

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

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1. Measurements of extracellular and total body water provide useful information on the nutritional status of surgical patients and may be estimated from whole-body bio-impedance measurements at different frequencies.

2. Resistance and reactance were measured at 50 frequencies from 5 kHz to 1 MHz in 29 surgical patients (17 males, 12 females) with a wide range of extracellular to total body water ratios.

3. A fit to the spectrum of reactance versus resistance data gave predicted resistances at frequencies zero and infinity. Values of extracellular and total body water determined by this bio-impedance spectroscopy technique were regressed against values obtained from radioisotope dilution. The standard errors of the estimate were 1.893 l and 3.259 l respectively.

4. Resistance indices (height²/resistance) at selected frequencies gave the highest correlations with extracellular and total body water at 5 kHz and 200 kHz respectively, and prediction equations derived from multiple stepwise regressions also showed these to be the optimum frequencies. The standard errors of the estimate for this multi-frequency bio-impedance analysis method were 1.937 l and 2.606 l for extracellular and total body water respectively.

5. To assess the ability of the two methods to measure changes in extracellular and total body water, reproducibility was assessed from repeat measurements 10 min apart in a subgroup of 15 patients. Bio-impedance spectroscopy gave mean coefficients of variation for extracellular and total body water of 0.9% and 3.0% respectively. For multi-frequency bio-impedance analysis the corresponding coefficients of variation were 0.9% and 0.6%.

6. It is concluded that a simple impedance analyser operating at only two frequencies compares favourably with the more complex spectroscopy technique for the determination of extracellular and total body water in surgical patients.

INTRODUCTION

Bio-impedance analysis (BIA) is a simple, non-invasive method for the assessment of total body water (TBW). In subjects with normal hydration, fat-free mass may also be estimated [1]. The method involves passing a small radiofrequency current, typically at 50 kHz, between surface electrodes placed on a hand and a foot, and measuring the impedance to the current flow using detection electrodes placed adjacent to the source electrodes. The impedance is usually normalized by the square of the subject's height and the prediction equation may also include other parameters such as age and weight. The principle of the BIA technique is that it is essentially the electrolyte-containing water which conducts the electrical current. At 50 kHz the current does not completely penetrate the cell membranes but in normal subjects this is not particularly important because the intracellular volume represents a relatively constant proportion of the TBW. In patients there may be significant variations in the proportions of intracellular and extracellular water and it is more important that the frequency is high enough to allow the current to completely penetrate the intracellular space.

However, the measurement of TBW is of limited value in the nutritional assessment of the seriously ill patient. Patients with trauma or sepsis may retain fluid in response to nutritional support and weight gain may simply reflect an expansion of the extracellular water space. Such weight gain cannot be considered as an improvement in nutritional status as it does not reflect an increase in protein stores [2]. A more useful assessment of nutritional status in such patients may be obtained from separate estimates of extracellular water (ECW) and TBW. ECW and TBW can be measured by stable or radioactive isotopes but the methods used are relatively complex and are not generally available. Various bio-impedance instruments are available

Key words: bio-impedance, extracellular water, nutrition, total body water.

Abbreviations: BIA, bio-impedance analysis; BIS, bio-impedance spectroscopy; CoV, coefficient of variation; ECW, extracellular water; MFBI, multi-frequency bio-impedance analysis; SEE, standard error of estimate; TBW, total body water.

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which attempt to measure ECW and TBW by measuring impedance at different radiofrequencies. These instruments vary from relatively simple ones which measure resistance at only two frequencies to more complex ones which measure resistance and reactance at a wide range of frequencies and require a computer to perform data analysis. The basic principle of these instruments is that at low frequencies the capacitive nature of the cell membranes does not allow the current to penetrate the cell so the impedance is related only to the ECW. At very high frequencies the current is able to penetrate the cell membranes and the impedance is a measure of the combined intracellular and extracellular spaces. Theoretically the optimum frequencies for the extracellular and total body water spaces are zero and infinity respectively. In practice it is not possible to make reliable impedance measurements below about 5 kHz or above about 1 MHz. Studies at various frequencies have been carried out in normal subjects [3, 4] but in patients with altered hydration status the optimum frequencies have not been established.

