Body Composition in Renal Transplant Patients: Bioimpedance Analysis Compared to Isotope Dilution, Dual Energy X-Ray Absorptiometry, and Anthropometry

EUGÉNIE C.H. VAN DEN HAM,* JEROEN P. KOOMAN, *
MAARTEN H.L. CHRISTIAANS,* FRED H.M. NIEMAN,[†]
BERNHARD K. VAN KREEL,[‡] GUIDO A.K. HEIDENDAL,[§] and
JOHANNES P. VAN HOOFF*

*Department of Internal Medicine, Division of Nephrology, [†]Methodology Section, [‡]Department of Clinical Chemistry, and [§]Department of Nuclear Medicine, University Hospital Maastricht, Maastricht, The Netherlands.

Abstract. Whether multifrequency bioelectrical impedance analysis (MF-BIA), a relatively new method for measuring body composition, is also applicable for accurate body composition measurements in renal transplant (RTx) patients is not known. Therefore, the use of MF-BIA is validated in 77 RTx patients with a stable renal function at least 2 yr posttransplantation. MF-BIA is compared to isotope dilution techniques for measurement of body water compartments, and to dual energy x-ray absorptiometry (DEXA) and anthropometry for measurement of fat and fat free mass. Finally, DEXA and anthropometry are compared to each other. Method agreement is assessed by intraclass correlation coefficients (ICC) and plotted by Bland and Altman analysis. MF-BIA significantly underestimates total body water (TBW, 0.7 ± 2.1 L) and overestimates the extracellular water (ECW, 3.3 ± 1.8 L) compared to isotope dilution; the ICC between both techniques is 0.943 for TBW and 0.846 for ECW. The percentage body fat (BF) measured by MF-BIA is significantly higher than both BF measured by DEXA (3.4 ± 4.7%) or by anthropometry (5.5 ± 5.2%). The ICC between MF-BIA and DEXA is 0.887 and between MF-BIA and anthropometry 0.856. BF measured by DEXA is significantly higher than BF measured by anthropometry (2.1 ± 4.4%); their ICC is 0.913. In conclusion, MF-BIA seems to be suitable for measurement of TBW in RTx patients; however, method agreement between isotope dilution and MF-BIA for the measurement of ECW is not satisfactory. In the assessment of fat and fat free mass, the reliability of MF-BIA appears to be questionable. Method agreement between DEXA and anthropometry seems to be slightly better.

Renal transplant patients are at risk for increased weight, centripetal obesity, and muscle atrophy because of their long-term glucocorticoid requirements (1–5). Such changes in body composition are associated with an increased risk of cardiovascular complications (1,6–10), a major cause of morbidity and mortality in renal transplant patients (11–16). Body composition data might provide insight into the relation with outcome, survival, and posttransplant complications; it might also affect approaches to nutritional therapy and to therapy in the field of physical activity.

Various techniques are available to measure body composition, among them isotope dilution, anthropometry, dual energy x-ray absorptiometry (DEXA), and multifrequency bioelectrical impedance analysis (MF-BIA). Multifrequency bioelectrical impedance analysis is recently introduced for measuring body composition. It is a relatively inexpensive, noninvasive, easy to use, and portable technique, and therefore suitable for routine clinical use.

MF-BIA is considered a useful technique for body composition analysis in healthy individuals (17). However, it is not known whether MF-BIA is also applicable for accurate body composition measurements in renal transplant patients. In this study, we validated MF-BIA for its use in renal transplant patients by comparing MF-BIA to isotope dilution, which is considered the gold standard for measuring body water compartments in healthy individuals. Furthermore, we compared MF-BIA, DEXA, and anthropometry to one another for measurement of the body fat and fat free compartment.

Materials and Methods

Patients

Renal transplant patients with a stable renal function and maintenance immunosuppressive therapy for at least 2 yr were studied. Exclusion criteria were insulin-dependent diabetes mellitus, metal implants (prostheses or pacemakers), and recent complications (*e.g.*, malignancies or surgery). All patients were receiving immunosuppres-

sive therapy, which consisted of combinations of prednisolone, aza-thioprine, and cyclosporine.

Informed consent was obtained from each patient, and the study was approved by the Ethics Committee of the Maastricht University Hospital.

Methods

In this cross-sectional study, body composition was measured by isotope dilution (deuterium (D_2O) and potassium bromide (KBr)), DEXA, anthropometry, and MF-BIA (Figure 1).

Isotope Dilution

In the early morning after an overnight fast, patients received an orally administered dose of D_2O of 25 ml (99%, Sigma Chemicals, St. Louis, MO) and KBr of 30 ml (150 mM). Dose bottles were rinsed out and the rinse water was also ingested by the patients to ensure that all D_2O and KBr was consumed. Enrichments of D_2O and KBr in the body fluid were measured in serum. Immediately before D_2O and KBr intake, a (background) blood sample was taken. After an equilibration time of 4 h, a second blood sample was collected. Food intake was not allowed until the second blood sample was collected; nevertheless, drinking some small amounts of water was allowed as all patients had to take their immunosuppressive medication just before or during measurements.

The concentration of deuterium and bromide in serum was determined by isotope ratio mass spectrometry and ion chromatography, respectively (18,19). D_2O and bromide dilution spaces were calculated from, respectively, the enrichment of D_2O and bromide after 4 h (20). Total body water (TBW) was calculated as the deuterium dilution space corrected for the exchange of D_2O with nonaqueous compartments and for the concentration of water in the serum by first dividing the dilution space by 1.04 and thereafter multiplying it by 0.94 (20,21). The extracellular water compartment (ECW) was calculated as the bromide dilution space corrected for intracellular penetration of bromide in erythrocytes, leukocytes, and secretory cells; for unequal bromide concentrations in the extracellular fluids (Gibbs–Donnan effect); and for the concentration of water in the serum. Therefore, bromide dilution space was multiplied by $0.90 \times 0.95 \times 0.94$, respectively (20,22–25).

Dual Energy X-Ray Absorptiometry

DEXA was used for measurement of whole-body composition, including fat mass, lean soft tissue mass (comprising muscle, inner organs, and body water), and bone mineral density. The equipment used in this study was DPX-L (Lunar Radiation Corp., Madison, WI).

DEXA measurements were performed in a standard manner while the patient was lying in a supine position on a table. From an x-ray source and K-edge filter below the patient, x-ray beams of stable energy radiation of 38 and 70 KeV were emitted. Attenuation of the x-rays was measured with a detector situated above the patient. Transverse scans of the body were made from top to toe. For each transverse scan, about 120 pixel elements with a size of approximately 5×10 mm yield data on the attenuation ratio. Approximately 40 to 45% of the pixels over the body contain bone and soft tissue, and 55 to 60% contain soft tissue alone (26). Bone mass was estimated from the ratio of the attenuation at low energy peaks relative to attenuation at high energy peaks through bone containing pixels after correction for the overlying soft tissue. The composition of the soft tissue was estimated by the ratio of beam attenuation at lower energy relative to the higher energy in soft tissue pixels; this ratio is inversely and linearly related to the percentage of fat (26,27).

Anthropometry

Body weight was measured to the nearest 0.1 kg using a balance scale. Body height was measured to the nearest 0.5 cm with the patient standing, back to a stadiometer.

Skinfold measurements were used to estimate total body fat mass. Skinfold thickness was measured to the nearest 0.1 mm by a skinfold caliper. Measurements were made at the nondominant side of the body at four sites: biceps, triceps, subscapula, and iliac crest. Skinfolds were measured three times. Thereafter, the sum of the individual skinfolds was averaged. The logarithm of the sum of the four skinfolds was used in age- and gender-specific regression equations of Durnin and Womersly (28) to compute body density (D). Fat mass was computed as: Body weight (kg) $\times [(4.95/D) - 4.5]$; fat free mass (kg) was calculated as: Body weight (kg) $-$ Fat mass (kg) (28,29).

Waist circumference was measured midway between the lower rib margin (costal margin) and the superior anterior iliac spine (iliac crest). Hip circumference was measured at the level of the great trochanters. Both waist and hip circumference were measured to the nearest millimeter with a flexible tape, with the patient in the standing position.

