98.60		58.92	8.05	48.10	72.90		75.63	15.65	32.60	114.50		67.24	14.45	38.00	93.00	
BMI	25.24	2.13	22.50	31.12		21.26	2.26	18.90	25.30		24.97	4.76	12.90	36.10		26.63	5.84	14.80	39.20	
Rzero [Ω]	544.78	51.30	480.87	691.38		709.09	59.98	621.25	806.72		529.43	85.81	290.97	753.28		600.73	94.12	432.03	764.34	
R_{int} [Ω]	368.57	34.01	332.43	462.34		496.53	47.81	425.65	562.89		388.45	70.69	234.00	591.82		449.74	78.76	261.23	596.54	
TBW_d [L]	45.24	4.06	35.82	51.53		30.54	3.52	24.45	35.47		41.95	6.89	26.45	65.61		32.15	5.78	23.96	46.90	
ECF_{Br} [L]	22.14	2.49	16.45	25.71		16.74	1.76	13.66	19.62		22.83	4.27	14.34	35.48		19.44	3.37	9.96	24.52	
TBW_{dxa} [L]	45.23	4.61	34.97	54.87	99.97%	30.08	3.58	24.22	35.61	98.50%	41.73	7.07	20.39	57.12	99.47%	32.16	5.60	22.46	44.83	100.04%
FM_{dxa} [kg]	16.61	4.03	9.79	26.36		16.36	4.32	10.30	23.12		17.27	8.07	3.85	47.53		22.13	8.84	5.64	38.57	
TBW_{SFB7} [L]	42.17	4.32	32.60	47.50	93.20%	26.87	3.40	21.30	31.80	87.99%	36.94	6.70	17.90	51.30	88.06%	27.96	6.03	20.20	46.90	86.99%
ECF_{SFB7} [L]	22.82	2.30	17.40	26.40	103.03%	15.39	1.80	12.70	18.90	91.89%	21.96	3.60	12.10	32.40	96.20%	17.08	2.86	12.30	22.80	87.87%
FM_{SFB7} [kg]	22.47	5.19	15.10	37.40	135.27%	22.21	5.19	14.00	31.10	135.71%	23.93	8.93	6.90	52.60	138.51%	29.04	9.78	7.20	44.30	131.19%
TBW_d [% BWt]	56.72	3.90	47.99	66.36		52.06	3.63	47.70	59.08		56.49	8.00	41.15	81.13		48.83	7.78	37.23	63.05	
ECF_{Br} [% BWt]	27.78	2.53	23.95	32.19		28.55	1.53	26.70	30.94		31.36	7.45	22.47	58.40		30.32	7.28	16.78	46.93	
TBW_{dxa} [% BWt]	56.57	2.55	52.03	62.30	99.74%	51.22	2.88	48.27	57.07	98.38%	55.85	5.29	40.70	70.88	98.86%	48.57	5.46	39.02	59.55	99.47%
FM_{dxa} [% BWt]	20.60	3.50	13.60	27.51		27.51	4.64	18.86	32.63		21.98	6.22	11.48	41.51		31.78	7.90	14.83	46.40	
TBW_{SFB7} [% BWt]	52.76	3.10	45.44	57.78	93.03%	45.79	3.81	41.98	54.61	87.95%	50.05	4.89	39.56	60.21	88.59%	42.19	6.80	33.67	60.05	86.40%
ECF_{SFB7} [% BWt]	28.56	1.75	24.54	30.69	102.83%	26.21	1.37	24.06	28.75	91.78%	29.78	3.55	23.94	38.38	94.94%	25.92	3.89	21.33	37.67	85.48%
FM_{SFB7} [% BWt]	27.91	4.27	20.97	37.93	135.46%	37.43	5.21	25.32	42.66	136.03%	31.25	7.01	14.98	45.94	142.17%	42.38	9.25	18.05	53.92	133.36%

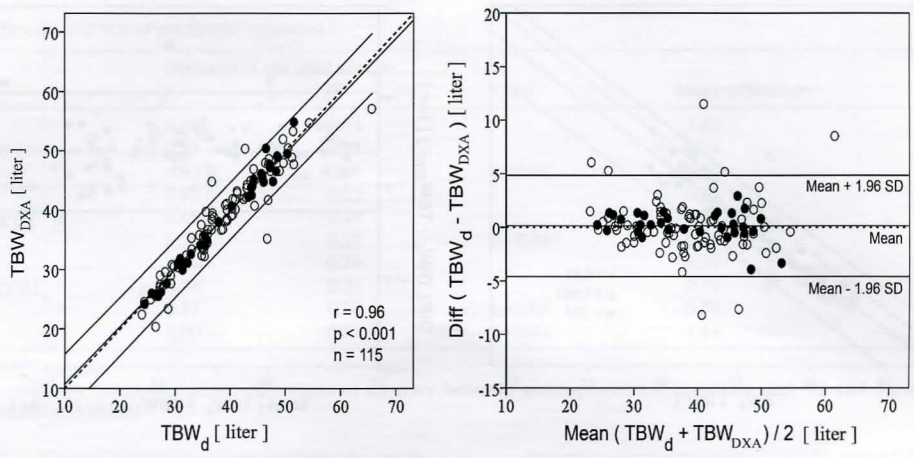

Fig. 1. Correlation between total body water by deuterium dilution (TBW_d) and by DXA (TBW_{DXA}) in healthy subjects and patients. Right panel: the corresponding Bland–Altman plot. ● Healthy subjects; ○ patients.

