Assessing Body Composition and Energy Expenditure in Children with Severe Neurological Impairment and Intellectual Disability

Rob Rieken

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Meten van de voedingstoestand en het energieverbruik bij kinderen met ernstige meervoudige beperkingen

Proefschrift

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	Prof.dr. D. Tibboel
Overige leden:	Prof.dr. W.F.M. Arts
	Prof.dr. H.A. Moll
	Prof.dr. H.N. Lafeber
Copromotor:	Dr. C. Penning
Paranimfen:	Anita Meulendijks
	Maaike van Os

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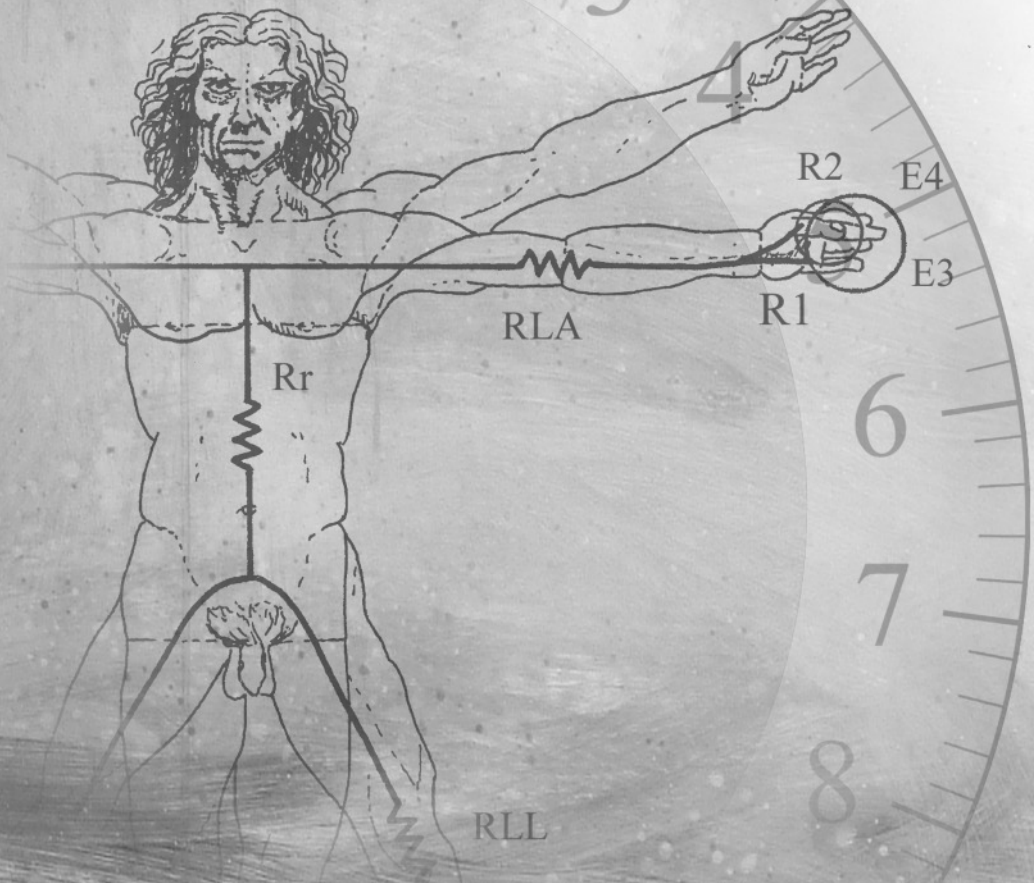
Chapter 1

General Introduction



INTRODUCTION

This thesis describes the measurement of body composition and energy expenditure in children with severe neurological impairment and intellectual disability (ID). These children have many health problems and constitute a very vulnerable paediatric population. While aetiology of the disorder is variable, there is little difference in extent of motor and intellectual disabilities. However, international consensus on the definition of this group of children is lacking. A wide range of terms is being used: for example severe (generalized) cerebral palsy (CP), profound and intellectual multiple disabilities (PIMD) and severe motor and intellectual disabilities (SMID), with subtle differences between these terms. Since none truly encompasses the group studied in this thesis, we will refer to them as children with severe neurological impairment and ID. Generally, these children have moderate to severe intellectual disability and a motor impairment, defined as hypertonic or hypotonic generalized cerebral palsy (CP), or a motor developmental delay to such an extent that the child can at best crawl. The motor impairment corresponds to Gross Motor Function Classification System (GMFCS) levels four and five.[1] The literature on the defined group of interest is scarce. However, the definition we use bears close resemblance to the definition of CP, which is why the theoretical framework in this thesis is mainly based on literature on children with CP.

The exact prevalence of severe neurological impairment and ID in children is unknown. Yeargin Allsop et al.[2] examined the prevalence of CP in 114,897 8-year-olds in three large states in the United States and found a prevalence of 3.6 per 1000 children. In 2000, the estimated number of children with neurological impairment and ID living in the Netherlands was 2000, most of whom live at home.[3] Since CP represents a wide range of physical disability and does not necessarily include an intellectual disability, it is logical to assume that the prevalence of severe neurological impairment and ID is lower than that of CP.

For decades, perinatal asphyxia was believed to be the predominant aetiology of neurological impairment. More recent findings point at multiple antenatal factors as likely causes in both the preterm and term infant, with birth asphyxia playing a minor role. These antenatal factors include prematurity or low birth weight, chorioamnionitis, multiple gestation, complications in pregnancy such as thrombophilias, viral infections, haemorrhage and preeclampsia, and iatrogenic causes. It is now believed that 70% to 80% of all cases of CP are due to antenatal factors; 10% to 28% of all cases, then, are due to birth asphyxia in term and near-term infants.[4-7] The aetiology of CP resembles that of severe neurological impairment and ID except for brain damage later in life as a result of traumatic brain injury, such as posthypoxic encephalopathy following cardiac arrest after resuscitation, infectious diseases such as meningitis

and viral encephalitis in childhood, or as a result of progressive disorders such as metabolic disease with progressive loss of neurological functions.

Children with neurological impairment and ID often show comorbidity, notably those with more severe disabilities. These morbid conditions include epilepsy, sensory impairment, recurrent pulmonary infections[8-11], dysphagia[12-14], gastro-oesophageal reflux disease[15-18], delayed gastric emptying[19, 20], osteoporosis[21] and scoliosis[22].

Children with neurological impairment and ID are also more at risk of developing malnutrition. Malnutrition has grave consequences for health and well-being. It leads to diminished immune function[23] and thus to increased susceptibility to infection, poor growth[24] and higher risk of postoperative complications[25, 26]. Nutritional deficiencies used to be one of the reasons why life expectancy in children with severe neurological impairment could be as low as 11 years.[27] However, recent years have seen greatly improved survival, especially for children with more severe CP. Survival rate increased from 53% before 1995 to 67% based on survival data up to and including 2002. Concomitantly, life expectancy increased by six years. Much of this improvement is due to earlier intervention with gastrostomy when children fail to thrive or fail to gain sufficient weight. This intervention used to be offered as a last resort but has gained more acceptance since animal models showed that it does not seem to promote gastro-oesophageal reflux disease[28, 29]. Acidic reflux can damage the mucosa of the upper gastrointestinal tract, so that swallowing becomes painful and discourages proper intake of food.

In spite of all that, nutritional problems still remain important health issues in these children. Detailed prevalence rates are lacking, however, since recruiting a sufficiently large, representative group of children is difficult and since there is no international consensus on the definition of malnutrition in these children. Still, there is consensus on the magnitude of the problem of undernutrition. In 1993, Stallings et al.[30] in a sample of 154 children with diplegic or hemiplegic CP found that about 30 per cent were undernourished, as documented by body weight or depleted subcutaneous fat stores at the triceps skinfold site. In contrast, and testifying to the wider acceptance of gastrostomy through the years, Sullivan et al.[31] in 2006 found higher fat percentages in 40 children receiving tube feeding and even warned against overfeeding.

In summary, undernutrition and overnutrition feature largely in children with severe neurological impairment and ID, and may present health problems. Through lack of scientific evidence, a guideline for diagnosing nutritional problems in this population is not yet available. The underlying aim of this thesis was to provide a framework for developing such a guideline. Since regular growth curves may not be applicable to these children because of their specific growth pattern[24, 32], other more sophisticated nutritional assessment methods will have to serve to accurately deter-

mine the nutritional state. In this thesis, therefore, nutritional state was evaluated by a dual approach, viz. measuring body composition and energy expenditure. The applied techniques are described below:

MEASURING BODY COMPOSITION

Body composition refers to quantifiable components of the body including fat mass (FM), fat free mass (FFM), total body water (TBW), protein and bone. Body composition reflects the body's energy reserves and is therefore excellently suited to measure nutritional state. Several "gold standard" methods such as the doubly labelled water (DLW) method and dual energy x-ray absorptiometry (DEXA) are considered most accurate.[33] However, these techniques are expensive and are mostly used in research settings to validate more practical, easy to use (field) methods, such as skinfold measurements and bioelectrical impedance analysis.

Skinfold thickness can be measured at several sites of the body (biceps, triceps, subscapular and suprailiacal) using a skinfold calliper. Indicative of subcutaneous fat reserves, skinfold thickness is thought to reflect total body fat. Equations for calculation of body fat are based on the measurement of two[34, 35] or four[36] skinfolds. It is important though that highly trained antropometrists perform these measurements since they are prone to large intraobserver and interobserver variability.[37]

Bioelectrical impedance analysis (BIA) is a technique in which a small electrical current is passed through the body and measured by electrodes on the hand and feet. The body offers two types of resistance (R): capacitive R (reactance) and resistive R. BIA measures a combination of both: impedance.[38] The measurement of body composition is based on the premise that fat mass has low electrical conductivity and high impedance relative to body water. The advantage over measuring skinfold thickness is that BIA measurements are less reliant on the exact position of fat in the body. It is also quick, easy and requires no training. Impedance measured with BIA can be transformed into body composition outcomes, such as body water and lean body mass. A great many equations exist for different age and ethnic groups, and for different clinical conditions with abnormal hydration states, signifying the population specific nature of these equations.[38] Furthermore, accuracy of BIA measurements relies on the adherence to a great number of standardized conditions.[39] For example, the children have to fast for at least four hours before the measurement. Furthermore, the body must be positioned with arms and legs extending from the trunk. Skin temperature should be no warmer than ambient temperature. Children should have urinated prior to the measurement. Although it might be hard to adhere to these ideal conditions

in children with neurological impairment and ID who have contractures, scoliosis and are incontinent, BIA has been found feasible in these children.[40]

Both skinfold thickness measurements and BIA recordings have been applied in children with neurological impairment.[40-43] However, aspects of validity have not been extensively studied. It is important to test validity of these methods in children with neurological impairment since they are of shorter stature[24], are thought to have more intra-abdominal fat than subcutaneous fat[44, 45], and have reduced muscle[46] and bone mass[21] compared with their healthy peers.

ENERGY EXPENDITURE

If a child is suspected of having malnutrition and needs nutritional rehabilitation, it is advisable to estimate energy expenditure. The outcome together with data on food intake may then serve as the basis for a plan to achieve adequate weight gain. A distinction is made between resting energy expenditure (REE) and total energy expenditure (TEE). REE is the energy expenditure necessary to support life and is usually measured after two to four hours of rest. Basal metabolic rate (BMR), a related concept, is measured in much more controlled circumstances: in the morning, 12 hours after the last meal, and in ambient temperature. Although REE and BMR might have slightly different outcome, they are used interchangeably in literature. TEE is the energy expenditure needed to sustain the bodily processes and for one to be active. It is expressed as a multiple of REE, also referred to as physical activity level (PAL).

REE is measured objectively with indirect calorimetry. This is a method by which metabolic rate is estimated from measurements of oxygen (O_2) and carbon dioxide (CO_2).[47] An equation by Weir et al.[48] then yields the expenditure. This equation is based on the assumption that O_2 is oxidized for energy and that all CO_2 evolved from those oxidative processes. The subject therefore must remain calm because extra gas exchange due to restlessness and hypo- or hyperventilation will invalidate the measurement. This might prove challenging in children with neurological impairment who cannot be instructed nor can actively cooperate.

TEE can be measured by the DLW method, in which two stable isotopes of deuterium and oxygen (^2H_2O or $H_2{}^{18}O$) are administered to the child. Both labeled deuterium and oxygen are slowly eliminated from the body water and their enrichments can be measured in urine, saliva or serum. However, in addition to clearance through these routes, labeled oxygen is also exhaled as CO_2 and is therefore eliminated faster than labeled deuterium. The difference in elimination rates represents the amount of exhaled CO_2 (Figure 1). The equations described by Schoeller et al.[49] can then be used to calculate TEE.

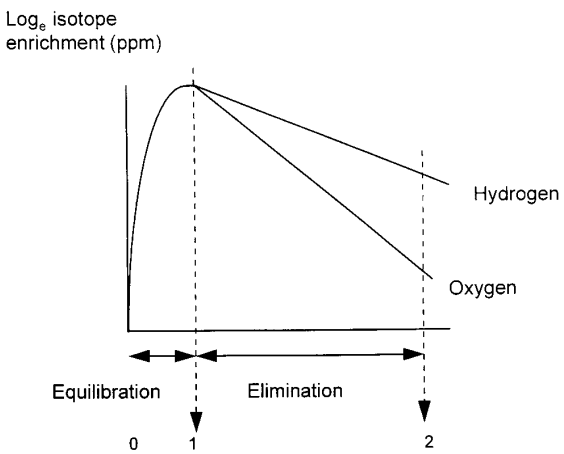


Figure 1: Elimination of oxygen and hydrogen in the DLW method

Both indirect calorimetry and the DLW method have been applied in children with CP to estimate energy expenditure. The largest group studied with indirect calorimetry comprised 61 subjects.[50] The DLW method has been applied in groups ranging from 13 to 32 children.[41, 42, 51] Children with neurological impairment tended to have lower energy expenditure and lower PAL than their healthy peers. Therefore, generic paediatric equations to predict energy expenditure, such as Schofield's[52], seem to have little value in children with severe neurological impairment and ID. Consequently, there are no guidelines on energy intake in these children needed to gain or lose weight.

THIS THESIS

In this thesis we aimed to identify valid instruments for measuring body composition in children with severe neurological impairment and ID. Furthermore, we focussed on measuring energy expenditure and identifying or developing the most reliable, easy to use equation to estimate caloric energy needs. Chapter 2 is a review of the literature on the validity of skinfold measurements and BIA in these children. In chapter 3 the feasibility of the DLW method and comparability of its clinical outcomes using urine and saliva samples was studied. Chapter 4 outlines whether the DLW method is technically sound in these children. In chapter 5, factors that may influence the feasibility of dual energy x-ray absorptiometry (DEXA) and the accuracy of bone density outcome were studied. In chapter 6 the validity was tested of both skinfold

measurements and BIA using established paediatric equations. If an equation proved invalid, as shown by comparing the outcome to that of the DLW method, a new equation was developed. In chapter 7 REE and TEE data were collected. Using variables that are known or hypothesised to influence energy expenditure, a new equation was created. Chapter 8 presents a discussion of the results and limitations of the studies in this thesis. Furthermore, a draft guideline to diagnose undernutrition and overnutrition is presented.