Multifrequency Bioelectrical Impedance Analysis

Bioelectrical impedance measurements were performed in a standard manner while the patient was lying supine on a flat, nonconductive bed. Multifrequency (5 to 500 kHz), imperceptible currents were introduced at distal electrodes on the hands (just proximal to the phalangeal-metacarpal joint in the middle of the dorsal side of the hand) and the feet (just proximal to the transverse [metatorsal] arch on
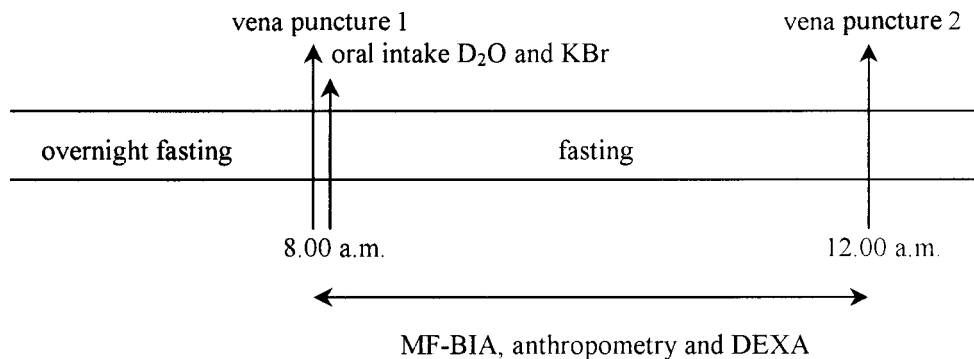

Figure 1. Study design.

the superior side of the foot), and resistances were measured by proximal electrodes (to the wrist midway between the styloid process, to the ankle midway between the malleoli). TBW and ECW were predicted from a general mixture theory (theory of Hanai) (30–32): Water compartments are directly calculated from resistance values, assuming specific resistances of ECW and ICW. Specific resistances of ECW and ICW are provided by the manufacturer (for men: ρECW = 215.0, ρICW = 824.0; for women: ρECW = 206.0, ρICW = 797.0). The bioelectrical impedance analyser used in this study was the Xitron 4000B (Xitron Technologies, San Diego, CA). Fat free mass (FFM) is derived from the ECW and ICW volume; fat mass is calculated as: Body weight − FFM (33).

To determine the reproducibility of MF-BIA measurements, the MF-BIA measurement was performed twice in a small number of patients ($n = 10$); precision was assessed by calculating the mean coefficient of variation of duplicate measurements (Coefficient of variation = [SD of the two measurements/Mean of the two measurements] × 100%).

Statistical Analyses

Paired t tests were used to compare TBW and ECW measured by isotope dilution to TBW and ECW measured by MF-BIA and to compare body fat (BF) measured by MF-BIA to BF measured by DEXA and anthropometry, respectively. Correlations between body compartments measured by different methods were estimated by the use of Pearson product moment correlations. A P value < 0.05 was considered significant. Bland and Altman plots (34,35) were used to visually assess agreement between the different methods to measure TBW, ECW, and body fat, respectively. In addition to these, the intraclass correlation coefficient (ICC) was calculated using variance components generated from repeated measures ANOVA. The intraclass correlation coefficient can be interpreted as a measure of agreement between methods and will vary from 0 (no agreement at all) to 1 (perfect agreement). Lowest acceptable method agreement was defined as 0.85. According to Fleiss (36), an approximate one-sided 95% lower confidence level for the ICC can be calculated to ascertain whether it satisfies the predetermined level of agreement. Smallest detectable differences are used to calculate the limits of agreement at a 5% level. To ascertain whether method agreement varied over important covariates or factors of the patient population, the included (among other things creatinine clearance, the use of calcium antagonists, and the presence of cystic kidneys in patients with polycystic kidney disease) intraclass correlation coefficients were also calculated for subcategories of covariates or factors to see whether method agreement increases or declines in certain subcategories. For this purpose, covariates were categorized into three classes containing one-third of the patients (about 22 to 25 per class), using cutting points as defined in Table 1. Statistical analysis was performed by SPSS for Windows, version 7.5.

Results

Patient Characteristics

Seventy-seven renal transplant patients (35 women, 42 men) with a stable renal function participated in the study. Patient characteristics are shown in Table 2. Mean age of the population was 51.0 ± 11.7 yr. Body mass index (BMI) of the transplant patients was 24.8 ± 4.8 kg/m². Mean follow-up time after transplantation was 9.1 ± 4.3 yr, and the mean creatinine clearance was 60.2 ± 20.5 ml/min. On average, patients used a cumulative prednisolone dose of 19.3 ± 21.5 g.

Reproducibility of MF-BIA Measurements

Repeated MF-BIA measurements were performed in 10 renal transplant patients (five women, five men), with a mean age of 59.0 ± 13.1 yr and a mean BMI of 23.7 ± 5.1 kg/m². The mean coefficient of variation (CV) of the TBW measurements is 1.0% (range individual CV, 0.0 to 2.9%) and of the ECW measurements 0.6% (range individual CV, 0.0 to 1.9%).

Total Body Water

Of 73 renal transplant patients, data of D_2O dilution are available; data of four patients were lost during laboratory analysis. Descriptive statistics (mean, SD, and range) of TBW measured by D_2O dilution (TBW_{D_2O}) and TBW measured by MF-BIA (TBW_{MF-BIA}) are shown in Table 3. TBW_{D_2O} compared with TBW_{MF-BIA} is 34.2 ± 6.1 L *versus* 33.5 ± 5.9 L ($P < 0.05$). TBW_{D_2O} is highly and significantly correlated to TBW_{MF-BIA} ($r = 0.943$, $P < 0.001$). The regression plot of TBW_{D_2O} and TBW_{MF-BIA} is given in Figure 2. In Figure 3, the Bland and Altman analysis is plotted. The mean difference between TBW_{D_2O} and TBW_{MF-BIA} is 0.7 L (95% confidence interval [CI], 0.2 to 1.2 L). The limits of agreement (mean ± 2 SD) show that TBW_{MF-BIA} may be 3.4 L higher or 4.8 L lower than TBW_{D_2O}.

Table 4 shows the intraclass correlation coefficient for measurement of agreement between TBW_{D_2O} and TBW_{MF-BIA} (ICC_{TBW}) and the calculation of this ICC. ICC_{TBW} is 0.943 (95% limits of agreement, −3.3 to 4.8 L). The approximate

Table 1. Definition of cutting points used to obtain three subcategories in covariates[a]

Parameter	Lowest 33%	Middle 33%	Highest 33%
Age (yr)	≤45.0	45.0 to 57.0	>57.0
BMI (kg/m²)	≤22.5	22.5 to 26.0	>26.0
Waist-to-hip ratio	≤0.83	0.83 to 0.93	>0.93
Hydration status LBM (L/kg)[b]	≤0.6900	0.6900 to 0.7292	>0.7292
Ratio ECW to ICW[c]	≤0.7651	0.7651 to 0.8950	>0.8950
Creatinine clearance (ml/min)	≤50.76	50.76 to 70.50	>70.50

[a] BMI, body mass index; LBM, lean body mass; ECW, extracellular water; ICW, intracellular water.
[b] Calculated as TBW measured by deuterium isotope dilution divided by LBM measured by dual energy x-ray absorptiometry.
[c] ECW measured by bromide isotope dilution; ICW calculated as TBW minus ECW (both TBW and ECW measured by isotope dilution).

Table 2. Patient characteristics ($n = 77$)

Characteristic	Mean ± SD	Range
Age (yr)	51.0 ± 11.7	25.5 to 78.2
Height (m)	1.68 ± 0.10	1.51 to 1.86
Weight (kg)	69.8 ± 12.8	43.5 to 99.5
Body mass index (kg/m²)	24.8 ± 4.8	16.7 to 42.2
Follow-up after transplantation (yr)[a]	9.1 ± 4.3	2.2 to 22.9
Creatinine clearance (ml/min)[b]	60.2 ± 20.5	19.2 to 114.0
Cumulative dose of prednisolone (g)	19.3 ± 21.5	0.5 to 83.4

[a] Follow-up after transplantation = time lapse posttransplantation.

[b] Creatinine clearance calculated by the formula of Cockcroft (37): for men: creatinine clearance = [(140 − age (yr)) × weight (kg)]/[0.81 × serum creatinine (μmol/L)]; for women: creatinine clearance = [(140 − age (yr)) × weight (kg)]/[0.85 × serum creatinine (μmol/L)].

Table 3. TBW, ECW, and BF measured by different techniques in renal transplant patients (mean, SD, and range)[a]

Parameter	Mean	SD	Range	n[b]
TBW_{D_2O} (L)	34.2	6.1	22.4 to 50.2	73
TBW_{MF-BIA} (L)	33.5	5.9	19.3 to 48.4	73
ECW_{KBr} (L)	15.5	2.9	10.4 to 24.3	72
ECW_{MF-BIA} (L)	18.7	3.6	11.1 to 29.0	72
BF_{DEXA} (%)	30.3	10.5	9.0 to 58.0	75
BF_{MF-BIA} (%)	33.7	9.2	8.0 to 54.2	75
BF_{anthr} (%)	27.9	10.2	8.4 to 48.0	74
BF_{MF-BIA} (%)	33.4	9.2	8.0 to 54.2	74
BF_{DEXA} (%)	30.2	10.7	9.0 to 55.8	72
BF_{anthr} (%)	28.1	10.3	8.4 to 48.0	72

[b] The number of patients in each comparison differs due to missing values (see text).

