Validation of Multifrequency Bioelectrical Impedance Analysis in Monitoring Fluid Balance in Healthy Elderly Subjects

Marcel G.M. Olde Rikkert,1 Paul Deurenberg,2 René W.M.M. Jansen,1 Martin A. van’t Hof,2 and Willibrord H.L. Hoefnagels1

Departments of 1Geriatric Medicine and 2Methodology, Statistics and Informatics, University of Nijmegen, The Netherlands.
2Department of Human Nutrition, Wageningen Agricultural University, The Netherlands.

Background. Multifrequency Bioelectrical Impedance Analysis (MFBIA) is a novel method to assess body composition in elderly subjects. However, it is unclear whether MFBIA can detect changes in body water compartments in elders. We aimed to determine the within-subject variability of MFBIA and the responsiveness to a diuretic intervention in aged subjects with a stable fluid balance.

Methods. We selected 12 healthy active elderly subjects (5 male, 7 female) with a mean age of 75 years. Total body water and extracellular fluid (ECF) were measured by deuterium oxide- and potassium bromide-dilution techniques. Within-subject variability in total body MFBIA was assessed by performing four measurements at 1, 5, 50, and 100 kHz within a 2-month period. Subsequently, responsiveness of MFBIA to the ECF loss caused by oral administration of 40 mg of furosemide was determined.

Results. Within-subject variability in MFBIA at 1, 5, 50, and 100 kHz expressed as standard deviations was 21, 19, 14, and 14 Ohm (Ω), respectively. Furosemide caused a mean weight loss of 1.8 ± 0.6 kg, which resulted in significant increases in impedance of 57 ± 24 Ω at 1 kHz and 37 ± 12 Ω at 100 kHz (p < .001). The responsiveness of MFBIA for the diuretic intervention was best at 5 kHz (responsiveness index = 1.98).

Conclusions. Within-subject variability of MFBIA was small in healthy elderly subjects with stable fluid balance. Responsiveness of MFBIA to 9% furosemide-induced ECF loss was excellent. These data support the necessity for further clinical assessment of the value of MFBIA in monitoring fluid balance in geriatric patients.

OVERHYDRATION and dehydration are extremely common problems in geriatric practice. This may be caused by a labile fluid balance in elderly people. A reliable assessment of a patient's fluid balance is very difficult to make, especially in mild and moderately severe disturbances of fluid balance. It has been shown that physical examination is not very sensitive in recognizing early stages of dehydration in the elderly (1–3). From the laboratory measures, plasma osmolality is most frequently used to assess dehydration. However, high normal or elevated serum osmolality may be present without dehydration in geriatric patients (4). Moreover, early stages of overhydration due to congestive heart failure are also difficult to diagnose (5). Therefore, an accurate diagnostic instrument to assess fluid balance, which is applicable to frail elderly subjects, is needed.

Recently, the bioelectrical impedance analysis has become a popular method of assessing body composition because it is a quick, safe, and noninvasive technique that does not require much cooperation from the subjects (6). It has been shown that the resistance or impedance to a weak electrical current at a frequency of 50 kHz is inversely proportional to the amount of total body water and the lean body mass (7,8). Multifrequency Bioelectrical Impedance Analysis (MFBIA) instruments have been developed because low and high frequency measurements could possibly distinguish between extracellular fluid (ECF) and total body water (TBW) (9). This can be explained by the fact that the alternating current is unable to penetrate the cell membrane at low frequencies, owing to the capacitive properties of the membrane. Apart from the impedances measured at various frequencies, low/high frequency impedance ratios are also often calculated. Increases in these impedance ratios correlated well with decreases in ECF/TBW ratios due to ECF loss caused by administration of a diuretic drug (10). Epidemiological studies have shown that MFBIA is a useful instrument to estimate fat-free mass, ECF, and TBW in elderly populations (11–13). Hence, the validity of MFBIA as a tool in measuring body composition is not seriously doubted. However, the sensitivity to detect changes in body composition has been debated (14). Data to assess the usefulness of MFBIA as a diagnostic tool in elderly patients are scarce. As a first step in the assessment of the clinical value of MFBIA, we studied MFBIA as a diagnostic measurement of fluid balance in healthy elderly subjects.

In the diagnostic process, MFBIA might be used as a discriminative or as an evaluative measurement of disturbances of fluid balance (15). As a discriminative tool, MFBIA needs to provide reliable data on the actual hydration state of unknown patients. However, the individual error in estimating ECF and TBW by means of population-
of this investigation was to assess within-subject variability changes in body water by using MFBIA (10,18,19). The aim was to determine whether the reliability of individual estimates of population-based reference values for MFBIA.