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Chapter 2

Validation of skinfold measurements and bioelectrical impedance analysis in children with severe cerebral palsy: a review

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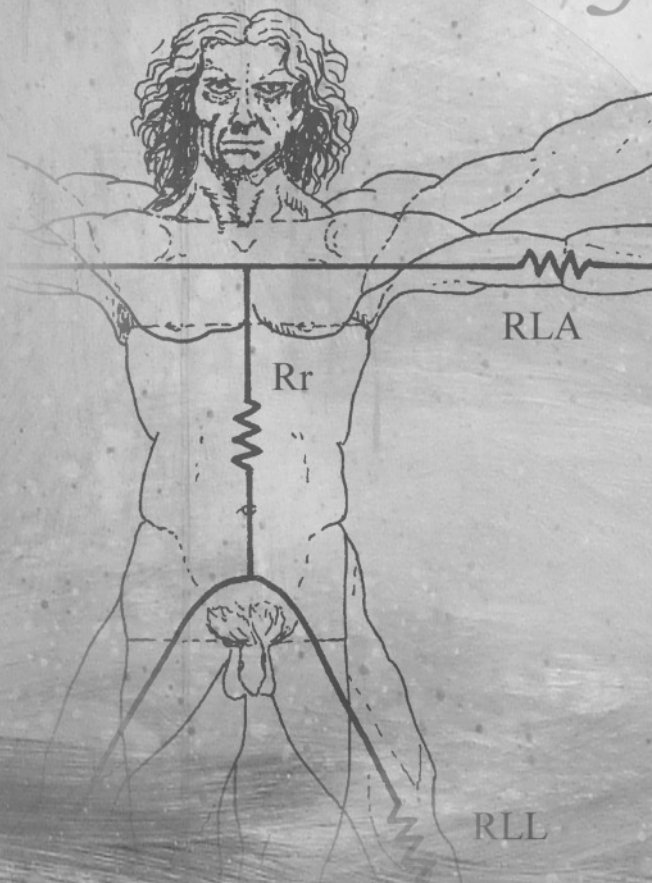

ABSTRACT

Background & Aims Accurately measuring nutritional status in children with severe cerebral palsy (CP) is a challenge. This review seeks to assess the validity of skinfold measurements and bioelectrical impedance analysis (BIA) for measuring body composition in children with severe CP.

Methods We executed a literature search on the validation of both methods in children with severe CP. To be eligible for inclusion, a study had to report on a statistical comparison between these two methods and any method of reference. The QUADAS tool was used for quality assessment.

Results The search strategy resulted in 1549 studies of which 5 studies eventually met the inclusion criteria. When comparing body composition outcomes of skinfold measurements to a reference method, correlation coefficients were found ranging from 0.406 to 0.988. Correlation coefficients between body composition data of BIA and a reference method ranged from 0.515 and 0.95.

Conclusions Although a number of authors found favorable agreement between skinfold measurements and BIA in comparison with reference methods, the small numbers studied, the lack of methodological quality measured by QUADAS, and the use of inappropriate analytical methods hamper solid conclusions.

INTRODUCTION

Nutritional problems are common in children with cerebral palsy (CP), especially in those children who are more severely disabled.[1] Malnutrition in these children is primarily caused by feeding problems. These problems, that include oral motor dysfunction, dysphagia, gastro-oesophageal reflux disease (GORD) and vomiting[2, 3], are secondary to the primary cause of disability in children with CP and can often lead to a reduction in nutrient intake. In many cases gastrostomy feeding is needed in order to reach or maintain a healthy weight. However, Sullivan et al.[4] have shown that children with CP who receive tube feeding are actually at increased risk of being overfed.

In order to prevent undernutrition and overnutrition in this population, careful monitoring of nutritional status is required. A well accepted way of evaluating nutritional status is by measuring body composition.

Body composition is most accurately evaluated using so-called reference methods such as isotope dilution, Dual Energy X-ray Absorptiometry (DEXA) or hydrostatic weighing. However, these methods are relatively complex and costly and therefore only used for research purposes. Two methods that are relatively easy to put into practice outside the hospital setting and which provide valid and reliable measures of body composition in children without disabilities are skinfold measurement and bioelectrical impedance analysis (BIA).[5, 6]

However, there are a number of differences between healthy children and children with CP that justify research into the validity of these two methods of nutritional assessment for this specific group. First, children with CP are often of smaller stature than their healthy peers.[7] Second, when compared with a control group, evidence was found indicating that children with CP have less subcutaneous fat and more intra-abdominal fat.[8, 9] Finally, children with CP often have contractures and scoliosis that complicate measurement of bioelectric impedance, which requires a symmetrical body configuration.

Therefore, the aim of this literature review was to report on the validity of skinfold measurements and bioelectric impedance analysis for nutritional assessment of children with severe CP.

METHODS

Literature search

Studies were identified by searches of the computerized bibliography databases Medline and Embase up to March 2009. Keywords were used for the target population, the disability, skinfold measurements and BIA – to also be referred to as index methods from this point forward – and the available reference methods (Table 1). There is no general consensus on the definition of the disability of these children, which is why the search for this term also contained specific features of CP (e.g. type of paralysis). On the basis of title and abstract, articles were excluded in which no comparison was made between the outcomes of the index and reference methods. Full texts of the remaining articles were assessed on eligibility.

Table 1. Literature search

Target population	Disability	Index methods	Reference methods
Child (E/M)	Cerebral palsy (E/M)	Body composition (E/M)	Isotope labeling (E/M)
Infant (E/M)	Central nervous system	Body constitution (E/M)	Oxygen isotopes (M)
Baby	diseases (E/M)	Nutritional assessment	Isotope
Babies	Spastic diplegia	(E/M)	Isotopes (E)
Teen	Spastic quadriplegia	Nutritional status (E/M)	Isotope dilution
Young person	Nervous system diseases	Anthropometry (E/M)	Doubly labeled water assay (E)
Young people	(M)	Skinfold Thickness (E/M)	Deuterium dilution
Youth	Neurologic disease (E)	Electric Impedance (E/M)	Body water (M)
Adolescent (E/M)	Neurologically disabled	BIA	Total body water (E)
	Neurologically impaired	Bioelectrical impedance	Absorptiometry, photon (E/M)
			DEXA
			Densitometry (E/M)
			Hydrostatic weighing
			40K counting
			Neutron activation analysis (E/M)
			In vivo neutron activation
			Plethysmography (E/M)
			Magnetic resonance imaging (E/M)

A MESH term is indicated with the letter M, an EMTREE term with the letter E. All MESH and ENTREE terms were also entered as text words. All terms without a designated letter were entered only as text words.

Eligibility

Studies were eligible for inclusion if (1) the study had been conducted in children with cerebral palsy, (2) at least half of the group studied was non-ambulatory, and (3) a statistical comparison had been made between the outcomes of either index method, and the outcome of any of the following reference methods: isotope dilution method, DEXA, 40K counting, hydrostatic weighing, neutron activation analysis,

plethysmography or MRI. Studies solely based upon people older than 18 years were excluded. Studies written in English, French, German or Dutch were considered eligible.

Table 2. Criteria for assessing quality (based on QUADAS)

Spectrum composition
Was the spectrum of patients representative of the patients who will receive the test in practice? (Q)
Were selection criteria clearly described? (Q)
Index test and reference standard: Selection and execution
Is the reference standard likely to correctly classify the target condition? (Q)
Is it possible that a change in the technology of the index test occurred since this paper was published? (Q)
Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? (Q)
Was the execution of the index test described in sufficient detail to permit replication of the test? (Q)
Was the execution of the reference standard described in sufficient detail to permit its replication? (Q)
Did the whole sample, or a random selection of the sample, receive verification using a reference standard of diagnosis? (Q)
Did all patients receive the same reference standard regardless of the index test results? (Q)
Was the reference standard independent of the index test? (Q)
Index test and reference standard: interpretation
Were the index test results interpreted without knowledge of the results of the reference standard? (Q)
Were the reference standard results interpreted without knowledge of the results of the index test? (Q)
Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? (Q)
Is data presented on observer or instrument variation that could have affected the estimates of test performance?
Analysis
Were appropriate results presented and where these calculated appropriately?
Was a measure of precision of the results presented (confidence intervals, standard errors)?
Were uninterpretable / intermediate results reported? (Q)
Were withdrawals from the study explained? (Q)
Research planning
Was an appropriate sample size calculation performed and were sufficient numbers of patients included in the study?
Were study objectives clearly reported?

Items that are part of the current QUADAS tool are marked with a Q.

Data collection

Data on (1) the characteristics of the recruited population (age, gender, presence of gastrostomy and extent of the motor and intellectual impairment), (2) the statistical methods used, and (3) the results of the statistical comparison of the outcomes of the index methods compared with those of the method of reference, were extracted.

Quality assessment

In order to assess the quality of the studies included, two researchers (RR and HE), scored them in accordance with a modified version of the QUADAS criteria developed by a Cochrane working group.[10] This is an evidence-based quality assessment tool for use in systematic reviews of diagnostic accuracy studies. After consulting a team of experts in the field, the Cochrane working group established a list of fourteen items using the Delphi procedure. While still under development, in our opinion this tool is well suited for providing insight into the quality of validation studies. Because this QUADAS tool is still under development, we introduced six additional items from an earlier stage of the working group's research. These items pertain particularly to accuracy of statistical analysis and appropriate sample size calculation. The final item set is listed in Table 2.

The items were scored "Yes", "No", "Unclear" or "Not applicable". The QUADAS tool in its current form does not provide an overall score because the developers believe the individual items do not carry equal weight in every individual diagnostic study. However, to provide insight into the quality of these studies, both researchers agreed on a verdict of one of five categories: studies of either inferior, insufficient, sufficient, good or excellent quality. The verdict was based upon the number of times an item scored "yes" within each subcategory (spectrum composition, selection and execution, interpretation and analysis). If all subcategories contained items that scored "no" on critical items, the verdict was "inferior quality". If the majority of items in a subcategory scored "yes", then that study was promoted a step higher in the quality assessment. If all four subcategories contained only "yes" scores, the qualification "excellent quality" was awarded.

It has to be noted, however, that only the methodology applied to answer the research question on validity was evaluated using the QUADAS tool. The overall quality of the studies was not assessed.

RESULTS

Study selection

The search strategy in Pubmed resulted in 598 studies and in Embase 1096 studies. After deduplication, 1549 unique studies remained. After the first eligibility screening based on title and abstract, seven potentially relevant articles were identified. After reviewing full text articles, six studies comparing either skinfold measurements or BIA with a reference method that fully met the inclusion criteria were found.

Table 3. Characteristics of the included studies

Authors	Publication Year	Population	N	Age (± SD years)	Gender (% male)	Ambulatory status	Motor disability	Assessment methods	Reference methods	Quality
Bandini et al.[14]	1991	Adolescents with CP	13	18.2 ± 1.6 (range 15-30)	31%	53.8% non ambulatory	SQ: 77%, SD:15%, H: 7%	SF	DLW	Inferior
Stallings et al.[12]	1995	Prepubertal children with SQCP	28	7.5 ± 2.3 (range 2-12)	61%	Non-ambulatory	All had SQ	SF	DLW	Insufficient
Azcue et al.[15]	1996	Children and adolescents with SQCP	14	8.4 ± 4.4 (range 2-16)	57%	Non-ambulatory	-	BIA	DLW	Insufficient
Van den Berg – Emons et al.[8]	1998	Children with CP	22	10.0 ± 1.5 (range 7-13)	50%	50% non-ambulatory	SQ: 19.2%, SD: 81.8%	SF	DLW	Good
Liu et al.[13]	2005	Children with CP	8	10 (range 4-21)	75%	Mean GMFCS=4,6	SQ: 63%, SD:25%, MQ: 13%	SF, BIA	DEXA	Insufficient

N=number of patients, SD=standard deviation, GMFCS=Gross motor function classification system, CP=Cerebral palsy, SQ=spastic quadriplegic, SD=spastic diplegia, MQ = mixed quadriplegia, Q=quadriplegia, T=Triplegia, D=Diplegia, H=hemiplegia, SF=skinfolds, DLW=doubly labeled water, BIA=bioelectrical impedance analysis.

Upon further examination five out of six studies focused on measures of body fatness, while a study by Arrowsmith et al.[11] compared outcomes of methods that measure total body protein. Because of the inability to compare the outcomes of this study with those of the other studies, the study by Arrowsmith was excluded.

In the study by Van de Berg-Emons et al.[8] the research question on validity was its only aim, in the other four[12-15] it was one of several.

Study population characteristics

The characteristics of the five study populations included in this review are presented in Table 3. All studies were published between 1991 and 2005. The numbers studied varied from eight to 28 children. In all studies at least half of the children were non-ambulatory. In four out of five studies at least half of the children had a spastic quadriplegia. Only Van den Berg-Emons et al.[8] and Stallings et al.[12] reported on intellectual functioning, with half of their respective study populations having a mild intellectual disability or cognitive delay. Stallings et al.[12] reported the presence of a gastrostomy in 47% of their population.

Nutritional assessment methods

Three authors studied the validity of skinfold measurements[8, 12, 14], one author the validity of BIA[15] and one author the validity of both these methods[13]. In four studies the assessment methods were validated against the outcomes of the isotope dilution method.[8, 12, 14, 15] Only Liu et al.[13] used a different method of reference: DEXA.

The outcomes used to validate the assessment methods were: fat mass (FM), fat free mass (FFM), percentage body fat (%BF), extracellular water (ECW) and total body water (TBW). Prediction equations had been used to transform skinfold thicknesses and impedance values from BIA into their primary outcomes. These primary outcome measures are the most direct result of the prediction equations, other outcomes are derivatives of that primary outcome.

In these studies, three prediction equations were used. Skinfold thicknesses were transformed into the primary outcome percentage body fat (%BF) using the formula by Slaughter et al.[5] for measurement of two skinfolds (triceps and subscapular), and the formula by Durnin et al.[16] for measurement of four skinfolds (triceps, biceps, suprailiacal and subscapular). Bioimpedance values were transformed into the primary outcome total body water (TBW) using the formula by Pencharz et al.[6] by both studies.

The isotope dilution method uses the principle of diluting stable isotopes in body water, then assessing the enrichment of these isotopes in serum, urine or saliva to calculate its primary outcome: total body water (TBW). DEXA provides both FM and FFM through software based calculations.

In order to compare the outcomes of the assessment methods with those of the method of reference, in many cases derivatives of the primary outcomes have to be used. For example, to compare the outcomes of skinfold measurements and isotope dilution, FFM can be used. FFM from skinfold measurements can be calculated by subtracting FM from body weight. FFM from isotope dilution can be calculated by using established proportions of water in FFM.