[a] TBW, total body water; ECW, extracellular water; BF, body fat; D_2O, deuterium; MF-BIA, multifrequency bioelectrical impedance analysis; KBr, potassium bromide; DEXA, dual energy x-ray absorptiometry; anthr, anthropometry.

Figure 2. Regression plot of TBW_{D_2O} and TBW_{MF-BIA}. Outliers (standardized z-residue scores < -2 or > 2) are given as open circles.

Figure 3. Assessment of TBW by D_2O dilution against MF-BIA (Bland and Altman analysis). The relative bias (dilution − MF-BIA) plotted against the size of the measurement (mean of dilution and MF-BIA).

one-sided 95% lower confidence level for the ICC according to Fleiss (36) turns out to be 0.916. Table 6 shows the ICC values for the subcategories. The agreement between the methods appears to vary little over age, BMI, waist-to-hip ratio, hydration status of the lean body mass (LBM), the ratio ECW to ICW, creatinine clearance, gender, or the use of calcium antagonists. All ICC values are larger than 0.892. The ICC_{TBW} in patients with cystic kidneys is 0.886, whereas the ICC_{TBW} in patients without cystic kidneys seems to be higher (0.950).

Extracellular Water

Data of bromide dilution are available for 72 renal transplant patients (data of five patients were lost during laboratory analysis). Descriptive statistics of extracellular water measured by bromide dilution (ECW_{KBr}) and ECW measured by MF-
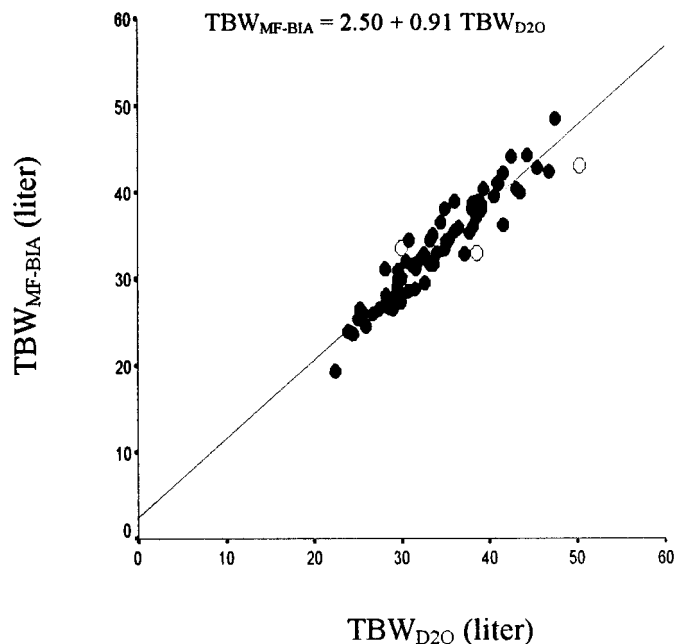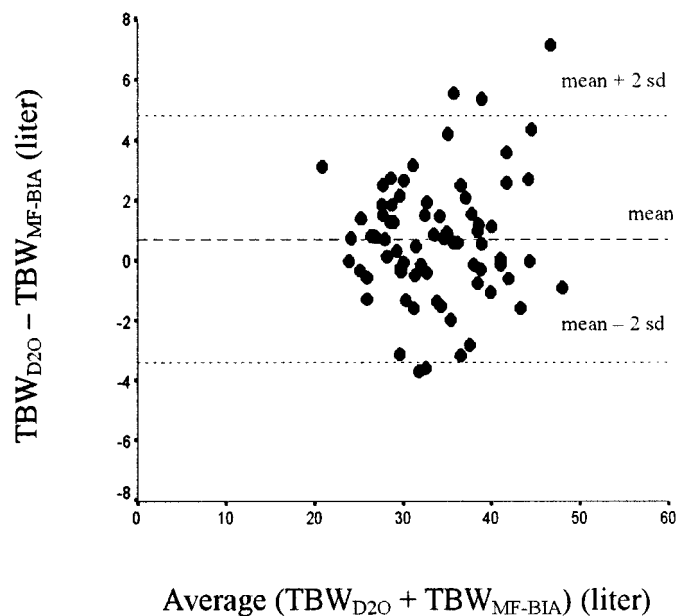
BIA (ECW_{MF-BIA}) are shown in Table 3. ECW_{KBr} compared with ECW_{MF-BIA} is 15.5 ± 2.9 L *versus* 18.7 ± 3.6 L ($P < 0.05$). ECW_{KBr} is highly and significantly correlated to ECW_{MF-BIA} ($r = 0.865$, $P < 0.001$). The regression plot of ECW_{KBr} and ECW_{MF-BIA} is given in Figure 4. The Bland and Altman analysis is plotted in Figure 5. The mean difference between ECW_{KBr} and ECW_{MF-BIA} is 3.3 L (95% CI, −3.7 to −2.8 L). Limits of agreement show that ECW_{MF-BIA} may be 0.4 L lower or 6.9 L higher than ECW_{KBr}.

Table 4. Analysis of variance for 73 renal transplant patients measured twice on TBW (by D$_2$O dilution and MF-BIA) supplemented with estimation of variance components[a]

Sources of Variation	SS	df	MS	EMS = σ^2	ICC/Proportion
Patients (P)	5157.97	72	71.64	34.76	0.943
Method (M)	19.00	1	19.00	(0.23)	
P × M (error)	152.65	72	2.12	2.12	0.057
Total		145		36.88	1.000

[a] SS, sums of squares; MS, mean sum of squares; df, degrees of freedom; EMS, σ^2 = expected mean sum of squares = (estimated) variance component (VC); ICC, intraclass correlation coefficient.

ICC = method agreement = proportion of patient VC to the total amount of patient VC plus the patient–by–method VC,

or: ICC = $\sigma^2_P/(\sigma^2_P + \sigma^2_{P \times M})$

For TBW: $\sigma^2_P = (MS_P - MS_{P \times M}) / 2$

$\sigma^2_M = (MS_M - MS_{P \times M}) / 73$

$\sigma^2_{P \times M} = MS_{P \times M}$

SDD = smallest detectable difference; with two repeated measurements and a probability of 95%

SDD = $\sqrt{MS_{P \times M}} \times \sqrt{2} \times 1.96$

For TBW: SDD = 4.04, so with a probability of 95% score differences lie between −3.3 and 4.8.

"Limits of agreement" are almost equivalent to SDD limits. Factor "patients" is considered to be a "random" factor, the factor "method" is fixed.

Table 5. Analysis of variance for 72 renal transplant patients measured twice on ECW (by KBr dilution and MF-BIA) supplemented with estimation of variance components[a]

Sources of Variation	SS	df	MS	EMS = σ^2	ICC/Proportion
Patients (P)	1396.83	71	19.67	9.02	0.846
Method (M)	379.76	1	379.76	(5.25)	
P × M (error)	116.48	71	1.64	1.64	0.154
Total		143		10.66	1.000

[a] SS, sums of squares; MS, mean sum of squares; df, degrees of freedom; EMS, σ^2 = expected mean sum of squares = (estimated) variance component (VC); ICC, intraclass correlation coefficient.

ICC = method agreement = proportion of patient VC to the total amount of patient VC plus the patient–by–method VC,
or: ICC = $\sigma^2_P/(\sigma^2_P + \sigma^2_{P \times M})$

For ECW: $\sigma^2_P = (MS_P - MS_{P \times M}) / 2$

$\sigma^2_M = (MS_M - MS_{P \times M}) / 72$

$\sigma^2_{P \times M} = MS_{P \times M}$

SDD = smallest detectable difference; with two repeated measurements and a probability of 95%

SDD = $\sqrt{MS_{P \times M}} \times \sqrt{2} \times 1.96$

For ECW: SDD = 3.55, so with a probability of 95% score differences lie between −6.8 and 0.3.

"Limits of agreement" are almost equivalent to SDD limits. Factor "patients" is considered to be a "random" factor, the factor "method" is fixed.

The ICC for measurement of ECW by KBr dilution and MF-BIA (ICC_{ECW}) plus the way it is calculated is given in Table 5; ICC_{ECW} turns out to be 0.846 (95% limits of agreement, −6.8 to 0.3 L). This is below the predetermined acceptable method agreement. The approximate one-sided lower 95% confidence level for the ICC according to Fleiss (36) is 0.779. Subcategory ICC values are given in Table 7. Many of the subcategories do not meet the lowest acceptable method agreement. Remarkably, method agreement appears to be acceptable in female patients, but not in male patients.

