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New equations for estimating body cell mass from bioimpedance parallel models in healthy older Germans

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Dittmar, Manuela, and Helmut Reber. New equations for estimating body cell mass from bioimpedance parallel models in healthy older Germans. *Am J Physiol Endocrinol Metab* 281: E1005–E1014, 2001.—The objectives of this study were to assess for elderly Germans the validity of existing equations for predicting body cell mass (BCM) and to develop from single- and multifrequency bioimpedance (SFBIA, MFBIA) models new prediction equations. In a data-splitting approach, validation and cross-validation were performed in 160 healthy elderly (60- to 90-yr) subjects. BCM was determined using a tetrapolar bioimpedance analyzer (800 μ A; 4 fixed frequencies: 1, 5, 50, and 100 kHz; electrodes placed to hand, wrist, ankle, and foot) and whole body ⁴⁰K counting as a reference method. New prediction equations were derived by multiple stepwise regression analysis. The Bland-Altman procedure was used for methods comparison. Relative to whole body counting, the manufacturer's equation overestimated BCM by 9% in men ($P < 0.0001$, paired t -test) and 4% in women ($P = 0.002$). Compared with the manufacturer's equation, the newly derived equations ($r = 0.92$, RMSE = 6–9%) improved accuracy (pure error = 13 vs. 7–8%) and reduced bias and limits of agreement. SFBIA and MFBIA equations did not differ in precision or accuracy. We conclude that the newly derived equations improved BCM estimates in the elderly compared with existing equations. There was no advantage of MFBIA over SFBIA equations.

body composition; whole body potassium-40 counting; validation; cross-validation; European ancestry

THE HUMAN BODY MASS can be partitioned into two main compartments, the fat-free mass and the fat mass. The fat-free mass comprises the cellular mass, bone mass, and extracellular water. The body cell mass (BCM) can be defined as the fat-free mass without bone mineral mass and extracellular water. The concept of BCM as the sum of oxygen-exchanging, glucose-oxidizing, work-performing, potassium-rich cellular components of the human body has been formulated by Moore et al. (31). BCM is the most metabolically active human body compartment, whose main fractions are the cellular components of muscles and viscera. It comprises those tissues that are most likely to be affected by physical activity, nutrition, or disease. The assessment and quantification of BCM is particularly important in el-

derly subjects, because BCM decreases in the course of natural aging processes (3, 11), primarily due to a loss in skeletal muscle mass. Because the amount of BCM decline is closely related to immobility, morbidity, disease, and mortality (37, 42), the accurate assessment of BCM has considerable clinical relevance.

For this reason, valid and reliable techniques for determining BCM are needed. BCM is considered difficult to quantify. Commonly used methods are the counting of naturally occurring ⁴⁰K in a whole body counter, the determination of total exchangeable potassium by isotopic dilution techniques with the use of the exchangeable sodium-to-potassium ratio (Na_e/K_e) (43), and, recently, bioelectrical impedance analysis (BIA).

Whole body counting of ⁴⁰K is considered a criterion method for measuring BCM (34). This method enables by noninvasive nuclear technique the in vivo determination of the naturally occurring radioactive isotope ⁴⁰K in the human body. Because ⁴⁰K is 0.012% of natural potassium, it provides the value of total body potassium (TBK). Potassium is almost exclusively an intracellular cation (95%), found chiefly in muscle and viscera (hence, essentially not found in fat, bone, or extracellular water), so that the TBK measurement serves as an index of BCM. However, this method is not suited for epidemiological and clinical studies, because access to whole body counters is limited, specialized teams are required, and measurement is sophisticated and costly.

In contrast, the new, noninvasive BIA is well suited for determining BCM in larger population groups, because the relatively inexpensive equipment is portable, simple, and quick to operate, and the measurement has little interobserver error, requires minimal cooperation from the subject, and can be done at bedside. This method is based on the principle that various tissues have different conductive and resistive properties at different frequencies of an electrical current (28). Bioimpedance analyzers apply a low-level, 500- to 800- μ A alternating current at one or several frequencies to the human body and measure the impedance of the body to the electrical flow. The impedance of the body is composed of two components, 1) the resistance of the tis-

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sues, which is proportional to the fluid volume in the body, and 2) the reactance, which is the reciprocal of the capacitance of cell membranes, tissue interfaces, and nonionic tissues (21). Most single-frequency (SF-)BIA analyzers operate at a fixed frequency of 50 kHz, it being the frequency of maximal reactance for most muscle tissues (41), and provide separate measurements of resistance and reactance. In contrast, multi-frequency (MF)BIA analyzers work at several fixed frequencies or across a spectrum of frequencies (bioelectrical impedance spectroscopy) and are based on the principle that low-frequency impedance measures extracellular fluid whereas high-frequency impedance measures both extra- and intracellular fluids.

BIA predicts BCM from the measured resistance and reactance data by use of equations that are based on specific models (23, 27). The existing equations and the underlying assumptions were developed primarily from younger and middle-aged population groups and, therefore, might not be applicable to elderly groups. Consequently, there is a need to test the validity of the existing prediction equations for their applicability to the elderly and, if necessary, to develop new age-specific equations. Deurenberg et al. (15) found that prediction equations that were developed in younger populations overestimated fat-free mass in the elderly by ~6 kg (8% of body wt). Bussolotto et al. (8) reported that the fat-free mass hydration variability in the elderly might be the principal variable that explains errors obtained in fat-free mass estimation by BIA equations in the elderly, emphasizing the need for more validation studies. So far, age-specific predictive BIA equations for elderly groups have been developed for estimating fat-free mass (2, 15, 30, 38), whereas validation studies for estimating BCM are still, to the authors' knowledge, missing.

The primary objectives of this study were 1) to evaluate in relatively healthy elderly men and women the accuracy of existing prediction equations for estimating BCM from BIA and 2) to develop new population-, age-, and sex-specific prediction equations for estimating BCM from SF- and MFBIA models by use of whole body counting of ^{40}K as a reference method.

