NUTRITION RESEARCH, Vol. 12, pp. 657-666, 1992
0271-5317/92 $5.00 + .00 Printed in the USA.

MEASUREMENT OF EXTRACELLULAR AND TOTAL BODY WATER OF RATS USING MULTIPLE FREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS

B. H. CORNISH, M.App.Sc.[1], L. C. WARD, Ph.D.[2], and B. J. THOMAS Ph.D.[1]
Centre for Medical and Health Physics, Queensland University of Technology[1] and the Department of Biochemistry, University of Queensland[2], AUSTRALIA.

ABSTRACT

Multiple frequency bioelectrical impedance analysis (MFBIA) was used to measure whole body impedance of rats between frequencies from 1 kHz to 100 kHz. The data were analysed, with reference to an equivalent electrical circuit for biological tissue, to estimate total body water (TBW) and extracellular water (ECW). TBW and ECW were independently determined by isotope dilution and desiccation. Correlation between MFBIA measurements and these alternative techniques were r = 0.984 (SEE = 6.5%) and r = 0.995 (SEE = 3.2%) for TBW and ECW respectively. These results establish the validity, accuracy and precision of MFBIA for the assessment of both TBW and ECW.

KEY WORDS: Total body water, extracellular water, body composition, bioelectrical impedance analysis, inulin, deuterium oxide

INTRODUCTION

Water is the most abundant chemical in the body and it plays a central role in nutrient transport, waste removal and thermal regulation. Water is distributed intra- and extracellularly, and these volumes are well regulated in the healthy individual. Achieving water and electrolyte homeostasis is essential in the management of critically ill neonates, and other patients with any condition where the fluid balance or level is in question. Although the importance of assessing body composition including the body water compartments has been established, the routine use of body compositional methods is limited by practical and ethical constraints imposed by relatively invasive procedures and/or costly equipment (1).

One inexpensive and non-invasive technique of body composition analysis that has recently come to prominence is the measurement of whole body impedance. Bioelectrical impedance analysis (BIA) was first used in 1969 (2) to estimate total body water (TBW) by assuming the complex geometry of a living body could be approximated by a simple cylindrical conductor of length L, cross-sectional area A, and volume V.

The impedance, Z, is given by:

$$Z = \rho\frac{L}{A} = \rho\frac{L^2}{V} \qquad eqn\ 1$$

$$\therefore \quad V = \rho\frac{L^2}{Z} \qquad eqn\ 2$$

where:
Z = impedance
ρ = resistivity
L = length
V = volume
A = cross-sectional area

This method for determining body impedance uses the application of a constant low level alternating current. The body contains intra- and extracellular fluids that behave as conductors, and cell membranes and tissue interfaces that act as imperfect capacitors. The total impedance, and phase angle, is therefore dependant on the frequency of the alternating current. At low frequencies (< 1 kHz), the current passes mainly through the extracellular fluids, while at higher frequencies, (100 kHz to 1 MHz), the current penetrates both the extra- and intracellular fluids (3). Hence L^2/Z should be a good predictor of extracellular water (ECW) and TBW, provided that Z is taken as the impedance at low and high frequencies respectively.

While a substantial amount of work has been performed using BIA at a fixed frequency of 50 kHz and the measured impedance used as a predictor of TBW (eg. 1, 3) very little attention has been paid to the use of impedance at other frequencies (4, 5). Recent research, (6, 7), has investigated BIA over a range of frequencies, (1 kHz to 500 kHz), and used the impedance - frequency variation to study fluid shifts between body water compartments following intensive exercise in atheletes. However literature searches have not revealed any study to validate estimates of body water compartments using multiple frequency bioelectrical impedance analysis (MFBIA).