Outcomes

In all five studies, correlation coefficients (either Pearson's or Spearman's) were used to compare the agreement between the index methods and the reference method. These outcomes are presented in Table 4. In this table the outcome measures used for statistical comparison in each assessment method are noted. The formula that was used to calculate the outcome measure is presented, as are the reference methods used for comparison.

Van den Berg-Emons et al.[8] had carried out an additional Bland and Altman analysis.

Table 4. Correlations of the index methods with the reference

Index method	Outcome measures	Formula	Reference method	Correlation with reference
Skinfold measurements	FM	b	DEXA	0.406°[13]
		a	DEXA	0.588*[13]
		a	DLW	0.69*[12]
	FFM	b	DEXA	0.952**[13]
		a	DEXA	0.988**[13]
	%BF	b	DEXA	0.758*[13]
		a	DEXA	0.891**[13]
		a	DLW	0.82***[8]
		b	DLW	0.84***[8]
Bioelectrical impedance analysis (BIA)	%BF	c	DEXA	0.515°[13]
	FM	c	DEXA	0.83°[13]
	FFM	c	DEXA	0.915°[13]
	ECW	c	DLW	0.92°[15]
	TBW	c	DLW	0.95°[15]

DLW=doubly labeled water, FM=fat mass, FFM=fat free mass, ECW=extracellular water, %BF=percent body fat.
*P<0.05, ** P<0.01, *** P<0.001, ° p value not mentioned
a Formula by Slaughter et al., based on 2 skinfolds[5]
b Formula by Durnin et al., based on 4 skinfolds[16]
c Formula by Pencharz et al.[6]

Correlation coefficients

In summary, when comparing the outcome measures of skinfold measurements with those of the reference methods, correlation coefficients (CC) were highest for FFM

(0.952-0.988). The CCs for percentage body fat (%BF) ranged from 0.758 to 0.891, and for FM from 0.406 to 0.69.

On comparing the outcome measures of BIA to those of the reference methods, CCs ranging from 0.515 (for %BF) to 0.95 (for TBW) were found. The CCs reported for other outcome measures (FM, FFM and ECW) were between 0.83 and 0.92.

Stallings et al.[12] and Bandini et al.[14] correlated a number of other anthropometric indices (such as additional skinfolds and body circumferences) with the outcome of the reference method. These data are not included in Table 4, but correlation coefficients for these outcomes ranged from 0.57 to 0.96.

Bland & Altman analysis

Van den Berg-Emons et al.[8] were the only authors that used Bland and Altman plots to present data on the agreement between the studied methods. The authors found a significant correlation ($r=-0.58$, $p<0.01$) between the difference in percentage of body fat (%BF) obtained from skinfolds and isotope dilution and the average %BF. This indicates that as the measured level of body fat of the children increased, the differences between %BF obtained from skinfold measurements and from isotope dilution also increased.

Quality assessment

The results of the quality assessment using QUADAS are included in Table 3. Only the study by van den Berg-Emons et al.[8] was of good quality, although they did not carry out a power analysis and did not report confidence intervals. The other studies were either of insufficient or of inferior quality, mainly because of the lack of appropriate statistical methods.

DISCUSSION

This review identified five studies investigating the validity of skinfold measurements and bioelectrical impedance analysis in children with severe cerebral palsy by comparing their outcomes with those of either the isotope dilution method or DEXA. Although most studies reported favorable agreement for skinfold measurements and BIA[12-15], these conclusions are hampered by small, heterogenic populations, the use of statistical methods that are considered weak when studying agreement, and the lack of methodological quality measured by QUADAS. Therefore, the validity of skinfold measurements and BIA in children with severe CP needs a more robust evaluation.

In general, population size remains an issue in studies in children with severe CP. In the studies we looked at, group size varied from 8[13] to 28[12] children. It is questionable whether these sample sizes provide enough power to draw reliable conclusions on the validity of the tests under study. For that matter, none of the studies reported power calculations. Further complicating the applicability of the conclusions of the studies is the fact that the populations under study differed considerably between studies. Distribution of age, sex, type of motor disability and other characteristics was diverse or had not been reported on at all. Not reporting these parameters hampers comparison between studies and the generalizability of their results in relation to the population of children with CP as a whole.

Another bias that was introduced – in particular in the older studies – is the use of correlation coefficients or regression analyses to study agreement between methods. Bland and Altman et al.[17] concluded that when assessing agreement between methods of clinical measurement, a limits of agreement method should be used instead of a correlation coefficient - a point further supported in the study by Stallings and colleagues.[12] The author found a reasonable correlation between FM from skinfold measurements and isotope dilution, while in absolute numbers FM from skinfolds was underestimated by 1.3 kg compared with isotope dilution. Only Van den Berg-Emons et al.[8] had applied the limits of agreement analysis in addition to reporting on the correlation coefficient, which led them to be critical of using skinfold measurements to study body composition in spite of favorable correlation coefficients.

Leaving aside suitability of methods, one observation is certainly interesting: correlation coefficients tended to be strongest for the outcome measure FFM. Those for FM and %BF were lower. This is probably best explained by the fact that FFM is a more direct outcome of BIA than are FM and in particular %BF. The latter two are derived measures of FFM. This observation would seem to suggest FFM is the most relevant clinical outcome measure for BIA, as suggested earlier by Wright et al.[18]

Mainly because weaker statistical methods were used to study agreement, the quality of four out of the five studies was deemed to be insufficient. The study by van den Berg-Emons et al.[11] was the only study of good quality. To be fair however, these authors' express purpose was to validate skinfold measurements, whereas for the others the question of validity was of lesser importance.

Conclusions

Measuring body composition in children with CP is a complex matter. Only a small number of studies have, in some form, studied the validity of methods of nutritional assessment in children with CP. The overall methodology used to answer the question

of validity in these studies was often found wanting, however, and this hampers solid conclusions with regard to validity.

Ideally, a future trial should compare the outcomes of skinfold measurements and BIA with those of a reference method, such as isotope dilution, in a larger number of children. Besides some exploratory statistics (including calculation of correlations), the data should most importantly be analyzed using Bland and Altman limits of agreement analyses. A larger trial might even provide enough statistical power to justify the development of specific prediction equations to determine body composition in children with severe CP.

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Chapter 3

Validation of deuterium and oxygen^{18} in urine and saliva samples from children using online continuous flow isotope ratio mass spectrometry

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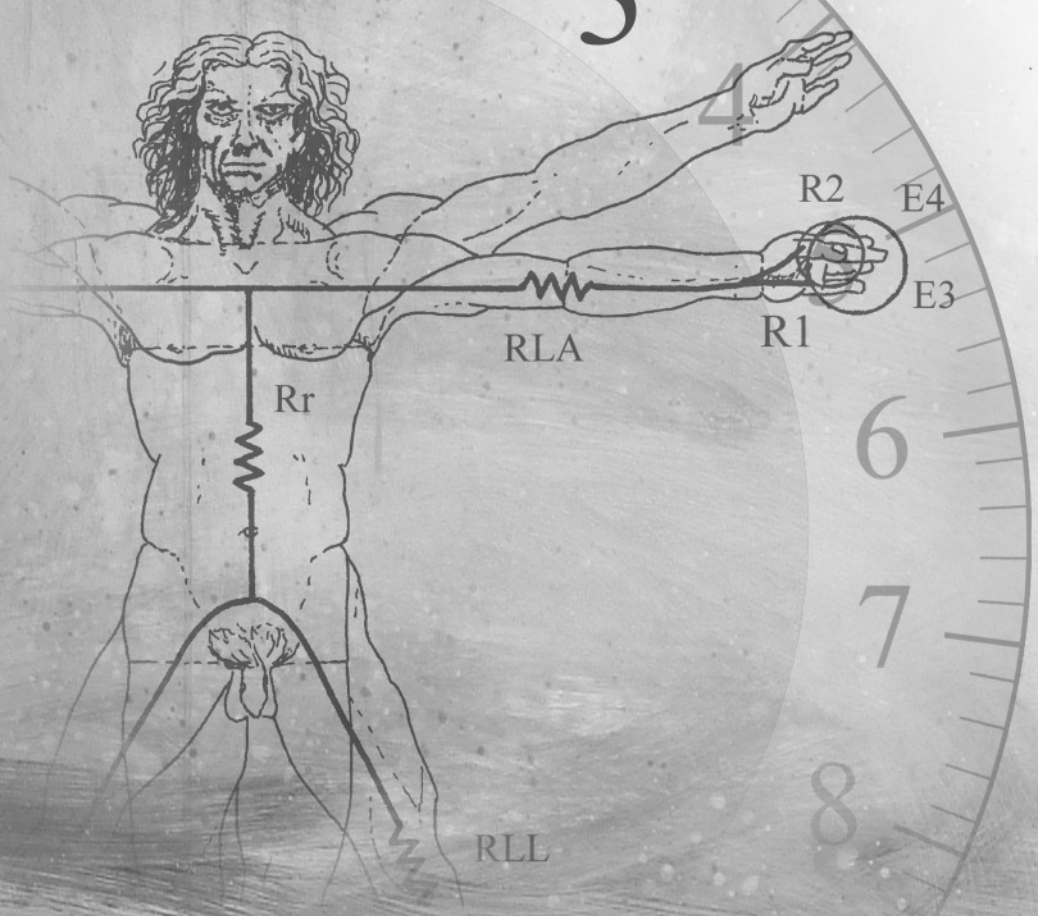

ABSTRACT

The doubly labeled water method is valuable for measuring energy expenditure in humans. It usually involves blood or urine sampling, which might be difficult in neonates and children with cerebral palsy or other disabilities. We therefore aimed to validate a method making use of saliva samples analyzed by automated thermal conversion elemental analyzer in combination with isotope ratio mass spectrometry (TC-EA/IRMS). The subjects received labeled water orally and urine and saliva samples were collected and analyzed. Deuterium as well as oxygen^{18} were measured in one single run using a peak jump method. Excellent linearity was found for measurement of enrichments of deuterium (R^2=0.9999) and oxygen^{18} (R^2=0.9999). Both the intra-assay precision and the inter-assay precision of the measurement of two standards were good for both deuterium and oxygen^{18}.

The variation between urine and saliva samples was small (4.83% for deuterium and 2.33% for oxygen^{18} n=40). Saliva sampling is to be preferred, therefore, as it can be easily collected and is non-invasive. Moreover, its time of production is almost exactly known. The TC-EA/IRMS method is a good alternative to the more laborious off-line IRMS measurements.

INTRODUCTION

The past decade has seen the development of new interfaces for measuring deuterium and ^{18}O enrichments.[1,2] In the classical methods, water is isolated from biological fluids (plasma and urine) by cryo-distillation and converted into HD/H_2 and $C^{18}O_2/C^{16}O_2$ gases.[3] The former are produced through reduction of the collected water using zinc, platinum or manganese for example.[4-10] The latter are produced by water equilibration overnight with un-enriched CO_2 gas to achieve ^{18}O enrichment in CO_2 gas similar to that present in the water samples. HD/H_2 and $C^{18}O_2/C^{16}O_2$ are then transferred off-line into the isotope ratio mass spectrometer for measuring their deuterium and ^{18}O enrichments. These doubly labeled water ($D_2{}^{18}O$) techniques are laborious, time-consuming and require relatively voluminous samples (a few hundred microliters or even a few millilitres). Yet they are frequently used in clinical nutrition to assess patients' total body water composition (TBW)[6, 8, 11-13] and total energy expenditure (TEE)[5, 14-17], following the administration of a single dose of $D_2{}^{18}O$ or D_2O. Quantities of fat free mass and fat mass can be derived from TBW estimates by assuming that all water is contained in the lean body mass and that the composition of the lean body mass is constant. TEE determination is accomplished by the following principle. Deuterium leaves the organism only as water, whereas ^{18}O leaves the organism as water and carbon dioxide. The difference in the elimination rates of deuterium and ^{18}O is therefore a measure of the body's CO_2 production rate, which in its turn can be converted into energy expenditure.

The usual sample medium is plasma, because full equilibration of the tracer in plasma occurs very quickly. As plasma sampling may raise ethical and practical concerns, urine and saliva have been validated as alternative media. Urine, however, has the disadvantage that it takes longer to reach an isotopic equilibrium. Furthermore, it may be harder to collect in specific populations such as children suffering from severe generalized cerebral palsy. This condition is characterized by a moderate to severe intellectual disability in combination with a severe motor handicap, and most of the patients are incontinent. Consequently the exact time of urine production is often difficult to determine, which has an impact on the precision of the results. By contrast, saliva is collected almost immediately after it is produced. Isotopic equilibration in saliva is also faster than in urine.[12] Generally, data on the feasibility of sample collection in children are lacking.

For paediatric studies there is a need to simplify sample preparation with a minimum of sample volume (a few microlitres). This allows a high-throughput (10 measurements / hour) Continuous-flow isotope ratio mass spectrometry (CF-IRMS) systems[18-21] are designed for high productivity and for streamlining the lengthy proce-

dures associated with off-line sample preparation and dual-inlet mass spectrometry measurements. To this aim a helium carrier transports the gas to be measured from the preparation device to the directly coupled isotope ratio mass spectrometer. The most recent system for the analysis of $^{2}H/^{1}H$ enrichments is the thermal conversion elemental analyzer (TC/EA), which converts micromoles of water sample into H_2 by reaction in a glassy carbon tube reactor at 1420° C.[1, 2, 22-24] This system was used to measure $^{2}H/^{1}H$ and $^{18}O/^{16}O$ in plasma samples of rats.[2] The procedure is largely automated, so that samples can be analysed on a routine basis. The major disadvantages of TC/EA are its high purchase price and costs of consumables, as well as the need to repack the ceramic tube with glassy carbon chips after every 300–400 samples.

We report a study aimed at developing and validating a robust and quick procedure using the TC/EA-IRMS online technique for accurate simultaneous measurement of deuterium and ^{18}O in body fluids of children obtained in a non-invasive way. The simultaneous determination of the enrichment in deuterium and ^{18}O of urine and saliva samples offers great advantages in terms of non-invasive sample collection, smaller sample size, minimal sample preparation and high throughput which will be a major advantage for total body water and energy expenditure studies in neonates and children with cerebral palsy or other disabilities.

EXPERIMENTAL

Chemicals and materials

$D_2{}^{18}O$ was purchased from Cambridge Isotope Laboratories (Buchem, Apeldoorn, Netherlands). Mini centrifuge filters 0.22 µm were bought from Millipore BV (Bedford, MA, USA). The H_2 and CO working reference gases (Linde, quality 6.0 and 4.7 respectively) were calibrated with known reference waters, i.e. Standard Light Antarctic Precipitation (SLAP) and Greenland Ice Sheet Precipitation (GISP), purchased from the International Atomic Energy Agency (IAEA, Vienna, Austria). Working standards GS 47, GS 49 and HDW1 were used for calculation of each batch of analyses.

