Pergamon

Neuromusc. Disord., Vol. 6. No. 1, pp. 55–60, 1996

0960-8966/96 $15.00 + .00

0960-8966(95)00015-1

MULTIFREQUENCY BIOELECTRIC IMPEDANCE MEASUREMENTS FOR PREDICTING BODY WATER COMPARTMENTS IN DUCHENNE MUSCULAR DYSTROPHY

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(*Received 8 December 1994; revised 27 March 1995; accepted 21 April 1995*)

Abstract—Body hydration and extra- to intra-cellular water ratio (ECW: ICW) have been studied in 12 duchenne muscular dystrophy (DMD) patients and 15 healthy controls. Subjects underwent total body water (TBW) and extracellular water (ECW) assessment by deuterium and bromide dilution, respectively. Multifrequency bioelectric impedance analysis (MFBIA) was performed on all subjects with the aim to establish its accuracy in predicting TBW and ECW in DMD. Body hydration was lower (51.8 ± 2.8 vs $58.5 \pm 5.9\%$, $P < 0.01$) and the ECW: ICW ratio higher (1.15 ± 0.25 vs 0.70 ± 0.23, $P < 0.001$) in DMD than in control subjects. Hence, control-generated formulae for predicting TBW and ECW from MFBIA gave inaccurate results in DMD subjects. Population-specific formulae were developed to obtain an accurate prediction of body water compartments in DMD patients.

Key words: Total body water, extracellular water, bioelectric impedance, Duchenne muscular dystrophy

INTRODUCTION

Assessment of hydration status is of great clinical interest. Body hydration and extra- to intra-cellular water ratio are known to change with a person's nutritional status. Undernourished subjects often exhibit an increase in extracellular water (ECW) relative to total body water (TBW), which may be responsible for the clinical appearance of oedema [1]. None the less, obese subjects often show an increase in ECW relative to TBW, whose clinical significance is currently under investigation [2, 3]. In addition to under- and over-nourishment, it has recently been shown that an increase in ECW relative to TBW may also occur in highly trained athletes [4]. Thus, body water distribution appears to be significantly linked to nutritional status in both physiological and clinical conditions. It is therefore clear that the measurement of water compartments is an essential part of body composition assessment and that it may be helpful in elucidating the mechanisms of disease [5]. However, measurements of ECW and TBW involve the use of invasive and time-consuming tracer-dilution techniques and are, therefore, not suitable for use in the clinical field. Multifrequency bioelectric impedance analysis (MFBIA) is a novel technique for the assessment of TBW and ECW. In this method, an alternating electrical current (a.c.) of constant frequency and low intensity (usually 800 μA) is applied to the body. The opposition of the body to the flow of the a.c., known as bioelectric impedance (Z), is recorded at low (< 50 kHz) and high (> 50 kHz) frequencies. As the a.c. is not able to penetrate the cell at low frequencies owing to the capacitive properties of cell membranes, Z is a measure of ECW at frequencies under 50 kHz (usually 1 or 5 kHz). At progressively higher frequencies, the contribution of cell capacitance to body impedance decreases and finally disappears so that Z provides a measure of TBW at frequencies higher than 50 kHz (usually 100 kHz) [6]. At a constant frequency, and assuming a constant conductor configuration, the impedance to the flow of current can

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be related to the volume of the conductor according to the formula [7]:

$$\text{volume} = a \times \text{length}^2/Z + b^*.$$

On the basis of this theoretical model a number of formulae have been developed for predicting body water from Z by substituting the length of the conductor with body height, thus giving the definition of the impedance index (height^2/Z or ZI) [6, 8, 9]. To date, no algorithm has been published for predicting TBW and ECW from MFBIA in children. However, some formulae which employ ZI at 50 kHz are available for predicting TBW in children [10]. Of these formulae, the more commonly employed is that of Davies *et al.* [11], which was developed on a sample of both healthy and sick children.

