Evaluation of multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients

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1. Multi-frequency bio-impedance analysis has been used to estimate extracellular and total body water in a heterogeneous group of 43 surgical patients (23 males, 20 females).
2. Radioisotope-dilution methods were used for the measurement of extracellular and total body water.
3. Resistance and reactance were measured between wrist and ankle at frequencies from 5 kHz to 1 MHz.
4. Extracellular and total body water were estimated by multiple stepwise regression using the radioisotope values as the dependent variables. The parameters included in the regression were: resistance and reactance at each frequency, body habitus parameters, plasma albumin and plasma sodium.
5. The standard errors of the estimates between the measured and estimated values were 1.73 litres (coeficient of variation 9.6%) and 2.17 litres (coefficient of variation 6.0%) for extracellular and total body water, respectively.
6. These errors represent a useful improvement relative to those obtained from anthropometric estimates. However, the improvements relative to the use of a single frequency (50 kHz) are not clinically significant.

INTRODUCTION

Bio-impedance analysis (BIA) may be used for the assessment of body composition in terms of fat-free mass (FFM) or total body water (TBW). The method is based on the conduction of an applied electrical current through the body. A constant radiofrequency current, typically 50 kHz, is passed between surface electrodes placed on a hand and a foot, and the impedance to the current flow is measured by separate electrodes placed adjacent to the injection electrodes. The basic principle of the method is that the lean tissues, which consist essentially of electrolyte-containing water, conduct the electrical current, whereas the fat acts as an insulator. The impedance of the body is therefore determined largely by the low-impedance lean tissues. Regression equations are then derived which relate impedance to FFM or TBW measured by independent techniques. The impedance is usually normalized by the square of the subject's height and the prediction may be further improved by including other parameters, such as weight and age [1]. The BIA technique has been assessed for the measurement of body composition in groups of healthy subjects. Kushner et al. [1] measured TBW by isotope dilution and used multiple regression analysis to derive BIA equations which gave a standard error of estimate (SEE) of 1.8 litres. When these equations were prospectively tested in males and females the errors were 2.3 litres and 2.9 litres, respectively, with differences between mean predicted and measured TBW of -1.4 ± 2.1 (SD) litres and -0.5 ± 2.8 (SD) litres for males and females, respectively. These results illustrate the importance of performing prospective measurements to fully evaluate equations which have been derived by regression analysis. Lukaski et al. [2] compared FFM predicted by BIA with values measured by hydrodensitometry and obtained an SEE of 2.1 kg.

At 50 kHz a proportion of the applied current is unable to penetrate the cell membranes and therefore passes only through the extracellular space. At this frequency BIA is only able to predict TBW and FFM in healthy subjects because of the close correspondence between extracellular volume and TBW in these subjects. The prediction equations derived for control subjects may therefore not apply to stressed or malnourished surgical patients with acute changes in the distribution of their body water. Schroeder et al. [3] derived BIA prediction equations for FFM and TBW in normal subjects and surgical patients. They obtained SEEs of 4.0 kg and 2.2 litres for FFM and TBW, respectively. More recently, we obtained a similar SEE of 2.7 litres for...
the prediction of TBW in surgical patients [4]. It is possible that an improvement in the prediction error for TBW could be obtained by using higher frequencies which adequately penetrate the intracellular space.

However, the measurement of TBW alone is only of limited value in the nutritional or functional assessment of the seriously ill patient. Patients with ongoing infections have been shown to retain fluid in response to nutritional support and weight gain is due to expansion of the extracellular water (ECW) space. It has been emphasized that this weight gain cannot be viewed as an improvement in nutritional status as it does not reflect an improvement in protein stores [5]. There is also evidence that surgical patients who respond to nutritional support with an increase in ECW have increased postoperative complication rates compared with patients who lose water, and that they may benefit from longer courses of nutritional support [6]. Hence a measure of the distribution of TBW between extracellular and intracellular spaces may provide a useful index of the well-being or response to feeding of critically ill patients.

It has been suggested that the ratio of exchangeable sodium to exchangeable potassium could provide such a marker of nutritional status [7]. This ratio, which is effectively the extracellular mass expressed as a function of the metabolically active body cell mass, has been shown to be a predictor of mortality in critically ill malnourished patients [8]. However, the measurements of exchangeable sodium and potassium involve the use of radioisotope-dilution methods. These are time-consuming to perform and cannot be readily repeated to follow changes in nutritional status.