It is more likely that MFBIA can be used in monitoring fluid balance in elderly patients. For this purpose, MFBIA needs to be responsive to individual changes in fluid balance. In addition, within-subject variability has to be small in subjects with a stable fluid balance. At present, there are conflicting data on the reliability of individual estimates of changes in body water by using MFBIA (10,18,19). The aim of this investigation was to assess within-subject variability of MFBIA and the responsiveness to a standardized diuretic intervention in fluid balance in healthy elderly subjects with a stable fluid balance.

METHODS

Subjects. — Out of 25 subjects aged 70 years and older who were invited for this study, 17 men and women were willing to participate. All subjects were recruited from the elderly participants of the Nijmegen Annual Four-Days Marches. We selected long-distance marchers because we assumed that these subjects were in good health, without pathological disturbances of fluid balance. In addition, these healthy elderly subjects could probably endure the stress of oral administration of a diuretic safely. None of the subjects showed either renal or cardiovascular diseases or disturbances of fluid balance as assessed by medical history and physical and laboratory examinations. Five subjects were excluded because they used drugs that might affect fluid balance (diuretics: 4 subjects; corticosteroids: 1 subject).

Finally, 12 eligible subjects (5 male, 7 female) took part in the present study. The study protocol was approved by the Committee for Experiments with Human Subjects of the University Hospital of Nijmegen. All subjects provided written informed consent.

Design. — Within- and between-subject variability in MFBIA were determined by performing impedance measurements on 4 days within a 2-month period. Consecutive measurements were separated by 7–28 days. All studies were performed in the morning, after an overnight fast from midnight. Four-electrode MFBIA was performed on the nondominant side, following the methodology for total body measurements (8), after emptying the bladder and supine rest for 45 min. Impedance was measured at 4 frequencies: 1, 5, 50, and 100 kHz, using a Human-Im Scan (Dietosystem, Milan, Italy). Adhesive electrodes were used (Red Dot 2330, 3M, Leiden, The Netherlands). On each occasion plasma sodium, urea, and creatinine were measured. To assess body composition in the subjects, TBW and ECF were determined by duplicate deuterium oxide- and potassium bromide-dilution techniques on the second and third visit. A cocktail of 10 g deuterium oxide (precision of 0.000 g) and 900 mg potassium bromide was taken orally before MFBIA measurements. After 3.5-h dilution time a venous blood sample was drawn. After sublimation of the plasma, the deuterium concentration was determined in the sublimate by infrared analysis (20). TBW was calculated from the given dose and the tracer concentration determined in plasma, using a correction of 5% for nonaqueous dilution (21). Bromide in plasma was determined after ultrafiltration by high liquid chromatography (22). A correction of 5% was used for the Donnan effect and a correction of 10% for nonextracellular dilution (21). Analytical measurement errors in bromide and deuterium dilution methods, as measured by within-run and between-run variability in two pairs of identical blood samples, were 2.2% and 2.5%, respectively. Validity of these dilution methods in measuring body fluid compartments is good compared to other dilution methods (21).

Responsiveness of MFBIA to changes in extracellular volume was studied by orally giving all subjects 40 mg furosemide (Lasix, Hoechst, Germany) following the fourth baseline measurement. Body weight and impedances were measured before and 5 h after the administration of furosemide. Body weight was measured on a digital scale to the nearest 0.05 kg (Indicat BII, Berkel, Rotterdam, The Netherlands). Before the diuretic intervention, the electrode positions were marked with indelible ink to facilitate an exact replacement of the electrodes. The urine voided during the 5 h after administration of furosemide was collected, and urinary sodium concentration was determined. The excreted ECF was calculated individually by dividing the excreted amount of urinary sodium by the plasma sodium concentration. After the last measurement, subjects were allowed to drink and eat freely. A final MFBIA was performed 40 h after administering furosemide.