We have measured impedance (resistance and reactance) from 5 kHz to 1 MHz in a heterogeneous group of surgical patients with a wide range of body habitus parameters and a wide range of ECW/TBW ratios. A modelling program supplied by the manufacturer of the analyser was used to predict the impedances at frequencies zero and infinity, from which ECW and TBW were estimated. This method is referred to as bio-impedance spectroscopy (BIS). In addition, resistance indices ($\text{height}^2/\text{resistance}$) were noted for selected frequencies and were regressed against ECW and TBW measured by radioisotope dilution. The optimum frequencies for ECW and TBW were established using multiple stepwise regression to develop appropriate prediction equations. This technique is referred to as multi-frequency bio-impedance analysis (MFBA). The aims of this work were (i) to assess the optimum frequencies for the measurement of ECW and TBW in surgical patients, (ii) to compare the errors obtained by the MFBA prediction equations with those obtained by the more complex BIS technique, and (iii) to assess the reproducibility of the BIS and MFBA methods.

METHODS

Subjects

The subjects consisted of 29 surgical patients (17 males, 12 females). All patients signed an informed consent form and the study had the approval of our hospital's Ethical Committee. Six patients were on total parenteral nutrition and five of the remaining patients were on intravenous fluids. The presence of oedema, indicated by pitting of the limbs, was noted in 11 subjects. The diagnoses were as follows: 11 patients had gastrointestinal cancer, eight had benign gastrointestinal disease, nine had pancreatitis and one had inflammatory bowel disease. In addition

Table 1. Mean values, SD and range for age, height, weight, body mass index, ECW, TBW and the ratio ECW/TBW for the 29 surgical patients

	Mean	SD	Range
Age (years)	62.2	15.9	27.5-91.0
Height (cm)	165.5	11.9	127.0-185.4
Weight (kg)	59.5	13.4	34.8-80.0
Body mass index (kg/m^2)	21.7	3.8	13.6-28.9
ECW (l)	16.3	3.7	12.0-26.2
TBW (l)	32.2	7.4	21.8-47.1
ECW/TBW	0.512	0.061	0.388-0.625

tion to the impedance measurements, height, weight and age were included in the regression analysis. Height (H) was measured using a stadiometer with the patient standing upright and weight (W) was measured on beam balance scales. The characteristics of the patients are summarized in Table 1. None of the measurements were performed within the first post-operative week.

An important consideration in the evaluation of bio-impedance methods is the ability to measure changes in ECW and TBW. To assess the reproducibility in patients, it is important to perform the repeat measurements within a relatively short time in order to eliminate the possibility of actual changes in ECW or TBW. However, possible effects introduced by differences in electrode placement should be included in such an assessment. To assess the reproducibility of the BIS and MFBA methods, repeat measurements were performed in a subgroup of 15 patients (nine males, six females). For convenience the reproducibility was assessed from two measurements 10 min apart with the electrodes replaced between measurements.

Radioisotope dilution

^3H -Labelled water (2 MBq; Amersham International, Amersham, U.K.) and ^{77}Br (0.7 MBq) as a carrier-free solution of bromide in isotonic saline (MRC Cyclotron Unit, Hammersmith Hospital, London, U.K.) were administered intravenously after an overnight fast. A 10 ml blood sample was taken at 3 h. The activity of ^{77}Br in 3 ml of plasma was determined by assaying the plasma sample and a standard of known activity in an automatic γ -counter. The bromide distribution volume was calculated by dividing the activity administered by the activity per litre of plasma. The ECW volume was calculated by correcting the bromide distribution volume for erythrocyte bromide concentration (0.90), the Donnan equilibrium effect (0.95) and the concentration of water in serum (0.94) [5]:

$$\text{ECW}(l) = \frac{[^{77}\text{Br}]_a}{[^{77}\text{Br}]_p} \times 0.90 \times 0.95 \times 0.94 \quad (1)$$

where $[^{77}\text{Br}]_a$ and $[^{77}\text{Br}]_p$ represent the activity of ^{77}Br administered and the activity per litre of

plasma respectively. The activity of ^3H in 1 ml of plasma and in a known standard were determined by assaying the samples in a liquid-scintillation counter with quench corrections. The contribution from ^{77}Br in the plasma sample was determined by counting the sample twice, approximately 3 days apart. Since there is no significant decay of ^3H during this period the reduction in count rate was attributed entirely to the decay of the ^{77}Br , and from the difference in count rate the initial contribution of ^{77}Br to the combined ^3H and ^{77}Br was determined. TBW was calculated from:

$$\text{TBW(l)} = \frac{[\text{}^3\text{H}]_s}{[\text{}^3\text{H}]_p} \times 0.94 \times 0.96 \quad (2)$$

where $[\text{}^3\text{H}]_s$ and $[\text{}^3\text{H}]_p$ represent the activity of ^3H administered and the activity of ^3H per litre of plasma respectively, 0.94 is the correction for the concentration of water in serum and 0.96 corrects for exchange of ^3H with non-aqueous hydrogen in the body.

