Body composition in adults with cerebral palsy by dual-energy X-ray absorptiometry, bioelectrical impedance analysis, and skinfold anthropometry compared with the \(^{18}\)O isotope-dilution technique\(^1\)\(^-\)\(^3\)

Heidi G Hildreth, Rachel K Johnson, Michael I Goran, and Stephen H Contompasis

ABSTRACT The aim of this study was to determine in adults with cerebral palsy the accuracy and practicality of standard methods used to estimate body composition. The sample consisted of 20 adults (13 men and 7 women) aged 20–55 y with various degrees of cerebral palsy. Percentage body fat was estimated from skinfold thickness, bioelectrical impedance analysis (BIA), and dual-energy X-ray absorptiometry (DXA), and compared with the reference measure of percentage body fat from total body water by \(^{18}\)O dilution. Values derived from use of BIA and skinfold thickness, estimated by using the Jackson-Pollock equation, were significantly different from those derived with use of \(^{18}\)O dilution (\(P < 0.001\) and \(P < 0.001\), respectively). There was no significant difference between percentage body fat measured with DXA and that measured with \(^{18}\)O. There was favorable agreement between DXA and \(^{18}\)O (mean difference: 0.06 ± 9.6%), but not between skinfold thickness (mean difference: 6.33 ± 12.3%) or BIA (mean difference: –6.55 ± 13.6%) and \(^{18}\)O. Although DXA was the best measure for predicting percentage body fat in the sample, its high cost prohibits its use as a practical method. The best-fitting regression equation specific for this sample by using anthropometric measures to predict percentage body fat was as follows: 8.76 – (7.34 \(\times\) sex) + (0.32 \(\times\) weight) + (0.38 \(\times\) biceps skinfold) (\(R^2 = 0.84\), \(P < 0.001\), SEE = 4.85). This equation needs to be cross-validated in an independent sample of adults with cerebral palsy.


KEY WORDS Body composition, body fat, total body water, dual-energy X-ray absorptiometry, bioelectrical impedance analysis, isotope dilution, skinfold anthropometry, cerebral palsy, adults

INTRODUCTION

Adults with cerebral palsy (CP) exhibit a broad range of leanness and fatness (1), suggesting that the nutrition problems noted in children with CP (2–6) persist with age. CP is the most common physical disability in humans and most persons with the disease survive to adulthood (7, 8). Therefore, it is essential for clinicians to monitor the nutritional status of adults with CP to provide appropriate nutrition intervention aimed at preventing the occurrence of over- and undernutrition. Measurement of body composition provides an overall assessment of the body’s energy stores and, in turn, nutritional status. Because of the lack of established prediction formulas for adults with CP, body-composition estimates thus far have been derived from equations developed for normal, able-bodied adults without disabilities (1, 9, 10). The accuracy of this approach is not known.

Stable isotopes of water offer the most accurate and convenient measure of total body water (TBW) and consequently body composition in a research setting (11). The neuromuscular complications associated with CP, such as joint contractures and involuntary movements, make accurate height measurements difficult to obtain and inhibit the subject from remaining motionless during measurements of body composition. Additionally, neuromuscular deficits may increase the deposition of fat over affected extremities (12), thereby causing asymmetrical body composition. Isotope dilution measures the water compartment of the whole body rather than a single area assumed to mimic the composition of the whole body. Thus, the use of a stable isotope to measure body composition is ideal for people with CP because it is noninvasive, does not require the subject to remain still for the measurement, and is independent of height and body symmetry. However, the prohibitive cost of the isotopes and the need for a mass spectrometry facility and highly trained technicians make this method impractical for routine clinical use. It is more appropriate for validating other less expensive, more practical techniques for the assessment of body composition in a research setting.

Bandini et al (6) examined the relation between anthropometric indexes and body fatness estimated from TBW through use of the \(^{18}\)O dilution method in adolescent females with CP.
These associations suggested that anthropometric indexes can be useful markers of body composition in persons with CP. In addition, Bandini et al’s work highlighted the need for developing specific regression equations that can easily assess body composition in persons with CP.