Statistical methods. — All statistical analyses were performed using SPSS for Windows, version 6.1, 1994 (SPSS Inc., Chicago). The usefulness of MFBIA as an evaluative instrument was determined by calculating Guyatt et al.'s responsiveness index for MFBIA and body weight (15). The responsiveness index for MFBIA can be derived by taking the ratio of the change in impedance after diuretic intervention to the between-subject variability in impedance before this intervention. This between-subject variability in within-subject changes in impedances during the first four MFBIA measurements is represented, according to Guyatt, by the square root of twice the mean square error (\(\sqrt{2 \times \text{MSE}}\)) in impedances as calculated by analysis of variance (ANOVA) of repeated MFBIA. This responsiveness index was calculated at 1, 5, 50, and 100 kHz. Similarly, the responsiveness for changes in body weight was determined. A responsiveness index exceeding 1 implies that the instrument tested is very sensitive to change and that only small sample sizes (< 20 subjects) are required in experiments in which change over time in test scores is the endpoint (15).

Between-subject variability was calculated for the mean impedance values of the first four standardized MFBIA measurements. Within- and between-subject variability are...
expressed in standard deviations (SD). For the whole group, significance of the change in MFBIA 5 h following drug administration compared to baseline was tested with a paired t-test. Linear regression was used to calculate prediction formulas for TBW and ECF from \( H/Z_f \), where \( H \) is the body height (m) and \( Z_f \) is the mean body impedance at different frequencies obtained before the use of the diuretic drug. The application of \( H/Z_f \) is generally recommended in calibration of MFBIA against dilution techniques (6). This generally results in prediction formulas for TBW and ECF: predicted body water compartment = \( a + b \times H \times 1/Z_f \), where \( a \) is the intercept, \( b \) is the regression coefficient, and TBW is best predicted by high frequency impedances and ECF by low frequency impedances. Using these formulas, the change (\( \Delta \)) in TBW and ECF was predicted by the formula: \( \Delta = b \times H \times (1/Z_{final} - 1/Z_{initial}) \). The predicted changes were compared with the observed changes in TBW and the changes in ECF calculated by urinary sodium excretion during the 5-h period by the paired t-test. Correlations between the collected amount of urine and weight loss and between ECF loss and increase in impedances were tested with Pearson’s correlation coefficients. Gender effects on within-subject variability were tested with F-tests. Data are presented as mean ± SD, unless indicated otherwise.

**RESULTS**

Some characteristics of the subjects are shown in Table 1. The within-subject variability in TBW (SD of the differences between the measurements divided by \( \sqrt{2} \)) was 3.8 L and in ECF, 0.8 L. Absolute differences in both measurements of TBW and ECF ranged from 0.1 to 10.0 and from 0.0 to 3.2 L, respectively. The within-subject variability for body weight during the first four measurements was 0.5 kg.

The MFBIA results from the first four measurements are summarized in Table 2. Total body impedances of the women were significantly larger compared to men, with \( p < .01 \). Between-subject variability was about 5 times larger than within-subject variability. There was no significant gender effect on within-subject variability.

The prediction formulae for ECF and TBW found in this population were: ECF = 0.314.5 \( \times H/Z_f + 5.82 \); \( r^2 = .70 \); SEE = 2.20 L ECF; TBW = 532.6 \( \times H/Z_f + 10.76 \); \( r^2 = .82 \); and SEE = 3.08 L TBW, where \( Z_f \) is the body impedance at 1 kHz and \( Z_s \) is the body impedance at 5 kHz, \( r^2 \) is the explained variance, and \( \text{SEE} \) is the standard error of the estimate.

The mean loss in body weight, 5 h following oral administration of furosemide, was 1.8 ± 0.6 kg (range: 0.7–2.7). This was in agreement with the mean urine volume (1.8 ± 0.7 L; range: 0.6–2.9; \( r = .94 \)). Hence, related to the body water compartments, the diuretic intervention caused a mean loss in TBW of 1.8 ± 0.7 L (5.1 ± 1.5%). The calculated ECF loss was 1.6 ± 0.5 L (range: 0.4–2.6), which corresponded with a decline in ECF of 8.7 ± 2% (range: 5.3–12.6%). Consequently, the mean ECF/TBW ratio decreased from 49.6% to 47.4%. The changes in impedance at 1, 5, 50, and 100 kHz caused by ECF loss were all statistically significant (Table 3). In all subjects impedances increased following the diuretic intervention. Apart from the 1-kHz measurements, the minimum increase in impedance was larger than the SD of within-subject variability. Even following 35 h, during which the subjects had free access to food and beverages, MFBIA still showed a significant increase at all frequencies. Impedance ratios of 1/100 kHz as well as 5/100 kHz also showed a significant increase at 5 and 40 h after the intervention (results not presented). Subsequently, the responsiveness indexes of MFBIA for the diuretic intervention were calculated (Table 3). Responsiveness indexes were greater than 1 at all frequencies, and responsiveness was best at 5 kHz. Impedance ratios had much smaller responsiveness ratios. The responsiveness index of weight changes was 2.35.