the quantitative conversion to hydrogen gas according to equation (1).

$$2Cr(s) + 3H_2O(l) = Cr_2O_3(s) + 3H_2(g) \quad (1)$$

The He stream carried the hydrogen gas generated in the Cr reactor through the GC column to an open split sampling capillary and into the source of a Finnigan DELTAplusXP continuous flow stable isotope ratio mass spectrometer to measure deuterium. The mean value of the quintuples was used for further calculation. The results were corrected by 4% based on the exchange of deuterium with non-aqueous hydrogen [18].

4.2. Sodium bromide measurements

The concentration of bromide in the serum water obtained by ultrafiltration of human plasma was measured by ion chromatography using a Dionex DX-500 ion chromatography system equipped with a GP40 gradient pump connected to a 250 mm × 4 mm (IonPac® AS9-HC) analytical column and 4 mm (AG-9-HC) guard column. An 18-min isocratic elution with 9 mM sodium bicarbonate was used for all chromatographic separations. The original method was modified in that bromide was detected with an ED 40 electrochemical detector working in conductivity mode [19]. The results were corrected for ECF as follows

$$ECF = \text{bromide (dose)} \times 0.90 \times 0.95 \times 0.94 \quad (2)$$

where 0.90 is the correction for nonextracellular distribution of bromide, 0.95 is the Donnan equilibrium factor and 0.94 is the correction of water in the plasma [20].

4.3. Statistics

In our laboratory the analytical variabilities of TBW_d, ECF_{br} are ≤1.5% and ≤1.3%, respectively. The coefficient of variation of the Hologic QDR 4500 A DXA instrument is <1.5%, one hour apart and <2.2%, 1-week apart [21]. The output of the SFB7 (TBW, ECF, and FM) was compared with the deuterium and bromide space by single correlation and Bland–Altman analysis. Anthropometric data and body compartments as assessed by gold-standard methods and by SFB7 are given in Table 1. For the prediction of TBW, ECF and FM we performed partial correlation analyses of anthropometric variables, Ht^2/R_{zero} and Ht^2/R_{inf} using a stepwise backward propagation procedure using only significant parameters ($p < 0.05$). The parameters of the prediction equations were determined by regression analysis as suggested by Moissl et al. [22]: "Since the estimated parameters naturally depend on the available data, especially for small datasets, we used the concept of cross validation to reduce the uncertainty of the estimation. This concept involved repeated random splitting of the data into a training and a generalization group." Both groups had an equal number of 16 healthy subjects and 16 patients ensuring that there was no bias toward a subpopulation. The splitting and estimation was carried out $n = 5000$ times and the mean parameters were chosen for the final prediction equation after making sure that the parameters were normally distributed [23]. These equations were then applied to healthy subjects and patients. All statistical analyses were performed with the SPSS statistics program (SPSS 18.0, IBM Corporation; NY, United States).

5. Results

5.1. TBW, ECF and FM measurements by the SFB7 (impedimed)

Descriptive statistics and the percentage differences between the gold standard methods and the SFB7 output are shown in Table 1. The correlation between the measurements of deuterium dilution and TBW as assessed by DXA in healthy subjects was $r = 0.99$, $p < 0.001$ in subjects with body homeostasis disorders it was $r = 0.94$, $p < 0.001$ (Fig. 1). The correlation coefficient between TBW_d and ECF_{br} for healthy subjects was $r = 0.94$, $p < 0.001$ (Fig. 2).

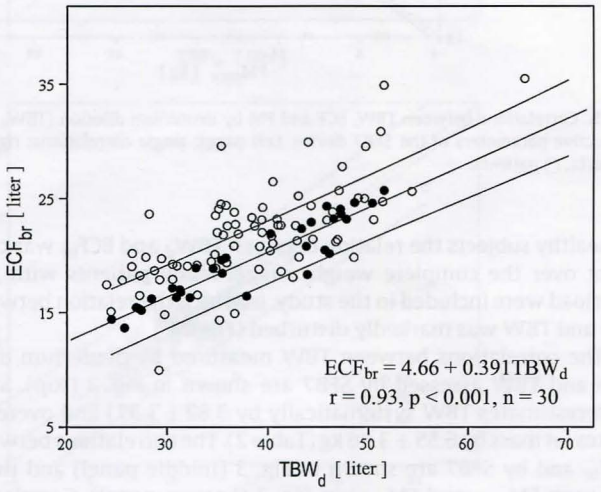

Fig. 2. Correlation between total body water by deuterium (TBW_d) and extracellular fluid volume (ECF_{br}) by sodium bromide dilution in healthy subjects and patients. ● Healthy subjects; ○ patients. Note the constant relation between both compartments in healthy subjects and the ECF overload in patients.