The principal aim of this study was to determine the accuracy and practicality of skinfold anthropometric measurements, dual-energy X-ray absorptiometry (DXA), and bioelectrical impedance analysis (BIA) in determining body composition in adults with CP by comparing these measures with body-composition measurements obtained from TBW as estimated by $^{18}$O dilution. The second objective was to develop a simple anthropometric equation to predict body composition in adults with CP by using $^{18}$O as a reference method.

**SUBJECTS AND METHODS**

**Subjects**

The sample consisted of 20 adults with CP (13 men and 7 women) ranging in age from 20 to 55 y. Subjects were recruited from community organizations serving persons with CP and through newspaper advertisements. All of the subjects or their guardians gave their informed written consent before participating in the study. The protocol was approved by the Committee on Human Research at the University of Vermont.

A physician (SHC) specializing in developmental disabilities conducted a neurodevelopmental exam on each subject to determine the extent of CP involvement. Subjects were classified according to type of motor impairment (spastic, athetoid, or mixed), degree of paralysis (diplegia, hemiplegia, triplegia, or quadriplegia), and ambulation status (ambulatory or nonambulatory). Oral motor impairment was assessed with a questionnaire adapted from Stallings et al (3) that determined the presence or absence of feeding problems (tongue thrust, fluid or food loss during eating, coughing or gagging during mealtime, excessive time required for meals, or difficulty in consuming textured foods).

**Anthropometry**

Body weight was measured to the nearest 0.1 kg with a calibrated digital scale (Scale-Tronix, Wheaton, IL) with subjects barefoot and in light clothing. Nonambulatory subjects were weighed in a digital sling scale (Scale-Tronix). Body height was recorded to the nearest 0.5 cm with a stadiometer (Scale-Tronix). For those subjects who were unable to stand, segmental height was recorded with a tape measure.

Skinfold-thickness measurements were taken from nine sites (triceps, biceps, subscapula, axilla, chest, abdomen, suprailium, thigh, and calf) with Lange calipers (Cambridge Scientific Instruments, Cambridge, MA) according to Lohman et al (13). All measurements were made in triplicate to the nearest 0.5 mm by a single investigator (HGH) and the mean of the measurements was used for analysis. If the subject had bilateral involvement, skinfold-thickness measurements were taken on the least affected side. The Jackson-Pollock (10) sex- and age-specific equations were used to estimate fat-free mass and percentage body fat. We reported a precision of $r = 0.87$ for the estimation of fat-free mass with use of skinfold-thickness measurements compared with DXA in normal, healthy children (14).

**Bioelectrical impedance analysis**

Whole-body resistance or impedance was measured with a bioelectrical impedance analyzer (101A; RJL Systems, Detroit) with subjects in a supine position and their limbs away from their trunk. Four surface electrodes were placed on the right side of the body as described by Lukaski et al (15). TBW was calculated from the equation of Kushner and Schoeller (16). We reported a precision of $r = 0.94$ for the estimation of fat-free mass with use of BIA compared with $^{18}$O in normal, healthy children (17). However, the inclusion of weight in the BIA predictive equation may reduce its accuracy in determining change in lean body mass (18).

**Dual-energy X-ray absorptiometry**

Body-composition measurements were made with Lunar DPX-L total-body scan computer software (version 1.3Y; Lunar Co, Madison, WI) while subjects were in a supine position as described by Mazess et al (19). Scan modes were chosen from the measure of anteroposterior tissue thickness as defined by the Lunar DPX manual (20). This method is based on the same principle as that of dual-photon absorptiometry, which has been described elsewhere (21–23), with two adjustments. First, the radioactive source is replaced with an X-ray beam tube behind a k-edge filter that converts the X-ray beam into two main energy peaks. Additionally, the attenuation of soft tissue is now measured, rather than assumed to be constant, which provides the simultaneous measurement of bone mineral, fat, and fat-free tissue (24). We reported a precision of $r = 0.98$ for the estimation of fat-free mass with use of DXA compared with pig carcass analysis (25). The measurements of BIA and anthropometric indexes were made after the DXA scan between 0900 and 1000.

