Estimation of Body Water Compartments in Cirrhosis by Multiple-Frequency Bioelectrical-Impedance Analysis

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Estimation of total body water by measuring bioelectrical impedance at a fixed frequency of 50 kHz is useful in assessing body composition in healthy populations. However, in cirrhosis, the distribution of total body water between the extracellular and intracellular compartments is of greater clinical importance. We report an evaluation of a new multiple-frequency bioelectrical-impedance analysis technique (MFBIA) that may quantify the distribution of total body water in cirrhosis. In 21 cirrhotic patients and 21 healthy control subjects, impedance to the flow of current was measured at frequencies ranging from 4 to 1012 kHz. These measurements were used to estimate body water compartments and then compared with total body water and extracellular water determined by isotope methodology. In cirrhotic patients, extracellular water and total body water (as determined by isotope methods) were well predicted by MFBIA ($r = 0.73$ and $0.89$, respectively). However, the 95% confidence intervals of the limits of agreement between MFBIA and the isotope methods were $\pm 14\%$ and $\pm 9\%$ for cirrhotics (extracellular water and total body water, respectively) and $\pm 9\%$ and $\pm 9\%$ for cirrhotics without ascites. The 95% confidence intervals estimated from the control group were $\pm 10\%$ and $\pm 5\%$ for extracellular water and total body water, respectively. Thus, despite strong correlations between MFBIA and isotope measurements, the relatively large limits of agreement with accepted techniques suggest that the MFBIA technique requires further refinement before it can be routinely used to determine the nutritional assessment of individual cirrhotic patients. *Nutrition* 2001;17:31–34. ©Elsevier Science Inc. 2001

Key words: multiple-frequency bioelectrical impedance, cirrhosis, ascites, total body water, liver

INTRODUCTION

Malnutrition is associated with increased morbidity and mortality in the pre- and posttransplant phases of management of patients with liver disease.1,2 Unfortunately, accurate nutritional assessment is difficult in patients with cirrhosis because standard laboratory methods are inaccurate and the techniques used to precisely assess metabolic compartments are complex, expensive, and of limited availability.3

Single-frequency bioelectrical impedance analysis (BIA) is safe, noninvasive, rapid, and inexpensive method of assessing total body water (TBW) and thus lean body mass in the healthy population.4 However, in situations where there are clinically important changes in intracellular and extracellular water distribution, such as cirrhosis,5,6 the value of single-frequency BIA is limited.7

Previous studies have indicated an improved prediction of body water compartments by BIA if measurements are made while the frequency of the applied current is changed.8–10 At low frequencies, the current passes through the extracellular fluids because of the capacitance effect of cell membranes and tissue interfaces, whereas at higher frequencies, the current is conducted through both the intra- and extracellular fluids.11 It is proposed that this measurement technique may be of particular use in patients with cirrhosis because they have altered distribution of body water even in the absence of ascites.6

Thus, the purpose of this study was to assess the accuracy of multiple-frequency bioelectrical-impedance analysis (MFBIA) in the measurement of TBW and extracellular water (ECW) in normal subjects and patients with cirrhosis.

MATERIALS AND METHODS

Study Population

The study population consisted of 21 subjects with chronic liver disease. Cirrhosis was confirmed by histologic examination of liver biopsy specimens in 18 patients. Prolonged prothrombin time precluded safe biopsy in the three remaining patients. However, each of these patients had clinical, biochemical, and radiologic evidence of cirrhosis. No patient had evidence of underlying renal dysfunction or cardiac failure. Liver-function tests, plasma albu-
program supplied by the manufacturer. The software performs a Cole–Cole analysis of the data and provides a plot of the reactive and resistive components of the measured impedance at each frequency. The frequency at the peak of this plot is known as the characteristic frequency and the impedance at this frequency is $Z_c$. This plot was extrapolated to zero frequency using linear least-squares regression analysis. Thus, the value of the body impedance at zero frequency (i.e., $Z_0$) was determined. At the characteristic frequency current flows through both intracellular water (ICW) and ECW, i.e., TBW, but at zero frequency, the current flows through the ECW alone. The predictive relationships between the impedance and the fluid-compartment volumes have been previously demonstrated.