STUDY POPULATION AND METHODS

Study Population

The elderly participants of this study were recruited by M. Dittmar from local community centers and associations for elderly people in the area of Mainz from October 1998 to July 1999. Their health status was evaluated by means of a medical questionnaire and by their private physicians. Their nutritional status was determined using a standardized food record based on a 7-day food diary. Subjects were selected for this study if they met entry criteria as follows: 1) no amputations, 2) no diseases and medications that affect body composition and potassium homeostasis, 3) no diarrhea or edema, 4) no current taking of diuretics, laxatives, or oral potassium supplements, 5) no alcohol abuse, 6) no malnutrition, 7) no adiposity [body mass index (BMI) <30 kg/m²]. Obese subjects were excluded because on the one hand, fat tissue tends to absorb γ -rays to a small extent so that ^{40}K

emission would be underestimated (12) and as a consequence BCM would be underestimated, and on the other hand, BIA overestimates fat-free mass, and thereby BCM, in obese subjects (3, 40). Of the 192 volunteers that met the entry criteria, 31 refused to participate in whole body counting of naturally occurring ^{40}K because of claustrophobia. There was no statistically significant difference in weight, height, BMI, and physical activity level of the elderly who agreed to participate compared with those who decided not to participate in the study. Finally, 160 apparently healthy, free-living, retired men ($n = 81$) and women ($n = 79$) of German ancestry, aged 60–90 yr, volunteered to participate in this study. Data on their physical activity level and dietary potassium intake were published in an earlier study (17). One of the original 161 participants who entered that study was excluded from this report because of a missing bioimpedance measurement. Written informed consent was obtained from each participant before investigation. The study protocol was approved by the ethics committee of the Medical Association of Rhineland-Palatinate.

Measurement Methods

Study design. To validate BIA in the elderly group, all subjects had BIA and whole body ^{40}K counting in a set order on the same day in the morning after an overnight fast of ≥ 12 h. Subjects were instructed to refrain from ingesting alcohol for 48 h and from strenuous physical activity for 24 h preceding the testing day to minimize perturbation of body fluid.

Anthropometry. Height and weight were measured following the technique of Martin (see Ref. 26). Standing height was measured, with subjects unshod and wearing light indoor clothes, to the nearest 0.01 m, by means of an anthropometer. Weight was determined on a calibrated electronic scale accurate to 0.1 kg. BMI was calculated as body weight divided by height squared (kg/m²).

Whole body counting of ^{40}K . TBK was determined by whole body counting of the radioactive isotope ^{40}K by H. Reber at the Department of Nuclear Medicine, University Hospital, Mainz. The ^{40}K activity was measured in a counting chamber with 33-cm-thick barite-concrete outer walls and 15-cm-thick steel inner walls from pre-World War II battleships; inside dimensions of the chamber are 1.2 m wide \times 1.2 m high \times 2.2 m long. The whole body counter is a fixed-array counter consisting of 12 NaI (T1) detectors mounted in pairs below the measuring couch, where the subject lies still in a supine position for 10-min counting time in the enclosed counting chamber after removal of eyeglasses, jewelry, and watches. Voice communication with the subject and ventilation are provided in the chamber. Analysis of the measuring data was computer based. TBK was calculated from the background-corrected ^{40}K counts as $\text{TBK (g)} = \text{Cf}_i \times ^{40}\text{K counts}$, where Cf_i is the specific calibration factor for the geometry of the whole body counter. For calibration, a homogeneous anthropomorphically shaped phantom filled with a known concentration of potassium chloride solution of known composition was used. The relative counting error of the TBK measurement, governed by the ^{40}K counts, is 2.1%. BCM was calculated from TBK by use of the formula of Moore et al. (31) as $\text{BCM (kg)} = 0.00833 \times \text{TBK (mmol)}$. It has been shown that the potassium concentration in both the BCM and the intracellular compartment is relatively constant in normal subjects, independent of age and sex (11).

MFBIA. BCM was determined by M. Dittmar at the Institute of Anthropology, University of Mainz, by using a tetrapolar whole body multifrequency bioelectrical impedance analyzer (BIA 2000-M, Data Input, Hofheim, Germany) that

is derived from the RJL analyzers. The device was calibrated each morning by means of a standard resistor supplied by the manufacturer. Measurements were performed in the subjects, within 30 min of voiding, in a supine position on a flat, nonconductive couch, with their limbs abducted from the trunk. Glasses, jewelry, and watches were removed. A tetrapolar arrangement of gel electrodes was placed at defined anatomical sites to one hand, wrist, ankle, and foot of each participant, following the instructions of the manufacturer. The minimal distance between the electrodes was 5 cm to avoid interactions between source and sensor electrodes. The BIA 2000-M analyzer applies an 800- μ A alternating current and measures resistance (R , Ω), reactance (Xc , Ω), impedance (Z , Ω), and phase angle (PA, $^\circ$) at four fixed frequencies (1, 5, 50, 100 kHz). At 1 kHz, only R and Z are measured, because Xc always equals zero. The precision error of the analyzer for the measurement of R is $\pm 0.5\%$, for Xc $\pm 2.0\%$, and for the PA $\pm 0.5^\circ$. R and Xc are the two vectors of the impedance that the human body opposes to the applied alternating electrical current, where Z is defined as $Z^2 = R^2 + Xc^2$. To differentiate between R and Xc , the BIA 2000-M device uses phase-sensitive electronics. Capacitors in the circuit of the alternating current cause a time shift, where the maximum of the voltage lags behind the maximum of the current. Because alternating current is sinusoidal, the phase shift is measured in degrees and is called the PA. The PA was calculated as a function of the ratio of the resistance of body fluid volumes to the reactance (capacitance) of cell membranes as $PA = \arctan(Xc/R)^{0.5} \times (180/\pi)$, with $\pi = 3.1416$ (19). Studies have shown that the PA at 50 kHz (1) enables discrimination between intra- and extracellular current distribution (1, 20) and (2) is proportional to the amount of BCM in the lean body mass (LBM) (19). Therefore, the manufacturer's analysis software (Body software version 4) calculated BCM as $BCM(\text{kg}) = LBM \times \ln(PA_{50}) \times 0.29$. The LBM was calculated from total body water (TBW) by assuming 73% hydration of LBM (33) as $LBM = TBW/0.732$. TBW was calculated from the resistivity index Ht^2/R at 50 kHz and weight using the published bioimpedance equation of Kushner and Schoeller (29), modified by the manufacturer's empirical constants.