Clinical study design

Ten children with severe cerebral palsy who participated in a larger nutritional study were selected to validate the method. They met the following inclusion criteria: age between 2 and 19 years; IQ<55; and a motor impairment, defined as hypertonic or hypotonic generalized cerebral palsy or a motor developmental delay to such an extent that the subject could at best crawl. Exclusion criteria were: active infection or an altered water balance (oedema or dehydration as confirmed by a physician).

The study design was approved by the Erasmus MC Medical Central Committee on Research Involving Human Subjects and informed parental consent was obtained prior to the study.

Doubly labeled water administration / urine and saliva sampling

One dose (3 g/kg) of doubly labeled water (^2H_2O: 10%, $H_2{}^{18}O$: 5%) was administered orally, or via gastrostomy. Saliva and urine samples were collected just before administration and after an equilibration period of 4 h, in which children remained fasted. Over the following two weeks another five urine and saliva samples (days 1, 5, 8, 11 and 15) were collected and stored until analysis. Patients were not allowed to drink for 30 min prior to saliva sampling. Urine was extracted from diapers with cotton batting pads and stored in 30 mL glass urine bottles. Saliva was sampled by swabbing a dry cotton rod in the child's mouth for 2-5 minutes and then putting the cotton rod in a plastic container (Salivette, Sarstedt, Etten-Leur, The Netherlands). The container was then centrifuged (3000 g) and a clear, fluid sample (0.25 – 1.5 mL) was transferred into a glass vial of 2 mL. Both urine and saliva sample bottles were flushed with nitrogen to reduce isotope exchange in the sample container. Also for this reason we stored the samples in glass rather than plastic containers, as the latter are semi-permeable. All sample containers were stored frozen at -20° C prior to analysis.

TC-EA/IRMS

Experiments were carried out on a high-temperature thermal conversion elemental analyzer (TC-EA) (Thermo Fisher, Bremen, Germany) coupled with a Delta XP isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany) via a Conflo-III Interface (Thermo Fisher, Bremen, Germany). The IRMS instrument was operated at an accelerating voltage of 5 kV. The ion source was held at a pressure of 3.0×10^{-6} Torr, and ions generated by electron impact at 70 eV. Subsequently, two sets of faraday cup detectors monitored signals for the ions at m/z 2 ($^1H/^1H$) and m/z 3 ($^2H/^1H$) ion beams of H_2 gas, as well as the m/z 28 ($C^{16}O$) and m/z 30 ($C^{18}O$) ion beams of CO. The $^2H/^1H$ ratios were corrected for the H3+ effect. The dynamic range of the instrument is between 0.2 and 50 V. The reactor consists of a glassy carbon tube filled with carbon chips (IVA, Meerbusch, Germany). The following conditions were used: reactor temperature 1420° C, GC column temperature 90° C, helium flow 110 mL/min, and two reference gases, hydrogen 6.0 and carbon monoxide 4.7.

Analytical conditions

Samples were thawed and aliquots of 50 µL were transferred to a mini centrifuge filter tube (0.22 µm) and centrifuged at 4000 g for 5 min. The filtrated fluid was collected

and transferred to a sample vial with a 100 µL insert. Aliquots of 0.1 µL were injected by an auto sampler into the TC-EA/IRMS system. Samples were analyzed in the dual measurement mode. Each analytical cycle consists of three pulses of the hydrogen reference gas introduced by the Con Flow III unit followed by measurement of the eluting hydrogen peak. After a quick swap to a different cup setting, the eluting carbon monoxide was measured, followed by three pulses of CO reference gas. Each sample was measured five times and calculated against the reference gases injected in the same run. The deuterium and oxygen isotope abundances of the water samples are expressed in delta per mil (δ pm). The $^{2}H/^{1}H$ ratios were corrected for the H3+ effect, which was determined before each sequence.

Calculations

TEE can be calculated by using the equation by De Weir[25,26]:

$$\text{TEE (kcal/day)} = \frac{3.9 rCO_2 \text{ (liter/day)}}{RQ} + 1.11 rCO_2$$

where rCO_2 is expressed in L/day and can be converted from mol/day by multiplying by 22.4. RQ is oxygen consumption/rCO_2.

RQ can be measured by performing indirect calorimetry for at least 20 minutes. rCO_2 can be calculated using the following equation:

$$rCO_2 = 0.4554N\ (1.01k_o - 1.04K_h)$$

where K_o and K_h the rate constants at which $^{2}H_2O$ and ^{18}O are lost from the pool.

The dilution space for deuterium or ^{18}O or TBW can be calculated using the following equation:

$$N \text{ (mol)} = \left[\frac{WA}{(18.02)a}\right] x \left[\frac{da - dt}{ds - dp}\right]$$

where N is the pool space, W is the amount of water used to dilute the labelled water, A is the weight of labelled water administered, a is the diluted dose for analysis, and d is the enrichment of dose (a), dilution water (t), post dose sample (s) and pre dose baseline (p) samples.[11, 25] Total body water can be determined using either deuterium

or ^{18}O. To correct for isotopic fractionation the deuterium dilution space must be divided by 1.04 and the ^{18}O diluting space by 1.01.[6]

Statistics

Calculations were made using Microsoft Office - Excel software (version 2003; Microsoft Corp, Redmond, WA, USA). Statistical analysis was performed using GraphPad Prism software (version 4.0; San Diego, CA, USA). The intra-assay precision was determined by a multiple measurement (n=10) of standards on a given day. Samples of these standards were also injected together with each series of analyses, and the enrichments determined in this way provided the inter-assay precision. The Bland-Altman method[27] was used for the comparison of the two different methods.

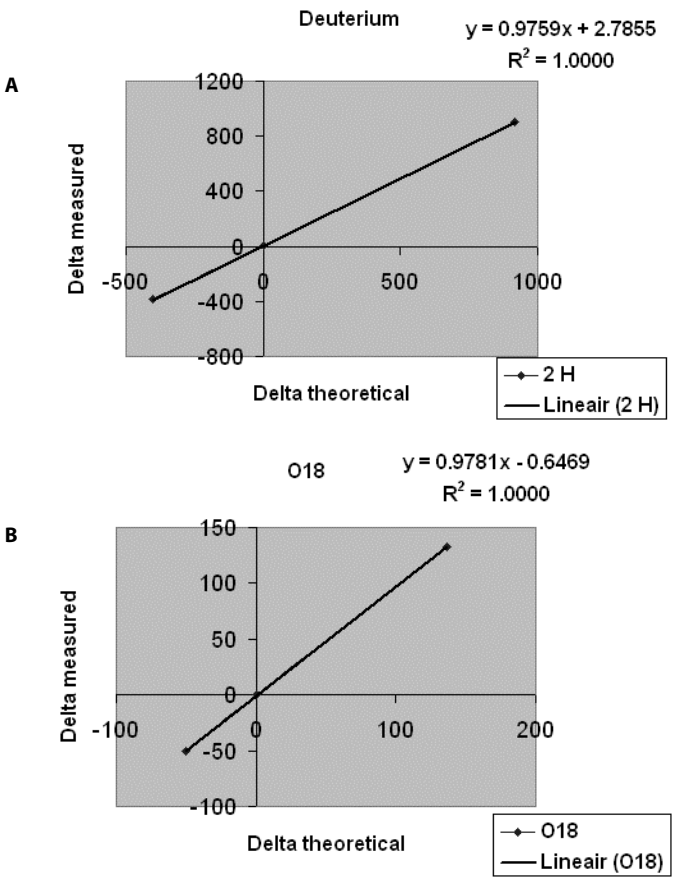

Figure 1 Calibration curve for measurement of (a) $^{2}H_{2}$ enrichment and (b) ^{18}O enrichment

RESULTS AND DISCUSSION

Calibration

Three well defined standard solutions of water with known deuterium and ^{18}O enrichments (GS 47, GS 49 and HDW1) were used to establish the calibration curve for correction of the values. They were in a range of -50.6 δ ‰ up to 136.7 δ ‰ for ^{18}O and of -400.2 δ ‰ up to 918.5 δ ‰ for HD respectively. These set of standards were analyzed at the beginning and end of each sequence of 28 samples. An excellent correlation was obtained for both ^{18}O and HD (Figures 1a and 1b).

Sample measurement

Memory effects are known to occur when measuring samples using this technique.[1, 22, 24, 28, 29] When injecting biological samples containing varying enrichments of D and ^{18}O into the IRMS, trace amounts of the previous sample will be carried over, resulting in a memory effect

To minimize this effect, each sample is injected five times. The first two measured enrichments are excluded from the final analysis. After each injection the full volume of the syringe is flushed five times with air in order to avoid cross contamination of the samples. Finally, the syringe is flushed with one syringe volume of sample prior to injection. Samples were analyzed in dual measurement mode with a jump calibration between H and CO measurements. After each 150 injections the septum of the injector was replaced and after each 300 injections, the glassy carbon reactor was exchanged with a new one.

Table 1. Intra-assay precision assessed by replicate analysis of specimen aliquots on a single day (n=9)

	δ Deuterium ‰	δ Deuterium ‰	δ Oxygen-18 ‰	δ Oxygen-18 ‰
	Gs 49	HDW-1	Gs 49	HDW-1
	6.985	873.745	2.341	132.226
	6.077	873.538	2.409	132.768
	6.09	874.994	2.331	133.271
	7.567	880.998	2.469	133.522
	7.238	881.97	2.489	132.879
	7.458	883.443	2.49	133.514
	5.841	878.854	2.648	133.799
	6.285	880.165	2.879	133.9
	5.899	880.871	2.712	133.74
Mean	6.60	878.73	2.53	133.29
Sd	0.701	3.713	0.182	0.560

Table 2. Inter-assay precision assessed by replicate analysis of specimen aliquots on several days (n=9)

	δ Deuterium ‰	δ Deuterium ‰	δ Oxygen-18 ‰	δ Oxygen-18 ‰
	Gs 49	HDW-1	Gs 49	HDW-1
	6.38	874.09	2.16	132.76
	7.42	882.14	2.15	133.31
	6.01	879.96	2.75	133.81
	5.80	860.44	2.75	132.90
	5.23	860.64	2.62	131.54
	6.57	881.28	2.54	131.10
	5.12	870.75	2.38	131.75
	6.06	865.55	2.44	133.67
	6.45	878.91	2.59	133.71
Mean	6.12	872.64	2.49	132.73
Sd	0.705	8.707	0.225	1.025

Accuracy and precision

The intra-assay precisions of the $^{18}O/^{16}O$ and $^{2}H/^{1}H$ analyses were determined using three well defined doubly labelled water standards. Standards were determined ten times on a given day, and these results provided the intra-assay precision (Table 1). Samples of this water were also injected together with each series of analyses, and the enrichments determined in this way provided the inter-assay precision (Table 2). The variability of the D enrichment was higher than that of the ^{18}O enrichment, in line with findings from the literature.[2] The accuracy of the TC-EA/IRMS instrument was determined by injecting the two certified reference waters, i.e. SLAP and GISP several times (Table 3). The measured D and ^{18}O enrichments for these two water standards are in close agreement with the values determined by the IAEA against the

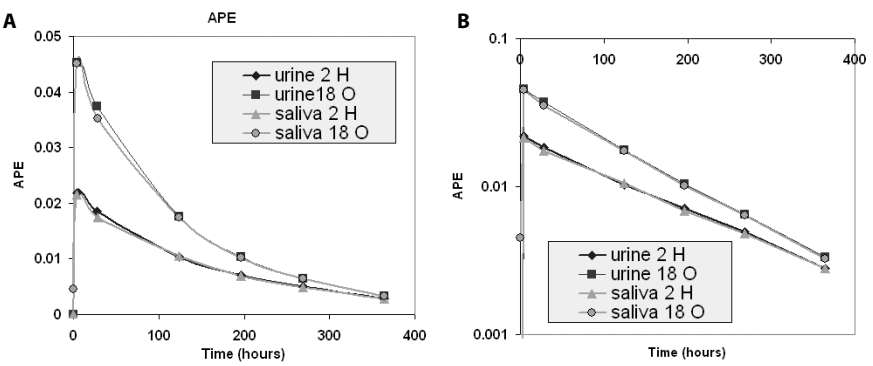

Figure 2 The decline of $^{2}H_{2}$ enrichment and ^{18}O enrichment after administration of (a) an oral dose $^{2}H_{2}{}^{18}O$ and (b) the same values expressed logarithmically.

Table 3. Two international standards, GISP and SLAP, were assessed by replicate analysis to determine the accuracy

Standard	δ Deuterium	δ Deuterium	Accuracy	δ Oxygen18	δ Oxygen18	Accuracy
	Value IAEA	Measured		Value IAEA	Measured	
	‰	‰	‰	‰	‰	‰
SLAP	-428.00	-425.12	2.88	-55.50	-54.83	0.67
		-425.46	2.54		-54.99	0.51
		-426.44	1.56		-54.91	0.59
		-426.32	1.68		-55.15	0.35
		-426.68	1.32		-55.21	0.29
GISP	-189.30	-188.47	0.83	-24.77	-24.27	0.50
		-188.09	1.21		-24.33	0.44
		-187.82	1.48		-24.38	0.39
		-187.37	1.93		-24.24	0.53
		-187-91	1.39		-24.46	0.31
Mean SLAP		-426.00	2.00		-55.02	0.48
Sd		0.675			0.160	
CV %		-0.16			-0.29	
Mean GISP		-187.93	1.37		-24.34	0.43
Sd		0.401			0.088	
CV %		-0.21			-0.36	

international reference, V-SMOW. These data demonstrate the reliable performance of the system at the level of accuracy reported in the literature.

Analysis of human saliva and urine samples

Deuterium and ^{18}O enrichments were measured in urine and saliva from 10 subjects during a period of two weeks. Values measured in saliva and urine both showed a linear decline in enrichment as shown in the logarithmic presentation (Figure 2). The enrichments of D and ^{18}O of the 20 saliva and 20 urine samples showed a good correlation with only a small variation of 5.9% (sd= 0.026) for deuterium and 0.95% for ^{18}O (sd= 0.002). The average enrichments for deuterium (4.83% n=40) and for ^{18}O (2.33% n=40) were consistently lower for saliva (Table 4, Figure 3). While saliva production and collection occur roughly at the same time, the time between urine production and collection is often unknown in incontinent subjects. However, it is safe to assume that the latter time is longer, resulting in a higher value for the enrichments of deuterium and ^{18}O in urine.