Duchenne muscular dystrophy (DMD) is an X-linked myopathy associated with profound changes in body composition. Body hydration of DMD patients has been reported to be reduced in comparison to healthy subjects [12]. Nevertheless, it has been suggested that an increase in the ratio of exchangeable sodium to total body potassium may reflect an expansion of the extracellular space of DMD patients [12].

The objectives of this study were, first, to ascertain if an expansion of ECW really occurs in DMD patients as compared to healthy children and, second, to verify the accuracy of the MFBIA method for predicting hydration status of these patients.

MATERIALS AND METHODS

Subjects

Twelve children affected by DMD and 15 healthy controls matched for age (mean ± S.D.: 11.6 ± 2.2, range: 6.1–15.1 yr), weight and height were recruited for this study. Of the DMD subjects, seven were ambulant and five non-ambulant. None of the subjects or controls had taken any drug and all were on a free diet. The aim of the study was explained and informed consent was obtained from the parents of all the children taking part. The experimental protocol had previously been approved by the Ethical Committee of "Rizzoli" Orthopaedic Institute.

*a and b represent the slope and intercept of the regression equation, respectively.

TBW and ECW assessment

TBW and ECW were measured by means of deuterium oxide (D_2O) and sodium bromide (NaBr) dilution techniques respectively. Subjects fasted for at least 8 h and voided the bladder before receiving orally a precisely weighed solution made up of D_20, NaBr and drinkable water. A preliminary study aimed at establishing the time needed by D_2O and NaBr to reach the equilibrium was done on the plasma of four unselected DMD patients. Both tracers reached the equilibrium 3.0 h after administration, a time very close to that obtained for the normal subject [13]. Following this finding, blood samples for deuterium and bromide measurements were taken just before the tracer load (background) and 3.5 h later in both DMD and control children. A detailed description of deuterium load and body fluid collection is reported elsewhere [14]. The deuterium load was sufficient to increase the isotopic excess to approximately 5000 δ units over the background. Deuterium enrichment in plasma samples was measured by Infrared Spectrophotometry according to the method of Lukaski and Johnson [13]. Bromide concentration in plasma was measured by High Performance Liquid Chromatography according to the method of Wang *et al.* [15] with minor modifications [4]. The final concentration in plasma was below one-tenth of the value regarded as toxic (6 mM) [16].

Anthropometry

On the same day that D_2O and NaBr were administered, body weight was recorded to the nearest 0.1 kg on a beam scale for controls and on a beam chair scale for DMD patients, as described in detail elsewhere [17]. Where possible, body height was recorded to the nearest 0.1 cm on a stadiometer with a standing subject. In the more disabled patients, body height was measured with the patient lying supine. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Relative weight (RW) was calculated as weight × 100/ideal weight, the latter corresponding to the 50th percentile of weight for age according to NCHS growth-charts [18]. Skinfolds (triceps, subscapular, suprailiac, abdominal) and circumferences (arm, waist, hip) were measured following the Anthropometric Standardization Reference Manual [17].

MFBIA

The determination of Z was made with a tetrapolar impedance plethysmograph (Human IM Scan, Dietosystem, Milan, Italy) at frequencies of 5, 50 and 100 kHz as described by Segal [8]. The intensity of the a.c. was set to 800 μA. TBW was calculated from ZI at 50 kHz (ZI_{50}) by applying the formula of Davies *et al.* [11]:

$$TBW = 0.6 \times ZI_{50} - 0.05$$

Formulae were developed on our control group for predicting TBW and ECW from ZI at 100 (ZI_{100}) and 5 (ZI_5) kHz, respectively. Davies' and control-generated equations were applied to the DMD patients to ascertain if formulae developed on healthy subjects would provide reliable results in DMD.

Statistics

Statistical analysis (means, standard deviations, ANOVA, least squares linear regressions) was performed on a Macintosh computer using the Statview 4.01 software. Statistical significance was set to a $P < 0.05$. Results are expressed as mean ± S.D.

RESULTS

Table 1 presents the values of weight, height, BMI, RW, circumferences, skinfolds, TBW standardized per kg of weight (TBW%), ECW standardized per l of TBW (ECW%), intracellular water (ICW) standardized per l of TBW (ICW%) and ECW : ICW ratio in DMD and control subjects.