Body Fat and Fat Free Mass: MF-BIA Compared to DEXA

For 75 renal transplant patients, DEXA total body scans are available. Descriptive statistics of body fat (BF, percentage of total body weight) measured by DEXA (BF_{DEXA}) and BF

Table 6. Subcategory intraclass correlation coefficients for measurement of TBW[a]

	ICC for Subcategories	
Factors		
gender	0.907 (men, $n = 41$)	0.902 (women, $n = 32$)
cystic kidneys (PCKD)	0.886 (PCKD, $n = 9$)	0.950 (non-PCKD, $n = 63$)
calcium antagonists	0.943 (users, $n = 27$)	0.940 (nonusers, $n = 45$)

	Lowest 33% ICC	Middle 33% ICC	Highest 33% ICC
Covariates			
age	0.980	0.967	0.915
BMI	0.924	0.949	0.933
waist-to-hip ratio	0.919	0.939	0.912
hydration status LBM	0.964	0.914	0.945
ratio ECW to ICW	0.938	0.978	0.892
creatinine clearance	0.948	0.906	0.959

[a] Original ICC for all patients ($n = 73$) is 0.943. PCKD, polycystic kidney disease.
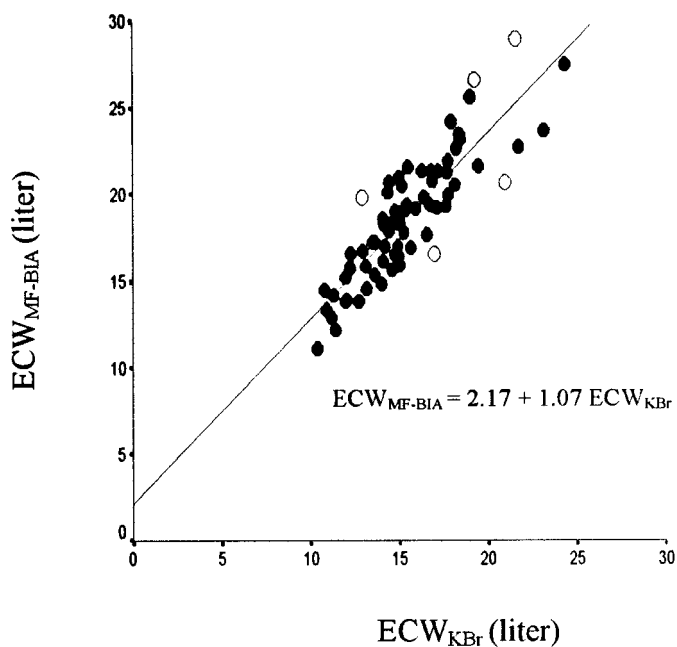

Figure 4. Regression plot of ECW_{KBr} and $ECW_{MF\text{-}BIA}$. Outliers (standardized z-residue scores < -2 or > 2) are given as open circles.
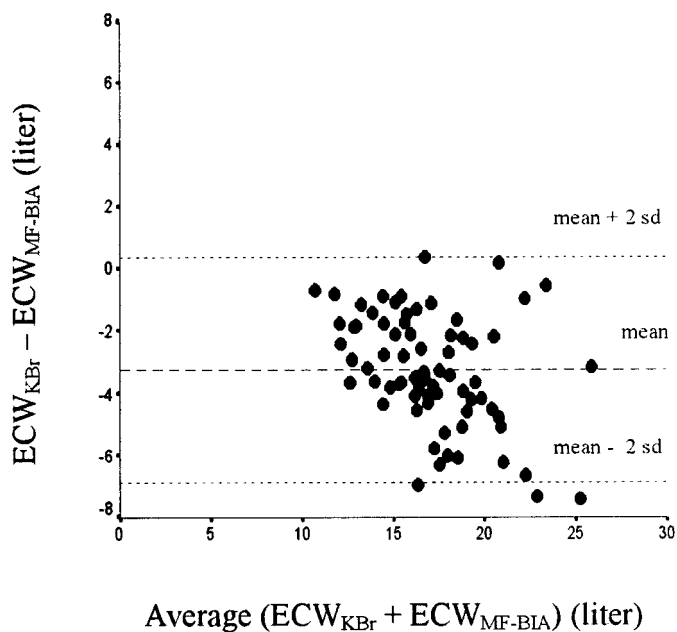

Figure 5. Assessment of ECW by KBr dilution against MF-BIA (Bland and Altman analysis). The relative bias (dilution − MF-BIA) plotted against the size of the measurement (mean of dilution and MF-BIA).

measured by MF-BIA ($BF_{MF\text{-}BIA}$) are shown in Table 3. BF_{DEXA} compared with $BF_{MF\text{-}BIA}$ is $30.3 \pm 10.5\%$ *versus* $33.7 \pm 9.2\%$ ($P < 0.05$). BF_{DEXA} and $BF_{MF\text{-}BIA}$ are highly and significantly correlated ($r = 0.895$, $P < 0.001$). The regression plot of BF_{DEXA} and $BF_{MF\text{-}BIA}$ is given in Figure 6. In Figure 7, the Bland and Altman analysis is plotted. The mean difference between BF_{DEXA} and $BF_{MF\text{-}BIA}$ is 3.4% (95% CI, -4.5 to -2.3%). Limits of agreement show that $BF_{MF\text{-}BIA}$ may be 6.0% lower or 12.8% higher than BF_{DEXA}.

The intraclass correlation coefficient for method agreement between BF_{DEXA} and $BF_{MF\text{-}BIA}$ ($ICC_{BF\text{-}D}$) is 0.887 (95% limits of agreement, -12.6 to 5.8%). According to the Fleiss formula

(36), the approximate one-sided 95% lower confidence level for the ICC turns out to be 0.836. The subcategory ICC values are shown in Table 8. Method agreement appears to be acceptable in most of the subcategories. Especially in patients with cystic kidneys, in patients with a BMI < 22.5 kg/m^2, and in patients with a high waist-to-hip ratio, method agreement appears to be below the predetermined lowest acceptable method agreement. Because fat free mass is calculated as 100% minus fat mass both in DEXA and in MF-BIA, the results of ICC and thus of method agreement with regard to fat free mass are identical.

Table 7. Subcategory intraclass correlation coefficients for measurement of ECW[a]

	ICC for Subcategories	
Factors		
gender	0.817 (men, $n = 40$)	0.877 (women, $n = 32$)
cystic kidneys (PCKD)	0.802 (PCKD, $n = 8$)	0.841 (non-PCKD, $n = 63$)
calcium antagonists	0.853 (users, $n = 27$)	0.845 (nonusers, $n = 44$)

	Lowest 33% ICC	Middle 33% ICC	Highest 33% ICC
Covariates			
age	0.778	0.869	0.887
BMI	0.829	0.852	0.786
waist-to-hip ratio	0.888	0.847	0.770
hydration status LBM	0.875	0.816	0.875
ratio ECW to ICW	0.871	0.916	0.857
creatinine clearance	0.838	0.858	0.820

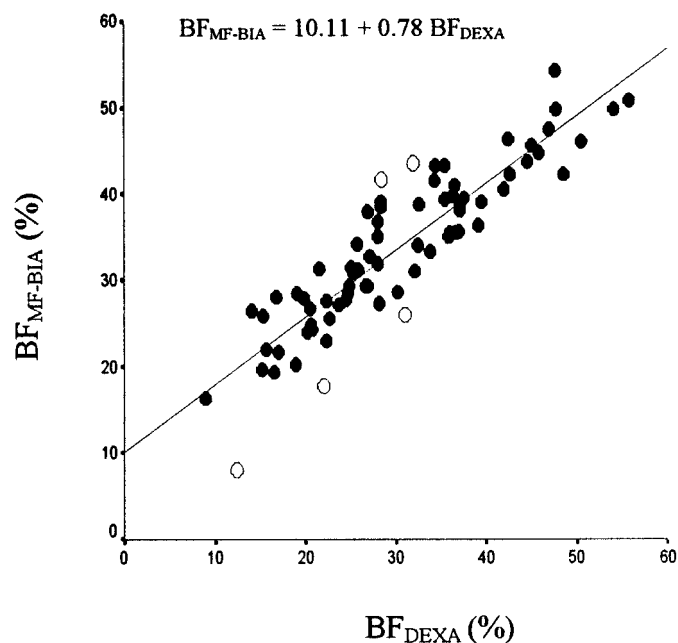[a] Original ICC for all patients ($n = 72$) is 0.846.