$\text{TBW} \propto \frac{H^2}{Z_c}$ and $\text{ECW} \propto \frac{H^2}{Z}$

**TABLE I.**

**CHARACTERISTICS OF STUDY GROUPS**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Cirrhotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>16:5</td>
<td>16:5</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>63.0 ± 15</td>
<td>64.0 ± 11</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>74.1 ± 12</td>
<td>84.1 ± 17</td>
</tr>
<tr>
<td>Height (cm)*</td>
<td>171 ± 7</td>
<td>172 ± 5</td>
</tr>
<tr>
<td>TBK (g)*</td>
<td>117 ± 26</td>
<td>105 ± 19</td>
</tr>
<tr>
<td>BCM (kg)*</td>
<td>25.1 ± 5</td>
<td>22.4 ± 4</td>
</tr>
<tr>
<td>TBW (kg)*</td>
<td>41.9 ± 8</td>
<td>44.6 ± 9</td>
</tr>
<tr>
<td>ECW (kg)*</td>
<td>23.6 ± 5</td>
<td>26.9 ± 4</td>
</tr>
<tr>
<td>ICW (kg)*</td>
<td>16.4 ± 3</td>
<td>18.3 ± 4</td>
</tr>
</tbody>
</table>

* Mean ± SD.

**Techniques of Body-Composition Analysis**

**MULTIPLE-FREQUENCY BIOELECTRICAL-IMPEDANCE ANALYSIS.** Impedance was measured in all subjects using an MFBI A model SFB2 (SEAC, Brisbane, Australia). This impedance analyzer uses a tetrapolar electrode system that measures the impedance and phase angle of alternating electric current at 496 different frequencies between 4 and 1012 kHz.

Impedance measurements were made with the subject lying in a supine position, on a nonconducting surface, with the legs not touching at the thighs and the arms not touching the torso. All subjects were fasted for at least 12 h before the measurement and had been lying supine for at least 5 min before measurement.

One pair of electrodes (Littman, EKG 3 M, Sydney, Australia) was placed on the right hand. The detector electrode was positioned midway between the radius and ulna at the level of the wrist joint. The source electrode was placed 5 cm distally on the dorsal surface of the hand overlying the third metacarpal. Another pair of electrodes was positioned on the left foot. The detector electrode was placed midway between the medial and lateral malleolus at the level of the ankle. The source electrode was placed 5 cm distally on the dorsal surface overlying the metatarsals. All electrode sites were precleaned using an alcohol swab.

Data analysis was performed using the SFBIM Data Reduction

**TOTAL BODY WATER.** TBW was determined by measuring urinary deuterium enrichment by mass spectrometry after an oral dose of deuterium oxide ($D_2O$, 0.1 g kg$^{-1}$ body weight (Cambridge Isotopes Laboratories, Canberra, Australia), as previously described.5 The deuterium was administered after an overnight fast and on the same day as the total body potassium (TBK) measurements. A background urine sample was collected before and 6 h after the administration of the deuterium and after voiding of the bladder.

Calculations of TBW was based on the isotopic dilution principle, allowing for corrections for urinary losses of $D_2O$ and incorporating the international Atomic Energy Agency standard reference, Vienna–Smow. Thus,

$$\text{TBW} (kg) = \frac{(D_2O \text{ dose (g)}) \times 0.999 \times 0.9025 \times 10^6}{\delta A \times 157.6 \times 1.04}$$

where $\delta A$ is urinary deuterium enrichment with respect to measured background values, 0.999 is the concentration of administered $D_2O$, 0.994 is the correction for loss of $D_2O$ in the urine, 0.9025 is density ratio of water to $D_2O$, 157.6 ÷ 10$^{-6}$ is the absolute deuterium/hydrogen (D/H) ratio of the standard, and 1.04 is the correction for isotopic exchange of deuterium with OH in macromolecules.