Predicted changes in TBW and ECF were 1.99 ± .84 L and 1.14 ± .49 L, respectively. Observed changes in TBW did not differ from these estimates; however, predicted changes in ECF differed significantly from the ECF changes calculated from collected urinary volume and sodium excretion ratio (\( p < .05 \)).

---

**Table 1. Physical Characteristics of the 12 Elderly Subjects**

<table>
<thead>
<tr>
<th>(5 male, 7 female)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>75 ± 3</td>
<td>72–80</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66 ± 10</td>
<td>50–81</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 ± .10</td>
<td>1.50–1.80</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 3.2</td>
<td>20.1–31.1</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>142 ± 2.4</td>
<td>137–144</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>6.2 ± 1.3</td>
<td>4.3–10.0</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>84 ± 15</td>
<td>50–105</td>
</tr>
<tr>
<td>TBW (l)</td>
<td>36.0 ± 8.1</td>
<td>26.5–50.2</td>
</tr>
<tr>
<td>ECF (l)</td>
<td>17.7 ± 3.7</td>
<td>13.3–22.6</td>
</tr>
<tr>
<td>ECF/TBW (%)</td>
<td>49.6 ± 4.3</td>
<td>44.1–59.4</td>
</tr>
</tbody>
</table>

Notes: Weight, length, body mass index (BMI), plasma sodium, urea, and creatinine as measured during initial assessment. Total body water (TBW), extracellular fluid (ECF), and ECF/TBW ratio are means of two measurements.

**Table 2. Mean Body Impedances at 1, 5, 50, and 100 kHz**

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Impedance (Ω)</th>
<th>SD</th>
<th>Between-Subject SD</th>
<th>Within-Subject SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>654</td>
<td>99</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>623</td>
<td>95</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>546</td>
<td>82</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>515</td>
<td>77</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Responsiveness Indexes of MFBIA for the Diuretic Intervention**

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Responsiveness Index</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/100</td>
<td>1.27</td>
<td>.03</td>
</tr>
<tr>
<td>5/100</td>
<td>1.21</td>
<td>.02</td>
</tr>
</tbody>
</table>

---

FLUID BALANCE AND BIOELECTRICAL IMPEDANCE

M139
**DISCUSSION**

To our knowledge, this is the first study investigating both variability and responsiveness of MFBIA, as a noninvasive diagnostic instrument of fluid balance, in healthy elderly subjects. Although we selected an exceptionally fit group of elderly subjects, 20% had to be excluded because their fluid balance could have been influenced by chronic drug use. TBW of the active elderly subjects was 54.7 ± 7.8% of their body weight. This is in agreement with the general decline of TBW with age (21). ECF was 50 ± 3.0% of TBW, which is within the wide range of 43–52% reported for the ECF/TBW ratio in the literature (12,23–25). Besides differences in subject selection, this wide range can be explained by considerable differences in the techniques used in assessing body composition. The considerable within-subject variability in the TBW (10%) and ECF (5%) is a combination of the analytical error in the measurement of body water volumes by bromide and deuterium dilution and the true differences in body water compartments between the two occasions caused by biological within-subject variability in fluid balance. Less than half of this variability may be caused by technical errors in the dilution assays.

The main findings of our study are: the small within-subject variability in MFBIA, a between-subject variability in MFBIA similar to that reported for younger age groups (19,26,27), and the good responsiveness of MFBIA to changes in fluid balance in healthy elderly subjects. The variability in MFBIA was expressed in SD because there was no correlation between the magnitude of the SD and the mean impedance value. To enable comparison with data from the literature, we also calculated coefficients of variation. The within- and between-subject variability at all frequencies, expressed as coefficients of variation, were 3% and 15%, respectively. The within-subject variability in MFBIA is comparable to the 2.3 and 2.7% day-to-day variability of 50 kHz impedances in healthy elderly subjects published recently (12). This suggests that biological within-subject variability in body water compartments may not differ in a cross-sectional comparison of elderly and younger populations. In younger subjects day-to-day variability ranged from 8.7% at 1 kHz to 2.0% at 100 kHz, and week-to-week variability ranged from 6.7% at 1 kHz to 2.4% at 100 kHz (27). Variability at 1 kHz was smaller in the present study because we used better electrodes with larger surface areas (± 4 cm²).