Figure 6. Regression plot of BF_{DEXA} and $BF_{MF\text{-}BIA}$. Outliers (standardized z-residue scores < -2 or > 2) are given as open circles.
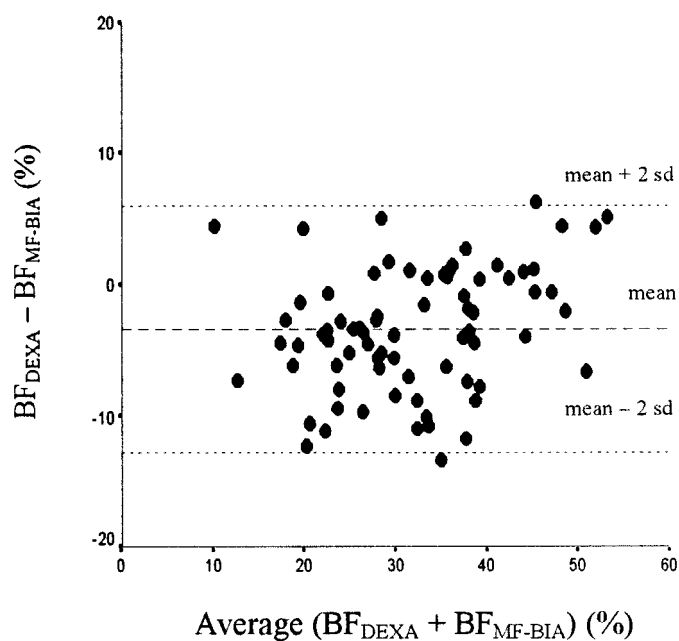

Figure 7. Assessment of BF by DEXA against MF-BIA (Bland and Altman analysis). The relative bias (DEXA − MF-BIA) plotted against the size of the measurement (mean of DEXA and MF-BIA).

Body Fat and Fat Free Mass: MF-BIA Compared to Anthropometry (Skinfolds)

Anthropometric data are available for 74 renal transplant patients (in three patients it was not possible to measure skinfold thickness of the iliac crest because of multiple scars). Descriptive statistics of the percentage body fat measured by anthropometry (BF_{anthr}) and $BF_{MF\text{-}BIA}$ are shown in Table 3. BF_{anthr} compared with $BF_{MF\text{-}BIA}$ is $27.9 \pm 10.2\%$ *versus* $33.4 \pm 9.2\%$ ($P < 0.05$). BF_{anthr} is highly and significantly correlated to $BF_{MF\text{-}BIA}$ ($r = 0.860$, $P < 0.001$). The regression plot of BF_{anthr} and $BF_{MF\text{-}BIA}$ is given in Figure 8. The Bland and Altman analysis is plotted in Figure 9. The mean differ-ence between BF_{anthr} and $BF_{MF\text{-}BIA}$ is $5.5 \pm 5.2\%$ (95% CI, -6.7 to -4.3%). Limits of agreement show that $BF_{MF\text{-}BIA}$ may be 15.9% higher or 4.9% lower than BF_{anthr}.

The intraclass correlation coefficient for method agreement of BF by anthropometry and by MF-BIA ($ICC_{BF\text{-}A}$) is 0.856; because this ICC lies only a fraction above the predetermined lowest acceptable method agreement, method agreement between anthropometry and MF-BIA for measuring body fat appears to be questionable. The approximate one-sided lower 95% confidence level for the ICC turns out to be 0.793. Table 9 shows the subcategory ICC values. Also in most subcatego-

Table 8. Subcategory intraclass correlation coefficients for measurement of percentage body fat by DEXA and MF-BIA[a]

	ICC for Subcategories	
Factors		
gender	0.833 (men, $n = 41$)	0.823 (women, $n = 34$)
cystic kidneys (PCKD)	0.829 (PCKD, $n = 9$)	0.889 (non-PCKD, $n = 65$)
calcium antagonists	0.894 (users, $n = 27$)	0.880 (nonusers, $n = 47$)

	Lowest 33% ICC	Middle 33% ICC	Highest 33% ICC
Covariates			
age	0.862	0.856	0.916
BMI	0.754	0.870	0.879
waist-to-hip ratio	0.885	0.903	0.847
hydration status LBM	0.865	0.915	0.919
ratio ECW to ICW	0.919	0.831	0.896
creatinine clearance	0.863	0.933	0.815

[a] Original ICC for all patients ($n = 75$) is 0.887.
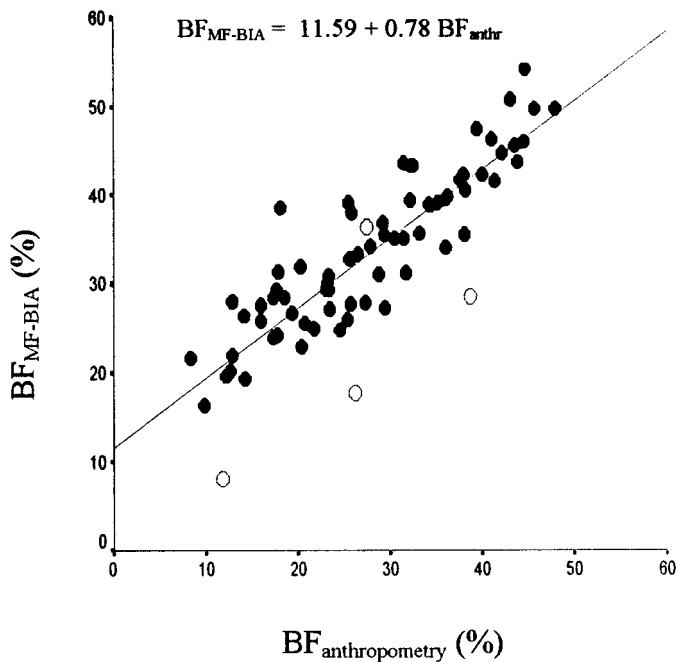

Figure 8. Regression plot of $BF_{anthropometry}$ and $BF_{MF\text{-}BIA}$. Outliers (standardized z-residue scores < -2 or > 2) are given as open circles.
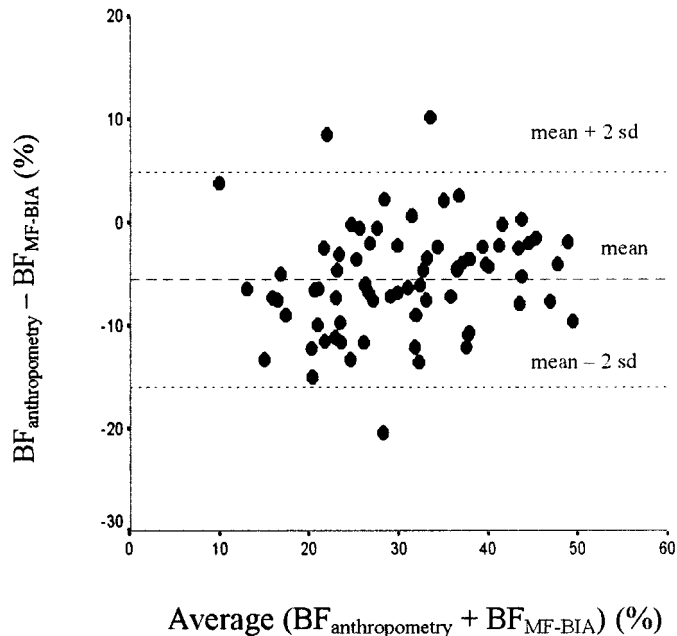

Figure 9. Assessment of BF by anthropometry against MF-BIA (Bland and Altman analysis). The relative bias (anthropometry − MF-BIA) against the size of the measurement (mean of anthropometry and MF-BIA).

ries method agreement is poor. Because fat free mass is calculated as 100% minus fat mass both in anthropometry and in MF-BIA, the results of ICC and thus of method agreement with regard to fat free mass are identical.

Body Fat and Fat Free Mass: DEXA Compared to Anthropometry (Skinfolds)

DEXA total body scans and anthropometric data are available for 72 renal transplant patients. Descriptive statistics of BF_{DEXA} and BF_{anthr} are shown in Table 3. BF_{DEXA} compared with BF_{anthr} is 30.2 ± 10.7% *versus* 28.1 ± 10.3% ($P < 0.05$). BF_{DEXA} is highly and significantly correlated to BF_{anthr} ($r =$

0.913, $P < 0.001$). The regression plot of BF_{DEXA} and BF_{anthr} is given in Figure 10. In Figure 11, the Bland and Altman analysis is plotted. The mean difference between BF_{DEXA} and BF_{anthr} is 2.1% (95% CI, 1.1 to 3.1%); limits of agreement show that BF_{anthr} may be 6.7% higher or 10.9% lower than BF_{DEXA}.

The intraclass correlation coefficient for method agreement between BF_{DEXA} and BF_{anthr} ($ICC_{BF\text{-}DA}$) turns out to be 0.913 (95% limits of agreement, −6.5 to 10.7%). The approximate one-sided 95% lower confidence level for the ICC is 0.872. The subcategory ICC values are given in Table 10. In most of

Table 9. Subcategory intraclass correlation coefficients for measurement of percentage body fat by anthropometry (skinfolds) and MF-BIA[a]

	ICC for Subcategories	
Factors		
gender	0.722 (men, $n = 40$)	0.827 (women, $n = 34$)
cystic kidneys (PCKD)	0.884 (PCKD, $n = 9$)	0.848 (non-PCKD, $n = 64$)
calcium antagonists	0.834 (users, $n = 28$)	0.866 (nonusers, $n = 45$)

	Lowest 33% ICC	Middle 33% ICC	Highest 33% ICC
Covariates			
age	0.735	0.857	0.897
BMI	0.602	0.859	0.897
waist-to-hip ratio	0.899	0.856	0.773
hydration status LBM	0.722	0.825	0.893
ratio ECW to ICW	0.841	0.794	0.890
creatinine clearance	0.784	0.910	0.776

[a] Original ICC for all patients ($n = 74$) is 0.856.