Weight, height, BMI, RW, skinfolds and circumferences do not differ in DMD and control subjects. TBW% and ICW% are lower ($P < 0.01$ and $P < 0.05$, respectively) and ECW% higher ($P < 0.05$) in DMD patients than in controls. It follows that the ECW : ICW ratio is higher in DMD patients than in controls ($P < 0.001$).

Table 2 presents the values of TBW and ECW obtained by dilution and by MFBIA in DMD and control subjects.

TBW predicted from Davies' formula is significantly lower than TBW obtained by D_2O dilution in both control and DMD subjects. Formulae generated from MFBIA on control subjects ($TBW = 7.249 + 0.452 \times ZI_{100}$, $r = 0.884$, $P < 0.0001$; $ECW = 1.052 + 0.280 \times ZI_5$, $r = 0.704$, $P < 0.001$) significantly underscored TBW and ECW in DMD patients (Table 2). The reason for the biased prediction can be appreciated from Figs 1 and 2 which show ZI_{100} and ZI_5 as a function of TBW and ECW respectively in control and DMD children. It can be seen that DMD patients have, on the mean, higher values of TBW and ECW for the same value of ZI of control subjects.

Population-specific formulae were developed for DMD children. As is to be expected for an homogeneous population such as that employed in the present study [19], body weight explained much of the variance of TBW (86%) and ECW (90%) in DMD subjects. However, when ZI was included with body weight among

Table 1. Characteristics of DMD and control subjects

	DMD	Controls
Weight (kg)	46.4 ± 18.6	43.5 ± 11.3
Height (cm)	148.1 ± 17.6	146.9 ± 12.7
BMI (kg/cm²)	19.0 ± 4.3	19.9 ± 3.4
RW (%)	110.8 ± 31.9	109.6 ± 31.7
AC (cm)	22.7 + 5.5	22.0 ± 2.7
WC (cm)	77.2 ± 16.5	68.6 ± 8.5
HC (cm)	85.4 ± 15.2	76.8 ± 15.7
TSF (mm)	19.5 ± 9.1	16.9 ± 8.6
SSF (mm)	16.7 ± 10.6	12.8 ± 9.3
SISF (mm)	17.7 ± 11.1	16.7 ± 12.4
ASF (mm)	20.0 ± 11.9	17.0 ± 11.4
TBW%	51.8 ± 2.8†	58.5 ± 5.9
ECW%	52.9 ± 5.6*	40.3 ± 6.4
ICW%	47.1 ± 5.6*	59.7 ± 6.4
ECW:ICW	1.15 ± 0.25††	0.70 ± 0.23

*$P < 0.05$, †$P < 0.01$ and ††$P < 0.001$ vs controls

Age, weight, height, body mass index (BMI), relative weight (RW), arm circumference (AC), waist circumference (WC), hip circumference (HC), triceps skinfold (TSF), subscapular skinfold (SSF), suprailiac skinfold (SISF), abdominal skinfold (ASF), total body water standardized per kg of body weight (TBW%), extracellular water standardized per litre of total body water (ECW%), intracellular water standardized per litre of TBW (ICW%) and extra- to intra-cellular water ratio (ECW:ICW) in children affected by Duchenne muscular dystrophy (DMD) and in healthy controls.

Table 2. Total body water (TBW) and extracellular water (ECW) of DMD and control subjects

	DMD	Controls
TBW by D_2O (I)	23.8 ± 9.1	25.0 ± 5.1
TBW by Davies (I)	14.2 ± 4.1†	21.3 ± 5.3*
TBW by ZI_{100} (I)	18.6 ± 3.3*	25.0 ± 4.5
ECW by NaBr (I)	12.8 ± 5.8	10.2 ± 3.2
ECW by ZI_5 (I)	7.6 ± 1.8*	10.2 ± 2.2

TBW was measured by deuterium oxide dilution (D_2O) and predicted by Davies formula (11) and by the impedance index at 100 kHz by means of a formula generated on control subjects. Extracellular water (ECW) was measured by sodium bromide dilution (NaBr) and predicted from the impedance index at 5 kHz by means of a formula generated on control subjects.