In contrast, BIA is non-invasive, simple to perform and may be repeated as frequently as required. Impedance to the flow of current through the body is dependent on the frequency of the applied voltage. It has been demonstrated that the impedance is largely dependent on the intracellular water (ICW) and ECW distribution [9]. At low radio-frequencies the current passes predominantly through the extracellular fluids, but at higher radio-frequencies it can penetrate the cell membranes and so is conducted by both the intra- and extra-cellular fluids. This raises the possibility that by measuring impedance at different frequencies it may be possible to predict both intra- and extra-cellular fluid volumes. ICW and ECW can be related to body cell mass and extracellular mass, respectively [10].

We have evaluated multi-frequency BIA (MFBIA) in a heterogeneous surgical population. Resistance and reactance were measured at a range of frequencies from 5 kHz to 1 MHz. TBW and ECW were measured by radioisotope-dilution methods. These measured values were then used as the dependent variables in a multiple stepwise regression. The parameters included in the regression were: resistance and reactance at each frequency, body habitus parameters, plasma albumin and plasma sodium. The measured and estimated values of TBW and ECW were used to calculate the SEE errors. These errors were compared with the corresponding errors obtained from estimates based on anthropometric variables and bio-impedance measurements at a single frequency (50 kHz).

### MATERIALS AND METHODS

#### Subjects

The total group consisted of 23 male and 20 female surgical patients. All patients signed an informed consent form, and the study had the approval of our hospital's Ethical Committee. Five patients were on total parenteral nutrition and none of the remaining 38 patients was on intravenous fluids. The presence of oedema, indicated by pitting of the limbs, was noted in five subjects. The diagnoses were as follows: 23 patients had gastrointestinal cancer; seven had benign gastrointestinal disease; 10 had pancreatitis and three had inflammatory bowel disease.

In addition to the MFBIA measurements, height, weight, age, antero-posterior thickness (APT), shoulder width, plasma albumin and plasma sodium were included in the regression analysis. The body habitus parameters may be expected to be of some predictive value in the estimation of TBW and ECW. Plasma albumin and plasma sodium may be influenced by fluid shifts [11–13] and were thus considered to have potential predictive value. The mean values and SDs for all of these parameters are listed in Table 1. The values of body mass index (weight/height²), ECW, TBW and the ratio ECW/TBW are also listed in Table 1.

The correlations between MFBIA measurements at each frequency were assessed in a sub-group consisting of 15 surgical patients.

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5</td>
</tr>
<tr>
<td>APT (cm)</td>
<td>21.2</td>
</tr>
<tr>
<td>Shoulder width (cm)</td>
<td>35.4</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>34.5</td>
</tr>
<tr>
<td>Plasma sodium (mmol/l)</td>
<td>137.0</td>
</tr>
<tr>
<td>ECW (litres)</td>
<td>18.1</td>
</tr>
<tr>
<td>TBW (litres)</td>
<td>36.4</td>
</tr>
<tr>
<td>ECW/TBW</td>
<td>0.497</td>
</tr>
</tbody>
</table>
MFBIA

Total body resistance \((R)\) and reactance \((X)\) were measured using a Xitron 4000B Multi-Frequency Bio-Impedance Analyser (Xitron Technologies, San Diego, CA, U.S.A.) operated at 200 \(\mu A\). This uses a four-cable technique with each cable individually shielded to minimize the effect of cable placement. Before each patient measurement the analyser was self-calibrated using a 422 \(\Omega\) test resistor supplied by the manufacturer. In addition, its performance was checked using a simple series/parallel circuit which we constructed. This consisted of a 1 k\(\Omega\) resistor in parallel with a 470 \(\Omega\) resistor and a 2.2 nF capacitor in series. Using the manufacturer’s analysis program predicted values for these components within 2\% of those measured by electrical test equipment.

Pre-gelled current injection electrodes (3M Littmann type 2325VP) with a surface contact area of 6\(\,cm^2\) were positioned on the right hand and foot just proximal to the third metacarpal and metatarsal bones, respectively. Similar detection electrodes were placed on the right wrist between the radius and ulna, and on the right ankle between the malleoli. The MFBIA measurement was made with the patient in a supine position with limbs and leads apart. Resistance and reactance were measured at frequencies of 5, 50, 100, 500 and 1000kHz. The electrodes were then replaced with new electrodes and the measurement was repeated. The mean value was used in the subsequent analysis. These MFBIA measurements were measured immediately before administration of the radioisotope-dilution materials.