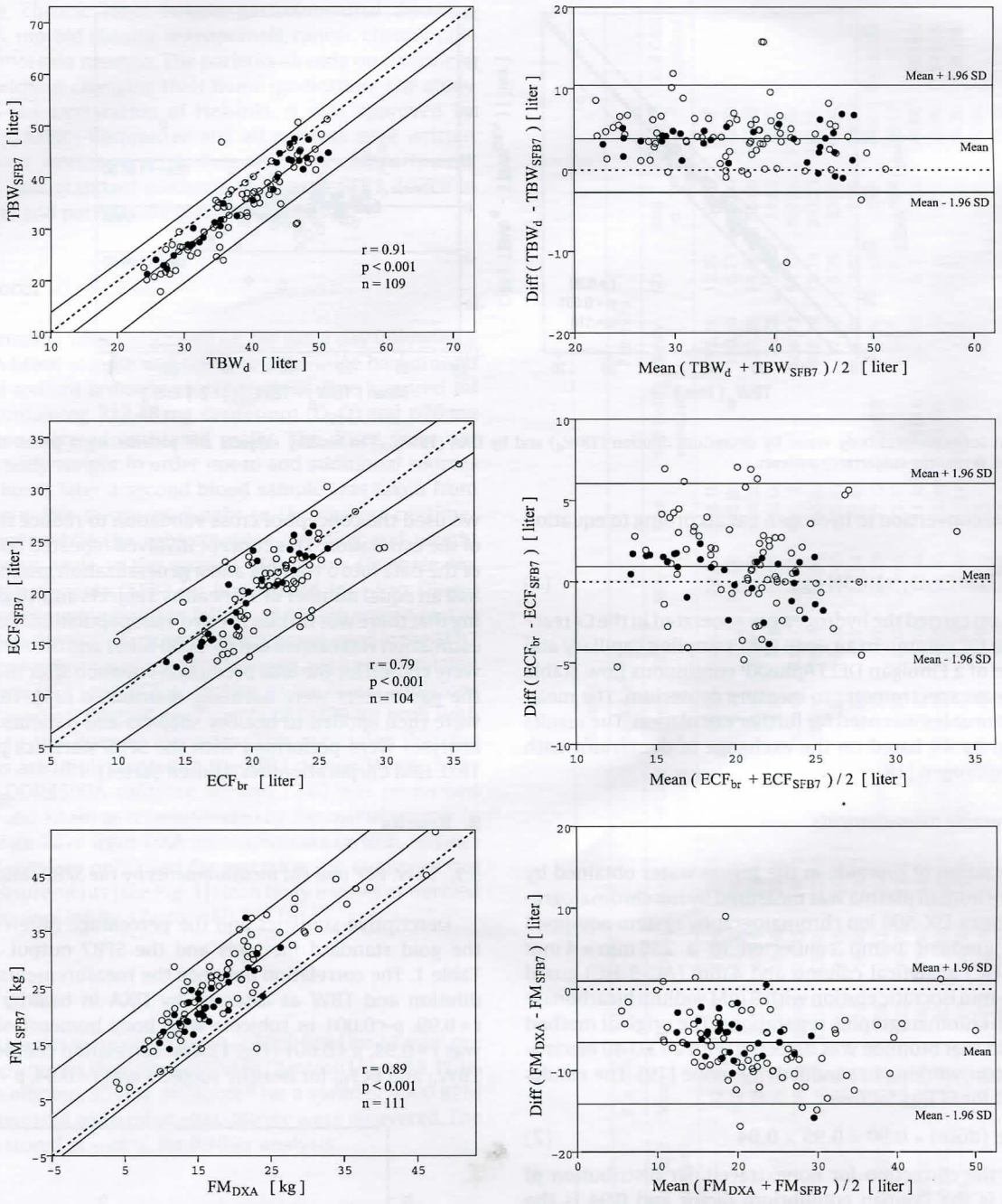

Fig. 3. Correlations between TBW, ECF and FM by deuterium dilution (TBW_d), sodium bromide dilution (ECF_{br}) and DXA (FM_{DXA}) and the output (built-in model) of the respective parameters of the SFB7 device. Left panel: single correlations; right panels: Bland–Altman plots. The lines of identity are shown as dashed lines. ● Healthy subjects; ○ patients.

In healthy subjects the relation between TBW_d and ECF_{br} was constant over the complete weight range. Many patients with ECF overload were included in the study, in which the relation between ECF and TBW was markedly disturbed (Fig. 2).

The correlations between TBW measured by deuterium dilution and TBW assessed by SFB7 are shown in Fig. 3 (top). SFB7 underestimates TBW systematically by 3.82 ± 3.37 l and overestimates fat mass by 6.55 ± 3.86 kg (Table 2). The correlations between ECF_{br} and by SFB7 are shown in Fig. 3 (middle panel) and those between FM_{DXA} and FM_{SFB7} in Fig. 3 (bottom panel). Correlation coefficients and the deviation of the estimates are shown in Table 2. The corresponding Bland–Altman analyses are shown in Fig. 3, right panel.