Bioimpedance Models

In this study, new equations for predicting BCM were developed from SFBIA and MFBIA models. Following De Lorenzo et al. (13), BCM was considered a concept that could be defined by intracellular water (ICW), because impedance is more closely related to the volume of intracellular ion-containing water fluids than to the corresponding body masses. For predicting ICW, one SFBIA and four MFBIA models were considered on the basis of parallel models described by Kotler et al. (27) and Gudivaka et al. (23), respectively. The SFBIA model was included to evaluate whether MFBIA predicts BCM better than SFBIA.

Single-frequency 50-kHz parallel model. This model uses resistance (R) and reactance (Xc) as measured at 50 kHz. The choice of predicting BCM on the basis of Xc at 50 kHz was performed in view of the results of Kotler et al. (27) and Gudivaka et al. (23). A parallel model was used because the 1997 follow-up to the 1994 Technology Conference of the National Institutes of Health (NIH) on the assessment of bioimpedance analysis technology for body composition measurement has stated that the parallel SFBIA model provides more acceptable estimates of BCM than the serial model (20). The parallel model is based on the assumption that the human body reacts as if the resistance-capacitance circuits were arranged in parallel (32). For this, the Xc value at 50

kHz is converted to its parallel value (Xcp) as $Xcp_{50} = Xc_{50} + (R_{50}^2/Xc_{50})$. The parallel reactance is linearly related to ICW as

$$ICW_{50} = mHt^2/Xcp_{50} + c \quad (1)$$

where m and c are constants derived from linear regression analysis (m is slope and c is intercept), and Ht means height, which estimates circuit length.

Multifrequency parallel models: 1/50, 1/100, 5/50, and 5/100 kHz. Multifrequency models are based on the observation that the injected current passes almost exclusively through extracellular water at low frequencies < 10 kHz, because it cannot pass the cell membranes, and through extra- and intracellular water at high frequencies > 10 kHz. In this study, the 1-, 5-, 50-, and 100-kHz frequencies were used, because these four frequencies were available on the BIA 2000-M bioimpedance analyzer. Parallel models were applied because Gudivaka et al. (23) stated that these models consider "that the specific resistivities (resistance per unit length of a conductor with a cross-sectional area of 1 cm^2) are quite different for extra- and intracellular fluids and that the resistance should be segregated into the extracellular (R_{ec}) and intracellular (R_{ic}) components by use of a parallel model." Four models were evaluated in this study, all based on the models described by Gudivaka et al., where R_f is resistance at f kHz

Multifrequency 1/50-kHz parallel model

$$ICW_{1/50} = mHt^2/R_{ic1/50} + c \quad (2)$$

where $R_{ec} = R_1$ and $R_{ic} = (R_1 \times R_{50})/(R_1 - R_{50})$

Multifrequency 1/100-kHz parallel model

$$ICW_{1/100} = mHt^2/R_{ic1/100} + c \quad (3)$$

where $R_{ec} = R_1$ and $R_{ic} = (R_1 \times R_{100})/(R_1 - R_{100})$

Multifrequency-5/50-kHz parallel model

$$ICW_{5/50} = mHt^2/R_{ic5/50} + c \quad (4)$$

where $R_{ec} = R_5$ and $R_{ic} = (R_5 \times R_{50})/(R_5 - R_{50})$

Multifrequency-5/100-kHz parallel model

$$ICW_{5/100} = mHt^2/R_{ic5/100} + c \quad (5)$$

where $R_{ec} = R_5$ and $R_{ic} = (R_5 \times R_{100})/(R_5 - R_{100})$

Statistical Methods

All statistical analyses of the data were performed by M. Dittmar using the SPSS/PC software package for MS Windows, release 8.0 (SPSS, Chicago, IL). For the analyses, an α -level of 0.05 was used as the criterion for statistical significance.

Development and Cross-Validation of New Predictive Equations

New population-, age-, sex-, and device-specific equations for estimating BCM from BIA were developed following the statistical methods described by Guo and Chumlea (24). BCM (kg) was chosen as the dependent variable estimated from TBK measured by whole body counting of ^{40}K . Validation and cross-validation were performed by a computer-based data-splitting approach. The prediction equation for BCM was derived from a random subset of the study population (model-building sample, 55 men, 55 women) and was internally validated on the remaining subset (cross-validation sample, 26 men, 24 women). Multiple linear regression

analysis (stepwise method) was performed to identify the best predictors of BCM by using as independent variables the bioimpedance indexes relating to the five BIA models (Ht^2/X_{CP50} , $Ht^2/R_{ic1/50}$, $Ht^2/R_{ic1/100}$, $Ht^2/R_{ic5/50}$, $Ht^2/R_{ic5/100}$), sex (as a dummy variable; 1 man, 0 women), weight, age, and further bioimpedance measures. The assumption of normality of the response variable was tested using the Kolmogorov-Smirnov test. The homogeneity of the response variable was tested by checking residual plots for trends or patterns. Presence of a linear relationship between the response variable and each predictor variable was tested by using scatterplots. Absence of multicollinearity of the predictor variables in the equations was examined by the condition number (CN) computed for standardized residuals with the intercept included. Absence of autocorrelation was judged by the Durbin-Watson test. The coefficient of determination (r^2) was used as a measure of goodness of fit of the generated equations. The precision of the predictive equations in the model-building sample was determined using the root mean square error (RMSE) as $RMSE = [\sum(X_1 - X_2)^2/(n - p - 1)]^{0.5}$, where X_1 and X_2 are the observed and predicted BCM values for an individual, n is the number of subjects in the model-building sample, and p is the number of predictor variables. To measure the performance of the predictive equations on cross-validation, the pure error (PE) between the TBK and BIA methods in the internal cross-validation sample was calculated as $PE = [\sum(Y_1 - Y_2)^2/n]^{0.5}$, where Y_1 is BCM predicted by BIA, Y_2 is BCM observed by the TBK method, and n is the number of subjects in the internal cross-validation sample.

Measurement Methods Comparison

Student's paired t -test and the statistical technique of Bland and Altman (6) were employed to examine the degree of agreement between the TBK and BIA methods to predict

BCM. The latter technique concentrates on individual differences and calculates bias and 95% limits of agreement between the TBK and BIA measures. The use of correlation coefficients or regression analysis has been shown not to be appropriate for the comparison of measurement methods (6, 44).