Experiments for measurement of deuterium and ^{18}O have also been assessed with other techniques, i.e. using a laser and IRMS[30, 31] and high temperature conversion

Table 4. Comparison of urine and saliva analysis of ten subjects after administration of an oral dose $D_2{}^{18}O$

Subject	Time (hours)	Type	At% D	Sd (n=2)	At% 18O	Sd (n=2)
506	0.0	Urine	0.0150237	0.0000113	0.1993150	0.0031997
	0.0	Saliva	0.0150397		0.2038400	
	4.2	Urine	0.0369177	0.0002854	0.2446730	0.0002006
	4.1	Saliva	0.0365140		0.2443893	
	196.2	Urine	0.0221397	0.0001706	0.2097630	0.0002001
	196.3	Saliva	0.0218983		0.2094800	
	364.2	Urine	0.0178130	0.0000067	0.2026390	0.0000622
	364.3	Saliva	0.0178225		0.2025510	
505	0.0	Urine	0.0153223	0.0000005	0.1995747	0.0001223
	0.0	Saliva	0.0153230		0.1997477	
	3.5	Urine	0.0407120	0.0012398	0.2502970	0.0014632
	3.5	Saliva	0.0424653		0.2523663	
	196.0	Urine	0.0247163	0.0003578	0.2133520	0.0003352
	195.0	Saliva	0.0242103		0.2128780	
	363.5	Urine	0.0190680	0.0001214	0.2038390	0.0001042
	363.5	Saliva	0.0188963		0.2036917	
516	0.0	Urine	0.0154283	0.0000181	0.1996250	0.0000207
	0.0	Saliva	0.0154540		0.1995957	
	3.8	Urine	0.0503500	0.0045839	0.2651190	0.0044354
	3.8	Saliva	0.0438673		0.2588463	
	196.5	Urine	0.0312137	0.0002041	0.2235687	0.0000431
	196.7	Saliva	0.0315023		0.2235077	
	365.8	Urine	0.0156610	0.0049578	0.2001403	0.0065219
	365.7	Saliva	0.0226723		0.2093637	
507	0.0	Urine	0.0155183	0.0000693	0.2002643	0.0000354
	0.0	Saliva	0.0154203		0.2003143	
	3.2	Urine	0.0468937	0.0030479	0.2606437	0.0057624
	4.0	Saliva	0.0425833		0.2524943	
	192.8	Urine	0.0257623	0.0000212	0.2158477	0.0001558
	192.9	Saliva	0.0257923		0.2160680	
	361.6	Urine	0.0205453	0.0000627	0.2064480	0.0000097
	360.8	Saliva	0.0204567		0.2064617	
515	0.0	Urine	0.0153497	0.0000057	0.1991937	0.0000354
	0.0	Saliva	0.0153417		0.1991437	
	3.8	Urine	0.0574973	0.0017147	0.2798440	0.0029974
	3.9	Saliva	0.0550723		0.2756050	
	196.3	Urine	0.0222773	0.0004106	0.2095947	0.0003460
	196.3	Saliva	0.0216967		0.2091053	
	362.8	Urine	0.0168043	0.0000790	0.2009243	0.0000387
	362.7	Saliva	0.0166927		0.2009790	
Mean			0.0260934	0.0015348	0.2191274	0.0020916
CV%			5.88		0.95	

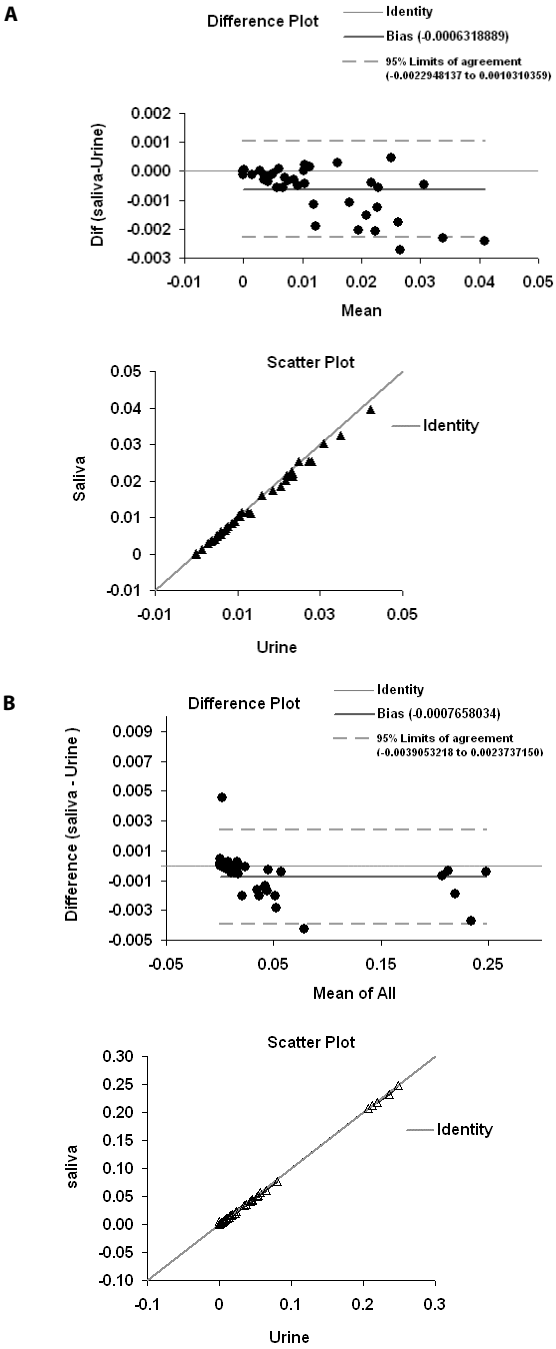

Figure 3 Scatter plot and Bland and Altman plot of the comparison of a) deuterium and b) oxygen[18] measurement using urine and saliva samples

IRMS[32, 33], but these techniques show to have less precision and accuracy compared to the technique using TC-EA/IRMS.

Conclusions

We have validated a method for simultaneous measurement of deuterium and ^{18}O enrichment in urine and saliva samples, enabling to determine children's total body water composition and energy expenditure. Sample preparation is much simpler than with the classical methods. The analysis process is fully automated, with very small samples (0.1 µL) directly injected into a TC-EA/IRMS system equipped with a liquid auto sampler. Samples are converted into hydrogen and carbon monoxide gases that are transferred on-line using helium gas into the directly coupled isotope ratio mass spectrometer. The TC-EA/IRMS system provides for accurate and simultaneous measurement of D and ^{18}O enrichment of saliva and urine samples. Although the results did not differ between the two sample types, sampling of saliva is preferred because its production time of saliva can be determined almost exactly. In addition, the sampling of saliva is less invasive than blood, which is an important issue in paediatric studies. This methodology is a good alternative to the laborious off-line IRMS measurements. This method is saving labour and analysis time and therefore also lowering the analysis costs. The accuracy, simplicity and robustness of the TC/EA-IRMS using the doubly labelled water dilution technique in saliva samples can be a great support to assess body composition and energy expenditure in all subjects in which blood collection is less desirable.

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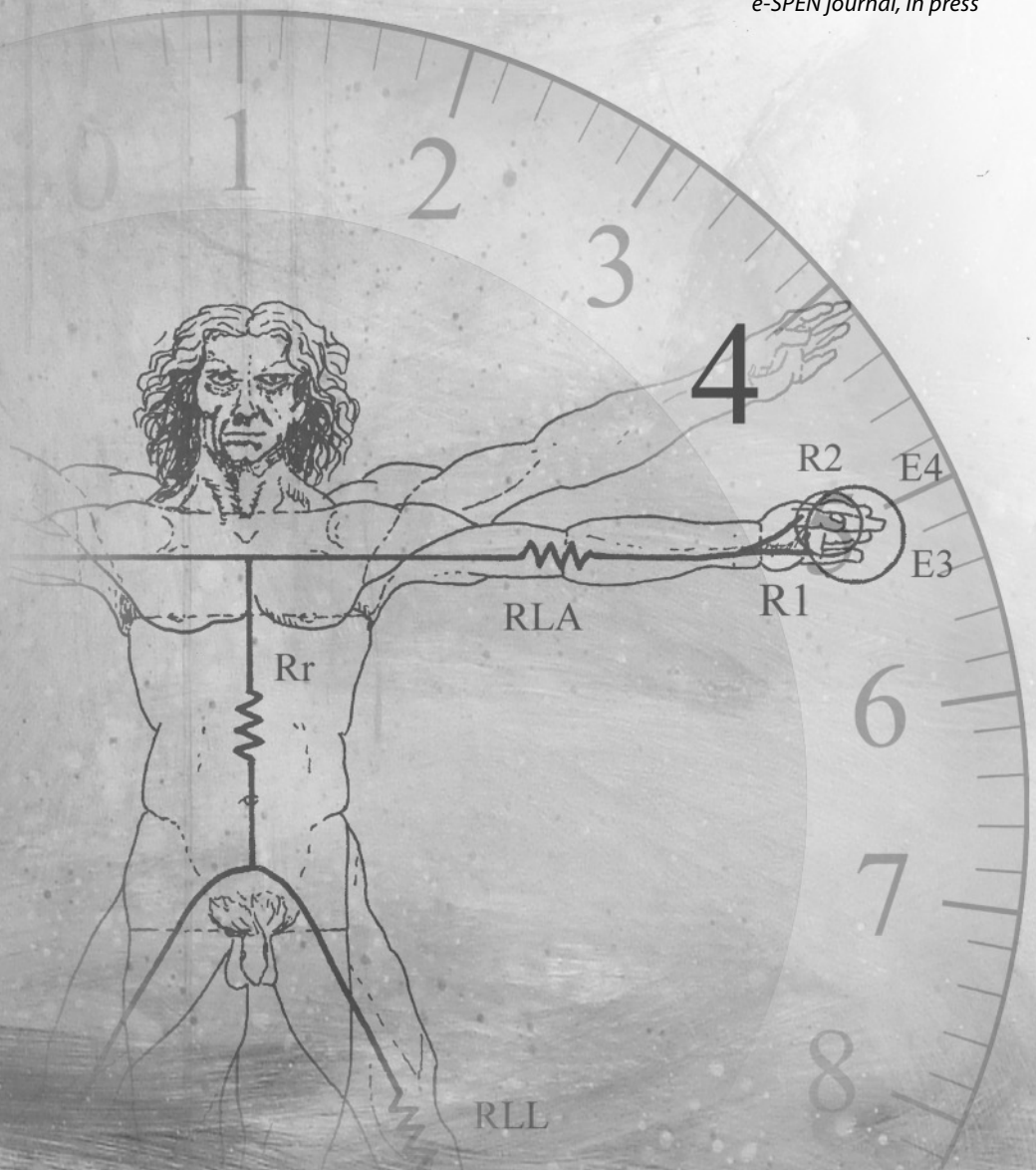
Chapter 4

Measuring energy expenditure and body composition in children with neurological impairment and intellectual disability using the doubly labeled water method: comparing urine and saliva sampling

e-SPEN journal, in press



ABSTRACT

Background & Aims Information is lacking on the comparability of the outcomes of the doubly labeled water method using urine and saliva samples in children with severe neurological impairment and intellectual disability (ID). Our aim is to compare outcomes and feasibility based on both sampling methods.

Methods Total energy expenditure (TEE) and total body water (TBW) were calculated using urine and saliva samples of thirteen children (aged 3-15 y). To correct for age and weight, TEE was expressed as a percentage of recommended daily allowance (%TEE of RDA) and TBW as a percentage of weight (%TBW). Agreement between methods was evaluated using Bland and Altman analyses.

Results 88.5% of urine and 79.5% of saliva samples were successfully analyzed. Limits of agreement between urine and saliva samples were favorable for the outcomes %TEE of RDA (mean difference –1.9% ± 7.5%) and %TBW (mean difference –1.9% ± 3.0%).

Conclusions Both urine and saliva are feasible sample media for the doubly labeled water method in children with severe neurological impairment and ID. Clinical outcomes based on either urine or saliva samples agree well. Thus, choosing either one of the sampling methods is justified if the other fails.

INTRODUCTION

Malnutrition is a major health problem in children with severe neurological impairment and intellectual disability (ID)[1] and has led to a considerable body of research into the variations in energy expenditure and body composition in this group of children. The technique that is considered to be a method of reference in this particular area of research is the doubly labeled water (DLW) method.[2-4] This method requires administering stable isotopes (tracers) of deuterium (^2H_2O) and labeled oxygen ($H_2{}^{18}O$) and provides accurate and reliable measurements of total energy expenditure (TEE) and total body water (TBW).[5, 6]

TEE and TBW can be calculated most accurately by measuring isotope enrichment in serum as full equilibration of the tracer in serum transpires very quickly.[7] However, due to the invasive nature of blood sampling and other practical concerns, for purposes of research urine and saliva sampling are more patient-friendly and in the past proved valid alternatives to sampling serum.[7, 8] In children with neurological impairment urine is the most commonly used sample medium for the DLW method,[2, 3, 9] but it has a couple of disadvantages. First of all, it takes longer for the tracers to equilibrate in the bladder contents.[6] Secondly, because almost all children with neurological impairment and ID are incontinent, urine has to be collected from a urine collection bag or cotton batting pads in the diaper. The time from initial production in the kidney to the actual collection of urine is therefore not known. Both problems add imprecision to the measurement.

However, saliva sampling is not affected by these issues since saliva can be collected almost immediately after it is produced. Isotopic equilibration in saliva is also faster than in urine.[7] However, some children with neurological impairment produce large amounts of saliva, which they do not swallow, and some of these children are treated with botulinum injections or surgery to restrict saliva production. Furthermore, saliva may be diluted by the large amounts of bronchial mucus that these children can cough up or by esophageal refluxate in the mouth. In addition to these problems, some children have oral hypersensitivity to such an extent that they might not even allow access to the mouth to collect saliva.

In the past, urine has generally been the sample medium of choice for the DLW method in children with neurological impairment[2-4, 9], but, more recently, Sullivan et al.[10] used both urine and saliva samples in their study evaluating the effect of gastrostomy tube feeding on weight gain and overfeeding in 40 children with spastic quadriplegic CP (cerebral palsy). However, they did not provide information on the comparability of the outcomes of the urine and saliva samples. The question is whether we may assume that in this group of complex children, in whom sampling

urine or saliva is not always easy, the outcomes of urine and saliva samples are comparable.

Therefore the aim of this study is to establish, when using the DLW method in children with severe neurological impairment and ID in a field setting, whether (1) saliva and urine sampling result in the same clinical outcomes, and (2) sampling these media is feasible in this specific group of children.

SUBJECTS & METHODS

Subjects

Thirteen children with severe neurological impairment and ID (seven boys and six girls) were recruited from a large children's care center in Rotterdam. Inclusion criteria were: age between 2 and 19 years; moderate to severe ID with an estimated IQ below 55; and motor impairment, defined as hypertonic or hypotonic generalized CP, or motor developmental delay to such an extent that a child could at best crawl. The motor impairment had to be the equivalent of Gross Motor Function Classification System (GMFCS) level four or five.[11] Children that had contracted an active infection or had an altered water balance (edema or dehydration) at the time of the measurements were excluded. The parents or legal guardians of the children provided written informed consent and the Dutch Central Committee on Research Involving Human Subjects approved the study protocol.