In the present study, whole body impedance of rats was measured using frequencies from 1 kHz to 100 kHz. MFBIA data were analysed by graphical analysis based on an equivalent electrical circuit. TBW and ECW were determined by standard isotope dilution procedures using deuterium oxide and tritiated inulin respectively. The animal carcasses were completely desiccated and TBW also determined as the difference between the live body weight and dry carcass weight.

Correlation of impedance measurements with the results of the accepted techniques establish the validity and accuracy of the MFBIA technique and demonstrate that high precision estimates of both TBW and ECW can be readily obtained using inexpensive and, potentially, non-invasive MFBIA.

MATERIALS AND METHODS

Animals

Seventeen healthy adult Wistar rats, both male and female, weights ranging from 200g to 650g, were used in validating the technique. The animals had ad libitum access to food and water prior to the procedure and were housed in an animal house maintained at 22°C on a 12 hour light 12 hour dark cycle. The animals were anaesthetised throughout the experimental procedure with sodium phenobarbitone (Fawns & McAllan Pty. Ltd. Victoria) with a dose of 100 mg kg^{-1} body weight. Weight of the anaesthetised animal was recorded to the nearest 0.1 g. Ethical clearance for all procedures was obtained from the University of Queensland's ethics committee.

Multiple Frequency Bioelectrical Impedance Analysis

The anaesthetised animal was positioned on a non-conductive surface with their dorsal surface upwards and limbs extended perpendicular to the longitudinal body axis. The MFBIA instrument manufactured by SEAC (Brisbane, Australia) uses a tetrapolar electrode arrangement (8). The electrodes were fabricated from 5 cm 20 gauge stainless steel hypodermic needles by bending the shaft perpendicularly 6 mm from the tip. The electrodes were inserted subcutaneously into the subdermal tissue avoiding penetrating the muscle with the tip directed distally along the dorsal midline of the body a distance of approximately 3 mm. One source electrode was positioned in line with the superior edge of the orbit, and the other 4 cm from the base of the tail. Measurement electrodes were positioned; one in line with the posterior opening of pinna, and the other in line with the iliac spines. The distance between the points of insertion of the measurement electrodes was measured to the nearest 0.1 cm.

A constant current of AC frequency, 1 kHz to 100 kHz, was applied to the source electrodes, and the corresponding impedance and phase angle detected by the measurement electrodes were recorded at six discrete frequencies (1.0, 3.25, 10.0, 32.5, 50.0, 100 kHz). The coefficient of variation for repeated measures on the same animal was $< 1.0\%$. The reactive and resistive components of the measured impedance at these six frequencies were graphed, and the resulting impedance plot forms part of a circular locus (9). The frequency at which the peak of this plot occurs is known as the characteristic frequency, and the impedance at this frequency is Z_c. Using the the geometric features of a circle the plot can be extrapolated, using linear least squares analysis, to zero frequency to obtain the body impedance to DC current, Z_0, otherwise immeasurable due to electrode capacitance. The quotients L^2/Z_c and L^2/Z_0, (where L is the distance between the measurement electrodes), were calculated and used in the correlation with TBW and ECW respectively.

Total Body Water by D_2O Dilution

The time required for deuterium oxide (D_2O) to equilibrate between all body compartments and tissues is approximately 2 hours in the rat (10). Each rat was injected intraperitoneally with an accurately weighed amount of D_2O (Cambridge Isotope Laboratories, Massachusetts), 1.1 g kg^{-1} body weight, 150 minutes prior to killing by anaesthetic overdose. At the time of death a 2 ml sample of blood was taken by cardiac puncture. Plasma from this sample was separated by centrifuge at 1500g for 6 minutes at 5°C.

The plasma concentration of D_2O was determined by Fourier transform infrared spectroscopy (FTIR). Using a set of standards of known D_2O concentration in plasma, and the principle of Beer's law, the concentration of D_2O in plasma was determined (11). During the procedure animals were constantly monitored and urination did not occur in any animal. Therefore, no correction for urinary loss of D_2O was necessary. Using a water content of plasma of 93.7% by volume (4, 10, 12), the corresponding D_2O concentration in water was calculated and hence the TBW estimated.