**$^{18}$O dilution**

TBW was measured by $^{18}$O ($H_2^{18}O$) dilution as described by Schoeller et al (11) with each subject receiving an oral dose of 0.15 g $H_2^{18}O$ per kg body wt (Cambridge Isotope Laboratories, Woburn, MA). The bottle was rinsed once with tap water, which was also consumed. For those individuals with severe oral-motor impairment, a sipping cup and lid were used in place of the normal dosing container to maximize consumption of the isotope. To account for any fluid loss, a preswallowed towel was placed around the bottle containing the dose. The towel was then reweighed to determine the amount of spillage, if any.

A baseline urine sample was collected before administration of the oral dose. After subjects had fasted overnight from 1800, two additional urine samples were collected the following morning and 10 d later. This time between the administration of the dose and the collection of the urine samples allowed for the isotope to become equilibrated with TBW. $^{18}$O in the water was measured in triplicate with an isotope-ratio mass spectrometer (model Sira2; VG, Cheshire, United Kingdom) at the Biomedical Mass Spectrometry Facility at the University of Vermont. TBW was calculated as the $^{18}$O dilution space di-
vided by 1.01, correcting for exchange of the $^{18}$O label with nonaqueous oxygen of body solids (11). Fat-free mass was derived from TBW with the assumption that fat-free mass is 73.2% hydrated (26).

**Statistical analysis**

Independent $t$ tests and Student’s paired $t$ tests were used to test for differences between means. The strength of the relation between variables was examined by simple regression. Agreement between the estimates of body composition (skinfold thickness, BIA, and DXA) and TBW by $^{18}$O dilution was assessed with the method of Bland and Altman (27). With this method, a pair-wise comparison is used to show the relative bias (mean difference) and the limits of agreement (mean difference ± 2 SD of the difference) between the estimates of body composition and the reference measure (TBW by $^{18}$O dilution) by plotting their mean difference against the mean of the two methods. Bivariate analyses were performed by using QUATTRO PRO FOR WINDOWS, version 6.0 (Borland, Scotts Valley, CA). Stepwise-multiple-correlation-regression analysis was used to determine the best predictors of percentage fat from $^{18}$O in the sample. Multivariate analyses were performed by using BMDP statistical software (BMDP Inc, Los Angeles). The level of significance for all analyses was specified at $P < 0.05$.

**RESULTS**

**Subject characteristics**

The physical characteristics of the study group are summarized in Table 1. The male and female subjects were comparable in age, weight, and height. The women had significantly higher BMIs (in kg/m$^2$) than the men (27.9 compared with 24.1, respectively). The extent of the manifestations of CP within the study group is presented in Table 2. Most of the group had spastic CP (75%), had diplegia (40%), were ambulatory (55%), and had no feeding difficulties (75%). The distribution of percentage body fat in the men and women as estimated from $^{18}$O is shown in Figure 1. On average, the women had higher percentages of body fat as estimated by $^{18}$O (38.1 ± 11.8%) than the men (25.2 ± 8.2%) ($P = 0.001$).

**Bivariate analysis**

On average, percentage body fat estimated by BIA (36.3 ± 11.4%) was significantly higher and that estimated by skinfold thickness (23.4 ± 12.2%) was significantly lower than percentage body fat estimated by $^{18}$O (29.8 ± 11.2%) ($P < 0.001$). Percentage body fat estimated by DXA (29.7 ± 13.9%) was not significantly different from that estimated by $^{18}$O. The strength of the relation as determined by simple regression was strongest between percentage body fat by $^{18}$O and that by DXA ($r^2 = 0.90$, $P < 0.001$), followed by skinfold thickness ($r^2 = 0.75$, $P < 0.001$), and BIA ($r^2 = 0.67$, $P < 0.001$). The level of agreement between percentage body fat by $^{18}$O and percentage body fat by DXA was favorable (Figure 2), with a relative bias of 0.06%. The 95% CI for the bias was 2.16%, -2.05%, and the limits of agreement ranged from 9.67% to -9.56%. DXA appeared to be negatively biased at the high end of body fatness (percentage body fat > 50%) (Figure 2). This observation was supported by the significant negative correlation between the mean of percentage body fat by $^{18}$O and by DXA and the difference in percentage body fat by $^{18}$O and by DXA ($r = -0.63, P = 0.002$).