Bio-impedance spectroscopy (BIS)

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 μA . Before each patient measurement the analyser was self-calibrated using a test resistor supplied by the manufacturer. The areas of the skin to which the electrodes were to be applied were wiped using swabs saturated with 70% isopropyl alcohol. The electrodes recommended by the manufacturer of the bio-impedance analyser were used (Xitron type IS4000); these are self-adhesive pre-gelled electrodes with a surface contact area of 13.5 cm^2 . The current source electrodes were positioned on the right hand and foot just proximal to the third metacarpal and metatarsal bones respectively. The detection electrodes were placed on the right wrist between the radius and ulna, and on the right ankle between the malleoli. The impedance measurements were made with limbs and leads apart after the patient had been lying supine for at least 5 min. Resistance and reactance were measured at 50 frequencies from 5 kHz to 1 MHz. The resistance and reactance measurements were transferred to a type 486DX laptop computer. Analysis was performed using a computer program supplied by Xitron Technologies (version 1.00D) in which the spectrum of reactance versus resistance values are plotted. A semi-circle function is then fitted to the data, and where the curve cuts the resistance axis the resistances at frequencies zero and infinity are predicted. These correspond to the extracellular resistance and the total body water resistance respectively and are combined with weight, height and resistivity of extracellular and intracellular water to calculate the extracellular, intracellular and total body water volumes based on the Hanai mixture theory [6]. The values of ECW

and TBW estimated by the BIS method were regressed against the values obtained by radioisotope dilution.

Multi-frequency bio-impedance analysis (MFBI)

These measurements were extracted from the BIS measurements by noting the resistance (R_f) and reactance (X_f) at frequencies (f) 5, 50, 100, 200 and 500 kHz. The resistances at frequencies zero and infinity, R_0 and R_∞ respectively, were also noted from the BIS analysis program. Impedances (Z_f) at 5, 50, 100, 200 and 500 kHz were calculated from the relationship: $Z_f = (R_f^2 + X_f^2)^{0.5}$. H^2/R_f , H^2/X_f and H^2/Z_f were regressed against ECW and TBW measured by radioisotope dilution. Multiple stepwise regressions were also performed by including weight, age and sex (1 for males, 0 for females) as well as H^2/R_f , H^2/X_f or H^2/Z_f .

Statistical analysis

Statistical analyses were performed using the Unistat Statistical Package version 4.5 (Unistat Limited, Highgate, London, U.K.) on a type 486/33 personal computer. Simple regression analysis was used to establish the correlations (r) of TBW and ECW with resistance indices (H^2/R_f). Multiple stepwise regressions were performed using ECW and TBW measured by radioisotope dilution as the dependent variables. For each regression the multiple correlation (R) and the standard error of the estimate (SEE) between the MFBI and the radioisotope values were obtained. Variables were only included in the final regression equations if they resulted in a significant improvement in the SEE. To determine the validity of the MFBI prediction equations, a double cross-validation study was performed. This involved randomly subdividing the patients into two subgroups of 14 and 15 subjects. Prediction equations for ECW and TBW were obtained by multiple stepwise regression for each subgroup. The prediction equations for each subgroup were then applied to the other subgroup and the predicted values for ECW and TBW were regressed against the values measured by radioisotope dilution. Reproducibility of the BIS and MFBI methods was estimated from the mean coefficient of variation (CoV) of duplicate measurements. A 5% level of significance was used for all data analyses.

RESULTS

The regression between extracellular water measured by the radioisotope dilution method (ECW) and the values predicted by BIS (ECW_{BIS}) was:

$$\text{ECW} = 0.701 \text{ ECW}_{\text{BIS}} + 4.2121 \\ (r = 0.861, \text{SEE} = 1.8931, \text{CoV} = 11.6\%)$$

For TBW the corresponding regression was:

Table 2. Correlations (r) between ECW and resistance indices (H^2/R_f) for various frequencies (f), and multiple correlation (R) and SEE from stepwise regressions. The variables are listed in the order in which they contributed to the prediction equations. H = height (cm), R_f = resistance (ohm) at frequency f (kHz), W = weight (kg), and C = constant.

	r	Variables	R	SEE (l)
H^2/R_5	0.832	$H^2/R_5, W, C$	0.859	1.945
H^2/R_0	0.839	$H^2/R_0, W, C$	0.860	1.937
H^2/R_{50}	0.828	$H^2/R_{50}, W, C$	0.842	2.045
H^2/R_{200}	0.813	$H^2/R_{200}, W, C$	0.830	2.118
HP/R_{500}	0.795	$HP/R_{500}, W, C$	0.815	2.201
HP/R_{1000}	0.801	$HP/R_{1000}, W, C$	0.818	2.181
HP/R_1	0.761	$HP/R_1, W, C$	0.814	2.206