Total Body Water by Desiccation

After the completion of the D_2O and inulin procedures the carcasses were sealed in polythene bags and stored at -70°C. The frozen carcasses were cut into smaller segments and homogenised using a Waring commercial blender. The complete homogenised remains of each animal were then quantitatively transferred to a metal dish and dried to a constant weight in an oven maintained at 60 to 65°C. Desiccation time was dependent upon the

size of the animal and varied from 3 to 5 days. TBW was determined by subtraction of the desiccated weight from the live body weight.

Extracellular Water by [^{3}H] Inulin Dilution

Inulin is a polysaccharide with a molecular mass of 5200 does not traverse the cell membrane. Hence its distribution space very closely approximates the ECW volume. The following procedure was conducted simultaneously with the previously described D_2O method such that both procedures concluded at the time the animal was killed.

Tritium labelled inulin (Amersham, England) was injected intravenously, via a lateral tail vein, providing a dose of 45 μCi kg^{-1} body weight, (in a volume 180 μl kg^{-1}). The catheter was flushed through with 50 μl of normal saline to ensure the complete dose was administered to the vein. The tip of the tail was clipped and 20 μl blood samples taken at timed intervals up to 75 minutes postinjection. From the final blood sample, taken via cardiac puncture, four 20 μl samples of plasma were obtained. The samples were treated with 1 ml hydrogen peroxide to bleach the colour and 0.1 ml 5% hydrochloric acid to neutralise the hydrogen peroxide (13). Scintillation fluid, volume 10 ml (ACS; Amersham, Illinois), was added and the activity determined by liquid scintillation spectrometry using an external standard ratio to correct for quenching.

After equilibration the fall in blood inulin concentration due to clearance by the kidneys approximates to a single exponential function (14), represented by a straight line on a semi-logarithmic plot. Using the slope of this line, fitted to the experimental data by least squares regression (Fig 1), and the mean activity of the final four plasma samples, the specific activity of the plasma at time zero was determined by extrapolation. This is the value of the specific activity of the plasma after equilibration but without the effect of clearance by the kidneys. From the total activity injected and the specific activity after equilibration the dilution factor was determined and using the water content of plasma, 93.7%, the ECW volume was calculated.

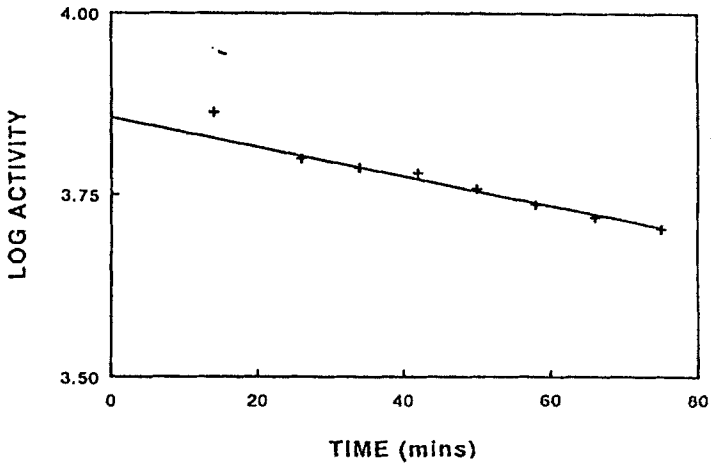

FIG. 1. Decrease in blood inulin concentration with time for a representative animal.