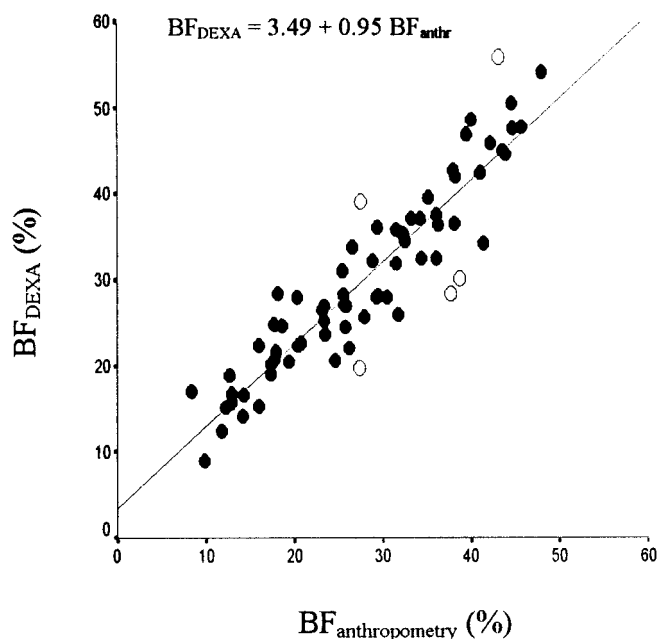*Figure 10.* Regression plot of BF_{DEXA} and $BF_{anthropometry}$. Outliers (standardized z-residue scores < -2 or > 2) are given as open circles.

Figure 11. Assessment of BF by DEXA against anthropometry (Bland and Altman analysis). The relative bias (DEXA − anthropometry) plotted against the size of the measurement (mean of DEXA and anthropometry).

the subcategories, the ICC is larger than 0.85; however, especially in patients with a BMI < 22.5 kg/m^2, patients with a high waist-to-hip ratio, and patients with a low hydration status of the LBM, method agreement does not meet the predetermined acceptable level. The results of ICC and thus of method agreement with regard to fat free mass are identical.

Discussion

In this study, we assessed in the first place the reproducibility of MF-BIA measurements, in the second place method agreement between MF-BIA and isotope dilution, DEXA, and
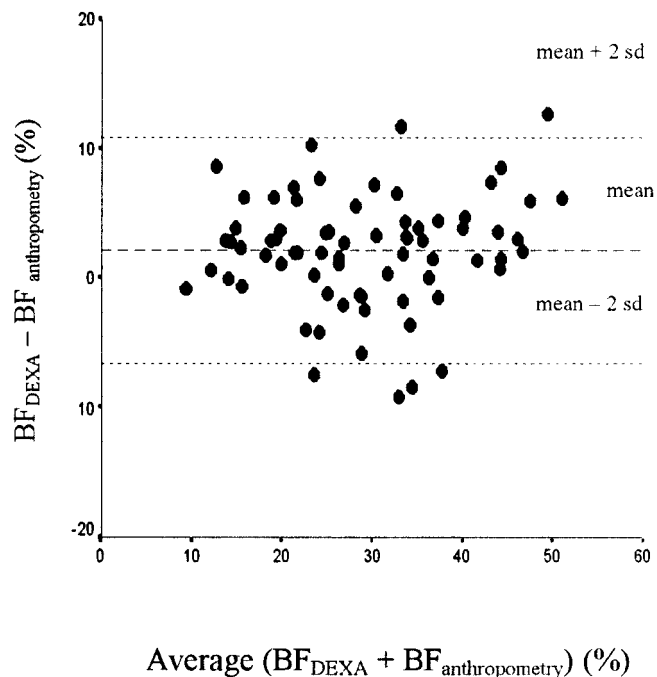
anthropometry, and finally method agreement between DEXA and anthropometry in renal transplant patients.

Reproducibility of MF-BIA Measurements

The reproducibility of the MF-BIA measurements was assessed by calculating coefficients of variation (CV) of duplicate measurements. The CV of the TBW measurements was 1.0%, and the CV of the ECW measurements was 0.6%. This

Table 10. Subcategory intraclass correlation coefficients for measurement of percentage body fat by DEXA and anthropometry (skinfolds)[a]

	ICC for Subcategories		
Factors			
gender	0.822 (men, $n = 39$)		0.850 (women, $n = 33$)
cystic kidneys (PCKD)	0.888 (PCKD, $n = 9$)		0.912 (non-PCKD, $n = 62$)
calcium antagonists	0.918 (users, $n = 27$)		0.906 (nonusers, $n = 44$)
	Lowest 33% ICC	Middle 33% ICC	Highest 33% ICC
Covariates			
age	0.886	0.903	0.932
BMI	0.841	0.902	0.851
waist-to-hip ratio	0.886	0.940	0.811
hydration status LBM	0.806	0.943	0.925
ratio ECW to ICW	0.947	0.842	0.931
creatinine clearance	0.912	0.935	0.839

[a] Original ICC for all patients ($n = 72$) is 0.913.

suggests a high reproducibility of the TBW and ECW measurements by MF-BIA, also reported by others (38–40).

Body Water Compartments: MF-BIA Compared to Isotope Dilution

MF-BIA is based on the different conductive and dielectric properties of various biologic tissues at various frequencies of current. Blood, muscles, and other tissues that contain a lot of water and electrolytes are highly conductive; in contrast, bone, fat tissue, and air-filled spaces are highly resistive. Currents will flow predominantly through materials with higher conductivities (41). The volume of the various tissues can be deduced from measurement of their resistances. In MF-BIA, the human body is considered as five conductive cylinders (*i.e.*, the trunk, two arms, and two legs), which are connected in series. In conductors connected in series, the conductor with the smallest cross-sectional area (*i.e.*, the arm) will determine most of the resistances in series. Although the trunk comprises nearly 50% of the total body weight, the trunk (conductor with the largest cross-sectional area) may have only little influence on the whole body resistance (40,41).

MF-BIA measures body impedance at a wide range of frequencies. The advantage of MF-BIA above the classic single frequency bioimpedance measurement is the possibility of MF-BIA to discriminate between the extracellular and intracellular water compartment. At low frequencies, cell membranes act like a condenser and completely block the flow of the current through the intracellular pathway. At higher frequencies, the condenser function of the cell membranes is lost, and the current flows to both the extra- and intracellular compartments and thus to the total body water (30,31,42). Another advantage of MF-BIA is that MF-BIA predicts the volume of TBW and ECW from a general mixture theory (theory of Hanai) (30–32). Water compartments are directly calculated from resistance values, assuming specific resistances of ECW

and ICW. So, empirically derived prediction formulas are no longer necessary for the estimation of TBW and ECW.

In this study, we validated MF-BIA against D_2O and KBr isotope dilution, a technique that is considered to be the gold standard for measurement of body water compartments in healthy subjects. The accuracy of isotope dilution for measurement of TBW is excellent; the accuracy is dependent on the uncertainty of the estimate of nonaqueous exchange, which is about 1% (20). For TBW measurements by continuous flow isotope ratio mass spectrometry, used in this study, the accuracy is 1.6% (18). The accuracy for the measurement of ECW by isotope dilution is about 5% (19).

Compared with D_2O dilution, MF-BIA underestimates TBW by 0.7 ± 2.1 L. ICC_{TBW} and the ICC values of the various subgroups are all >0.886. These high ICC values suggest good method agreement between D_2O dilution and MF-BIA for measuring TBW; therefore, for measuring TBW the D_2O dilution technique might be substituted by MF-BIA.

Dihydropyridine calcium antagonists, frequently prescribed for treatment of hypertension in renal transplant patients, can lead to peripheral edema. We investigated whether the use of these calcium antagonists influenced the reliability of MF-BIA; the ICC_{TBW} values in the group of patients using calcium antagonists and in the group of patients not using this medication are almost equal: 0.943 and 0.940, respectively. So, the use of calcium antagonists does not correlate with the method agreement for TBW.

We also investigated whether the presence of polycystic kidneys in the abdomen is related to method agreement. In patients with polycystic kidneys, the trunk contains a higher amount of body water than in patients without cystic kidneys. As mentioned earlier, in MF-BIA the trunk contributes hardly to the whole-body resistance, which is a major parameter in the estimation of body water compartments by MF-BIA (40,41). Therefore, in patients with polycystic kidney disease, MF-BIA

might underestimate the total body water compared to isotope dilution, and method agreement might be lower. The ICC_{TBW} between isotope dilution and MF-BIA in patients without polycystic kidneys is excellent (0.950); in patients with cystic kidneys the ICC_{TBW} appears to be somewhat lower, but still acceptable (0.886). So, the presence of cystic kidneys seems to be related to the method agreement between isotope dilution and MF-BIA for TBW.