Doubly labeled water method

Children were studied in the daycare center at least 3 h after their regular morning meal. Body weight (BW) was measured with an electronic wheelchair scale (Universal PM 7050, Lopital, Oisterwijk, Netherlands) to the nearest 0.1 kg. They then received a single dose of DLW (^2H_2O: 10%, $H_2{}^{18}O$: 5%, Cambridge Isotope Laboratories, distributed by Buchem BV, Apeldoorn, The Netherlands) of 3 g/kg BW orally or via a gastrostomy tube. The dose container was washed out with 50 mL of plain tap water and its contents administered to the child. Special care was taken to avoid spillage in children receiving the DLW orally. Saliva and urine samples were obtained just before administration of the DLW on day 0. In the subsequent two weeks, preferably in the morning, five additional urine and saliva samples (day 1, 5, 8, 11 and 15) were taken. The researcher (RR) collected most samples, but some were collected by the daycare professionals.

Urine and saliva sampling

Diapers with cotton batting pads were used for collection of urine samples. Approximately 5 mL of urine was retained at each time point and stored in 30 mL glass urine bottles. Saliva was sampled by swabbing the mouth of the child with a cotton mouth swab for 2-5 minutes and then putting it in a plastic container (Salivette, Sarstedt, Nümbrecht, Germany). To avoid dilution of the sample, no drinks were allowed during the 30 minutes prior to the saliva sampling. The saliva container was then centrifuged (3000 rpm) and a clear, fluid sample (0.25 – 1.5 mL) was pipetted into a 2 mL glass vial. The vial containing saliva, and the urine bottle were flushed rapidly with nitrogen in order to reduce isotope exchange inside the sample containers. A glass vial was opted for because plastic is a semi-permeable material. All sample containers were stored frozen at -20° C prior to analysis.

To assess the feasibility of both urine and saliva sampling in these children, the occurrences and reasons for failed sampling or failed analysis were recorded.

Analysis and calculations

Enrichments of the DLW given and the collected urine and saliva samples were measured in a high-temperature conversion elemental analyzer (TC-EA) coupled with a Delta XP isotope ratio mass spectrometer (IRMS) via a Conflo-III Interface (Thermo Fisher, Bremen, Germany). Each sample was injected five times into the IRMS, the first two of which were excluded from the analysis to avoid possible memory effects.

Only data of children, for whom a minimum of three out of a possible five successfully analyzed samples of both urine and saliva could be obtained, were entered into the final analysis. In a child with the minimum requirement of three samples, at least the samples on day one and fifteen had to be successfully analyzed for the child to be included. Excluded samples contained either insufficient material or caused a spike in the residuals of the elimination curves of the isotopes. A sample causing a residual in either isotope that exceeded 0.03 in the log transformed enrichment data and was more than 1.5 standard deviations removed from the mean of all residuals was excluded from analysis.

TBW was calculated by estimating isotope dilution spaces (IDS) using the multipoint-procedure. In this procedure, distribution space is calculated by determining the isotope dilution at time zero by back extrapolation using the same data as that used to measure the slopes of the elimination of both isotopes. The isotope dilution spaces of ^{2}H and ^{18}O were calculated by using the following formula:

$$IDS(kg) = \frac{d}{MW} \times \frac{APE}{100} \times 18.02$$

Where d is the dose of the isotope in grams, MW is the molecular weight of the tracer, and APE is the atom percent excess.

Using the IDS of both ^{2}H and ^{18}O, TBW is calculated using the formula:

$$TBW(kg)=\frac{\frac{IDS_{2H}}{1.041}+\frac{IDS_{180}}{1.007}}{2}$$

The constants 1.007 and 1.041 were included to adjust for the differences between the isotope dilution spaces and TBW due to isotope exchange.[12] The observed $IDS^{2}H$ and $IDS^{18}O$ values were normalized by a fixed factor of 1.034.[13]

TEE can be calculated by using the following equation, adapted from DeWeir[14]:

$$TEE\ (kcal/day)=\frac{3.9rCO_2\ (liter/day)}{RQ}+1.11rCO_2$$

Where rCO_2 is expressed in liter/day and Respiratory Quotient (RQ) is oxygen consumption/rCO_2.

For all children but one an individual Food Quotient (FQ) was calculated on the basis of a three-day food questionnaire. FQ most closely approximates RQ.[15] In one child a food questionnaire was unavailable. For this child the mean FQ of the study population was used, which was 0.84.

rCO_2 was calculated using the following equation, which is an adapted version by Racette[12] of the original formula by Schoeller[7]:

$$rCO_2 = (N / 2.078)(1.007K_O - 1.041K_D) - 0.0246R_{Gf}$$

where N is total body water (TBW); K_O and K_D are the ^{18}O and ^{2}H isotope disappearance rates, respectively; and rGf is the rate of water loss through gaseous routes subject to isotope fractionation. The latter is estimated as 1.05 N (1.007 K_O – 1.041 K_D).

To account for the broad age range, TEE was also expressed as a percentage of Recommended Daily Allowance (%TEE of RDA) based on national recommendations of daily energy intake published by the Health Council of the Netherlands.[16] To account for body size, TBW was expressed as a percentage of total body weight, calculated by the multi-point procedure (%TBW).

Statistical analysis

The absolute differences of TEE and TBW between urine and saliva samples were expressed as percentages of the outcome based on the urine samples. Correlations of these absolute outcomes and those corrected for age and body weight (TEE of RDA and %TBW) of the two sampling methods were calculated using intraclass correlation coefficients (ICCs). To study the agreement between these urine and saliva outcomes, Bland and Altman limits of agreement analyses were performed.[17] This method uses a pair-wise comparison to show the mean difference and the limits of agreement (mean difference ± 2 SD of the difference) between the outcomes of urine and saliva samples by plotting their mean difference against the mean of the two sampling methods. All analyses were done using SPSS 15.0 software (SPSS Inc, SPSS for Windows, Chicago, Illinois, United States).

RESULTS

Characteristics of subjects

General characteristics of the thirteen subjects are summarized in Table 1. Ages ranged from 3 to 15 years. Eleven children were non-ambulatory (GMFCS 5), while two could walk with a walking aid for a short distance or crawl (GMFCS 4). Twelve children received food through a gastrostomy tube and had a severe intellectual disability (ID). One child received food orally and had a moderate ID.

Table 1. General characteristics

Total number (n)	13
Mean (± SD) age (y)	7.1 ± 4.0
Sex	7 m, 6 f
Mean (± SD) weight (kg)	21.8 ± 9.6
Aetiology (n)	
Congenital	3
Perinatal	6
Acquired	2
Combination	2

SD = standard deviation.

Feasibility

Urine was collected and analyzed successfully in 69 out of 78 cases (88.5%) and saliva in 62 out of 78 cases (79.5%). Table 2 shows the numbers of successfully collected and analyzed urine and saliva samples for each subject.

Table 2. Raw data of clinical outcomes

Patient	Age (y)	No of urine samples	No of saliva samples	TEE (kcal) urine	TEE (kcal) saliva	TBW (kg) urine	TBW (kg) saliva
1	15	5	4	864	816	11.7	12.3
2	7	3	4	529	494	9.0	8.9
3	5	4	5	741	721	7.1	7.2
4	3	5	3	838	871	6.6	6.8
5	4	4	3	430	455	5.2	5.4
6	11	5	3	1147	1185	17.5	18.3
7	5	4	3	688	689	8.8	8.6
8	13	5	5	945	1104	15.6	17.1
9	5	5	4	1430	1558	10.1	10.9
10	4	4	3	814	907	7.3	7.6
11	6	3	4	576	523	8.8	9.3
12	11	5	5	735	742	11.2	11.9
13	3	4	4	957	1059	7.6	7.9

TEE = total energy expenditure, TBW = total body water.

Collecting urine samples failed in four cases. In three cases the child had not urinated at the time of the measurement. Once a caregiver forgot to collect the sample. Five urine samples were excluded because they caused a spike in the residuals of the elimination curves. Saliva collection failed in five cases. The amount of saliva collected was insufficient in three cases. Once the caregiver forgot to sample and in one case the child had drunk just before sampling. Sampling failed for unknown reasons in one additional case. Ten saliva samples were excluded because of a spike in the residuals of the elimination curves.

Comparison of clinical outcomes

The absolute outcomes TEE and TBW for all 13 children are listed in Table 2. Table 3 shows the group means of the absolute outcomes and those corrected for age and weight for urine and saliva sampling. Furthermore, it describes the limits of agreement and intraclass correlation coefficients (ICCs) for the comparisons between the outcomes of urine and saliva.

Table 3. Statistical comparison of clinical outcomes

Outcome	Mean ± SD urine	Mean ± SD saliva	Mean difference ± 2 SD	ICC
Absolute				
TEE (kcal)	822 ± 265	856 ± 312	-33.1 ± 137.2	0.972
TBW (kg)	9.7 ± 3.6	10.2 ± 3.9	-0.5 ± 0.9	0.993
Relative to age and body size				
%TEE of RDA	47.1 ± 17.0	49.0 ± 19.7	-1.9 ± 7.5	0.979
%TBW	46.2 ± 6.0	48.0 ± 6.5	-1.9 ± 3.0	0.971

N = 13, Mean difference = urine minus saliva, TEE = total energy expenditure, TBW = total body water, SD = standard deviation, ICC = intraclass correlation coefficient, %TEE of RDA = percentage of expected TEE based on Dutch guidelines, %TBW = percentage total body water of body weight.

ICCs for the clinical outcomes of TEE and TBW were all above 0.9 as were the corrected outcomes %TEE for RDA and %TBW. Figure 1 depicts Bland and Altman plots of the comparison between outcomes of urine and saliva for %TEE for RDA (1a) and %TBW (1b).

DISCUSSION

This study shows that when using the DLW method, urine and saliva samples can be reliably obtained from most children with severe neurological impairment and ID, and successfully analyzed in the laboratory. Energy expenditure and total body water outcomes of urine and saliva samples agreed favorably.

The amount of total body water determined in saliva samples seems to be slightly higher than that determined in urine samples. This observation is at least partly corroborated by two studies by Wong et al.[18, 19] These two studies reported that IDS based on urine samples were consistently lower than those based on saliva samples. This trend will therefore also be present in TBW, since IDS divided by the isotope fractionation results in the TBW outcome. However, the differences in the calculated TBW outcomes from IDS based on urine and saliva samples were small and were not significantly different.

Besides the fact that other authors have found small differences in TBW outcomes between both sample media, an additive explanation for the slightly lower TBW outcomes using urine samples in the present study may be because, in contrast to Wong et al.[18], we collected urine using cotton batting pads in the diaper. The time between the production of urine in the diaper and its collection is unknown, whereas saliva is immediately collected after its production. In addition, the diaper may act as a delay

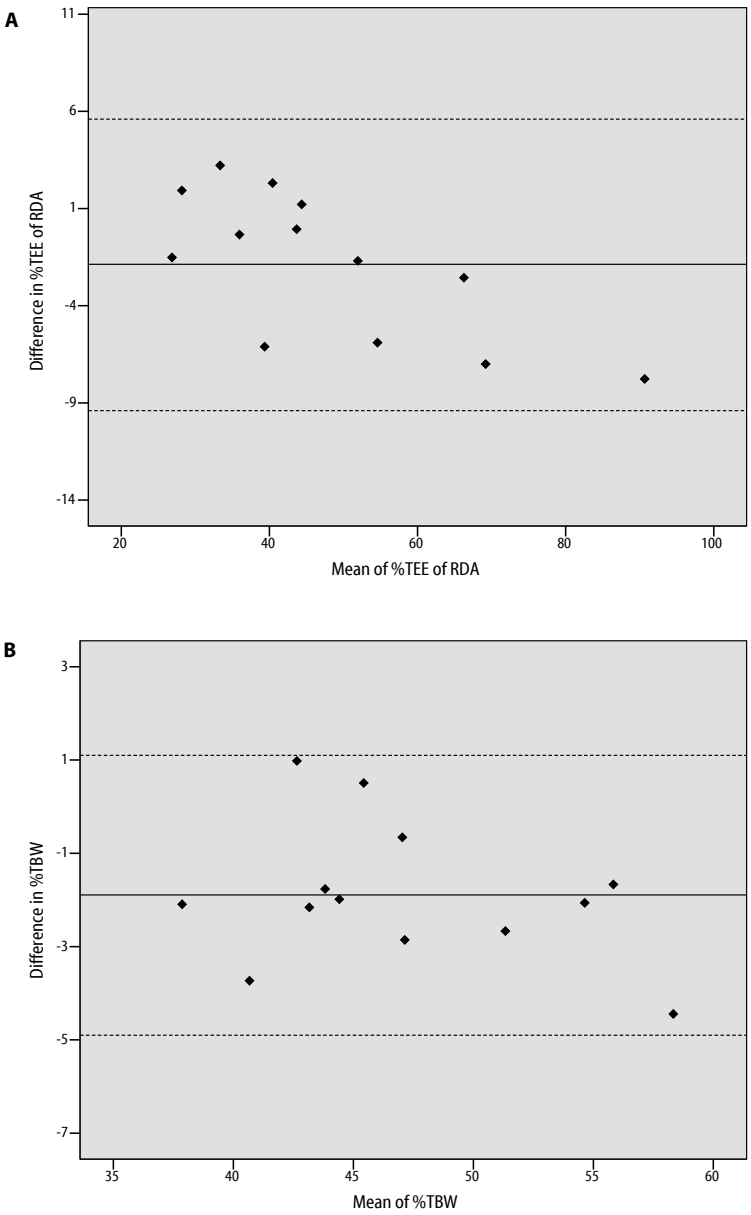


Figure 1. Bland and Altman plots of the differences between urine and saliva samples
Bland and Altman plots of the differences (urine minus saliva) of (a) percentage total energy expenditure of recommended daily allowance (%TEE of RDA) and (b) percentage total body water (%TBW) between urine and saliva samples (n=13).
On the x-axis the mean value of both recordings is displayed; the y-axis shows the difference between both values. The central dotted line indicates the mean difference of the study population, while the upper and lower dotted lines indicate the mean difference ± two standard deviations.

volume that pools older, more enriched urine with new production, analogous to the situation in the elderly where urine retention slows down equilibration of DLW isotopes.[20] Indeed, enrichments of urine samples were higher than those in saliva samples in a subset of ten children from the current study population. Data on these enrichments have been published in a separate paper by Schierbeek et al.[21] Consequently, the quantity of tracer eliminated will appear to be lower in urine samples, because of the time differential between the recorded time of collection, which was used for analysis, and the actual production, which might have taken place some time before collection. As a result the clinical outcomes will appear to be slightly lower than the outcomes for saliva. Accuracy of TBW estimates using urine samples might improve if time of collection is replaced with the mean time between putting the cotton batting pads in the diaper and the collection of a urine sample by the researcher.