5.2. Contribution of anthropometry and BIS in estimating TBW, ECF and FM

In order to predict TBW_d, ECF_{br} and FM_{DXA} from anthropometry and resistance measurements, provided as raw data by SFB7, stepwise multiple-regression analysis was performed by the repeated randomization procedure. The derived equations are shown in Table 3. These equations were then used in the normal subjects and the patients to predict the body compartments. Initially, we included only the anthropometric data height (H), weight (W), sex (S), and age (A). For inclusion into the prediction equations, a 0.05 level was required (Table 3). The additional inclusion of Ht^2/R_{zero} and Ht^2/R_{inf} [12] did not improve the coefficients of determination

Table 2
Comparison of correlation coefficients and SDs of prediction equations.

	Statistics of the total sample					
	r	R^2	t-Test†	Mean difference	SD	n
TBW_{SFB7} [L]	0.91*	0.83		3.82	3.37	109
pred TBW_d by Anthropometry [L]	0.88*	0.77		−0.24	3.67	113
pred TBW_d by BIS [L]	0.93*	0.86	$p = 0.31$	−0.90	2.97	110
ECF_{SFB7} [L]	0.79*	0.62		0.93	2.62	104
pred ECF_{br} by Anthropometry [L]	0.66*	0.44		−0.16	2.93	105
pred ECF_{br} by BIS [L]	0.76*	0.58	$p = 0.34$	−0.14	2.44	103
FM_{SFB7} [kg]	0.89*	0.79		−6.55	3.86	111
pred FM_{dxa} by Anthropometry [L]	0.88*	0.77		0.79	3.47	114
pred FM_{dxa} by BIS [L]	0.87*	0.76	$p = 0.07$	−0.70	3.75	110
pred FM_{dxa} by R_{50} [L]	0.90*	0.81	$p = 0.44$	1.14	3.32	113

* $p < 0.001$; % BWt = percent body weight.

† p-values refer to unpaired Student's t-tests comparing differences of accuracy between anthropometrical equations with BIS and R_{50} equations, respectively Mean difference = gold standard method − prediction.