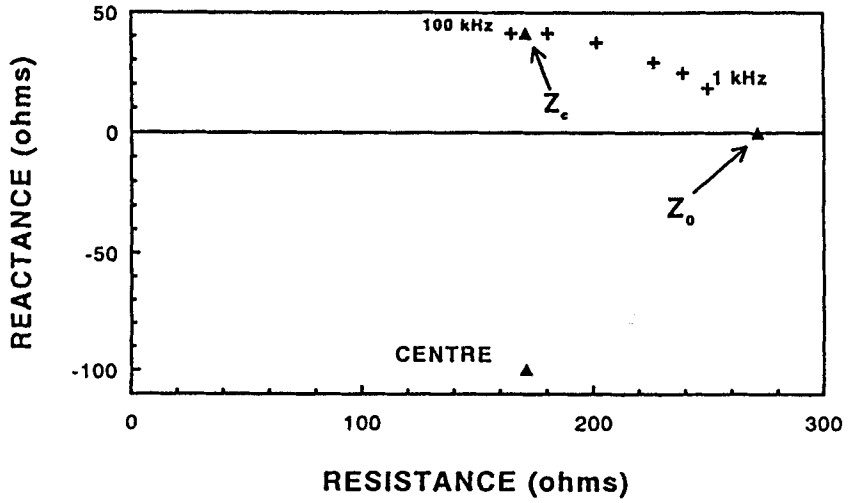

FIG. 2. Impedance plot showing the variation of reactance with resistance for frequencies 1 kHz to 100 kHz. Data depicted are for the same animal as the data shown in FIG 1.

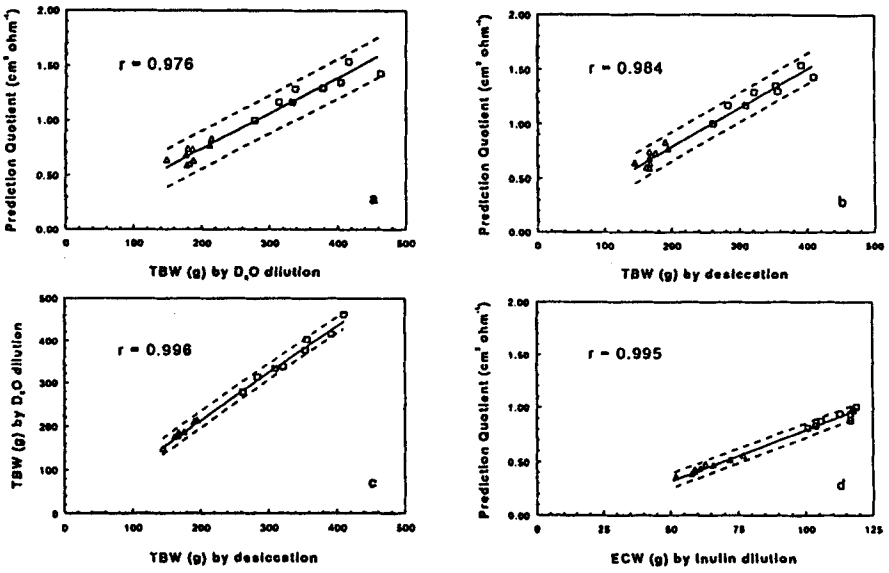

FIG. 3. Regression analysis indicating 95% confidence limits and corresponding correlation coefficients. △ = females; □ = males.

RESULTS

A typical impedance plot showing the measured resistance and reactance at the six frequencies is shown in Fig 2. The impedance at the characteristic frequency Z_c and zero frequency Z_0 and the centre of the circular locus are also shown. For this animal the impedance values at zero, and the characteristic frequency were 271 ohms and 176 ohms respectively, while the distance between the measurement electrodes was 15.4 cm. The corresponding MFBIA prediction quotients (L^2/Z_c & L^2/Z_0) for TBW and ECW were 1.347 and 0.875 cm^2ohm^{-1} respectively. For this animal the specific activity of the plasma at time zero was 385 dpm μl^{-1} (Fig 1). The dose administered was 4.380×10^7 dpm, and using the water content of plasma, 93.7%, the ECW volume for this animal was determined to be 105.9 ml (19.8% of the total body weight). Using a similar method of calculation for all animals the following prediction quotients L_2/Z_c and L^2/Z_0 were obtained (Table 1).