Radioisotope-dilution measurements

\(^3\text{H}\)-labelled water (2 MBq) and 0.7 MBq of \(^{77}\text{Br}\) as a carrier-free solution of bromide in isotonic saline was administered intravenously. Blood samples were taken at 3 h and 18 h to allow equilibration of the \(^3\text{H}\) and \(^{77}\text{Br}\), respectively. Urine was collected during the two equilibration periods.

The activity of \(^{77}\text{Br}\) in 3 ml of plasma, obtained from the 18 h blood sample, and the activity in an aliquot of urine were determined by assaying the samples in a liquid scintillation counter using appropriate quench corrections. The contribution to the count rate from \(^{77}\text{Br}\) was determined by counting the samples twice, approximately 3 days apart. During this period there was no significant decay of \(^3\text{H}\) and the difference in count rate could be attributed entirely to the decay of the \(^{77}\text{Br}\). From the difference in count rate the initial contribution of \(^{77}\text{Br}\) to the combined \(^3\text{H}\) and \(^{77}\text{Br}\) count rate was determined. No correction was applied for the exchange of \(^3\text{H}\) with non-aqueous hydrogen in the body. Since there was no significant difference between the \(^3\text{H}\)-labelled water distribution volumes at 3 h and 18 h after administration it was assumed that the magnitude of this effect during the initial 3 h was negligible. TBW was therefore obtained by dividing the activity of \(^3\text{H}\) retained by the activity of \(^3\text{H}\) per ml of plasma.

Anthropometry

Height was measured with the patient standing upright. APT was measured using calipers and was taken as the maximum thickness along the full length of the sternum. Shoulder width was similarly measured using calipers. Weight was measured on beam balance scales.

Plasma albumin and plasma sodium

Plasma albumin and plasma sodium were measured by standard automated techniques (Hitachi 747 Discrete Analyser, Boehringer Mannheim Lewes, Brighton, East Sussex, U.K.).

Statistical analysis

Statistical analyses were performed using the Unistat Statistical Package version 4.5 (Unistat Limited, PO Box 383, Highgate, London N6 5UP, U.K.) on a Personal Computer (Research Machines PC-486/33). It is apparent that both TBW and ECW will be related to a patient’s weight and body stature. When assessing the independent contribution of bio-impedance measurements to estimates of TBW and ECW, it is therefore appropriate to include these simple parameters in the regression analysis. Multiple correlation matrices for TBW and ECW were applied to establish the relationship of each of these to resistance \((R)\), reactance \((X)\), height \((H)\), weight, age, shoulder width, APT, plasma albumin and plasma sodium. Since prediction equations for single-frequency BIA often include the term \(H^2/R\) [15], each correlation matrix also included the parameters \(H^2/R\) and \(H^3/X\). Values for resis-
tance and reactance were included for each frequency. A 5% level of significance was used for all data analyses. Multiple stepwise regressions were performed using TBW and ECW measured by radiisotope dilution as the dependent variables. For each regression the correlation coefficient and SEE between the measured and estimated values were obtained. Variables were only included in the final regression equations if they resulted in a significant improvement in the SEE.

RESULTS

When the body habitus parameters only were included in the regression analysis the lowest error which could be achieved for TBW was 4.02 litres (coefficient of variation 11.1%) and for ECW the lowest error was 3.51 litres (coefficient of variation 19.4%).

When the resistance and reactance measurements at 50 kHz were included in the stepwise regression the following regression equations were obtained:

\[
\begin{align*}
TBW &= 0.497H^2/R_{50} + 0.5APT + 0.275 \\
& \quad (r = 0.957, \text{SEE} = 2.37 \text{litres, coefficient of variation } 6.5\%) \\
ECW &= 0.0119H^2/X_{50} + 0.123H^2/R_{50} + 6.15 \\
& \quad (r = 0.932, \text{SEE} = 1.72 \text{litres, coefficient of variation } 9.5\%)
\end{align*}
\]

where \( H \) = height (cm), \( R_{50} \) = resistance (\( \Omega \)) at 50 kHz, \( APT \) is in cm, \( X_{50} \) = reactance (\( \Omega \)) at 50 kHz, \( r \) is the multiple correlation coefficient and the coefficient of variation is defined as SEE as a percentage of the mean value. The variables are listed in the order in which they contributed to the final regression equations.