Compared to KBr dilution, MF-BIA overestimates ECW by 3.3 ± 1.8 L. ICC_{ECW} is 0.846 and thus below the lowest acceptable method agreement. The ICC values in most of the subgroups of patients do not meet the acceptable level of method agreement either. The low method agreement in male patients ($ICC_{ECW} = 0.817$) compared to the acceptable method agreement in female patients ($ICC_{ECW} = 0.877$) is remarkable. An explanation for this acceptable method agreement in female transplant patients cannot be given as yet. So, in renal transplant patients method agreement for measuring ECW by KBr dilution and by MF-BIA is not acceptable; method agreement is only reached in some very specific subgroups of patients. However, these results should be interpreted with caution. As discussed before, TBW determination by MF-BIA is satisfactory. TBW_{MF-BIA} is calculated as the sum of ECW and ICW. In contrast to the ICW, the ECW is measured directly by MF-BIA (33). The excellent agreement between TBW_{D_2O} and TBW_{MF-BIA} suggests that some of the disagreement between ECW_{KBr} and ECW_{MF-BIA} might be due to errors with the bromide dilution technique. Errors with the bromide isotope dilution technique, especially in patients with an abnormal ECW, might be due to the uncertainty in the correction constants for plasma water and Gibbs–Donnan equilibration as well as to penetration into the intracellular space due to changes in plasma protein concentrations and hematocrit (20).

Body Fat: MF-BIA Compared to DEXA

MF-BIA derives fat free mass from the measured volume of TBW. Fat mass is calculated as body weight minus fat free mass. In MF-BIA, it is assumed that fat is anhydrous and that the lean body mass contains a relatively constant proportion of water (73.2%), a proportion that is assumed to be true in healthy individuals (43). A disturbed water status can significantly reduce the accuracy of predictions of fat free mass and fat mass by MF-BIA. In renal transplant patients, the assumption of a constant hydration status is not invariably true.

As mentioned earlier, MF-BIA measures TBW with sufficient accuracy in renal transplant patients. The hydration status of the lean body mass (calculated as TBW_{D_2O} divided by lean body mass DEXA) in our study population is 0.709 ± 0.04 L/kg LBM (range, 0.596 to 0.836 L/kg LBM) and significantly different from the assumed hydration status. Therefore, MF-BIA probably overestimates the actual percentage of body fat.

We compared the values of BF_{MF-BIA} to the values of BF_{DEXA}. DEXA is a very useful technique for directly assessing soft tissue as well as bone; it has been shown to be of relatively high accuracy and very high precision (26). However, DEXA is not assumption-free: DEXA too assumes that the hydration status of the LBM is uniform and fixed at 0.732

L/kg LBM (26,27,44). The degree to which DEXA measurements of soft tissue are sensitive to changes in hydration in adult humans is unknown, and therefore further research is needed. Hence, DEXA cannot be considered as the gold standard for measuring body fat at this moment.

Compared to DEXA, MF-BIA overestimates the percentage of body fat by 3.7 ± 4.7%. The original ICC for method agreement between BF_{DEXA} and BF_{MF-BIA} is 0.887. So, method agreement seems acceptable. However, when our renal transplant population is divided into males and females, method agreement is unacceptable in both groups; the reason for this is that given an equal patient-by-method error, the ICC is generally lower within relatively homogeneous subgroups. The ICC values in some other specific subcategories also do not meet the acceptable level of method agreement. An explanation for the low method agreement in these groups cannot be given as yet. So, in renal transplant patients, method agreement between DEXA and MF-BIA for measuring body fat is not acceptable; therefore, results of DEXA and MF-BIA fat measurements may not be substituted for each other.

Body Fat: MF-BIA Compared to Anthropometry

We also compared MF-BIA to anthropometry (skinfolds) for measuring body fat. Skinfold thickness measurements are rapid, simple, noninvasive, and inexpensive. In skinfold thickness measurements, it is assumed that the majority of body fat resides in subcutaneous regions, that there is a consistent relationship between subcutaneous and visceral fat, and that body fat distribution is stable (45–47). It is also assumed that the density of the lean body mass is normal, *i.e.*, that the hydration status of the LBM is normal (0.732 L/kg LBM) and that the bone mineral content represents a fixed fraction of the LBM (47,48). The reliability of skinfold measurements is approximately 5%; it largely depends on the sites of the skinfold measurements and the experience of the examiner (45). Anthropometric evaluation of body fat is most appropriate for population surveys. Skinfold measurements cannot be considered a gold standard for measurement of body fat content.

Compared to anthropometry, MF-BIA overestimates the percentage body fat in renal transplant patients by 5.5 ± 5.2%. The original ICC for measurement of agreement between BF_{anthr} and BF_{MF-BIA} is 0.856 and thus only a fraction above the lowest acceptable level of method agreement. Therefore, method agreement seems to be questionable. Both in men and women the ICC for method agreement is unacceptable. In most of the other subgroups, ICC values also indicate questionable, poor, or even very poor method agreement. The reason(s) for the low method agreement in these subgroups are not known.

Body Fat: DEXA Compared to Anthropometry

At present, DEXA is used more and more for the measurement of total body fat content. However, as mentioned earlier, DEXA is not considered the gold standard for measurement of body fat. We finally compared DEXA to the relative simple anthropometric (skinfold thickness) measurements. Compared to DEXA, anthropometry underestimates the percentage of body fat in renal transplant patients by 2.1 ± 4.4%. Hart *et al.*

(49) also reported that skinfold measurements underestimate the percentage of body fat compared to DEXA in renal transplant patients. The original ICC for method agreement between DEXA and anthropometry found in our study is high, *i.e.*, 0.913. So, method agreement seems to be very good. Nevertheless, there are a few subcategories of renal transplant patients in which the ICC is below the predetermined acceptable level and in which method agreement thus is questionable. In patients with a low ($<$22.5 kg/m^2) and high ($>$26.0 kg/m^2) BMI, the poor method agreement can be explained by the fact that measurement of skinfolds in very lean and very obese subjects (who have respectively a low or high BMI) is technically difficult; therefore, the validity of skinfold measurements in these groups could be affected (50). In patients with a high waist-to-hip ratio (apple configuration), the abnormal body fat distribution might play a role. Skinfold measurements, in which a stable body fat distribution is assumed, might be invalid in this patient group. In patients with a low hydration status ($<$0.6900 L/kg), method agreement is probably low because both anthropometry and DEXA assume a normal hydration status (0.732 L/kg LBM).

Method Comparison for the Measurement of Fat Free Mass

In MF-BIA and anthropometry (skinfold thickness measurements), the body is considered to be composed of two compartments: the fat compartment and the fat free compartment (bone mass included) (41,47). In DEXA, the body is divided into the fat compartment, the lean soft tissue compartment, and the bone mineral content (fat free mass equals lean soft tissue mass plus bone mineral content) (27). Thus, in MF-BIA as well as in DEXA and anthropometry the percentage of fat free mass equals 100% minus the percentage of body fat. Therefore, when the percentage of body fat is overestimated in the method comparisons, the percentage of fat free mass will be underestimated, and conversely, when the percentage of body fat is underestimated, the percentage of fat free mass will be overestimated. Furthermore, the conclusions about the degree of method agreement between MF-BIA, DEXA, and anthropometry for the measurement of body fat are the same for the measurement of fat free mass.

In conclusion, MF-BIA seems to be suitable to measure TBW with sufficient accuracy in renal transplant patients. The agreement between ECW_{KBr} and ECW_{MF-BIA} is not satisfactory. However, possible limitations of the bromide isotope dilution technique have to be taken into account.

Because neither DEXA nor anthropometry can be considered a gold standard, the interpretation of the results of the method comparisons regarding fat and fat free mass should be done with caution. Still, the poor agreement for measuring body fat and fat free mass between MF-BIA and DEXA and between MF-BIA and anthropometry, and the reasonable agreement between DEXA and anthropometry, makes the reliability of MF-BIA in the assessment of fat and fat free mass in renal transplant patients questionable.