RESULTS

Table 1 summarizes the anthropometric and bioimpedance characteristics, measured TBK, and predicted BCM for the entire elderly sample and the validation and cross-validation subsamples. BMI ranged for the entire sample from 19.0 to 29.9 kg/m². BIA using the manufacturer's equation significantly overestimated BCM relative to the TBK method by 2.4 kg (8.9%) in older men ($t = -8.131$, $P < 0.0001$) and by 0.8 kg (4.1%) in women ($t = -3.149$, $P = 0.002$; paired t -tests, entire sample). To reduce the bias between the BIA and TBK methods in estimating BCM, new prediction equations for estimating BCM from BIA were developed.

Development of New Predictive Equations for Estimating BCM from BIA

Selection of predictor variables for the best fitting prediction equation for estimating BCM from BIA. For each of the five bioimpedance models under consideration, a multiple stepwise linear regression analysis was performed to develop prediction equations for BCM in the model-building subsample. Strongest correlations with BCM_{TBK} were found for the SF BIA

Table 1. Descriptive characteristics of 160 German elderly study participants by sample and sex

Variable	Whole Sample		Validation Subsample		Cross-Validation Subsample	
	Men (n = 81)	Women (n = 79)	Men (n = 55)	Women (n = 55)	Men (n = 26)	Women (n = 24)
Age, yr	68.6 ± 6.12	68.8 ± 5.67	68.6 ± 5.41	68.9 ± 5.72	68.7 ± 7.53	68.6 ± 5.66
Weight, kg	75.8 ± 7.14	63.0 ± 8.33	75.6 ± 7.46	62.7 ± 8.18	76.4 ± 6.52	63.7 ± 8.80
Height, m	1.72 ± 0.06	1.60 ± 0.06	1.73 ± 0.07	1.60 ± 0.05	1.73 ± 5.93	1.60 ± 0.06
BMI, kg/m ²	25.4 ± 1.94	24.5 ± 2.71	25.3 ± 2.08	24.5 ± 2.81	25.6 ± 1.61	24.6 ± 2.52
TBK, kg	0.126 ± 0.012	0.091 ± 0.008	0.126 ± 0.012	0.091 ± 0.008	0.128 ± 0.013	0.092 ± 0.008
Resistance, Ω						
1 kHz	576.1 ± 74.0	672.6 ± 73.1	584.1 ± 72.0	677.2 ± 62.1	559.2 ± 76.7	662.1 ± 94.4
5 kHz	555.9 ± 56.0	675.2 ± 59.4	564.0 ± 54.9	680.5 ± 60.5	538.8 ± 55.6	663.2 ± 56.0
50 kHz	490.3 ± 49.9	605.8 ± 55.3	497.4 ± 49.2	610.9 ± 58.0	475.2 ± 49.0	594.1 ± 47.6
100 kHz	466.2 ± 48.1	578.5 ± 53.8	472.9 ± 47.2	583.4 ± 57.0	451.9 ± 47.5	567.0 ± 44.7
Reactance, Ω						
5 kHz	18.0 ± 5.37	23.7 ± 4.82	17.8 ± 5.74	23.5 ± 4.81	18.4 ± 4.53	24.0 ± 4.93
50 kHz	47.0 ± 6.67	53.5 ± 6.17	47.7 ± 6.75	53.7 ± 5.57	45.7 ± 6.42	53.1 ± 7.49
100 kHz	39.8 ± 5.45	46.5 ± 5.34	40.3 ± 5.53	46.7 ± 4.81	38.7 ± 5.22	45.9 ± 6.48
Impedance, Ω						
1 kHz	576.1 ± 74.0	672.6 ± 73.1	584.1 ± 72.0	677.2 ± 62.1	559.2 ± 76.7	662.1 ± 94.4
5 kHz	556.2 ± 56.1	675.7 ± 59.4	564.3 ± 54.9	680.9 ± 60.5	539.1 ± 55.7	663.6 ± 56.0
50 kHz	492.5 ± 50.1	608.2 ± 55.4	499.7 ± 49.4	613.3 ± 58.0	477.4 ± 49.1	596.5 ± 47.9
100 kHz	467.9 ± 48.2	580.3 ± 53.9	474.7 ± 47.3	585.3 ± 57.1	453.6 ± 47.6	568.9 ± 45.0
Phase angle, °						
5 kHz	1.84 ± 0.51	2.00 ± 0.39	1.79 ± 0.55	1.98 ± 0.39	1.94 ± 0.39	2.06 ± 0.39
50 kHz	5.49 ± 0.61	5.07 ± 0.51	5.48 ± 0.60	5.05 ± 0.54	5.50 ± 0.65	5.10 ± 0.45
100 kHz	4.89 ± 0.54	4.60 ± 0.44	4.88 ± 0.52	4.60 ± 0.47	4.91 ± 0.57	4.61 ± 0.40
BCM_{TBK} , kg	26.9 ± 2.56	19.4 ± 1.67	26.7 ± 2.50	19.3 ± 1.66	27.4 ± 2.67	19.6 ± 1.72
BCM_{BIAMAN} , kg	29.3 ± 3.29	20.2 ± 2.36	29.0 ± 3.10	20.1 ± 2.55	29.9 ± 3.64	20.5 ± 1.87

Values are means ± SD. BMI, body mass index; TBK, total body potassium; BCM_{TBK} , body cell mass calculated from TBK; BCM_{BIAMAN} , BCM calculated from multifrequency bioelectrical impedance analysis (MF BIA) using the manufacturer's equation.