When the resistance and reactance measurements at all frequencies were allowed in the multiple stepwise regression the following regression equations were obtained:

\[
\begin{align*}
TBW &= 0.45H^2/R_{500} + 0.46APT + 0.0119H^2/X_{50} - 0.0106X^2/X_{500} - 1.04 \\
& \quad (r = 0.967, \text{SEE} = 2.17 \text{litres, coefficient of variation } 6.0\%) \\
ECW &= 0.01H^2/X_{50} + 0.165H^2/R_{5} + 5.75 \\
& \quad (r = 0.933, \text{SEE} = 1.73 \text{litre, coefficient of variation } 9.6\%)
\end{align*}
\]

where \( R_F \) and \( X_F \) are resistance and reactance, respectively, at frequency \( F \) kHz and the variables are again listed in the order in which they contributed to the final equations.

Neither the single- nor multi-frequency regression equations for TBW include the patient’s weight, even although this was included as one of the variables in the multiple stepwise regressions. This may be an advantage where it is difficult to weigh an acutely ill surgical patient. However, the regression equations do require the measurement of APT and it is recognized that this requires specifically designed calipers, which may not be readily available. Alternative single- and multi-frequency regression equations for TBW were therefore derived by excluding APT from the stepwise regression analysis. This provided the regressions equations:

\[
\begin{align*}
TBW &= 0.446H^2/R_{50} + 0.126W + 5.82 \\
& \quad (r = 0.951, \text{SEE} = 2.54 \text{litres, coefficient of variation } 7.0\%) \\
TBW &= 0.399H^2/R_{500} + 0.114W + 5.69 \\
& \quad (r = 0.952, \text{SEE} = 2.54 \text{litres, coefficient of variation } 7.0\%)
\end{align*}
\]

where \( W \) = weight (kg) and the terms are again listed in the order in which they contribute to the final equation.

The values for TBW and ECW estimated from the multifrequency equations are shown in Figs. 1 and 2, respectively. The possibility of bias between the measured and estimated values was investigated by plotting the difference against the mean [16]. For both ECW and TBW there was no significant bias and the estimated values agreed with the measured equally well over the entire range. However, since the equations were derived from a multiple regression analysis an assessment of their general applicability will require them to be used prospectively in a different surgical population.

All of the above regression equations were derived using the mean of two sets of resistance and
Multi-frequency bio-impedance analysis in surgical patients


Fig. 2 Comparison of ECW estimated by MFBIA with the values measured by the isotope-dilution method in 43 surgical patients. The solid line represents the least-squares fit to the data \( y = 0.886x + 2.09 \). The correlation coefficient was 0.933 \((P < 0.001)\) and the SEE was 1.73 litres (coefficient of variation 9.6%).

The correlations between the values of resistance and reactance measured at each frequency are summarized in Table 2. Although the actual values of resistance and reactance varied as a function of frequency these were highly correlated over much of the range. In particular the correlations between resistance measured at 50 kHz \( (R_{50}) \) and those measured at all higher frequencies are such \((r > 0.98)\) that there is unlikely to be any independent predictive value in the resistances at the higher frequencies. This is consistent with the minimal improvement in the SEEs relative to those obtained from the single-frequency (50 kHz) regression equations.