References

1. Moore LW, Gaber AO: Patterns of early weight change after renal transplantation. *J Ren Nutr* 6: 21–25, 1996
2. Merion RM, Twork AM, Rosenberg L, Ham JM, Burtch GD, Turcotte JG, Rocher LL, Campbell DA: Obesity and renal transplantation. *Surg Gynecol Obstet* 172: 367–376, 1991
3. Yanovski JA, Cutler CB: Glucocorticoid action and the clinical features of Cushing's syndrome. *Endocrinol Metab Clin North Am* 23: 487–509, 1994
4. Horber FF, Zuercher RM, Herren H, Crivelli MA, Robotti G, Frey FJ: Altered body fat distribution in patients with glucocorticoid treatment and in patients on long-term dialysis. *Am J Clin Nutr* 43: 758–769, 1986
5. Danovitch GM: Immunosuppressive medications and protocols for kidney transplantation. In: *Handbook of Kidney Transplantation*, 1st Ed., edited by Danovitch GM, Boston, Little Brown and Company, 1992, pp 67–104
6. Chan MK, Varghese Z, Persaud JW, Fernando ON, Moorhead JF: The role of multiple pharmaco-therapy in the pathogenesis of hyperlipidemia after renal transplantation. *Clin Nephrol* 15: 309–313, 1981
7. Vathsala A, Weinberg RB, Schoenberg L, Grevel L, Dunn J, Goldstein RA, Van Buren CT, Lewis RM, Kahan BD: Lipid abnormalities in renal transplant recipients treated with cyclosporine. *Transplant Proc* 21: 3670–3673, 1989
8. Isoniemi H, Tikkanen M, Hayry P, Eklund B, Hockerstedt K, Salmela K, Ahonen J: Lipid profiles with triple drug immunosuppressive therapy and with double drug combinations after renal transplantation and stable graft function. *Transplant Proc* 23: 1029–1031, 1991
9. Lapidus L, Bengtsson C, Pennert K, Rybo E, Sjöström L: Distribution of adipose tissue and risk of cardiovascular disease and death: A 12 year follow-up of participants in the population study of women in Gothenburg, Sweden. *Br Med J* 289: 1257–1261, 1984
10. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G: Abdominal adipose tissue distribution, obesity and risk of cardiovascular disease and death: 13 year follow-up of participants in the study of men born in 1913. *Br Med J* 288: 1401–1404, 1984
11. Gunnarsson R, Lofmark R, Nordlander R, Nyquist O, Groth CG: Acute myocardial infarction in renal transplant recipients: Incidence and prognosis. *Eur Heart J* 5: 218–221, 1984
12. Raine AEG, Margreiter R, Brunner FP, Ehrich JHH, Geerlings W, Landais P, Loirat C, Mallick NP, Selwood NH, Tufveson G: Report of management of renal failure in Europe, XXII 1991. *Nephrol Dial Transplant* 7[Suppl 2]: 7–35, 1992
13. Hyperlipidemia after renal transplantation [Editorial]. *Lancet* 1: 919–920, 1988
14. Markell MS, Friedman EA: Hyperlipidemia after organ transplantation. *Am J Med* 87: 61N–67N, 1989
15. Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ: Cardiovascular disease after renal transplantation. *J Am Soc Nephrol* 7: 158–165, 1996
16. Ibels LS, Alfrey AC, Weil R: Hyperlipidemia in adult, pediatric and diabetic renal transplant recipients. *Am J Med* 64: 634–642, 1978
17. Bioelectrical impedance analysis in body composition measurement. National Institutes of Health Technology Assessment Conference Statement. *Am J Clin Nutr* 64[Suppl 3]: 524S–532S, 1996
18. Van Kreel BK, Van der Vegt F, Meers M: Determination of total

body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 31: 108–111, 1996

19. Van Kreel BK: An improved bromide assay for the estimation of extracellular water volume by capillary gas chromatography. *Clin Chim Acta* 231: 117–128, 1994

20. Schoeller DA: Hydrometry. In: *Human Body Composition*, 1st Ed., edited by Roche AF, Heymsfield SB, Lohman TG, Champaign, IL, Human Kinetics, 1996, pp 25–44

21. Schoeller DA, Van Santen E, Peterson DW, Dietz W, Jaspen J, Klein PD: Total body water measurements in humans with ^{18}O and ^{2}H labeled water. *Am J Clin Nutr* 33: 2686–2693, 1980

22. Edelman IS, Leibman J: Anatomy of body water and electrolytes. *Am J Med* 27: 256–277, 1959

23. Donnan FG, Allmand AJ: Ionic equilibration across semi-permeable membranes. *J Chem Soc* 105: 1941–1963, 1914

24. Miller ME, Cosgriff JM, Forbes GB: Bromide space determination using anion-exchange chromatography for measurement of bromide. *Am J Clin Nutr* 50: 168–171, 1989

25. Bell EF, Ziegler EE, Forbes GB: Corrected bromide space. [Letter]. *Pediatr Res* 18: 392–393, 1984

26. Mazess RB, Barden HS, Bisek JP, Hanson J: Dual-energy x-ray absorptiometry for total body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 51: 1106–1112, 1990

27. Lohman TG: Dual energy x-ray absorptiometry. In: *Human Body Composition*, 1st Ed., edited by Roche AF, Heymsfield SB, Lohman TG, Champaign, IL, Human Kinetics, 1996, pp 63–78

28. Durnin JVGA, Womersly J: Body fat assessed from total body density and its estimation from skinfold thickness measurements in 481 men and women aged from 16 to 72 years. *Br J Nutr* 32: 77–97, 1974

29. Siri WE: The gross composition of the body. In: *Advances in Biological and Medical Physics*, 1st Ed., edited by Tobias CA, Lawrence JH, New York, Academic Press, 1956, pp 239–280

30. Van Loan MD, Kopp LE, King JC, Wong WW, Mayclin PL: Fluid changes during pregnancy: Use of bioimpedance spectroscopy. *J Appl Physiol* 78: 1037–1042, 1995

31. De Lorenzo A, Andreoli A, Matthie J, Withers P: Predicting body cell mass with bioimpedance by using theoretical methods: A technological review. *J Appl Physiol* 82: 1542–1558, 1997

32. Hanai T: Electrical properties of emulsions. In: *Emulsion Science*, 1st Ed., edited by Sherman PH, London, Academic Press, 1968, pp 354–377

33. Operating manual Xitron 4000B: Bio-impedance spectrum analyser system. Xitron Technologies INC, San Diego, CA

34. Altman DG, Bland JM: Measurement in medicine: The analysis of method comparison studies. *Statistician* 32: 307–317, 1983

35. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1: 307–310, 1986

36. Fleiss JL: *The Design and Analysis of Clinical Experiments*, 1st Ed., New York, John Wiley and Sons, 1986, pp 1–32

37. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976

38. Hannan WJ, Cowen SJ, Plester CE, Fearon KCH, DeBeau A: Comparison of bio-impedance spectroscopy and multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients. *Clin Sci* 89: 651–658, 1995

39. Van Marken Lichtenbelt WD, Westerterp KR, Wouters L, Luijendijk SCM: Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. *Am J Clin Nutr* 60: 159–166, 1994

40. Van Loan MD, Withers P, Matthie J, Mayclin PL: Use of bio-impedance spectroscopy (BIS) to determine extracellular fluid (ECF), intracellular fluid (ICF), total body water (TBW) and fat free mass (FFM). In: *Human Body Composition: In Vivo Methods, Models and Assessment*, 1st Ed., edited by Ellis KJ, Eastman JD, New York, Plenum, 1993, pp 67–70

41. Baumgartner RN: Electrical impedance and total body electrical conductivity. In: *Human Body Composition*, 1st Ed., edited by Roche AF, Heymsfield SB, Lohman TG, Champaign, IL, Human Kinetics, 1996, pp 79–107

42. Kushner RF: Bioelectrical impedance analysis: A review of principles and applications. *J Am Coll Nutr* 11: 199–209, 1992

43. Pace N, Rathbun EN: Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* 158: 685–691, 1945

44. Roubenoff R, Kehayias JJ, Dawson-Hughes B, Heymsfield SB: Use of dual-energy x-ray absorptiometry in body composition studies: Not yet a "gold standard." *Am J Clin Nutr* 58: 589–591, 1993

45. Lukaski HC: Methods for the assessment of human body composition: Traditional and new. *Am J Clin Nutr* 46: 537–556, 1987

46. Jensen MD: Research techniques for body composition assessment. *J Am Diet Assoc* 92: 454–460, 1992

47. Roche AF: Anthropometry and ultrasound. In: *Human Body Composition*, 1st Ed., edited by Roche AF, Heymsfield SB, Lohman TG, Champaign, IL, Human Kinetics, 1996, pp 167–190

48. Going SB: Densitometry. In: *Human Body Composition*, 1st Ed., edited by Roche AF, Heymsfield SB, Lohman TG, Champaign, IL, Human Kinetics, 1996, pp 3–24

49. Hart PD, Wilkie ME, Edwards A, Cunningham J: Dual energy x-ray absorptiometry versus skinfold measurements in the assessment of total body fat in renal transplant recipients. *Eur J Clin Nutr* 47: 347–352, 1993

50. Gray DS, Bray GA, Gemayel N, Kaplan K: Skinfold thickness measurements in obese subjects. *Am J Clin Nutr* 51: 571–577, 1990