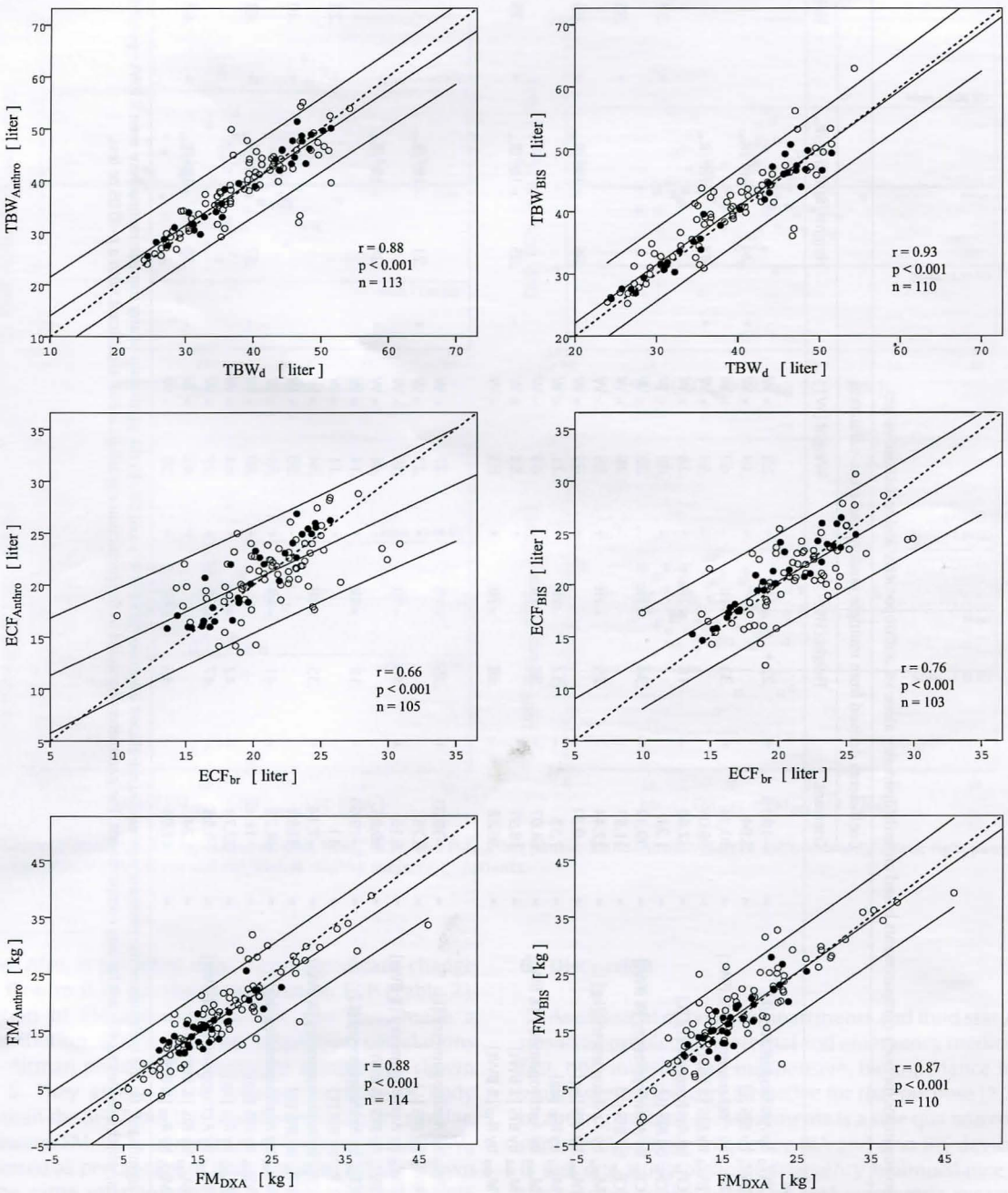

Fig. 4. Correlations between TBW, ECF and FM by deuterium dilution (TBW_d), sodium bromide dilution (ECF_{br}) and DXA (FM_{DXA}) and the predicted values by anthropometry (left panel) and anthropometry including Ht^2/Rzero and Ht^2/Rinf, respectively (right panel). The lines of identity are shown as dashed lines. ● Healthy subjects; ○ patients.

Table 3
Prediction equations for body compartments based on anthropometry alone vs. anthropometry and resistance values.

	Coefficients derived from multiple randomized cross-validation																	
	Constant		Height (Ht)			Weight (W)			Height^2/Rinf (Ht^2/R_∞)			Height^2/Rzero(Ht^2/R_0)			R_{50} (R_{50})			r
Females																		
pred TBW_d by Anthropometry [L]	= −19.18	+	.22	× Ht	+	.23	× W											.88
pred TBW_d by BIS [L]	= 5.94				+	.19	× W	+	.24	× Ht^2/R_∞								.89
pred TBW_d by Anthropometry [% BWt]	= 20.34	+	.33	× Ht	−	.40	× W											.86
pred TBW_d by BIS [% BWt]	= 59.09				−	.52	× W	+	.43	× Ht^2/R_∞								.85
pred ECF_{br} by Anthropometry [L]	= 32.90	−	.16	× Ht	+	.18	× W											.85
pred ECF_{br} by BIS [L]	= 1.24				+	.09	× W				+	.28	× Ht^2/R_0					.89
pred ECF_{br} by Anthropometry [% BWt]	= 99.32	−	.34	× Ht	−	.22	× W											.77
pred ECF_{br} by BIS [%]	= 31.57				−	.46	× W				+	.65	× Ht^2/R_0					.83
pred FM_{dxa} by Anthropometry [kg]	= 22.44	−	.25	× Ht	+	.59	× W											.94
pred FM_{dxa} by BIS [kg]	= −9.81				+	.65	× W	−	.66	× Ht^2/R_∞	+	.65	× Ht^2/R_0					.98
pred FM_{dxa} by R_{50} [kg]	= 7.53	−	.32	× Ht	+	.71	× W							+	.03	× R_{50}		.96
pred FM_{dxa} by Anthropometry [% BWt]	= 64.07	−	.38	× Ht	+	.43	× W											.82
pred FM_{dxa} by BIS [% BWt]	= 18.97				+	.53	× W	−	.95	× Ht^2/R_∞	+	.79	× Ht^2/R_0					.95
pred FM_{dxa} by R_{50} [% BWt]	= 36.88	−	.48	× Ht	+	.63	× W							+	.06	× R_{50}		.89
Males																		
pred TBW_d by Anthropometry [L]	= −38.81	+	.35	× Ht	+	.27	× W											.90
pred TBW_d by BIS [L]	= 8.75				+	.23	× W	+	.21	× Ht^2/R_∞								.91
pred TBW_d by Anthropometry [% BWt]	= 6.15	+	.46	× Ht	−	.39	× W											.80
pred TBW_d by BIS [% BWt]	= 69.85				−	.48	× W	+	.28	× Ht^2/R_∞								.84
pred ECF_{br} by Anthropometry [L]	= −19.65	+	.18	× Ht	+	.14	× W											.85
pred ECF_{br} by BIS [L]	= .11				+	.11	× W				+	.24	× Ht^2/R_0					.87
pred ECF_{br} by Anthropometry [% BWt]	= −7.14	+	.35	× Ht	−	.34	× W											.81
pred ECF_{br} by BIS [%]	= 33.55				−	.36	× W				+	.40	× Ht^2/R_0					.84
pred FM_{dxa} by Anthropometry [kg]	= 48.29	−	.41	× Ht	+	.51	× W											.89
pred FM_{dxa} by BIS [kg]	= −18.42				+	.60	× W	−	.57	× Ht^2/R_∞	+	.62	× Ht^2/R_0					.93
pred FM_{dxa} by R_{50} [kg]	= 22.79	−	.42	× Ht	+	.64	× W							+	.04	× R_{50}		.93
pred FM_{dxa} by Anthropometry [% BWt]	= 75.56	−	.47	× Ht	+	.37	× W											.77
pred FM_{dxa} by BIS [% BWt]	= 1.24				+	.42	× W	−	.59	× Ht^2/R_∞	+	.62	× Ht^2/R_0					.85
pred FM_{dxa} by R_{50} [% BWt]	= 47.88	−	.49	× Ht	+	.50	× W							+	.04	× R_{50}		.86

NS = not significant; by the inclusion of the resistance values no significant improvements of R^2s and SDs in the prediction of any compartments were seen % BWt = percent body weight; it was attempted to include age into the equations, which was not significant for any compartment. The parameters included in the prediction equations are all significant with a $p < 0.05$ or less.