Table 2. Comparison of the five bioimpedance models for predicting BCM (ICW) in the elderly: results of 5 separate stepwise multiple regression analyses (validation sample, n = 110)

Model No.	Predictor Variables*	r	r ²	RMSE, kg	P Value
Single-frequency model					
1	Ht ² /X _{cp50}	0.868	0.754	2.126	
	Ht ² /X _{cp50} , sex	0.915	0.838	1.736	<0.001
	Ht ² /X _{cp50} , sex, weight	0.919	0.844	1.711	<0.001
Multifrequency models					
2	Ht ² /R _{ic1/50} †	0.420	0.176	3.892	
3	Ht ² /R _{ic1/100} †	0.507	0.257	3.700	
4	Ht ² /R _{ic5/50}	0.862	0.743	2.173	
5	Ht ² /R _{ic5/50} , sex	0.915	0.838	1.734	<0.001
	Ht ² /R _{ic5/100}	0.867	0.751	2.140	
	Ht ² /R _{ic5/100} , sex	0.916	0.839	1.729	<0.001

ICW, intracellular water; r, multiple; r², coefficient of determination; R, resistance; X_c, reactance; X_{cp}, parallel X_c; RMSE, root mean square error; Ht², height squared; X_{cp50} = X_{c50} + (R₅₀²/X_{c50}); R_{ic1/50} = (R₁ × R₅₀)/(R₁ - R₅₀); R_{ic1/100} = (R₁ × R₁₀₀)/(R₁ - R₁₀₀); R_{ic5/50} = (R₅ × R₅₀)/(R₅ - R₅₀); R_{ic5/100} = (R₅ × R₁₀₀)/(R₅ - R₁₀₀). *Order of independent variables entered in the regression analyses: bioimpedance index, sex, age, weight, further bioimpedance variables. †When sex (r = 0.869, r² = 0.755, RMSE = 2.1 kg) was included in the regression analyses, the indexes Ht²/R_{ic1/50} and Ht²/R_{ic1/100} were removed by the stepwise method.

index Ht²/X_{cp50} (model 1) and for the MFBIA indexes Ht²/R_{ic5/50} (model 4) and Ht²/R_{ic5/100} (model 5), whereas the MFBIA indexes Ht²/R_{ic1/50} (model 2) and Ht²/R_{ic1/100} (model 3) displayed only weak correlations (Table 2). In addition, sex was strongly correlated with BCM_{TBK}. Separate stepwise regression analyses showed that the inclusion of sex as a predictor variable improved significance for the indexes Ht²/X_{cp50}, Ht²/R_{ic5/50}, and Ht²/R_{ic5/100}, whereas the indexes Ht²/R_{ic1/50} and Ht²/R_{ic1/100} were removed from the analyses. The additional inclusion of weight improved significance for the SFBIA index but not for the remaining MFBIA indexes. Further inclusion of bioelectrical impedance variables (R at 1, 5, 50, and 100 kHz; X_c and PA each at 5, 50, and 100 kHz) as predictors did not significantly alter the precision of the MFBIA equations. The resulting best fitting regression equations for estimating BCM from SFBIA (model 1) and MFBIA (models 4 and 5) were

SFBIA model

$$\text{BCM}_{\text{BIANEW1}} (\text{kg}) = 1.898 \times (\text{Ht}^2/\text{X}_{\text{cp50}}) - 0.051 \times \text{Wt} + 4.180 \times \text{sex} + 15.496 \quad (6)$$

(r² = 0.84, RMSE = 1.71 kg)

MFBIA models

$$\text{BCM}_{\text{BIANEW2}} (\text{kg}) = 1.118 \times (\text{Ht}^2/\text{R}_{\text{ic5/50}}) + 4.250 \times \text{sex} + 14.457 \quad (7)$$

(r² = 0.84, RMSE = 1.73 kg)

$$\text{BCM}_{\text{BIANEW3}} (\text{kg}) = 0.822 \times (\text{Ht}^2/\text{R}_{\text{ic5/100}}) + 4.158 \times \text{sex} + 14.096 \quad (8)$$

(r² = 0.84, RMSE = 1.73 kg)

where Ht is in cm, Wt is in kg, sex is coded as 1 for men and 0 for women, X_{cp50} is parallel reactance at 50 kHz

in ohms, R_{ic5/50} is [(R₅ × R₅₀)/(R₅ - R₅₀)] in ohms, and R_{ic5/100} is [(R₅ × R₁₀₀)/(R₅ - R₁₀₀)] in ohms. The precision of the three new equations is indicated by the RMSE that reached from 1.71 to 1.73 kg (6% for men, 9% for women).

Checking for violation of assumptions. The assumptions for performing linear regression analysis were fulfilled. The response variable was normally distributed in both sexes, as shown by the Kolmogorov-Smirnov test. The assumption of homogeneity of the response variable was fulfilled, because the variance of the response variable was constant for all values of the predictor variables, as indicated by the absence of trends or patterns in the residual plots. Scatter plots demonstrated linear relationships between the response variable and the predictor variables (shown for Eqs. 6 and 7 in Fig. 1). A scatter plot also indicated linear relationship among the independent variables X_{cp50} and weight. Absence of multicollinearity of the predictor variables in the equation has been indicated by the condition numbers (CN) that were smaller than 30. The CN were computed for standardized residuals with the intercept included. According to Belsley et al. (5), a CN of 30 indicates probable collinearity in the model. Absence of autocorrelation of the residual values was fulfilled, as shown by the Durbin-Watson test.

Cross-Validation of the New Developed Equations

The three new equations derived from the data set in the validation subsample were applied to the data set of the cross-validation subsample. The accuracy of the equations for predicting BCM from BIA in the cross-validation subsample, as indicated by the PE, was 1.61 kg (6.8%) for the SFBIA equation (BCM_{BIANEW1}), and 1.82 kg (7.7%) and 1.78 kg (7.5%) for the MFBIA equations (BCM_{BIANEW2} and BCM_{BIANEW3}), respectively (Table 3). The PEs were similar to the corresponding RMSEs of the same equations. The accuracy

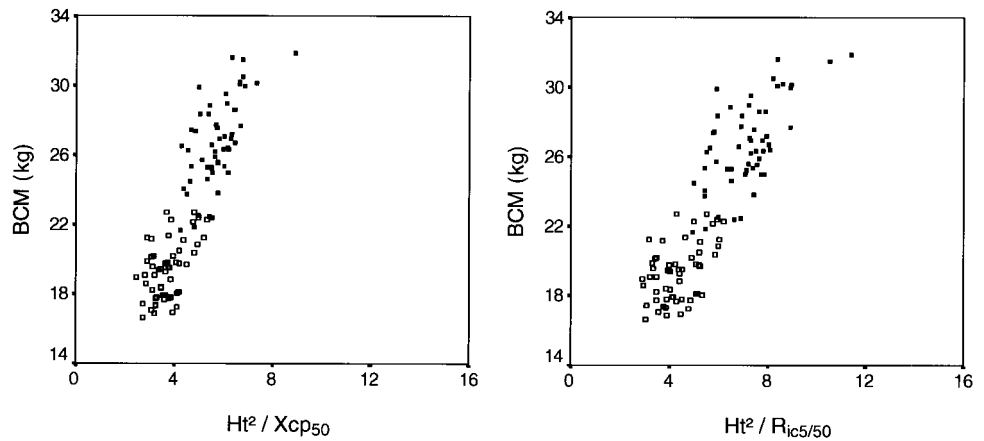


Fig. 1. Relationship between body cell mass (BCM) measured by ^{40}K and 2 indexes calculated from single- and multi-frequency bioimpedance parallel models, in men (■) and women (□) (validation subsample). Ht, height; X_{cp} , parallel reactance; R_{ic} , intracellular resistance.