The mean difference between percentage body fat estimated by $^{18}$O and that estimated by BIA was -6.55%, indicating that BIA was biased toward overestimating percentage fat (Figure 3). The limits of agreement ranged from 7.06% to -20.15%. Another bias can be applied to the measurement of percentage body fat from skinfold thickness, which underestimated percentage body fat by $^{18}$O with a mean difference of 6.33% and limits of agreement ranging from 18.62% to -5.97% (Figure 4).

**Multivariate analysis**

Stepwise-multiple-correlation-regression analysis was used to determine the strongest predictors of percentage body fat by $^{18}$O in the sample. Percentage body fat from BIA, skinfold thickness, and DXA, as well as height, weight, sex, age, degree of paralysis, ambulation status, and oral motor impairment were entered as independent variables in the analysis. The only variable selected by the model was percentage body fat from DXA, which explained 90% of the variation in percentage body fat estimated by $^{18}$O. Forcing in the other variables did not significantly add to the amount of variation explained in percentage body fat by $^{18}$O. Because measurement of percentage body fat by DXA may be impractical in nonresearch settings, a second analysis was performed omitting DXA and entering the individual skinfold measures as potential independent variables in addition to age, sex, weight, and height. The first variable selected by this model was biceps skinfold thickness ($r^2 = 0.78$), followed by weight ($R^2 = 0.80$), and sex ($R^2 = 0.84$), generating the following equation:

**Percentage body fat**

$$8.76 - (7.34 \times \text{sex}) + (0.32 \times \text{weight})$$

$$+ (0.38 \times \text{biceps skinfold thickness})$$

where sex is coded as 0 = female and 1 = male, weight is in kg, and biceps skinfold thickness is in mm ($R^2 = 0.84, P < 0.001, \text{SEE} = 4.85$). When this multiple-regression equation was applied to the present sample, it increased the predictive power of skinfold thicknesses for estimating percentage body fat ($R^2 = 0.84$) compared with the original Jackson-Pollock equation ($r^2 = 0.75$).

**DISCUSSION**

This study evaluated the accuracy and practicality of existing techniques of measuring body composition in adults with CP.

**TABLE 1**

Characteristics of adults with cerebral palsy

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 13)</th>
<th>Women (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>34.5 ± 10.9</td>
<td>37.4 ± 9.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.1 ± 8.5</td>
<td>152.1 ± 4.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.3 ± 13.7</td>
<td>64.1 ± 18.6</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.1 ± 4.6</td>
<td>27.9 ± 8.6</td>
</tr>
</tbody>
</table>

$^1$ ± SD; n = 20.  

$^2$ Significantly different from men, $P < 0.05$ (independent $t$ test).
The major findings were as follows: 1) BIA overestimated percentage body fat and the limits of agreement were wide, making it unacceptable for clinical use; 2) the Jackson-Pollock skinfold prediction equation underestimated percentage body fat and the limits of agreement were wide, making it also unacceptable for clinical use; 3) DXA had no bias and good agreement with percentage body fat from $^{18}$O, making it an accurate measure of body composition; and 4) biceps skinfold, weight, and sex were the best, easily measured predictors of percentage body fat in this sample.

### Isotope dilution as a reference measure of body composition

Because there is no true measure of body composition unless death occurs, a criterion method must be used to determine the accuracy of other easily applied measures. In this sample, for the reasons discussed in the Introduction, isotope dilution was chosen as the criterion measure of body composition. One possible limitation of this method within this sample may have been the presence of a compromised hydration status, which would interfere with the ratio of extracellular water to TBW. If this were true, the hydration of fat-free mass would deviate from the assumed value of 73.2% (26). However, there is no reason to believe that the hydration status of this population should differ from a normal, able-bodied population except in the most extreme cases of leanness and obesity, in which the ratio of extracellular water to TBW may be increased.