*$P < 0.05$ and †$P < 0.001$ vs the reference value by deuterium oxide (D_2O) or sodium bromide (NaBr) dilution

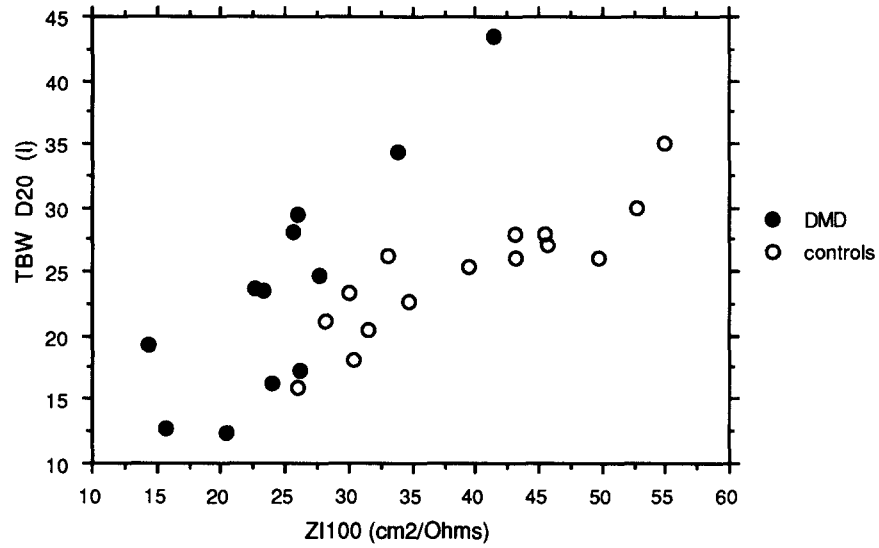

Fig. 1. The relationship between the impedance index at 100 kHz (ZI_{100}) and TBW in DMD and control children.

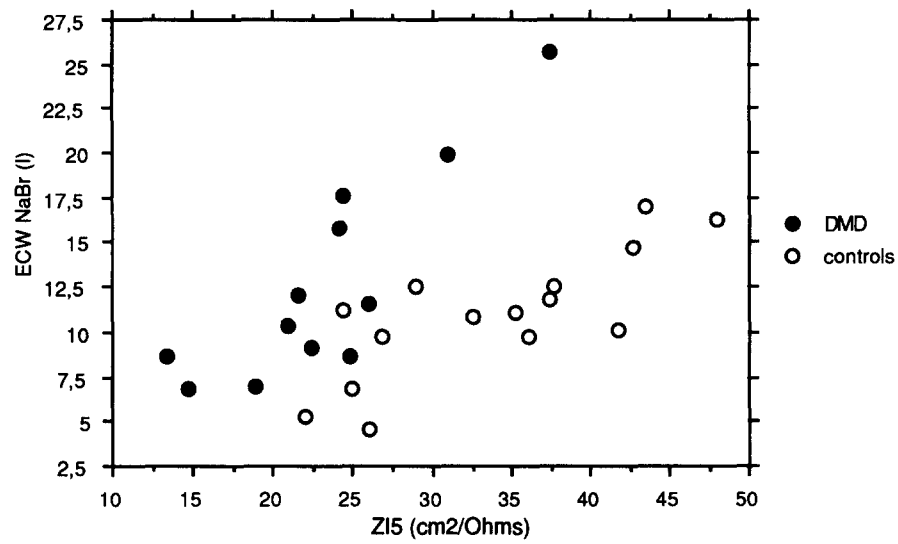

Fig. 2. The relationship between the impedance index at 5 kHz (ZI_5) and extracellular water (ECW) in DMD and control children.