TABLE 1

TBW estimated by D_2O dilution and desiccation, ECW by inulin dilution and the MFBIA prediction quotients calculated for each animal.

Sex	Body weight (g)	Carcass weight (g)	Total body water Dessication† (g)	Total body water D_2O†† (g)	Total body water MFBIA quotient	Extracellular water Inulin# (g)	Extracellular water MFBIA quotient
F	229.2	84.7	144.5	149.3	0.642	51.6	0.364
F	243.6	81.9	161.7	182.4	0.596	58.7	0.407
F	245.5	78.6	166.9	177.5	0.594	57.5	0.392
F	257.6	90.9	166.7	180.0	0.737	62.5	0.483
F	259.3	92.2	167.1	177.0	0.677	61.3	0.435
F	265.8	97.6	168.2	188.0	0.634	59.0	0.434
F	267.5	91.2	176.3	187.5	0.730	65.5	0.471
F	284.1	92.8	191.3	215.0	0.829	72.1	0.521
F	308.9	114.1	194.8	211.8	0.765	76.5	0.559
Mean (F)	262.4	91.6	170.8	185.4	0.690	62.7	0.452
± SD	23.6	10.3	15.2	19.5	0.081	7.7	0.063
M	456.1	194.5	261.6	278.2	1.004	101.1	0.812
M	492.5	208.6	283.9	314.3	1.172	104.5	0.832
M	497.8	176.3	321.5	338.0	1.290	117.0	0.945
M	534.5	180.2	354.3	404.2	1.347	105.9	0.875
M	550.9	240.5	310.4	333.9	1.175	119.2	0.999
M	608.6	252.1	356.5	378.2	1.303	117.5	0.974
M	627.1	216.8	410.3	462.6	1.428	112.7	0.935
M	661.4	269.8	391.6	415.7	1.545	116.6	0.875
Mean (M)	553.6	217.4	336.3	365.6	1.283	111.8	0.906
± SD	72.5	34.1	51.4	60.5	0.167	7.0	0.067
Mean (all)	399.4	150.8	248.7	270.2	0.969	85.8	0.665
± SD	158.2	68.9	92.3	101.9	0.330	26.2	0.242

†† TBW determined by D_2O dilution; † TBW determined by desiccation; # ECW determined by inulin dilution

The correlation between the MFBIA measurements and the results of the desiccation and isotope dilution methods were calculated and the corresponding regression lines (15) with 95% confidence intervals are shown in Fig 3. From these correlations the following prediction equations were derived.

$$TBW = 309.9 \frac{L^2}{Z_c} - 30.0 \quad SEE = 22 = 8.0\% \qquad eqn\ 3$$

$$TBW = 280.3 \frac{L^2}{Z_c} - 22.8 \quad SEE = 16 = 6.5\% \qquad eqn\ 4$$

$$ECW = 108.3 \frac{L^2}{Z_0} + 13.8 \quad SEE = 2.7 = 3.2\% \qquad eqn\ 5$$

where: L = distance between measurement electrodes (cm).
Z_c = impedance at the characteristic frequency (ohm).
Z_0 = impedance at zero frequency (ohm).
TBW & ECW = body water masses (g).
SEE = standard error of the prediction.

Predicted values for TBW and ECW calculated using these equations together with the measured estimates of these parameters are presented in Table 2. As observed by others (16,17) the mean value for TBW estimated by D_2O dilution was approximately 5% greater than that estimated by desiccation.

DISCUSSION

Although equations 3 and 4 differ slightly, in both cases MFBIA measurement is a similarly good predictor of TBW. The difference in the prediction equations is considered to be due to the overestimation of TBW by the deuterium dilution method (slope of the regression line in Fig 3c equals 1.1). Previous research (16,17) has attributed this overestimation when using isotopes of hydrogen in water for estimating TBW to be due to hydrogen ion exchange with body tissues.