of the newly derived equations was further compared with the accuracy of the manufacturer's equation and two published equations of Kotler et al. (27) for estimating BCM from BIA (equations are given in full in Table 3). Because the equations of Kotler et al. calculated TBK, they were multiplied by 0.00833 by use of the formula of Moore et al. (31) to convert TBK to BCM. The PEs of the manufacturer's and Kotler's linear equations were larger than the PEs found for the new generated equations. The manufacturer's equation significantly overestimated BCM relative to the TBK method, whereas the linear equation of Kotler et al. significantly underestimated BCM.

Measurement Method Comparison at an Individual Level

The Bland and Altman technique was applied to determine the level of agreement between the two methods TBK and BIA for predicting BCM at an individual level (Fig. 2). Comparisons were restricted to the three newly derived equations and the manufacturer's equation because they relate to the same bioimpedance analyzer. The limits of agreement (± 2 SD from mean bias) were for the manufacturer's BIA equa-

tion for men, ± 5.18 kg (19.3%), and for women, ± 4.52 kg (23.3%). The new equations BIA_{NEW1} , BIA_{NEW2} , and BIA_{NEW3} showed smaller limits of agreement, indicating better agreement between the BIA and TBK methods for predicting BCM: for men ± 3.84 kg (14.3%), ± 3.98 kg (14.8%), and ± 3.92 kg (14.6%), respectively; for women ± 2.74 kg (14.1%), ± 2.94 kg (15.1%), and ± 2.96 kg (15.3%), respectively.

The difference of BCM predicted from the TBK and BIA methods reached from 0.002 to 0.3 kg (0.01 to 1.1%) for the three new BIA equations. When the difference in BCM was plotted against age and weight, no systematic bias was detected for the new equations. In contrast, positive and negative relationships resulted when the difference in BCM was plotted against BCM_{TBK} and BMI, respectively, in any of the three new equations (shown for equation BIA_{NEW1} in Fig. 3).

DISCUSSION

This study developed, and cross-validated for the first time, equations for predicting BCM from BIA in relatively healthy elderly Germans aged 60–90 yr. Whole body counting of ^{40}K was used as a reference method. To obtain precise results by the TBK method,

Table 3. Cross-validation and significance of the difference (bias) between BCM calculated from TBK and from various BIA equations (cross-validation sample, $n = 51$)

Equation	PE		Men		Men		Women		Women					
			(n = 26)		(n = 24)		Bias ¹		95% CI					
	kg	%	Mean	SD	kg	%	kg	kg	%	kg				
$BCM_{BIA_{NEW1}}$	1.61	6.8	27.3	± 1.77	19.6	± 0.75	0.1	0.4	-0.6	to 0.8	0.002	0.01	-0.6	to 0.6
$BCM_{BIA_{NEW2}}$	1.82	7.7	27.1	± 1.50	19.5	± 0.80	0.3	1.1	-0.5	to 1.2	0.05	0.2	-0.6	to 0.7
$BCM_{BIA_{NEW3}}$	1.78	7.5	27.1	± 1.58	19.5	± 0.75	0.3	0.9	-0.6	to 1.1	0.04	0.2	-0.6	to 0.7
BCM_{BIAMAN}	3.08	13.0	29.9	± 3.64	20.5	± 1.87	-2.6‡	-9.3	-3.7	to -1.5	-1.0*	-5.0	-1.8	to -0.1
$BCM_{BIAKOTLIN}$	2.63	11.1	25.2	± 2.17	17.7	± 0.80	2.1‡	7.7	1.3	to 2.9	1.9‡	9.6	1.2	to 2.5
$BCM_{BIAKOTEXP}$	2.87	12.2	28.7	± 2.72	17.2	± 1.73	-1.4†	-5.0	-2.3	to -0.4	2.4‡	12.0	1.5	to 3.2

PE, pure error of cross-validation; 95% CI, 95% confidence interval for the bias; %, percentage of BCM_{TBK} ; $BCM_{BIA_{NEW1}}$, $BCM_{BIA_{NEW2}}$, $BCM_{BIA_{NEW3}}$, BCM (kg) calculated from single-frequency BIA (SF BIA) and MF BIA using new Eqs. 6, 7, and 8, respectively, developed in this study; BCM_{BIAMAN} , BCM (kg) calculated from BIA with the manufacturer's equation; $BCM_{BIAKOTLIN}$ and $BCM_{BIAKOTEXP}$, BCM (kg) calculated from SF BIA with the sex-specific linear (KOTLIN) and exponential (KOTEXP) equations of Kotler et al. (27) as follows: linear: $TBK = 254 \times (Ht^2/X_{cp50}) + 1,493$ for men; $TBK = 204 \times (Ht^2/X_{cp50}) + 1,336$ for women; exponential: $TBK = 0.76 \times [(Ht^{1.6}/X_{cp50}^{0.5}) \times 59.06] + 18.52$ weight - 386.66 for men; $TBK = 0.96 \times [(Ht^{2.07}/X_{cp50}^{0.36}) \times 1.30] + 5.79$ weight - 230.51 for women. TBK was multiplied by 0.00833 to obtain BCM: BCM (kg) = 0.00833 \times TBK (mmol). ¹Compared with BCM_{TBK} by paired *t*-test. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