### DISCUSSION

Body composition may be assessed by various techniques, including isotope dilution, hydrodensitometry, total body potassium and neutron activation analysis [17]. However, even where these techniques are available they are often not appropriate for critically ill surgical patients. There is therefore a need for a simple method for assessing the nutritional status of surgical patients which can also be used repeatedly to follow their response to treatment. A bioimpedance analyser is a simple portable instrument which can be used at the patient's bedside. The method is non-invasive and may be repeated readily. However, it is often not clear what additional predictive value, if any, is provided by BIA relative to predictions based on anthropometry alone. The use of the term \( H^2/R \) in BIA prediction equations is often claimed to have some explicit rationale by illustrating its derivation from simple conduction theory [1]. However this theory applies to a single homogeneous medium of constant cross-sectional area. This is far removed from the application of BIA to human subjects, where the resistance measured is the sum of the resistances in one arm, the trunk and one leg. It has been shown [17] that at the frequency of 50 kHz normally used for BIA the total body impedance is dominated by the arm (46%) and leg (44%).
trunk, which represents an average of 46% of the body weight, accounts for only 10% of the total body impedance. It may therefore not be surprising if a measure of total body impedance adds little predictive value in the calculation of TBW or ECW. Fuller and Elia [18] used densitometry as the reference method and concluded that even in normal subjects estimates of body composition by BIA were associated with only slightly smaller limits of agreement than those made by anthropometry. This is in agreement with our observations in anorexic females, where we derived prediction equations for FFM using radioisotope dilution, total body potassium and neutron activation analysis as reference methods [19]. The SEE obtained by BIA (1.27 kg) was only slightly lower than that calculated from weight alone (2.14 kg).

The role of BIA for the nutritional assessment of hospital patients has been discussed by Van Itallie and Segal [20], who concluded that adequate nutritional assessment required measurements of both ICW and ECW. Since the ratio of intra- to extracellular conduction is a function of frequency this suggested the possibility of estimating ECW and ICW by measuring impedance at different frequencies. However, the optimum frequencies for such measurements have not previously been established. Pullicino et al. [21] used 1 kHz and 50 kHz and found that at these frequencies BIA was not superior to anthropometric estimates of ECW and ICW. Segal et al. [22] measured bioelectrical impedance at 5, 50 and 100 kHz in a group of healthy males and obtained SEEs for ECW and TBW of 1.9 litres and 2.6 litres, respectively.

In the present study we have measured resistance and reactance at frequencies from 5 kHz to 1 MHz in a heterogeneous group of surgical patients. The regression errors obtained for TBW and ECW are lower than can be obtained using body habitus parameters alone, but are not significantly better than can be achieved by including resistance and reactance measurements at 50 kHz only. This is probably because of high correlations for both resistance and reactance measured at the various frequencies. Such high correlations may be expected for control subjects, where the extracellular volume represents a relatively constant proportion of the TBW. However, in the present study the ratio of ECW to TBW varied from 0.38 to 0.63. The failure to reduce the errors further by measuring impedance at multiple frequencies may therefore be attributable to the fundamental problems associated with total body impedance measurements. If this is the case it may prove possible to overcome these problems by performing segmental measurements.

A component of the prediction errors can in fact be attributed to the radioisotope-dilution measurements. Because of the relatively long half-life of the radioisotopes, it is difficult to assess the reproducibility of these methods in surgical patients. However, repeat measurements of TBW in control subjects indicates that the component of the prediction error attributable to the radioisotope-dilution measurement is in fact significant. Since the error due to counting statistics was negligible, this error may be attributed to the combination of errors in pipetting of the standard, urine and plasma samples. It therefore also provides a good indication of the likely error in the measurement of ECW by the radioisotope-dilution method.

In the present study we used only five discrete frequencies covering the range 5 kHz–1 MHz. We have previously observed poor reproducibility below 5 kHz, particularly in the reactance measurements. The instrument used is capable of measuring resistance and reactance at 50 frequencies in the range 1 kHz–1.3 MHz, and the manufacturers now recommend the use of a curve-fitting model which makes use of the entire frequency range. However, using this model SEEs of 2.5 litres and 0.97 litre have recently been reported for TBW and ECW, respectively, in a group of 24 healthy adults [23]. It seems unlikely therefore that any significant reduction in the SEEs obtained in the present study of surgical patients may be expected using the curve-fitting model.

We conclude that measurements of total body resistance and reactance at multiple frequencies do not provide any clinically significant improvement on measurements at only 50 kHz for the estimation of ECW and TBW in surgical patients. Similarly, the inclusion of plasma albumin and plasma sodium does not reduce the errors. However, the estimated contributions of the independent radioisotope measurements to the errors suggests that the 'true error' in the bio-impedance regression equations may be significantly lower than the apparent SEE. The possibility of reducing the error by performing segmental impedance measurements should be investigated. The role of MFBIA in the nutritional management of surgical patients should be examined.

REFERENCES

Multi-frequency bio-impedance analysis in surgical patients