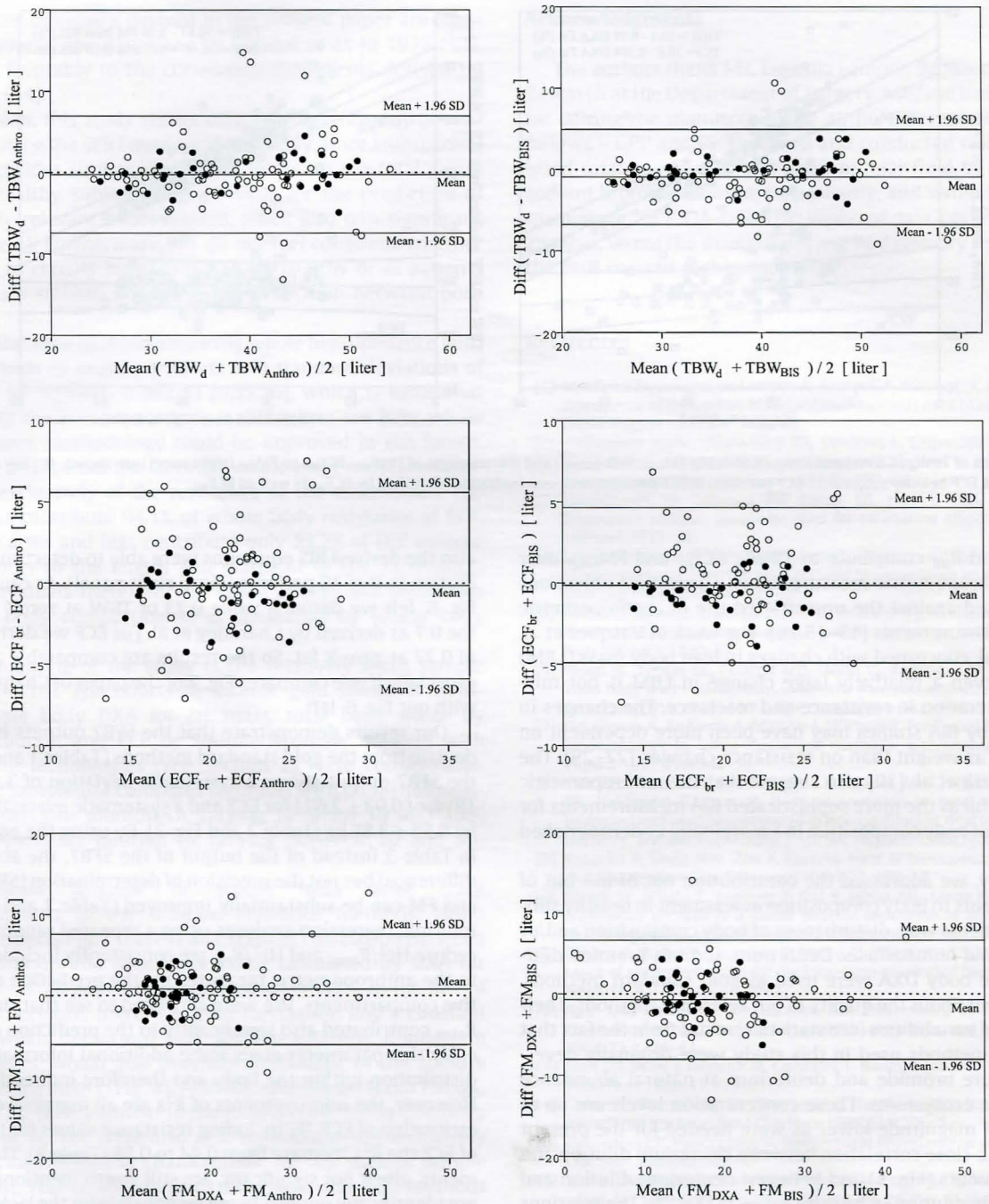

Fig. 5. Agreement between TBW, ECF and FM estimates with TBW_d, ECF_{br} and FM_{DXA} left panels: Bland–Altman plots of anthropometry alone; right panels: Bland–Altman plots of anthropometry including Ht^2/Rzero and Ht^2/Rinf. ● Healthy subjects; ○ patients.

of TBW and FM. Also, it provided only a non-significant change of total r from 0.66 to 0.76 for the determination ECF (Table 2). For the prediction of FM not only R_{inf} but also R_{zero} made a significant contribution (Table 3). The respective correlations and the Bland–Altman plots in the complete sample are shown in Figs. 4 and 5. They give a more accurate estimate of body compartments than the SFB7, but the SDs of prediction are similar. The relation between FM_{DXA} as percentage of bodyweight to TBW_d and ECF_{br} expressed as percentage of body weight [24] are shown in Fig. 6 and the same relations for the values predicted by BIS are shown in Fig. 6 right. 9 of 16 patients with ECF overload as identified by ECF_{br} could also be identified by BIS.