The standard deviations obtained using the prediction equations compared with that from the isotope dilution and desiccation procedures, suggest that MFBIA has an uncertainty which is greater than that associated with desiccation, but comparable to the uncertainty associated with widely used dilution techniques. MFBIA also has the advantage of being easy to implement with a minimal processing time, capital costs are low and running costs are negligible, capable of being repeated many times and its portability makes it suitable for field studies. MFBIA also has the capacity of being non-invasive when used with metal foil electrodes on the skin surface, i.e. in man (unpublished data).

The correlation of MFBIA with TBW, ($r = 0.984$), compares very favourably with the BIA measurements at a single frequency of 50 kHz, where a correlation of $r = 0.950$ was obtained. A significant ($P<0.025$) improvement in precision of prediction was also noted, SEE = 6.5% and 11.1% for MFBIA and single frequency measures respectively. The correlation of MFBIA using Z_0 with ECW, $r = 0.995$ and SEE = 3.2%, was not significantly different from the correlation obtained at a single frequency of 1 kHz ($r = 0.993$, SEE = 3.4%). This, however, is not unexpected when using needle electrodes thereby obviating skin surface capacitance effects at 1 kHz.

TABLE 2

TBW and ECW expressed as a percentage of body weight measured[††] and calculated[†].

Sex	Body weight (g)	% TBW				% ECW	
		D_2O[††]	MFBIA[†] (Eqn 3)	Drying[††]	MFBIA[†] (Eqn 4)	Inulin[††]	MFBIA[†] (Eqn 5)
F	229.2	65.2	73.8	63.0	68.6	22.5	23.2
F	243.6	74.9	63.5	66.4	59.2	24.1	23.7
F	245.5	72.3	62.8	68.0	58.5	23.4	22.9
F	257.6	69.9	77.1	64.7	71.4	24.3	25.6
F	259.3	68.3	69.4	64.4	64.4	23.6	23.5
F	265.8	70.7	62.6	63.3	58.3	22.2	22.9
F	267.5	70.1	73.4	65.9	68.0	24.5	24.2
F	284.1	75.7	79.9	67.3	73.8	25.4	24.7
F	308.9	68.6	67.1	63.1	62.1	24.8	24.1
Mean (F)	262.4	70.6	70.0	65.1	64.9	23.9	23.9
± SD	23.6	3.3	6.4	1.9	5.8	1.0	0.9
M	456.1	61.0	61.7	57.4	56.7	22.2	22.3
M	492.5	63.8	67.7	57.6	62.1	21.2	21.1
M	497.8	67.9	74.3	64.6	68.0	23.5	23.3
M	534.5	75.6	72.5	66.3	66.4	19.8	20.3
M	550.9	60.6	60.6	56.3	55.6	21.6	22.1
M	608.6	62.1	61.4	58.6	56.3	19.3	19.6
M	627.1	73.8	65.8	65.4	60.2	18.0	18.4
M	661.4	62.9	67.9	59.2	62.0	17.6	16.4
Mean (M)	553.6	66.0	66.5	60.7	60.9	20.4	20.4
± SD	72.5	5.9	5.1	4.1	4.6	2.1	2.3
Mean (all)	399.4	68.4	68.3	63.0	63.0	22.2	22.3
± SD	158.2	5.1	5.9	3.8	5.5	2.4	2.4

The merits of MFBIA over single frequency BIA are:

(a) the ability to estimate by extrapolation the impedance of biological tissue to DC current, which passes only through the ECW;

(b) the ability to identify the characteristic frequency of an individual animal at which there exists a fixed ratio between the intracellular and extracellular currents;

(c) the use of a graphical technique to analyse a number of impedance measurements on the same animal, thereby decreasing the inherent uncertainty associated with a single measurement at a predetermined frequency.