TEE estimates should be relatively non-affected by the delay in collection as this outcome is based upon the difference between the elimination rates of both tracers, which is independent of the precise enrichments at the different sampling times. However, in the present study TEE is also slightly higher using the saliva samples. The relative inaccuracy of assessing the exact time of urine production might also play a role here. While not dismissing the differences of TBW and TEE in urine and saliva seen in this dataset, they are relatively small and clinically acceptable.

The observed variability is most probably not due to the material used to collect the urine and saliva samples. In the doubly labeled water technique, cotton balls are suitable for urine collection in children, if the volume of urine that is expressed from the cotton ball exceeds 5 mL.[22] Schoeller et al.[7] used cotton rolls to collect saliva samples in order to validate the enrichment of deuterium and labeled oxygen of the sample expressed from these rolls to those of serum samples. Enrichment levels were slightly higher in saliva than in serum, but the authors did not attribute the difference to the collection method. It is probably the result of evaporation of water in the mouth that preferentially removes the lighter isotopes of hydrogen and oxygen and results in a relatively more enriched saliva sample.[23] The only difference between their experiments and ours is that we were not able to dry our cotton sampling material in an oven and store it in a dessicator before sampling. This procedure was not possible in the daycare center, where we collected samples. Schoeller's results indicate that failure to dry the cotton rolls could have resulted in a 1% to 2% relative error in total body water determination.[7] However, since we used cotton sampling material for both urine and saliva, it probably did not add to the variability between these two media. It may, however, have produced a small error compared with more controlled hospital settings.

Conclusion

Both urine and saliva sampling are acceptable choices for measuring the clinical outcomes TEE and TBW using the DLW technique in children with severe neurological impairment and ID. However, in our view saliva sampling deserves slight preference, primarily because the time between production and collection is minimal and therefore circumvents the inaccuracy in sampling urine using diapers as discussed above. However, in some children issues such as hyposalivation can impair proper collection and analysis. Similarly, urine sampling may be difficult in children with little urine output. Such conditions were not encountered in the present study. Ideally, we recommend sampling of both media in isotope studies in children with neurological impairment and ID.

If time or money is constrained, either sampling method for an individual child is justified since we have demonstrated that clinical outcomes based on urine and saliva sample agree well.

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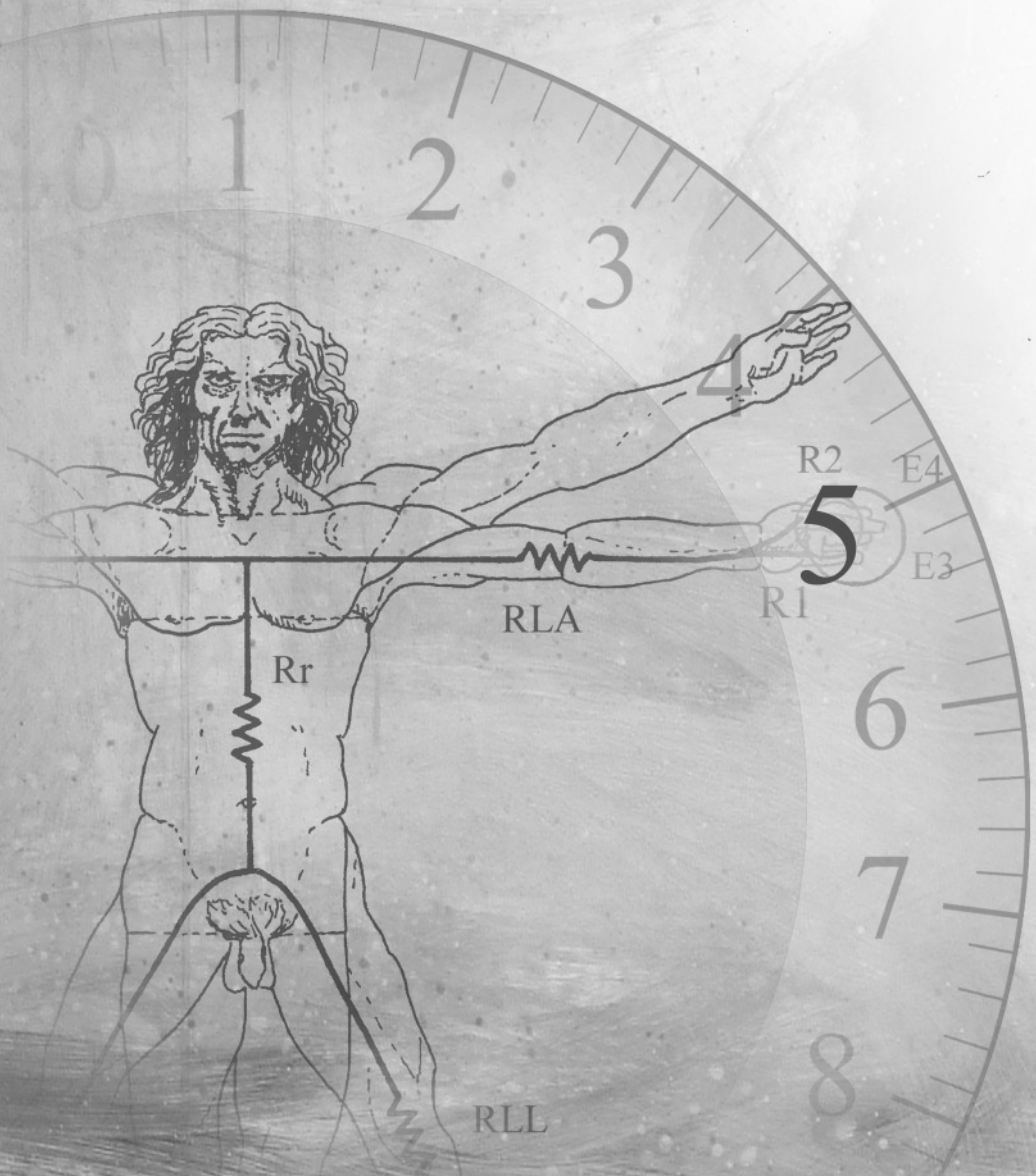
Chapter 5

Dual energy x-ray absorptiometry in children with severe neurological impairment and intellectual disability: artefacts and disrupting factors

Submitted



ABSTRACT

Aim To describe the occurrence of factors that may influence the feasibility of dual energy X-ray absorptiometry (DXA) and the accuracy of bone mineral density (BMD) outcome in children with severe neurological impairment and intellectual disabilities (ID).

Methods Based on literature and expert opinion a list of disrupting factors was developed. Occurrence of these factors was assessed in 27 children with moderate to severe ID and level IV or V on the gross motor function classification scale that underwent measurement of lumbar spine and total body BMD with DXA.

Results Disrupting factors that occurred most frequently were movement during measurement (81.5%), aberrant body composition (66.7%), small length for age (55.6%) and scoliosis (37%). The mean amount of disrupting factors per child was 5.3 (range 1-8).
The mean bone mineral density z-score for total body was -1.30 (SD 1.79) and for the lumbar spine (L2-L4) -2.41 (SD 1.18). No correlations were found between BMD z-scores and the number of disrupting factors.

Conclusion Factors that may negatively influence the accuracy of DXA bone mineral density outcome are frequently present in children with severe neurological impairment and ID. No structural deviation of BMD outcome in coherence with the amount of disrupting factors was found, but physicians should be aware of the possible negative influence of disrupting factors on the accuracy of BMD outcome in children with severe neurological impairment and ID, when interpreting the test results.

INTRODUCTION

Reduced bone health in children with severe neurological impairment and intellectual disability (ID) has become a topic of increased concern and research over the last years.[1-6] Nowadays it is established that children with moderate to severe cerebral palsy (CP), who usually experience many additional health problems, have an increased risk of developing low bone mineral density (BMD). Their risk of low impact fractures is therefore increased.[1, 7, 8]

To determine BMD, dual energy x-ray absorptiometry (DXA) is generally accepted as the method of choice. However, it is known that in children the accuracy of its outcome is diminished by several factors, such as variability in skeletal size and body composition.[9-11] Complementary, several studies have reported on additional artefacts and their influence on DXA results in the general population or in other patient groups.[12-16] Disrupting factors may lead to both underestimation and/or overestimation of BMD.[9, 13, 15, 17-19] Operator-related artefacts, e.g. using an incorrect region of interest or an inappropriate reference database, can be overcome by using an experienced and trained operator who is familiar with the applied DXA equipment and software.[13, 16] However, patient-related artefacts are more difficult to deal with, e.g. severe contractures or orthopaedic hardware following scoliosis operation.[15, 16, 20] While performing DXA measurements in children with severe neurological impairment and ID we noticed that disrupting factors are frequently present. As far as we are aware, there are no studies on the frequency of factors that may negatively influence accuracy of DXA outcomes in children with severe neurological impairment and ID. Likewise it is not clear whether presence of these factors may lead to a structural under or over diagnosis of low BMD in this group. This limits our knowledge of the feasibility of DXA in this specific group of children.

Objectives

Our main objective was to describe which factors should be considered to reduce accuracy of DXA BMD outcome and to determine their frequency in a group of children with severe neurological impairment and ID that underwent DXA examination. Supplementary we studied the correlation between the individual number of disturbing factors and lumbar spine or total body BMD values to observe whether these factors might lead to structural deviation of BMD outcomes.

MATERIALS AND METHODS

Study design

This study consisted of two separate parts. First, a checklist with known disrupting factors was developed. Secondly we cross-sectionally assessed the presence of these disrupting factors in 34 children with severe neurological impairment and ID who underwent DXA examination within the framework of a larger study on validation of nutritional assessment techniques, that had been approved by the Dutch Central Committee on Research Involving Human Subjects (The Hague, the Netherlands, P05.0102C).

Checklist

A literature study was carried out using Medline, in order to develop an overview of reported disrupting factors and artefacts. Disrupting factors according to five experts on (paediatric) DXA measurements were added. The respondents were a paediatric DXA operator, a paediatric-endocrinologist, a paediatric radiologist, an internist-endocrinologist and a radiotherapist. All had vast experience with DXA measurements for diagnostic and research purposes (mean years experience 17 years) and two of them were familiar with the target population (mean years experience 5.5 years). They were asked to answer the following questions in a questionnaire:

Which factors negatively influence the accuracy of DXA results?

To what extent do these factors disrupt the DXA results in children with severe neurological impairment and ID (hardly – extremely disturbing, 5-point scale).

All disrupting factors and artefacts were recorded in a checklist for clinical purposes (supplement 1).

Study population

Children, aged between 2 and 19 years, with severe neurological impairment and ID, known to have a moderate to severe intellectual disability (IQ< 55) and a gross motor function classification scale (GMFCS)[21] of level IV or V were recruited through children's day care centres.

DXA scan

Measurements of bone mineral density were performed by DXA (Lunar, DPXL/PED, Winconsin, USA). DXA values of the lumbar spine and total body were compared with normative data of healthy Caucasian children obtained by Van der Sluis et al.[22] and were converted to age and gender related z-scores. Low BMD was diagnosed if a z-score of -2.0 or below was obtained.[22] All DXA measurements were done by the

same well trained operator, who is experienced in working with children with intellectual disabilities. None of the children received sedating medication prior to the measurement. One of the researchers (RR) assisted during all DXA measurements and a parent or carer was present as well to reassure the child. In order to prevent movement during the recording the child was manually immobilized by the researcher and parent/carer. Attention was paid not to influence the DXA measurements. The operator aimed at obtaining the most optimal scanning result; therefore artefacts were removed if possible (eg metal objects on clothing) or otherwise excluded from the scan results (eg projection of the gastrostomy catheter onto a lumbar vertebra). All artefacts for which adjustment of the scan was needed were counted.

Evaluation of artefacts and disrupting factors included in the checklist

After the scan, the operator recorded specific details and presence of artefacts on the test outcome form. During DXA measurements, the child's level of movement was recorded on a four point scale, ranging from four points allocated to a child that was lying completely still, to one point allocated to the extremely movable patient.

Factors with regard to growth and nutritional status had been assessed within the framework of the larger study on nutritional assessment techniques.[23] Shortly, body height (cm) was measured with a flexible tape line and compared to Dutch reference values as provided by Growth Analyser 3.5 (Dutch Growth Foundation, 2007). A child was diagnosed with "small bones" if it had a body height below the 5th percentile for its age group. Complementary, triceps and subscapular skinfold thicknesses (mm) were measured with a Harpenden skinfold calliper (John Bull, England); these sites are most commonly included in equations on body fatness. Skinfold thickness was measured three times at each site, mean values were calculated and used for further analyses. Percentile scores in comparison with healthy corresponding gender and age groups were calculated using Dutch reference values of Gerver and de Bruin[24] and categorized as low (≤ 3rd percentile), normal (between 3rd and 97th percentile) or high (≥ 97th percentile). If there was a discrepancy in outcome between percentiles of subscapular and triceps skinfolds (e.g. triceps in the low and subscapular in the normal percentile group or triceps in the normal and in the subscapular high percentile group), the child was considered to have an aberrant subcutaneous fat distribution.

Medical history and medication were recorded from patient files; data on operations on the lumbar spine, presence of intracorporal devices, use of contrast agents, presence of calcinosis and use of calcium tablets were recorded.

Mobility according to the Gross Motor Function Classification System[21] and the presence of contractures or scoliosis were assessed by observation and performing physical examination if necessary.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences for Windows 15.0 (SPSS Inc., Chicago, IL., USA).

Descriptive statistics are reported as number of cases and percentages. Pearson correlations coefficients were calculated for bone density outcome measures of total body and spine and the number of disturbing factors per child. After dichotomizing BMD z-scores in low (≤ -2) and normal (> -2), an unpaired t-test was performed to assess the difference in mean number of disturbing factors in these groups.

A p-value of less than 0.05 was considered statistically significant.

RESULTS

The list of disrupting factors and artefacts according to literature findings and expert opinion is presented in Table 1.

Information on the presence or absence of all disrupting factors and outcome of the DXA measurement were available from 27/34 children. In three children skinfold thickness was not assessed and in four children no information was present on movement during examination, therefore they were excluded from analysis. Patient characteristics are summarized in Table 2. The children all had moderate to severe intellectual disabilities as well as severe motor disabilities: most scored level V on the Gross Motor Function and Classification Scale[21] and were unable to walk independently.