Table 3. Correlations (r) between TBW and resistance indices (H^2/R_f) for various frequencies (f), and multiple correlation (R) and SEE from the stepwise regressions. The variables are listed in the order in which they contributed to the prediction equations. H = height (cm), R_f = resistance (ohm) at frequency f (kHz), W = weight (kg), S = sex (male 1, female 0) and C = constant.

	r	Variables	R	SEE (l)
H^2/R_0	0.808	$W, H^2/R_0, S, C$	0.927	2.947
H^2/R_5	0.826	$H^2/R_5, W, S, C$	0.929	2.900
H^2/R_{50}	0.874	$H^2/R_{50}, W, S, C$	0.937	2.727
H^2/R_{100}	0.882	$H^2/R_{100}, W, S, C$	0.939	2.691
HP/R_{200}	0.896	$HP/R_{200}, W, S, C$	0.943	2.606
HP/R_{500}	0.893	$HP/R_{500}, W, S, C$	0.942	2.637
HP/R_1	0.826	$HP/R_1, W, S, C$	0.941	2.649

$$TBW = 0.947 TBW_{BIS} + 1.501$$

($r = 0.902$, $SEE = 3.259$ l, $CoV = 10.1\%$).

The correlation coefficients between H^2/R_f and the values of ECW and TBW measured by radioisotope dilution are summarized in Tables 2 and 3 respectively.

There was no significant improvement in the correlations when H^2/Z_f was used instead of H^2/R_f at any frequency. For ECW and TBW the highest correlation coefficients were obtained for the resistance indices measured at 5 kHz and 200 kHz respectively. The multiple correlations and standard errors obtained when weight and sex were included with H^2/R_f in the stepwise regressions are also summarized in Tables 2 and 3. The variables in the stepwise regression are listed in the order in which they contributed to the final prediction equation. The highest multiple correlations and lowest standard errors for ECW and TBW were again obtained with the resistance indices measured at 5 kHz and 200 kHz respectively.

The optimum prediction equations for ECW and TBW were:

$$ECW = 0.1782 H^2/R_5 + 0.0688 W + 3.771$$

($R = 0.860$, $SEE = 1.937$ l, $CoV = 11.9\%$), and

$$TBW = 0.2391 H^2/R_{200} + 0.1889 W + 2.971 S + 5.4641$$

($R = 0.943$, $SE = 2.606$ l, $CoV = 8.1\%$)

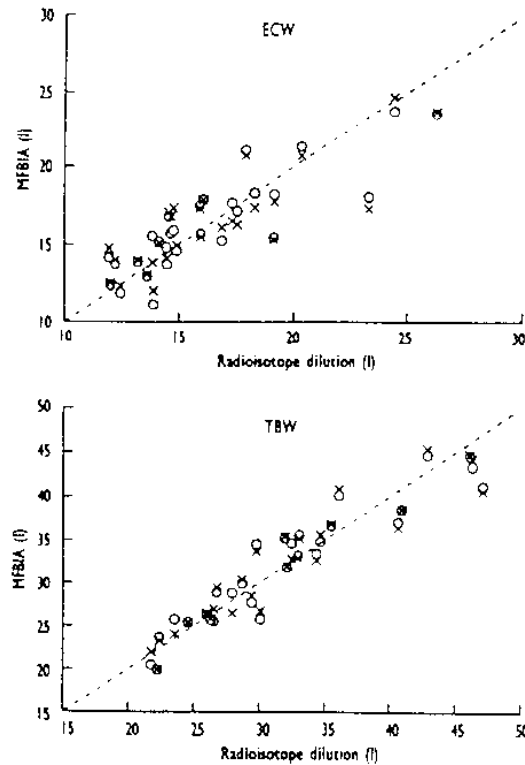


Fig. 1. ECW and TBW calculated from MFBIA prediction equations at two frequencies and the corresponding values obtained from radioisotope dilution. For ECW the frequencies are 5 kHz (\times) and zero (\circ) and for TBW the frequencies are 200 kHz (\times) and infinity (\circ). The broken lines are the lines of identity.

where H = height (cm), R_f = resistance (ohm) at frequency f (kHz), W = weight (kg) and S = sex (1 for males, 0 for females).