**TOTAL BODY POTASSIUM.** TBK was measured in a shadowshield–type whole-body counter (Accuscan, Canberra Industries, Boston, MA, USA). Subjects were measured for 40 min while supine on a scanning bed. TBK was determined by comparing the intensity of a 1.465-MeV 40 K γ-ray measured using a suitable phantom containing a known amount of 40K. The coefficient of variation for repeated measurements on a human subject is 4%.5

Body cell mass (BCM) was calculated from measurements of TBK using the relation

$$\text{BCM} (kg) = \frac{\text{TBK (g) } \times 8.33}{39.1}$$

The factor 8.33 for the calculation of BCM from TBK assumes cellular tissue to have an average potassium-to-nitrogen ratio of 3 mmol/g and a protein content of 25%.12 ICW was estimated by assuming that water constitutes 73.2% of the BCM, and ECW was subsequently estimated by subtracting ICW from TBW.5,12

Different techniques of body-composition analysis were compared by determining the Pearson correlation coefficient and the standard error of the estimates. Because all techniques of body-composition analysis used in the present study involve measurement error, Deming’s regression method was used to provide the line of best fit.3

When assessing the clinical utility of a new test, the estimated limits of agreement between the new test and a gold-standard
method of measurement are more important than the correlation coefficient. In the present study, this value is based on the difference between the value of fluid volumes obtained by MFBI and that obtained by isotope methods for each subject. Limits of agreement between the different techniques were assessed by the method of Bland and Altman.13,14

ETHICAL DETAILS. The conduct of this study was approved by the Hospital Ethics Committee, Greenslopes Hospital.

RESULTS

Patient Profiles

The mean age and height of the cirrhotic patients were not significantly different from those of control subjects (64 ± 11 y versus 63 ± 15 y, 172 ± 5 cm versus 171 ± 7 cm, respectively). The mean body weights were significantly different (P = 0.05, 84 ± 17 kg versus 74 ± 12 kg, respectively). The cirrhotic subjects differed widely with regard to TBW (44.6 ± 8.8 kg) and ECW (26.9 ± 5.6 kg). Similarly, there was a wide variation in TBW (41.9 ± 7.8 kg) and ECW (23.6 ± 5.0 kg) in the control subjects. The range of the characteristic frequency was similar in control subjects and patients with cirrhosis (24 to 46 kHz and 27 to 38 kHz, respectively). It should be noted that the characteristic frequency was not 50 kHz (the frequency at which single-frequency BIA is measured).

Body Water Compartments in Cirrhotic and Control Subjects

For TBW, there was a highly significant correlation between measurements using isotope methodology and MFBI in control subjects, cirrhotic subjects, and cirrhotic subjects without ascites (0.96, 0.89, and 0.90, respectively; Table II). Similarly for ECW, there was a highly significant correlation between isotope methodology and MFBI in control subjects, cirrhotic subjects, and cirrhotic subjects without ascites (0.89, 0.73, and 0.85, respectively; Table II). It should be noted that the consistently lower values of the correlation coefficient obtained with the ECW may well have been due to a compounding of uncertainties associated with the method of determining the ECW by the isotope methodology (namely ECW = TBW − ICW).

The slopes of the regression lines between the MFBI prediction (HfZc, dependent variable) and TBW (independent variable) were significantly different (P < 0.05) between control subjects and cirrhotic patients (1.37 and 1.01, respectively). However, the slopes of the regression lines were not significantly different (P > 0.95) between control subjects and cirrhotic patients without ascites (Table II). This same relation was also apparent in the regression analysis of the MFBI predictor (HfZc) and ECW (Table II).

Estimated Limits of Agreement

Comparison of the two methods were performed to determine the precision of the limits of agreement, as described by Bland and Altman.15 Using the regression equation derived between the MFBI predictor and the fluid volume determined by the isotope methodology, the predicted values of TBW and ECW were calculated for each subject in both control and cirrhotic groups. The standard deviations of the difference between the values obtained by each method (i.e., MFBI and isotope methodology) was calculated for each subject group and for both TBW and ECW and are shown in Table III.

The 95% confidence interval for the limits of agreement between the two methods for the determination of TBW was ±5%; for control subjects and ±9% for cirrhotics. The 95% confidence interval for the determination of ECW was ±10% for both controls and cirrhics without ascites but ±14% for cirrhotics (including those with and without ascites).