6. Discussion

Assessment of body compartments and fluid status is one of the unsolved problems in internal and emergency medicine [25]. Being fast, non-invasive and inexpensive, bioimpedance measurements would seem to be very attractive for that purpose [2,3,6]. The input of anthropometric measurements is a sine qua non condition for all commercial single frequency BIA and also BIS devices [12]. There is just one study of single frequency bioimpedance that assessed the relative contributions of anthropometric measurements and impedance measurements to estimation of body composition [13]. We are not aware of any investigation as to whether and to what

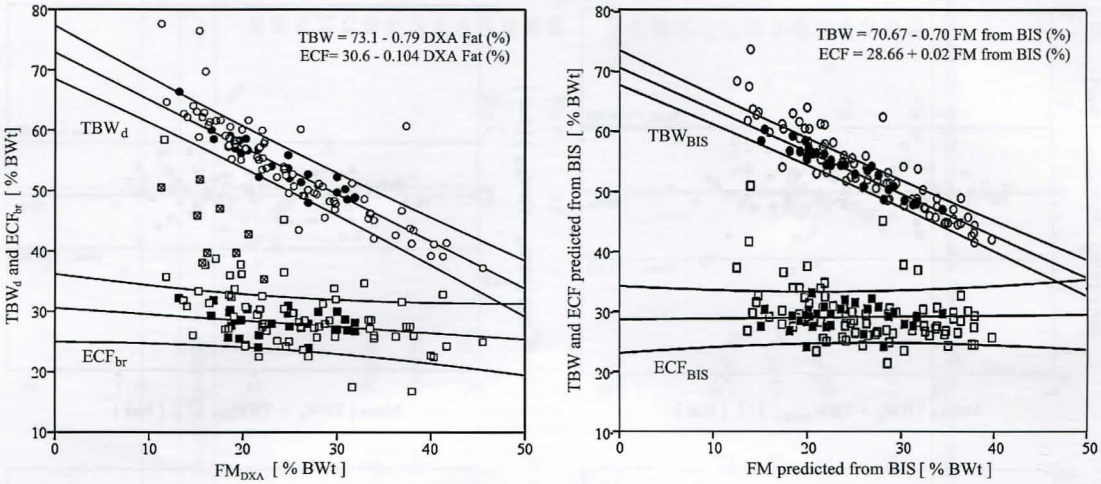

Fig. 6. The relations of TBW_d (% BWt) and ECF_{br} (% BWt) to FM_{DXA} (left panel) and the relations of TBW_{BIS}, ECF_{BIS} to FM_{BIS} (right panel) are shown. ● TBW healthy subjects; ○ TBW patients; ■ ECF healthy subjects; □ ECF patients; ⊠ ECF patients with overhydration identified by ECF_{br} as well as ECF_{BIS}.

extent R_{zero} and R_{inf} contribute to TBW_d, ECF_{br} and FM_{DXA} over and above anthropometric measurements. Theoretical objections have been raised against the unrestricted use of anthropometric data for BIA measurements [13–15,26]. The work of Vasquez et al. and Forbes et al. concerned with changes in lean body mass (LBM) showed that even a relatively large change in LBM is not mirrored by an alteration in resistance and reactance. The changes in LBM reported by BIA studies may have been more dependent on variables such as weight than on resistance changes [27–29]. The results of Conlisk et al. [30] also suggest that the anthropometric data are as useful as the more sophisticated BIA measurements for the prediction of body composition in a marginally undernourished population.

In our study, we addressed the contribution not of BIA but of BIS measurements to body composition assessment in healthy subjects and in patients with disturbances of body composition and/or disorders of fluid homeostasis. Deuterium, sodium bromide dilution and whole body DXA were used as gold standard methods. We are confident about the quality of our reference methods. Their small analytical variabilities (see statistics) result from the fact that the analytical methods used in this study were originally developed to measure bromide and deuterium at natural abundance level in aquatic ecosystems. These concentration levels are up to three orders of magnitude lower as were needed for the present study. There is a close correlation between deuterium dilution and DXA measurements (Fig. 1) and between deuterium dilution and sodium bromide dilution in healthy subjects (Fig. 2). The relations between FM_{DXA} and TBW_d, ECF_{br}, respectively, all expressed as percentage of body weight are very comparable to the results obtained by Chamney et al. [24]. Also, we were able to detect ECF accumulation sensitively in patients with edema, e.g. in chronic heart failure, by bromide dilution (Fig. 2). We used the DXA method only for estimating FM, since the use of DXA for estimating TBW requires an assumption of a constant hydration of FFM [31].