the predictor variables, an increase in the explained variance was seen (99% for TBW and 92% for ECW). Moreover, the SEE decreased from 1.1 to 1.0 l for TBW and from 1.9 to 1.6 for ECW with respect to the predictive algorithm including body weight alone. The generated equations are the following:

$$\text{TBW (l)} = 0.026 + 4.54 \times \text{weight (kg)} + 0.109 \times ZI_{100}\ (\text{cm}^2/\Omega)$$
$$(r = 0.995,\ P < 0.0001,\ \text{SEE} = 1.0\ \text{i}),$$

$$\text{ECW (l)} = -3.308 + 0.233 \times \text{weight (kg)} + .225 \times ZI_5\ (\text{cm}^2/\Omega)$$
$$(r = 0.960,\ P < 0.0001,\ \text{SEE} = 1.6\ \text{l}).$$

DISCUSSION

The present study shows a lowered TBW% in patients affected by DMD in comparison to control subjects, confirming the results of Edmonds *et al.* [12]. These authors have hypothesized that the decreased TBW% of their patients may be the result of an excess of fat mass (FM). However, they have calculated FM as difference between weight and lean body mass (LBM), the latter being obtained from TBW by assuming a LBM hydration (LBMH) equal to 73%. Since LBMH is likely to be reduced in DMD, this calculation, as the authors point out, will overestimate to some

extent the fat mass [12]. Unfortunately, there is no simple and indirect method available to assess body fatness of DMD patients, since, as emphasized by Willig *et al.* [20], formulas for calculating limb areas and FM which have been developed on normal subjects, will not work properly in DMD children, at least for the progressive fibrous and fat infiltration of muscle mass which characterizes the disease. However, as suggested by Willig *et al.*, skinfold thickness may be used to assess the level of adiposity of DMD children [20]. In this study, BMI, RW and triceps skinfold, which are adiposity indexes widely employed in paediatrics [21, 22], do not differ between controls and patients. Furthermore, no other measured skinfold or circumference differs between control and DMD children thus suggesting a similar adiposity level. It, therefore, seems reasonable that the decreased TBW% of our DMD patients may result from a reduction of LBM, highly hydrated, rather than an expansion of FM.

The present study also demonstrates a significant increase of the ECW : ICW ratio in DMD in comparison to healthy children. To our best knowledge, no other data have been published on the distribution of TBW into the extra- and intracellular space in DMD patients. However, on the basis of an increased ratio of exchangeable sodium to total body potassium, Edmonds *et al.* have suggested that an expansion of extracellular space occurs in DMD patients [12]. Our data, obtained by means of the reference method of bromide dilution, confirm the hypothesis of these authors and offer additional information about the cell hydration in DMD. In fact, not only is the ECW% of our DMD patients significantly increased but also their ICW% is significantly decreased with respect to control subjects. It appears therefore that TBW% of DMD patients may be reduced due to both an in increase in ECW% and a decrease in ICW%. Cell dehydration of DMD patients needs to be further investigated to assess its role in the natural history of DMD and the possibility of therapeutic intervention. A longitudinal study might enhance our understanding of the pathophysiological mechanisms underlying the abnormal ECW : ICW ratio in DMD.

With reference to the utilization of MFBIA for predicting the hydration status of DMD patients, our study suggests that TBW and ECW of these subjects may be adequately predicted using population-specific algorithms. Thus, due to its safety, low cost and high portability, MFBIA appears to be an expedient and reliable technique for predicting body water compartments of DMD children. Further studies and a cross-validation are needed to confirm this hypothesis. Finally, it is noteworthy that anthropometric parameters are not necessarily effective for assessing nutritional status of DMD patients because, even when apparently normal, they can mask a body composition radically different from normal, which is exemplified in the present study by an altered body water distribution. Our data suggest that assumptions regarding the chemical composition of the standard human body may not be valid in DMD children. As a consequence, body composition models based on standard values and body composition methodologies developed for normal populations would require adjustment for use in subjects affected by DMD.

Acknowledgements—This work was supported by TELETHON 1994 grants (Project Number 250).

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