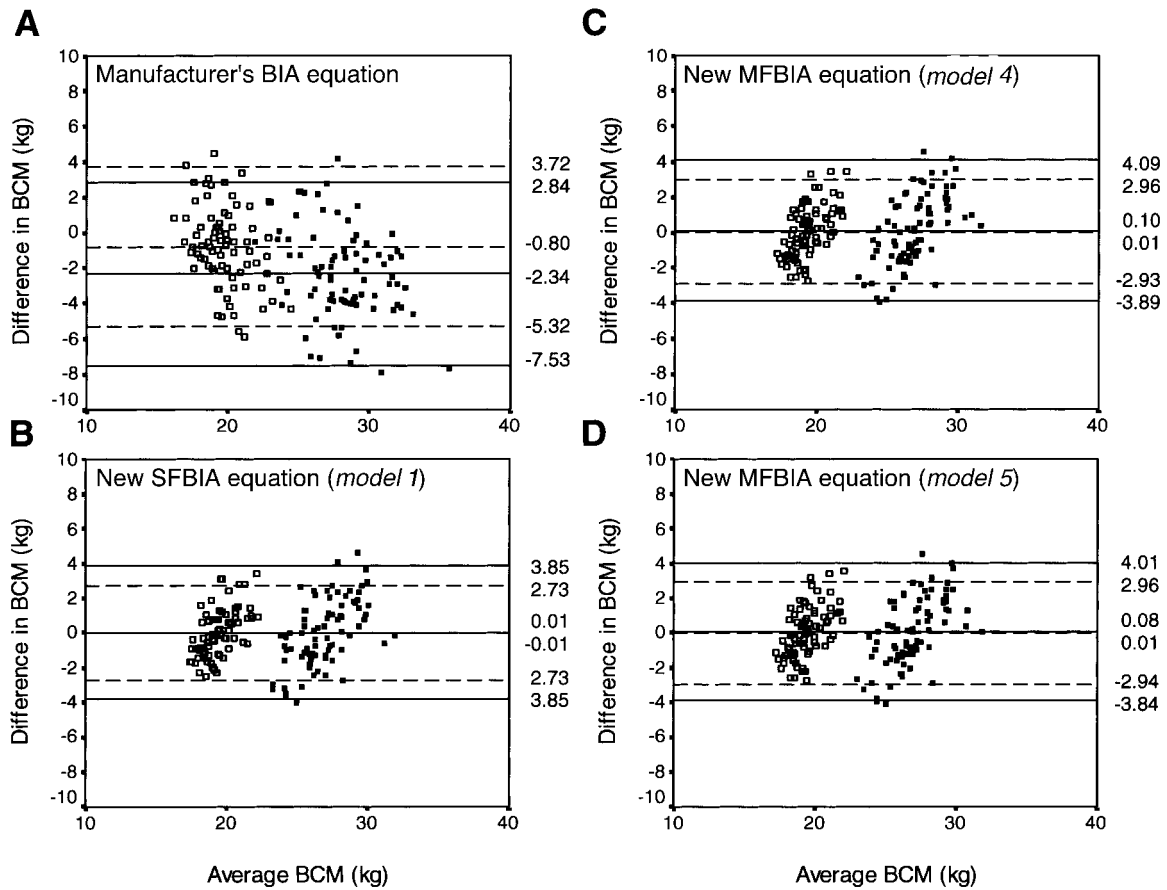


Fig. 2. Bland-Altman analysis of the difference between the total body potassium (TBK) and bioelectrical impedance analysis (BIA) methods for predicting BCM against the average measurement of both methods by use of the manufacturer's and the new BIA equations, on the basis of single-frequency (SF)BIA and multifrequency (MF)BIA models. Horizontal lines indicate the mean difference (bias, kg) \pm 2 SD (limits of agreement, kg) of the difference. Data are shown separately for men (■, solid line) and women (□, broken line).

elderly volunteers with potassium-wasting conditions or taking oral potassium supplements were excluded from this study. For precise BIA results, elderly with altered electrolyte concentration or hydration status were excluded. In addition, obese elderly were excluded

because the TBK method may underestimate BCM and the BIA method may overestimate BCM in the obese.

The development and cross-validation of the new equations were performed in a data-splitting approach, subdividing the entire sample into a validation and a

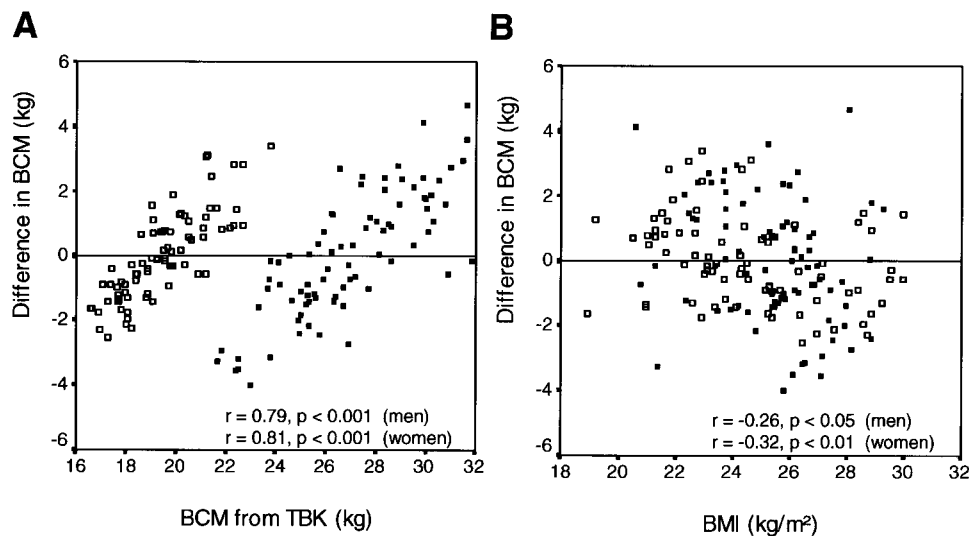


Fig. 3. Relationship between the prediction error of BCM (difference in BCM from TBK and BIA) from the developed prediction equation BIA_{NEW1} plotted against BCM_{TBK} and body mass index (BMI) for men (■) and women (□).

cross-validation subset. In the validation sample, three new prediction equations for estimating BCM were developed from five single- and multifrequency bioimpedance parallel models. Results show that BCM was best predicted from the single-frequency 50-kHz model (parallel reactance) and the multifrequency 5/50- and 5/100-kHz models, whereas the multifrequency 1/50- and 1/100-kHz models displayed much lower correlations with BCM from TBK. The lower correlations found for multifrequency models involving the 1-kHz frequency compared with those involving 5 kHz could be explained in part by the larger within-subject variation of resistance at 1 kHz compared with 5 kHz in the present elderly group, as can be seen from the larger standard deviation for 1 kHz in Table 1. This agrees with findings obtained in other studies in elderly groups where a larger standard deviation of the impedance at 1 kHz compared with other frequencies has been reported (43). The precision for each of the three new equations, as indicated by the RMSE, was 6% for men and 9% for women. These percentages are consistent with those reported from BIA validation studies that used parallel reactance models at 50 kHz and TBK as a reference method (27). The accuracy of the three new equations, as indicated by the PE in the cross-validation sample, was similar to the RMSE of the same equation in the validation sample. The new equations improved accuracy compared with the manufacturer's equation, reducing the PE in BCM from 13.0 to 6.8 (SFBIA equation), 7.5, and 7.7% (MFBIA equations).