The sample population was also administered a stable isotope of deuterium as part of another study examining energy requirements in adults with CP by using the doubly labeled water technique (28). Hence, TBW was measured with both the $^{18}$O and deuterium isotope-dilution techniques. $^{18}$O was chosen as the reference method in the present study over deuterium because of its accuracy and precision in determining TBW (11). The proposed reasoning for this superiority is that less $^{18}$O than deuterium exchanges with nonaqueous organic compounds. The dilution space for $^{18}$O is proposed to be $\approx$1% greater than TBW, whereas deuterium has a dilution space that is up to 5% greater (29). In our study, calculations with use of the data from deuterium analysis overestimated TBW in the extreme cases of leanness even after nonaqueous exchange of hydrogen was corrected for. This generated values of fat-free mass that were greater than body weight, consequently yielding negative values for percentage body fat. This outcome was also observed in Berg and Isaksson’s (30) study on children with CP and could indicate an analytic error of the type described by Ritz et al (31). Using $^{18}$O as a measure of body composition in adolescents with CP, Bandini et al (6) obtained reliable calculations for TBW indicative of malnutrition. Despite the increased cost of $^{18}$O isotope dilution compared with deuterium, the accuracy and precision of $^{18}$O made this method the superior research technique.

### Bioelectrical impedance analysis

The inability of BIA to accurately predict percentage body fat in the sample may be related to several factors. The main principle underlying the BIA method, that the impedance of a geometrical system (ie, the human body) is dependent on the length of the conductor and its configuration (32), raises the biggest question when considering the use of this method in this population because these components cannot be altered. It is extremely difficult to obtain an accurate height in individuals with CP because of their muscle contractures. Height (length of the conductor) is an important variable in the statistical relation of BIA to percentage body fat. An over- or underestimation of height by 2.5 cm can result in a 1.0-L error in the estimation of TBW (32), producing a small error in the estimation of percentage body fat (<5%). The second major problem is the assumption of a symmetrical configuration of the human body (32). The body asymmetry observed in this population renders this assumption of a constant configuration invalid.

While this study was being conducted, a statement was released from the National Institutes of Health regarding the

### Table 2

Number of patients in the sample with a given level of cerebral palsy involvement

<table>
<thead>
<tr>
<th>Motor involvement</th>
<th>Hemiplegia</th>
<th>Diplegia</th>
<th>Triplegia</th>
<th>Quadriplegia</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spastic</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Athetoid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Mixed</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Ambulatory</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>Nonambulatory</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>45</td>
</tr>
</tbody>
</table>

$i = 20$. 

![FIGURE 1. Distribution of percentage body fat as estimated by $^{18}$O isotope dilution in adult men (♂, $n = 13$) and women (♀, $n = 7$) with cerebral palsy.](image-url)
standardization and control of numerous variables for the accurate and reliable assessment of body composition by BIA (32). The impedance value is believed to be affected by the amount of time that the subject is recumbent. The longer the length of time that the subject remains in the supine position, the higher the impedance measure. This measure is dramatically increased within the first 10 min of a subject assuming the supine position and continues gradually thereafter (32). All of our subjects were measured after a DXA scan, which lasted 20–50 min depending on the size of the individual. In relation to body positioning, some obese subjects were unable to separate their legs completely, which may have interfered with the conductance of the electrical current through the body.

The remaining variables that affect the reliability and accuracy of BIA were unknowingly controlled, but may not be as easy to apply in a clinical setting. Hydration status and postprandial measurement were controlled to the best of our ability by requiring the subjects to remain in the Clinical Research Center overnight, thereby prohibiting any excessive activity and allowing for the consumption of only water after administration of the isotope. Any disturbances in intracellular hydration status associated with the condition of CP could obviously not be controlled, nor could changes in hydration status related to menstruation for the female subjects. When controlling for all these variables, a clinician can no longer consider BIA an easily applied, practical measure.