The between-subject variability in this study is similar to the between-subject variability reported earlier in healthy adult populations with ages ranging from 19 to 56 years (19,26,27). Even in 100 pubertal children a between-subject variability of 9–14% was found for total body impedance at 50 kHz (28). Hence, the generally postulated increase in differences between subjects with age does not seem to be true for bioelectrical impedance measurements when cross-sectional data for different age groups are compared.

The intervention with furosemide resulted in a statistically significant increase in the mean impedance of all subjects. Applying the concept of responsiveness as developed by Guyatt et al. (15), MFBIA seems very useful in monitoring changes in fluid balance. Responsiveness of MFBIA in detecting the effect of 40 mg furosemide was best at 5 kHz. This can be explained by the furosemide-induced decline in ECF, which is measured best at the lower frequencies. The finding that responsiveness of MFBIA was not the highest at 1 kHz may be due to a somewhat larger within-subject variability at this frequency. Although overall low/high frequency impedance ratios also showed a statistically significant increase following the drug-induced ECF loss, these increases were too small to be useful as an evaluative parameter of changes in body water compartments or changes in the ECF/TBW ratio.

Responsiveness of MFBIA to changes in total body impedance in healthy elderly subjects caused by an intervention of known efficacy is a prerequisite to the study of the usefulness of MFBIA to detect clinically important changes of geriatric patients. In clinical practice, these changes in fluid balance may be smaller and may develop less rapidly than the effect of the diuretic intervention that was used in this study. Additionally, the responsiveness of MFBIA in monitoring fluid balance may be influenced by changes in body temperature (29), electrolyte distribution and hemocrit (30), ECF/TBW ratio, and cachexia (31). The effects of conductive implants, significant body asymmetry, and non-inflammatory diseases such as diabetes mellitus on MFBIA need to be investigated (6).

The derived prediction formulas have a prediction error, expressed as a coefficient of variation, of about 10%, which is too large to enable accurate predictions of changes in fluid balance in individual subjects. This may explain the significant difference between observed and predicted ECF. The fact that MFBIA at 5 kHz and not at higher frequencies turned out to be the most important in predicting TBW is unexpected and might be due to the relatively large ECF compartments in these subjects. However, the prediction formulas were only designed to facilitate the translation of MFBIA into clinical practice. The sample size was too small to allow use of these formulas in other populations unless they are cross-validated.

In conclusion, within- and between-subject variability of MFBIA in healthy aged, physically active subjects with a stable fluid balance does not seem to be larger than in

---

**Table 3. Mean Differences in Total Body Impedance (Δ Impedance) and Impedance Ratios (Δ Impedance Ratio) Just Before and 5-h After Administrating 40 mg Furosemide Orally**

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Δ Impedance (Ω)</th>
<th>Responsiveness Index</th>
<th>Δ Impedance Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57 ± 24* (17–97)</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>54 ± 20* (25–90)</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>41 ± 13* (23–65)</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>37 ± 12* (21–60)</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>1/100</td>
<td>0.03 ± 02* (−02–05)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>5/100</td>
<td>0.02 ± 01* (−01–04)</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

*Notes: Ranges within parentheses. *p < .001 (paired t-test).
younger age groups. MFBA had an excellent responsiveness on group and individual level: the mean change in impedance was highly significant and Guyatt et al.'s responsiveness index was high. Moreover, in all subjects there was an increase in impedance during the monitoring of fluid balance at all frequencies. Our study underscores the importance of body weight, which is currently the best parameter in measuring a rapid loss of body water caused by a loop-diuretic drug. However, pathological disturbances of fluid balance in geriatric practice occur less rapidly, and changes in body weight might often be caused by malnutrition as well. In conclusion, MFBA seems to be a promising method to monitor changes in fluid balance in the elderly. The safety and simplicity of MFBA make this method extremely suitable as a diagnostic tool for frail elderly patients. Further studies are needed to determine the clinical usefulness of MFBA in geriatric patients.

ACKNOWLEDGMENTS

We thank F. J. M. Schouten (Department of Human Nutrition of the Wageningen Agricultural University) for measuring plasma concentrations of deuterium and bromide in this study. This work was supported by The Netherlands Programme for Research on Aging, NESTOR, funded by the Ministry of Education, Culture and Sciences, and the Ministry of Health, Welfare, and Sports.

Address correspondence to Dr. M. G. M. Olde Rikkert, Department of Geriatric Medicine, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

REFERENCES


Received February 26, 1996
Accepted August 30, 1996