It can be seen from Tables 2 and 3 that there are only relatively small differences in the errors for ECW and TBW over a wide range of frequencies. Typical results for individual patients are shown in Fig. 1, where prediction formulae obtained for two different frequencies are plotted as a function of the corresponding radioisotope dilution values. The MFBIA results for ECW correspond to frequencies zero and 5 kHz, and for TBW the frequencies are 200 kHz and infinity. It is apparent from Fig. 1 that there are no significant differences between the results at the fixed frequencies and those obtained from fits to the reactance versus resistance spectra.

In the cross-validation study the same variables were selected as listed in the above prediction equations. The prediction equations for each subgroup were applied to the other subgroup and the results were regressed against ECW and TBW measured by radioisotope dilution. Figure 2 shows the results for ECW and TBW when the prediction equations obtained for each subgroup were applied to the other subgroup. In all cases the regression

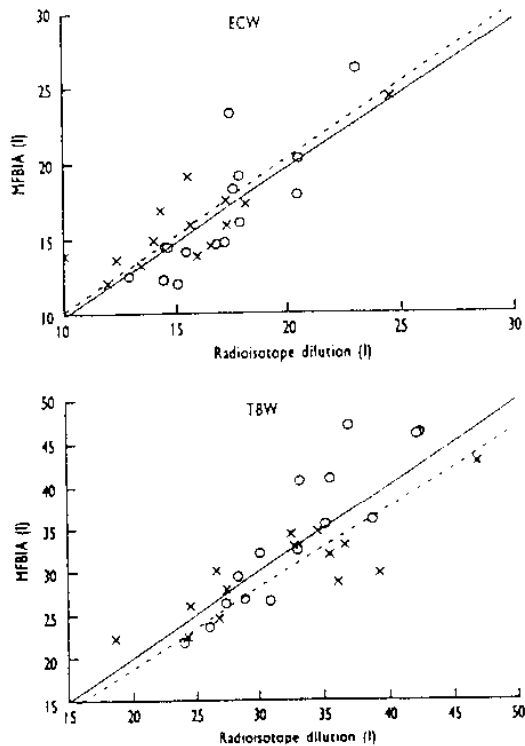


Fig. 2. Results of the double cross-validation study. The solid lines represent the least-squares fits for subgroup 1 (\circ) when the MFBIA values were derived from the subgroup 2 prediction equations. The broken lines represent the least-squares fits for subgroup 2 (\times) when the MFBIA values were derived from the subgroup 1 prediction equations. In all cases the lines are not significantly different from each other or from the lines of identity.

lines were not significantly different from the line of identity.

When the above MFBIA prediction equations were applied to the groups with and without oedema the SEEs for ECW were 2.451 and 1.561 respectively, and the SEEs for TBW were 2.981 and 2.351 respectively. For the BIS method the SEEs for ECW were 2.451 and 1.571 for the oedema and non-oedema groups respectively, and for TBW the SEEs were 4.301 and 2.691 respectively. The presence of oedema therefore significantly increases the errors for both the MFBIA and BIS methods.

The reproducibility of the BIS and MFBIA methods was estimated from the repeat measurements in the subgroup of 15 patients. For the BIS method the mean CoVs for ECW and TBW were 0.9% and 3.0% respectively. The mean CoVs using the MFBIA prediction equations were 0.9% for ECW and 0.6% for TBW.

One would expect that, in normal subjects, with a relatively constant ECW/TBW ratio, the choice of frequency would not be particularly critical. However, even in our surgical patients, where there was

Table 4. Correlation matrix for resistances (R_i) measured at frequencies (f) of 5, 50, 100, 200 and 500 kHz and the values estimated by the BIS fitting program corresponding to frequencies zero and infinity

	R_0	R_{50}	R_{100}	R_{200}	R_{500}	R_{∞}
R_0	0.997	0.976	0.965	0.954	0.947	0.883
R_5		0.988	0.979	0.969	0.963	0.892
R_{10}			0.998	0.993	0.990	0.881
R_{20}				0.998	0.996	0.876
R_{50}					0.996	0.875
R_{100}						0.880

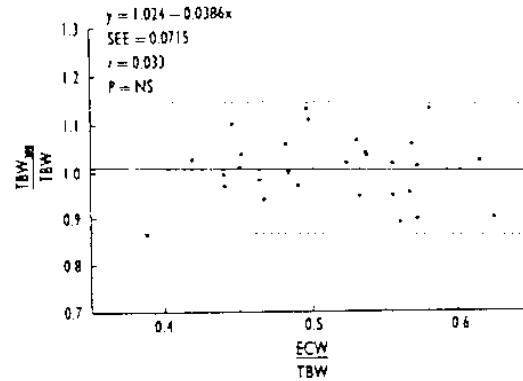


Fig. 3. Ratio of total body water from the 200 kHz MFBIA prediction equation (TBW_{200}) to total body water measured by radioisotope dilution (TBW), plotted as a function of ECW/TBW ratio measured by radioisotope dilution. The solid line is the least-squares fit to the data and the broken lines are the 95% confidence intervals. The parameters of the fit are also shown. TBW_{200}/TBW was independent of the ECW/TBW ratio.