**Skinfold anthropometry**

Indirect measures of body composition such as BIA and skinfold thicknesses have to rely on the statistical association...
of the method with percentage body fat derived from a reference population rather than on a biophysical model. For this reason, these methods are usually not effective for a population that differs substantially from the original reference population. Although the Jackson-Pollock equation (10), used to predict percentage fat from skinfold thicknesses, was validated with the well-established method of hydrostatic weighing, it was derived from a healthy adult population. The suppressed correlation coefficient \( r^2 = 0.75 \) and poor agreement with the reference measure of percentage body fat from \(^{18}\text{O}\) confirm the inability of the Jackson-Pollock equation to accurately predict percentage body fat in this sample. In this population, suprailiac, abdomen, midthigh, and subcapular skinfold thicknesses, required for the estimation of percentage body fat from the Jackson-Pollock equation, were difficult to obtain in the wheelchair-bound subjects. This could have been a potential source of error in estimating percentage body fat in this sample with use of the Jackson-Pollock equation.

**Dual-energy X-ray absorptiometry**

Of all the body-composition techniques examined, DXA was by far the best method for predicting percentage body fat in comparison with TBW by \(^{18}\text{O}\). The high correlation with percentage fat from \(^{18}\text{O}\) \( r^2 = 0.90, P < .001 \), supported by the strong agreement as determined by the method of Bland and Altman (27), made this an acceptable method to substitute for \(^{18}\text{O}\) isotope dilution. However, there are several limitations to consider before this method is advocated for routine use in adults with CP.

DXA was more likely to be negatively biased at the high end of percentage body fatness (percentage body fat > 50%) in this sample. However, this observation was based on measures from only three individuals and should be replicated in a larger sample.

Besides the burden placed on the subject from attaining a certain position within the dimensions of the scan table or remaining motionless during the 20-50-min scan, the clinical feasibility of this technique is also questionable. The expense of both the instrument and the actual scan is a limiting factor in the accessibility of DXA in a clinical setting. If a DXA machine is readily available for clinical use, its use is still limited by the requirement of a licensed radiologic technician to administer the scan. Finally, the amount of time required for the DXA scan and interpretation of the results could be anywhere from 0.5 to 2 h. On the basis of these limiting factors, DXA was rejected as a practical, easily applied measure of body composition in adults with CP.

**Prediction equation**

In clinical practice, anthropometric data are more practical for the assessment of body composition. The ease of administration and lack of highly sophisticated equipment required make anthropometric measurements ideal in terms of convenience and cost. Skinfold anthropometry has been criticized because of its high susceptibility to interobserver error. However, this can be controlled by having a properly trained individual perform the measurements. A precision within 5% can be achieved in this manner (33). Because skinfold-thickness measurements correlated strongly with percentage body fat as determined by \(^{18}\text{O}\) in the Bandini et al (6) study, individual measures of skinfold thicknesses were entered into a stepwise-multiple-regression model to determine whether the predictive power of skinfold thicknesses could be increased by generating a regression equation specific to adults with CP. A strong correlation, combined with a lack of bias and favorable limits of agreement, was found between percentage fat as estimated by \(^{18}\text{O}\) and three simple measures (biceps skinfold thickness, sex, and weight). The inclusion of sex in the equation adjusted for any variation in the body fat estimate that would be attributable to sex differences in the sample. However, because of the study’s small sample size, it is vital that this new equation be cross-validated to determine its ability to predict percentage body fat in adults with CP for whom the formula was not derived.

In summary, several body-composition techniques were compared with \(^{18}\text{O}\) as a reference standard in adults with CP.
Neither BIA nor predictive equations for skinfold thickness generated from normal, able-bodied adults accurately determined percentage body fat in the sample. DXA was an accurate and precise method but is not practical for routine clinical use. A new prediction equation including biceps skinfold thickness, sex, and weight was developed that explained 84% of the individual variation in percentage body fat, thus providing some direction for the development of new equations for predicting body composition in adults with CP. With an accurate indicator of body composition, clinicians can monitor changes in nutritional status and evaluate the effectiveness of nutrition intervention to improve quality of life for persons with CP.

We thank the nursing staff of the Clinical Research Center, John Hiser and Kathleen Ollwell from the Physiology Laboratory of the Soms Obesity and Nutrition Research Center, Dave Ebenstein and Bruce O’Rourke from the Biomedical Mass Spectrometer Facility at the University of Vermont for their technical expertise, and Diantha Howard for her statistical insight in the development of this manuscript. Most importantly we thank the volunteers, their families, and their caregivers, who participated with enthusiasm in this research project.

REFERENCES