The predictive precision and accuracy were similar for the three new equations in the healthy elderly group. With regard to the multifrequency bioimpedance equations, this implies that the use of 100 kHz over 50 kHz had no advantage. This further gives evidence that the equations derived from multifrequency bioimpedance did not perform better than the equation derived from the single-frequency 50-kHz data in the present elderly group. This indicates that single-frequency bioimpedance analyzers, operating at 50 kHz, may be well suited for predicting BCM in healthy elderly groups when the appropriate equation is used. Similarly, authors who performed validation studies in patients with HIV (39) and cystic fibrosis (36) did not observe an advantage of multifrequency over single-frequency BIA in predicting BCM. This can be explained by the observation that relations between extra- and intracellular fluid spaces are relatively constant in healthy and diseased subjects who are not characterized by altered body hydration. Nevertheless, in elderly with altered states of hydration, multifrequency bioimpedance might be superior to single-frequency bioimpedance (35). Future studies should address this question.

The newly derived BIA equations reduced the mean difference in BCM between TBK and BIA to 0.01–1.1% in both sexes in the cross-validation subsample. In contrast, the manufacturer's equation significantly overestimated BCM in the elderly men and women by 9.3 and 5.0%, respectively. The new equations also

improved the validity of bioimpedance analysis at an individual level, reducing the limits of agreement (± 2 SD from mean) for BCM estimates from ± 19 –23% (manufacturer's equation) to ± 14 –15%. The difference in accuracy between the new BIA equations and the manufacturer's equation can be explained by the use of different predictor variables and by possible effects of sample characteristics, particularly by differences in age ranges, on the development of the equations. The gender difference characterized by higher overprediction of BCM in men by the manufacturer's equation can be explained on the one hand by the larger decline of BCM in men with advancing age being attributable to a higher loss of muscle mass compared with women (11, 22, 25). As a consequence, older men probably differ in BCM more strongly from young and middle-aged reference populations than older women. On the other hand, body height is used in the equation for estimating BCM from BIA. Generally, height will be underestimated in elderly subjects, because there often occurs an age-related decline in height, due to senile kyphosis and vertebral collapse. Broekhoff et al. (7) reported that underestimation in height by five centimeters can cause an underestimation of fat-free mass of ~ 0.7 –1.9 kg, in case of its use in a prediction equation. Because BCM is a fraction of the fat-free mass and because the age-related decline in height is larger in women than in men, the lower BCM values in women estimated by the manufacturer's BIA equation could partly be attributed to the higher underestimation of height in women. A further explanation for the differences in accuracy between the sexes is provided by Deurenberg et al. (16), who reported a higher ratio of extra- to intracellular water in women resulting in a lower body impedance by BIA.

This study showed that the difference between the BIA and TBK methods in predicting BCM was positively related to BCM from TBK for each of the new equations. This indicates that the prediction error of BCM depends on the amount of BCM, in that the equations overestimated low BCM and underestimated high BCM in the elderly. Such a positive relationship has also been reported for intracellular water (14) and has been explained with the observation that, at higher levels of intracellular water, the extracellular water is overestimated. Subsequently, the difference between predicted total body water and extracellular water changes at increasing levels of intracellular water or BCM. Further factors that might influence the prediction error are altered cell membrane integrity and vascular permeability in the elderly. Future studies remain to be undertaken to investigate cell membrane capacitance in elderly groups.

Whole body counting of ^{40}K was used as a reference method to predict BCM. Cohn et al. (11), on the basis of a cross-sectional study in normal individuals aged 20 to 79 yr, found that the TBK-to-BCM ratio did not change significantly with age and that the intracellular potassium concentration was not affected by age, being similar in both sexes. Nevertheless, there might be a potential limitation to this study. The calculation of

BCM from TBK assumes for adults an average K-to-N ratio (K/N) of 3 mmol potassium per gram of nitrogen, and a 0.04-g nitrogen content/g wet wt for lean tissues, resulting in the equation $BCM \text{ (kg)} = 0.00833 \times TBK \text{ (mmol)}$ (31), as used in this study. This poses the question whether K/N changes with age. Cohn et al. (9) reported that both TBK and total body nitrogen (TBN) decrease with age in both sexes. They found that the TBN-to-TBK ratio (TBN/TBK) tended to increase for males aged 50–79 yr, reflecting the more rapid loss of TBK with age compared with the corresponding loss of TBN (10). In women, TBN/TBK showed only a slight tendency to increase with age. However, the K/N ratio of the entire body may not be instructive, because the various human body tissues vary widely in terms of their K/N ratios, and BCM comprises mainly skeletal muscle mass and viscera. Future long-term research with the use of appropriate methodology should address this question by quantifying age-related changes of the K/N ratio in the respective tissues in elderly groups. This study excluded subjects with altered hydration status due to diseases, medications, diarrhea, and edema. However, the hydration status of the subjects was not estimated; this is a potential source of error.

In conclusion, the newly derived age-specific regression equations improve prediction of BCM in elderly aged 60–90 yr compared with the generalized equation of the manufacturer. The new equations should be used for normal-weight and overweight elderly (BMI 19.0 to 29.9 kg/m²) where they have been developed. They might not be applicable to the obese elderly, because this study excluded subjects with a BMI >30 kg/m². Because the new equations were internally cross-validated, future studies are needed that will externally cross-validate the equations in independent population groups.

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