a wide variation in ECW/TBW ratio, the choice of frequency does not appear to be critical. For example the optimum frequency for TBW is 200 kHz but the error is only marginally lower than the error at 50 kHz. This simply reflects the fact that the resistances at the two frequencies are very highly correlated. Simple regression gave the relationship:

$$R_{50} = 1.0827 R_{200} + 5.7 \text{ ohms} \\ (r = 0.9931, \text{SEE} = 13.2 \text{ ohms}, P < 0.0001).$$

For ECW the multiple correlations and standard errors at zero and 5 kHz are not significantly different. This is again not surprising since the correlation between the resistances at frequencies zero and 5 kHz was also very high:

$$R_5 = 0.935 R_0 + 10.4 \text{ ohms} \\ (r = 0.9968, \text{SEE} = 9.9 \text{ ohms}, P < 0.0001).$$

The correlation matrix for resistances at the various frequencies is shown in Table 4.

It is important to establish if the MFBIA prediction equations apply equally well over the full range of ECW/TBW ratios. Figure 3 shows the ratio of TBW from the MFBIA prediction equation to the value measured by radioisotope dilution, plotted as

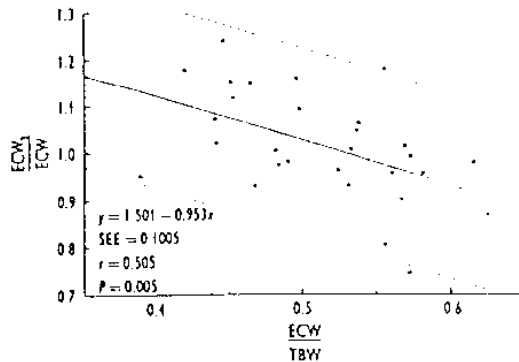


Fig. 4. Ratio of extracellular water from the 5 kHz MFBIA prediction equation (ECW_1) to extracellular water measured by radioisotope dilution (ECW_2), plotted as a function of ECW/TBW measured by radioisotope dilution. The solid line is the least-squares fit to the data and the broken lines are the 95% confidence intervals. The parameters of the fit are also shown. ECW_1 significantly overestimated ECW_2 at low ECW/TBW ratios and underestimated it at high ECW/TBW ratios.

a function of the ECW/TBW ratio. The solid line is the least-squares fit to the data and the broken lines represent the 95% confidence intervals. It is apparent that the MFBIA and radioisotope values for TBW agree equally well over the entire range of ECW/TBW ratios.

Figure 4 shows the ratio of ECW estimated from the MFBIA prediction equation to the value measured by radioisotope dilution, again plotted as a function of the ECW/TBW ratio. The solid line is the least-squares fit to the data and the broken lines represent the 95% confidence intervals. The relationship between the MFBIA and radioisotope values depends on the ratio ECW/TBW ($r=0.505$, $P=0.005$). Similar results were obtained when ECW was predicted by the BIS method. It is apparent that both MFBIA and BIS systematically overestimate ECW at low ECW/TBW ratios and underestimate it at high ECW/TBW ratios but the regression equation shown in Fig. 4 may be used to correct this effect.

DISCUSSION

Malnutrition has been associated with increased septic-related mortality in surgical patients. Body composition assessed using a multiple isotope dilution technique in normal subjects and patients with clinical malnutrition showed that malnutrition resulted in a loss of body cell mass, accompanied by an expansion of the extracellular mass [7]. Because of this expansion of the extracellular mass the loss of the cellular mass was not accurately reflected by the change in weight. Tellado et al. [8] studied the ability of various nutritional assessment techniques to define the final outcome in a population of surgical patients. They showed that the exchangeable sodium to potassium ratio is the best method

for identifying malnourished patients at risk of dying. This is equivalent to the ratio of extracellular mass to body cell mass and similar information may be expected from the use of the ratio ECW/TBW as an index of nutritional status.