In order to be useful in clinical medicine, values for all body compartments should be given as a percentage of body weight [32], since clinicians are not so much interested in body size and geometry which are provided by absolute values of TBW, ECF and FM. Only when body compartments are expressed as percentage of body weight, it is possible to assess the degree of obesity, malnutrition or to discriminate between over- and under-hydration. As can be seen in Fig. 6, left panel, the plot proposed by Chamney et al. [24], ECF_{br} is able to detect the overhydration characteristic for a large proportion of our patient sample. It is interesting to see that also the derived BIS equations were able to detect the ECF overhydration in 9 of 16 patients (Fig. 6 right panel). As can be seen from Fig. 6, left we derive a value 0.73 of TBW at zero% fat instead of the 0.7 as derived by Chamney et al. For ECF we derive 0.3 instead of 0.27 at zero % fat. So the results are comparable and plausible, especially if one compares Fig. 2 of the paper of Chamney et al. [24] with our Fig. 6, left.

Our results demonstrate that the SFB7 outputs except for ECF deviate from the gold standard methods (Tables 1 and 2, Fig. 3). So, the SFB7 output shows a systematic deviation of 3.82 ± 3.37 l for TBW, of 0.93 ± 2.62 l for ECF and a systematic overestimation of FM by 6.55 ± 3.86 kg (Table 2 and Fig. 3). By using the equations given in Table 3 instead of the output of the SFB7, the accuracy (mean difference) but not the precision of determination (SD) of TBW, ECF and FM can be substantially improved (Table 2 and Fig. 3). In the multiple regression analyses using a repeated randomization procedure Ht^2/R_{zero} and Ht^2/R_{inf} are consistently included in addition to the anthropometric measures for the prediction of the respective compartments. We were surprised to see that the inclusion of R_{zero} contributed also significantly to the prediction of FM. Apparently this parameter gives some additional information on water distribution within the body and therefore indirectly also on FM. However, the improvements of R^2s are all marginal except for the estimation of ECF. By including resistance values for the prediction of ECF the R^2s increase from 0.44 to 0.58 (Table 2). These improvements, albeit not significant, are still worth mentioning. We could not identify any outliers being responsible for the lack of significant improvement of the regression equations (Figs. 4 and 5). It appears that the relatively wide SDs for the estimation of TBW, ECF and FM are not a problem of non-linearity. Even though the new equations give smaller mean differences of body compartments to gold standard methods (Table 2), the SDs of prediction shown in Fig. 5 are so large that a meaningful assessment on an individual basis is not feasible, either for healthy subjects or for patients with disturbed body homeostasis. This implies that some important information for the prediction of compartments is missing. This might be the variable relation of the lengths and diameters of extremities and trunk in the individual subjects, which is believed erroneously to be obtainable by the individual heights of the subjects. In whole body impedance spectroscopy this relation is erroneously assumed to be constant. We are confident about the generalizability of the derived equations since we used a multiple ($n = 5000$) repeated randomization procedure of which the significant mean coefficients were used (Table 2). The correlation coefficients of the

anthropometric equations derived in the present paper are comparable to those of the equations by Skrabal et al. in 1973 [33], but compare favorably to the correlation coefficients derived by Watson et al. [34].

In conclusion, this study shows only non-significant improvements of BIS using the SFB7 over anthropometry, since anthropometry alone provides similar confidence limits for the total group as well for healthy subjects (Fig. 5). Only for the prediction of ECF a possibly relevant improvement, albeit also non-significant is seen (Table 2). Furthermore, we do not feel confident whether it is justified to classify humans simply as healthy or as patients since there is a smooth and subjective transition between both states.

In most validation studies comparing whole bioimpedance with dilution methods in healthy subjects two standard deviations of the estimate lie between 3 and 5 l [6,35,36], which is somewhat comparable to the present study. It is difficult to see how whole body impedance methodology could be improved in the future. The problem arises from the fact that whole body resistance consists nearly exclusively of the resistance of the extremities. The arms and legs contribute 94.1% of whole body resistance of ECF, although the arms and legs contribute only 52.2% of ECF volume [37]. Differences in the length and diameter of the extremities between individuals therefore will cause a large and erroneous variation of body compartments as measured by whole body impedance.

One shortcoming of the study is the relatively small sample size (n total = 116). The efforts to include gold standard methods like whole body DXA for fat mass, total body water by deuterium dilution and ECF by sodium bromide dilution with very elaborate technologies (see Section 2) were considerable. However, the sample size compares favorably with published studies using deuterium dilution (n varying between 10 and 139) and sodium bromide dilution (n varying between 10 and 90) [5,12].

A significant amount of scatter may arise from errors/assumptions in the dilution reference methods. However, if one inspects Fig. 1 there is an excellent correlation between deuterium dilution and total body water calculated by DXA for healthy subjects, all points being located on or close to the line of identity. Furthermore, there is a good correlation and a constant relation between deuterium and sodium bromide dilution in healthy subjects, which also provides confidence in the sodium bromide measurements (Fig. 2). Also the relation between FM_{DXA}, TBW_d and ECF_{br} is very similar to the results reported by Chamney et al. [24] (Fig. 6). We were also able to demonstrate sensitively the ECF overload, which is characteristic for our patient sample (Figs. 2 and 6). This all ascertains that our gold standard methods were carefully executed.

Competing interests

None declared.

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Ethical approval

Ethical Aproval was given by the Ethical Committee, Krankenhaus Barmherzige Brüder, Teaching Hospital University Graz, Reference: "Messung der Körperzusammensetzung mit Hilfe der Impedanzspektroskopie".

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