DISCUSSION

Estimation of body composition is important in the assessment and monitoring of cirrhotic patients. The development of a non-invasive, inexpensive, and accurate technique to assess body water and nutritional compartments in cirrhosis would be of great clinical value to identify those patients with impaired nutrition. Malnutrition is associated with increased morbidity and mortality in cirrhosis, and enhanced nutritional support is indicated in those patients with persisting nutritional deficits.

It has been suggested that single-frequency BIA may be useful in the estimation of metabolic compartments in cirrhotic patients if its use is restricted to patients without fluid accumulation and if it is used in combination with an isotopic determination of ECW.6 The problems with these recommendations are twofold. First, complex, invasive, and expensive technology is still required. Second, the technique is of little use in the subgroup of patients with ascites, and it is these patients who have the most severe malnutrition.7 Thus, there is still a requirement for the development of a bedside technique that reliably estimates body composition in patients with liver disease.

The development of an instrument that measures impedance at
different frequencies may satisfy this requirement. Previous studies in animals and healthy humans have indicated that measurements of TBW and ECW can be reliably predicted by the use of MFBIA.\textsuperscript{7,16} Reports of the use of MFBIA for the assessment of body water compartments in patients with disorders of fluid balance are limited. Studies of the use of MFBIA in surgical patients for the estimation of TBW and ECW have concluded that MFBIA does not provide any significant improvement on measurements at 50 kHz.\textsuperscript{16} However, studies of the use of MFBIA in postmastectomy lymphedema have suggested that this method may offer a means of earlier definitive diagnosis and more accurate monitoring of extracellular fluid changes.\textsuperscript{16} Borghi et al.\textsuperscript{17} observed that the published MFBIA formula for the prediction of TBW and ECW, although accurate, exhibited higher standard errors of the estimate compared with dilutional measurements. Furthermore, the magnitude of these errors was greater in Child’s class B patients than in those with class A cirrhosis. It was of interest, therefore, to extend these studies to the development of MFBIA-derived prediction equations in patients with liver disease, a group in whom disordered body hydration is common.

Our results are in agreement with previous single-frequency BIA studies\textsuperscript{3-7} that showed an excellent correlation between BIA and deuterium dilution for determining TBW in control subjects and cirrhotic patients without ascites. However, when assessing the clinical applicability of a new method of measurement, the estimated limits of agreement between a proposed new method and a gold-standard method should be determined. The 95\% confidence interval for the limits of agreement between the MFBIA technique and TBW was acceptable for the control group (±5\%) but was significantly higher for the cirrhotic group (±9\%). A similar trend was observed in the 95\% confidence interval for ECW estimated by MFBIA, with a significantly higher value for the cirrhotic group (±8\%).

The reasons MFBIA does not accurately predict water compartments in patients with cirrhosis and particularly those with ascites are unclear. The known altered distribution of water in patients with cirrhosis may alter the capacitance effect of all membranes on conductance through the fluid compartments, although this is highly speculative. Alternatively, in cirrhotic subjects, excess fluid may be primarily located in the trunk, particularly so for those with ascites. It is recognized that whole-body BIA is insensitive to fluid changes in the trunk. This is supported by the observation that applying the regression equation, derived in the nonascitic subjects only, for ECW to the ascitic subjects underestimates ECW by an average of 12\% (26.77 versus 30.4 kg by dilution). This is also supported by the lower value for the slope of the regression line for the cirrhotic subjects versus that of the controls. This highlights the need for further studies, particularly in those subject with more severe disease (Child’s class B and C cirrhosis) with a higher prevalence of ascites.

The cirrhotic patients were significantly heavier than the control subjects. This difference appears not to be attributable to a difference in BCM based on TBK measurements because these were not significantly different. Because BCM represents the major component of fat-free mass, this suggests that there was equally no significant difference in fat-free mass. Thus, the difference in weight is presumably due to difference in fat mass. Because of the low hydration of fat, any differences in fat mass will have an insignificant effect on both BIA and dilution measurements.

In summary, the accurate evaluation of the nutritional state is an important aspect of the care of patients with cirrhosis. MFBIA does offer some advantages over other techniques because it is noninvasive and inexpensive. Serial measurements may be readily performed, allowing the continual monitoring of fluid status of patients and thus providing important clinical information to the clinician. However, further refinement is required before it can eventually be used to determine nutrition in cirrhotic patients.

REFERENCES