Stable bromine may be used to measure ECW and analytical methods have included X-ray fluorescence [9], neutron activation analysis [10] and HPLC [11]. TBW may be measured by stable isotopes using ^{18}O - or 2H -labelled water and mass spectrometry [12]. All of these methods involve the use of analytical techniques which are not generally available. In the present study we used ^{77}Br and 3H to measure ECW and TBW respectively and assayed the radioactive samples in automatic sample counters which are available in most large hospitals. The effective dose from the 3H and ^{77}Br is only 0.09 mSv but ^{77}Br is not ideal because it has a half-life of only 56 h and it is not readily available. In addition, all of these stable and radioactive isotope methods are time consuming and therefore have limited usefulness for routine monitoring of severely ill patients.

Bio-impedance measurements are non-invasive, simple to perform and may be repeated at frequent intervals. Single frequency instruments, typically operating at 50 kHz, are designed to measure only TBW although it has been claimed that information on fluid distribution may be obtained from separate measurements of resistance and reactance [13]. Although 50 kHz is not the optimum frequency for patients with abnormal hydration status the present study suggests that the additional error caused by using this frequency is not likely to be clinically significant. However, measurement of TBW alone does not provide adequate information on the nutritional status of malnourished patients. Several instruments are available which attempt to measure both ECW and TBW using multiple frequencies. Segal et al. [3] estimated ECW and TBW using MFBIA in 36 healthy males. They concluded that ECW was best predicted by resistance measured at 5 kHz corrected for height and weight ($R=0.930$, $SEE=1.941$) and TBW was best predicted by resistance at 100 kHz corrected for height and weight ($R=0.947$, $SEE=2.641$). Van Loan and Mayclin [4] used MFBIA to estimate ECW in 60 normal subjects (40 males, 20 females). They developed a prediction equation for ECW which included the resistance index (H^2/R_t) at 224 kHz, weight and sex, and obtained a multiple correlation (R) of 0.961 and a SEE of 1.061. For TBW they derived an equation which included a resistance index at 224 kHz, weight, sex and age, and obtained a multiple correlation (R) of 0.927 and a SEE of 3.581.

BIS involves the measurement of resistance and reactance over a wide range of frequencies. It uses a computer program to obtain the best fit to the data from which the extracellular and intracellular resistances are estimated. The Xitron software uses these

resistances together with height, weight and a gender term to calculate ECW and TBW. Thomas et al. [14] reviewed the various bio-impedance methods and concluded that the BIS technique was preferred to the use of impedance measured at discrete frequencies. Van Loan et al. [15] evaluated the use of BIS in 24 normal subjects (10 males, 14 females) and concluded that the estimates of ECW and TBW were not significantly different from the isotope dilution results. For ECW they obtained a correlation coefficient of 0.893 and a SEE of 0.947; for TBW the correlation coefficient was 0.924 and the SEE was 2.276l. However, Deurenberg et al. [16] compared MFBIA and BIS in 48 normal subjects (23 males, 25 females) and assessed the errors introduced by the BIS fitting technique when estimating the resistances at frequencies zero and infinity. They concluded that because of these errors, modelling of the impedance data has no advantage over impedance values measured at fixed frequencies.

The errors obtained in the present study of surgical patients are within the range of those obtained by other groups in normal subjects. The errors in patients with oedema are greater than those in patients without oedema. In considering these errors it is important to estimate the contribution from the independent radioisotope measurements. Unfortunately the reproducibility of the radioisotope methods cannot be assessed from repeat measurements in surgical patients since their fluid status may change within a relatively short period. Instead we estimated the reproducibility by performing simulated studies in which the radioisotopes were uniformly dispersed in known volumes equivalent to typical ECW and TBW volumes. Fifteen duplicate measurements were made for each in which all weighing, pipetting and radioisotope counting aspects of the normal patient procedures were performed. The mean CoVs were 0.65% and 0.52% for the bromide and tritiated water distribution volumes respectively. The errors in ECW and TBW will also depend on the adequacy of the equilibrium time, the losses in the urine and the errors in the various assumed correction factors. A 3 h equilibrium time may appear to be rather short, particularly in patients with oedema. In normal subjects, Vaisman et al. [10] found no significant difference in corrected bromide space when samples were obtained at 2, 3 and 4 h although they did observe a significant difference depending on whether the bromide was administered orally or intravenously. In a previous study to investigate the optimum radioisotope dilution method, we obtained blood samples at 3 and 18 h; urine collections were also obtained. These measurements were performed in 43 surgical patients (23 male, 20 female); 23 patients had gastrointestinal cancer, seven had benign gastrointestinal disease, 10 had pancreatitis and three had inflammatory bowel disease. These patients were similar in terms of age, weight, ECW