Since no other anatomical measurements or physiological factors have been incorporated, the prediction equations estimate the volume of body water compartments using only the electrical properties of body tissues. The prediction equations were derived by considering the body to be a simple cylindrical conductor of length L, and with reference to the extrapolated impedance at two frequencies. Clearly this is not an accurate representation of the geometry of a living body. The accuracy of the technique may be further improved by the use of a better geometrical model.

In conclusion, the present data confirm the validity, accuracy and improved precision of MFBIA compared with single frequency BIA for the determination of TBW and its subcompartment ECW. To achieve this degree of precision without the need for the inclusion of other anatomical or physiological factors, (such as age, weight, sex, etc.), suggests that MFBIA will become the preferred method for determination of body water and its sub-compartments in animals and warrants further investigation in animals with abnormal intra- and extracellular water balance.

REFERENCES

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

2 Hoffer EC, Meador CK, Simpson DC. Correlation of whole body impedance with total body water volume. J Appl Physiol 1969; 27: 531, 534.

3 Jenin P, Lenoir J, Roullet C, Thomasset AL, Ducrot H. Determination of body fluid compartments by electrical impedance measurements. Aviat Space Environ Med 1975; 46: 152, 155.

4 Espejo MG, Neu J, Hamilton L, Eitzman B, Gimmotty P, Bucciarelli RL. Determination of extracellular fluid volume using impedance measurements. Crit Care Med 1989; 17: 360, 363.

5 Segal KR, Burastero S, Chun A, Coronel P, Pierson RN, Wang J. Estimation of extracellular and total body water by multiple frequency bioelectrical-impedance measurement. Am J Clin Nutr 1991; 54: 26, 29.

6 Kanai H, Haeno M, Sakamoto K. Electrical measurement of fluid distribution in legs and arms. Medical Progress Through Technology 1987; 12: 159, 170.

7 Gerth WA, Montgomery LD, Wu YC. A computer based bioelectrical impedance spectroscopic system for noninvasive assessment of compartmental fluid redistribution. Third annual IEEE symposium on computer-based medical systems 1990; 446, 453.

8 Hall CB, Lukaski HC, Marchello MJ. Estimation of rat body composition using tetrapolar bioelectrical impedance analysis. Nutr Rep Int 1989; 39: 627, 633.

9 Ackmann JJ, Seitz MA. Methods of complex impedance measurements in biologic tissue. Crit Rev Biomed Eng 1984; 11: 281, 311.

10 Rothwell NJ, Stock MJ. In vivo determination of body composition by tritium dilution in the rat. Br J Nutr 1979; 41: 625, 628.

11 Blagojevic N, Allen BJ, Gaskin KJ, Baur LA. Determination of total body water by fourier transform infrared analysis. Australasian Phys Eng Sci in Med 1990; 13: 110, 116.

12 Lukaski HC, Bolunchuk WW. Estimation of body fluid volumes using tetrapolar bioelectrical impedance measurements. Aviat Space Environ Med 1988; 59: 1163, 1169.

13 Holleman DF, Dieterich RA. Body water content and turnover in several species of rodents as evaluated by the tritiated water method. J of Mammology 1973; 54: 456, 465.

14 Lesser GT, Deutsch S, Markofsky J. Fat-free mass, total body water, and intracellular water in the aged rat. Am J Physiol 1980; 238: R82, R90.

15 Deming WE. Statistical Adjustment of Data. John Wiley and Sons, New York. 1943.

16 Schloerb PR, Friis-Hansen BJ, Edelman IS, Solomons AK, Moore FD. The measurement of total body water in the human subject by deuterium oxide dilution. J Clin Invest 1950; 29: 1296, 1310.

17 Culebras JM, Moore FD. Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. Am J Physiol: Regulatory Integrative Comp Physiol 1977; 232: R54, R59.

Accepted for publication January 27, 1992.