and TBW to the patients in the present study. ECW and TBW measured from blood samples taken at 18 h were $2.1 \pm 0.5\%$ (SE) and $3.5 \pm 0.4\%$ (SE) respectively greater than those measured at 3 h. Since the apparent increases at 18 h may be largely explained by the presence of insensible losses, we concluded that 3 h represented an adequate equilibrium period even in surgical patients. We also found that the corrections for losses in the urine during the first 3 h were insignificant; the mean correction factors were 1.0044 ± 0.0012 (SE) for ECW and 1.0014 ± 0.0003 (SE) for TBW. This is consistent with the results of Brans et al. [17] who found that the bromine losses in the urine were less than 1% of the administered dose in the first 3 h. Our standard radioisotope dilution method therefore consists of administering the radioisotope intravenously and obtaining a blood sample at 3 h, with no urine collection. The hydrogen dilution volume is greater than the total body water space because of the exchange with labile hydrogen of protein and other body constituents. However, there have been considerable variations in the estimated magnitude of this effect. Schoeller and Jones [18] reviewed this and concluded that the hydrogen dilution space is $4 \pm 1\%$ (SD) larger than TBW. We have therefore applied this correction in the present study. The factor used to correct for the protein content of plasma (0.94) has been widely used but it is an average value for normal subjects. In a separate group of 31 surgical patients, we observed a mean value of total plasma protein of 69.1 ± 6.5 g/l (SD) (range 52–82 g/l). The use of the average correction factor for normal subjects (0.94) may therefore have introduced a random error of approximately 0.6% to both ECW and TBW. This error could be avoided if the protein is removed from the plasma by ultrafiltration or if individual correction factors are applied. We have assumed random errors of $\pm 1\%$ each for the Donnan correction factor and erythrocyte concentration factor. The total errors for the radioisotope methods, determined by summing the individual errors in quadrature, are therefore estimated to be 1.7% for ECW and 1.3% for TBW, corresponding to approximately 0.31 and 0.41 respectively.

We have shown that the MFBIA method is highly reproducible, with mean CoVs of 0.9% for ECW and 0.6% for TBW, corresponding to 0.151 and 0.191 respectively. However, this does not necessarily imply that the method is capable of measuring small changes in ECW or TBW. The measured total body resistance is effectively the sum of the resistances in three major anatomical regions, namely arm, trunk and leg. Since resistance is inversely proportional to cross-sectional area it is largely dominated by the resistances in the arm and leg, with the trunk contributing least to the total resistance. In the present study, separate impedance measurements were obtained of the 'trunk' by positioning the electrodes close to the sternum and on

the thigh. At 5 kHz and 200 kHz the trunk resistance was $18.8 \pm 0.4\%$ (SE) and $17.8 \pm 0.4\%$ (SE) of the total body resistance respectively. Therefore, a change in volume which affects predominantly the trunk, such as the removal of ascites, may result in only a minimal change in the total body resistance. The change in ECW and TBW predicted by MFBIA or BIS is therefore likely to underestimate the true change.

The good reproducibilities of the radioisotope and bio-impedance methods suggests that these cannot account for the differences between the methods and we therefore conclude that the SEEs can be attributed largely to errors in the indirect bio-impedance methods. These errors are perhaps not surprising considering the inhomogeneous nature of the various body compartments and the large variations in cross-sectional area between them. These errors limit the clinical usefulness of the MFBIA and BIS methods for the assessment of ECW and TBW in individual patients, although both methods may be adequate for defining the characteristics of groups of patients. The ability of the MFBIA and BIS methods to monitor changes in ECW and TBW cannot simply be assessed from their reproducibility. This is because relatively large changes in the volume of the trunk may result in relatively small changes in total body resistance. The ability of the methods to follow changes in patients undergoing nutritional support requires further investigation.

We conclude that, in surgical patients, MFBIA measurements at only two frequencies provide estimates of ECW and TBW which have comparable errors to the more complex BIS technique. The reproducibility of the MFBIA method is the same as BIS for the measurement of ECW and is significantly better for the measurement of TBW. MFBIA measurements could be obtained using a simple bio-impedance analyser designed to measure only resistance at two frequencies. A simple analyser of this type, which does not require an associated computer, should offer considerable advantages in terms of reduced cost and reduced time to acquire and calculate the results. The optimum frequencies for ECW and TBW are 5 kHz and 200 kHz respectively, but the very high correlations between resistances at these and similar frequencies suggests that prediction equations based on alternative frequencies will be equally appropriate. The role of MFBIA

in assessing the nutritional status of surgical patients should be evaluated and its ability to measure changes in individual patients should be carefully assessed.

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