The mean amount of distorting factors and artefacts per child was 5.3 (range 1-8) (Figure 1). Five children (18.5%) had a mean of 4.6 contractures (range 2-8). In 10 children scoliosis was apparent (37.0%) and in one child (3.7%) the scoliosis was corrected with osteosynthesis materials in situ. Fourteen children (58.8%) had an intracorporal medical device, all of them in the form of a gastrostomy catheter, but only in 2 cases (7.4%) the catheter projected upon the lumbar spine. Additionally one of these children had an intrathecal pump for baclofen medication. During DXA examination five children (18.5%) were completely immobile and 26 children (81.5%) were moving with severity of movement varying from some movement till extreme movement. In our study population 15 children (55.6%) had a Z-score lower than –2 SD for length for age and 13 out of these 15 children had a Z-score lower than –2.5 SD. Nine children (29.0%) had a subscapular skinfold above the 97th percentile for their age and gender group and 15 children (48.5%) had a triceps skinfold on or below the 3rd percentile for their age and gender group. After categorizing skinfold outcomes of subscapular and triceps measuring sites in either low (≤ 3rd percentile), normal

Table 1. List of factors that might disrupt outcome of DXA measurements in children with severe neurological impairment and ID, including observed frequencies in the study group (N=27)

Factors		N	%
Contractures*		5	18.5
Scoliosis*		10	37.0
Movement during measurement*	Complete immobilisation	5	18.5
	Some movement	11	40.7
	Considerable movement	7	25.9
	Extreme movement	4	14.8
Orthopaedic hardware*		1	2.9
Small bones (length for age)	<p5 height for age	15	55.6
Intracorporal medical devices	Intrathecal pump	1	3.7
	Gastrostomy catheter	14	51.9
	With projection on lumbar spine	2	7.4
(Crush) fractures		0	0
Other vertebral anomalies (e.g. spondylodesis, osteoarthritis, spinal implants, laminectomy)		2	7.4
Jewellery or objects on clothing		1	3.7
Dense metal objects (e.g. bullet, large collection of clips)*		0	0
Metastatic lesions		0	0
Vascular/aortic calcification or calcified tendonitis and anostosis		0	0
Calcinosis or calculi		1	3.7
Use of contrast agents or undissolved calcium tablets in GI-tract		0	0

* Factors considered very or extremely disturbing by expert opinion.

Table 2. Patient characteristics (N=27)

		N	%	Mean (range)
Gender	Female	14	51.9	
	Male	13	48.1	
Age in years				8.5 (3-17)
Severity of intellectual disability	Moderate (IQ <50)	2	7.4	
	Severe (IQ< 35)	25	92.6	
GMFCS*	Level IV	2	7.4	
	Level V	25	92.6	

* GMFCS: Gross Motor Function Classification System[21].

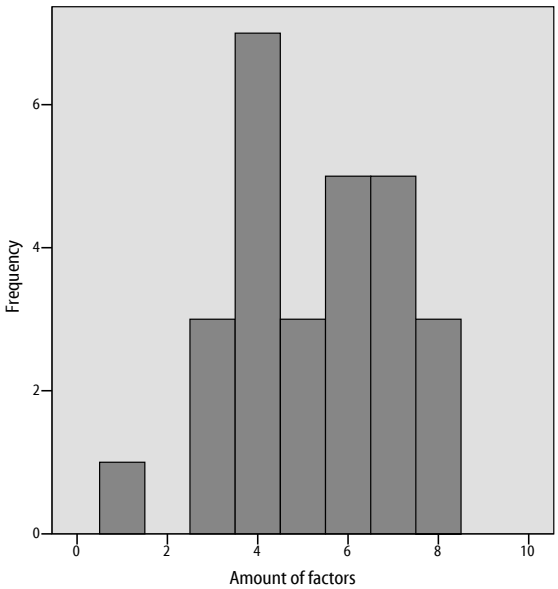


Figure 1. Distribution of disrupting factors in children in 27 children with severe neurological impairment

(between 3rd and 97th percentile) or high (≥ 97th percentile), in 21 children (67.6%) an aberrant body composition was identified. In none of the children the triceps skin fold thickness exceeded the subscapular skin fold thickness.

In spite of the disrupting factors, BMD results were present for lumbar spine and total body in all 27 children. The mean BMD z-score for total body DXA was -1.30 (SD 1.79) and the mean BMD z-score for the lumbar spine (L2-L4) was -2.41 (SD 1.18). A significant correlation between absolute BMD values of the total body and of the lumbar spine was observed (p=0.001). This correlation was not present between both BMD z-scores (p=0.455).

There was no significant correlation between the amount of disturbing factors and the BMD value of the total body (p=0.226), the BMD z-score of the total body (p=0.755), the BMD value of the lumbar spine (p=0.492) or the BMD z-score of the lumbar spine (p=0.192). Comparison of children with and without low BMD (defined as Z-score < -2.0) in total body or lumbar spine showed no significant difference in mean number of disrupting factors (Table 3).

Table 3. Mean number of disrupting factors in children with and without low BMD (N=27)

	Total body BMD Z-score		Lumbar spine BMD Z-score	
	Low (≤ -2.0)	Normal (> -2.0)	Low (≤ -2.0)	Normal (> -2.0)
	(N=11)	(N=16)	(N=18)	(N=9)
Mean number of disturbing factors	5.5 (SD 2.25)	5.1 (SD 1.50)*	5.6 (SD 1.98)	4.7 (SD 1.32)**

* p=0.65
** p= 0.24

DISCUSSION

As expected, factors that may negatively influence the accuracy of DXA measurements are frequently present in children with severe neurological impairment and ID, with a mean of 5.3 factors in 27 children. The most frequently occurring factors are movement during measurement, scoliosis, contractures, gastrostomy catheters, aberrant body composition and a height below the 5th percentile for age. We found no indication for a structural overestimation or underestimation of BMD outcome in coherence with the amount of disrupting factors. Therefore, it remains unknown to what extent DXA outcomes are disrupted if one or more artefacts are present.

Published studies on disrupting factors in DXA examination are mostly descriptive and frequently performed in older patients or postmenopausal women.[14, 17, 18, 25] Most studies describe only one of the factors in detail and we found no studies determining the total amount of disrupting factors present in specific patient groups. Therefore, it remains unknown whether the number of disrupting factors is higher in this population than in others. Our finding of a mean of five disrupting factors per child however implicates that DXA outcomes in children with severe neurological impairment and ID run a risk to be inaccurate. The lack of correlation between the amount of disrupting factors and BMD values can be explained by the relatively small study population (n=27) and the fact that the disrupting factors may both lead to overestimation and underestimation of bone density.[3, 9, 13, 15, 17-19] The question whether BMD outcome in children with severe neurological impairment and ID deviates in a structural way as a result of disrupting factors, can therefore not be thoroughly answered. Additional information on the presence of artefacts is however important to interpret the results of the individual bone density measurements, not only for single BMD measurements but especially for repeated measurements in which the presence of disrupting factors may differ.

Most of the reported disrupting factors are hard to avoid but movement during measurement might be diminished by giving sedative medication in advance[3] or by

placing sand cushions or straps to prevent movement. However, considering that these measures impose restraints on the children that undoubtedly will increase stress and knowing that sedatives might cause side effects like cardio respiratory depression or vomiting and aspiration[26], the advantages and disadvantages of those measures should be regarded individually.

As we stated earlier, the accuracy of DXA is largely dependent on the experience of the operator, by using appropriate regions of interest and removing artefacts whenever possible. Therefore we highly recommend that all clinical centres where DXA recordings are performed in children with severe neurological impairment and ID, should designate an operator with special interest, who gains experience while performing all measurements in this group. In addition, this operator should routinely record present disrupting factors and send them together with the scan results to the referring physician. For this purpose we developed the checklist added as a supplement to this article (supplement 1).

In the Netherlands it is common to measure bone density in children by performing DXA of the lumbar spine and total body.[22] However, Henderson and colleagues have indicated that measurement of the BMD of the distal femur projected in a lateral plane in children with moderate to severe CP or muscular dystrophy has a strong correlation with fracture history.[27] This specific scanning technique may diminish the amount of disturbing factors as well, e.g. no projection of scoliosis or intracorporal devices on the lumbar spine, less positioning problems due to contractures and probably less movement during examination because patients are lying on their side. Development of reference values for distal femur BMD for the different DXA devices and standardization of the measurement procedure may be an important step in standardizing diagnosis of low bone mineral density in children with severe neurological impairment and ID.

In conclusion, in children with severe neurological impairment and ID the frequently present disrupting factors may influence the feasibility of DXA and the accuracy of its outcome. While treatment of low bone density in practice is reserved for children with (low impact) fractures, this distortion presumably has had limited effect on treatment frequency. However, alterations in artefacts over time may complicate comparison of successive outcome values in the individual child. In addition, the effectiveness of preventive measures can only be determined if accurate and reliable bone density measurements are available. Therefore, either more information on the impact of individual disrupting factors is necessary or other methods or localisations for bone density measurement that are less prone to distortion, should be introduced for this population.

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SUPPLEMENT 1

Checklist of artefacts and disrupting factors during
DXA in children with severe neurological impairment and ID

Name patient:
Date of birth:
Date of examination:
Name operator:

Location DXA measurement:

- ☐ Total body
- ☐ Lumbar spine
- ☐ Proximal femur
- ☐ Distal femur

Movement during measurement *: yes/no

- ☐ Completely immobile
- ☐ Some movement
- ☐ Considerable movement
- ☐ Extreme movement

Aberrant body composition *:

Triceps skinfold: …. mm
Percentile for age: p … p value <p5: yes/no
Subscapular skinfold: …. mm
Percentile for age: p … p value <p5: yes/no

INTRACORPORAL MEDICAL DEVICES (E.G. GASTROSTOMY CATHETER, INTRATHECAL BACLOFEN PUMP)

Present: yes/no
Kind of device: ……
Projection on region of interest: yes/no

Scoliosis *: yes/no

Height: …….. cm

Percentile height for age: p… P value < p5: yes/no

Contractures *: yes/no

Location(s) of contracture(s): …….

Orthopaedic operations in patient history: yes/no

Orthopaedic hardware present *: yes/no

Vertebral crush fracture(s) present: yes/no

OTHER VERTEBRAL ANOMALIES PRESENT

(e.g. spondylodesis, osteoarthritis): yes/no

DENSE METAL OBJECTS PRESENT*

(e.g. bullets, collection operation clips): yes/no

Calcinosis of calculi present: yes/no

Recent use of contrast agent: yes/no

Calcifications present (e.g. vascular/aorta, tendinitis): yes/no

Metastatic lesions: yes/no

* Factors considered very or extremely disturbing by expert opinion.

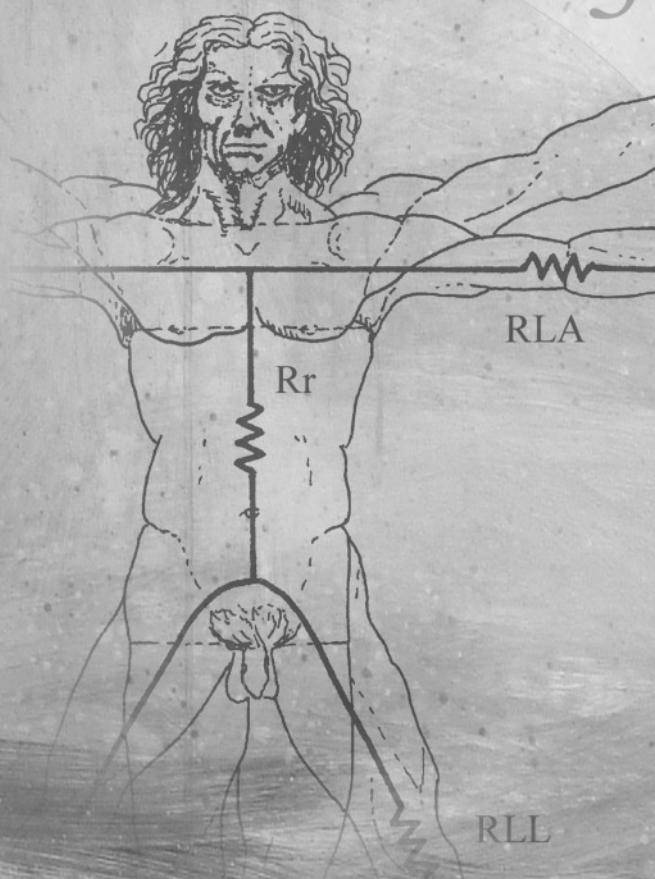
One or more yes answers on this checklist may implicate that the DXA outcome is less reliable; the extent of disruption depends on the factor involved and the degree of disturbance (e.g. movement).

In case of doubt concerning the interpretation of these results contact the operator for consultation.

Chapter 6

Developing new equations to predict body composition from bio-impedance and skinfold thickness in children with severe neurological impairment and intellectual disability

Submitted



ABSTRACT

Background Undernutrition and overnutrition are relevant problems in children with severe neurological impairments and intellectual disability (ID). However, accurate interpretation of commonly applied nutritional assessment methods is hampered by the lack of group-specific equations.

Objective The aim of this study was to develop new group-specific regression equations for predicting body fat based on skinfold measurements and bioelectrical impedance analysis (BIA).

Design We included 61 children with severe neurological impairments and ID (age: 10.1±4.3 yrs; 32 m). Percentage of body fat, estimated by current equations using two or four skinfolds, and total body water, estimated by a current BIA equation, were compared with the outcome of the doubly labeled water method (reference method). The strength of these comparisons was tested using intraclass correlation coefficients (ICC). Group-specific regression equations were developed using multivariate analyses.

Results Percentage of body fat was underestimated when using current skinfold equations (ICC: 0.47 – 0.56). The new equation, based on the sum of four skinfold measurements, did not improve agreement (ICC=0.61). TBW was overestimated by the BIA equation (ICC=0.94). A newly-developed equation included tibia length as an alternative for standing height. This new equation improved agreement (ICC=0.96).

Conclusions The current prediction equations using skinfold measurements underestimate the percentage of body fat in children with severe neurological impairments and ID, rendering them of little use in clinical practice. The agreement of the newly-developed BIA equation and the reference method was high. BIA is a more accurate method of assessing nutritional status in this population than measurement of skinfold thickness.

INTRODUCTION

Both undernutrition and overnutrition are major health concerns in children with severe neurological impairment and intellectual disability (ID).[1] However, measuring nutritional state in these children is challenging. While they are easily weighed, accurately measuring their height is often not possible. As height is used in parameters to describe nutritional state (i.e. weight for height) alternative ways of measuring body height in these children, i.e. segmental measures such as knee height and tibia length, have been proposed.[2, 3]

To complement measurements of weight and height, body composition is often used to measure the nutritional state of children with neurological impairments.[4] The components of body composition that are frequently measured include fat mass (FM), fat free mass (FFM), total body water (TBW) and percentage of body fat (%BF). The only way body composition can be directly measured is after death through carcass incineration; all other methods measure body composition indirectly. Of these methods, the doubly labeled water (DLW) method and dual energy x-ray absorptiometry (DEXA) measure body composition components most accurately and have been used as reference methods to evaluate the validity of other methods that are more easily applied outside the hospital setting.[4-8] These include measuring skinfold thickness and bioelectrical impedance analysis (BIA). The latter two methods are considered valid when applied in healthy children[9-11], but their validity in children with neurological impairments is a matter of debate. Earlier studies compared outcomes of skinfold measurements and BIA different reference methods in this population.[4-8] In a recent review by our group[12], we concluded that no definitive conclusions can be drawn from these studies about the validity of either skinfold measurements or BIA, because sample sizes were small, methodological quality was often insufficient and the statistical methods used were often inappropriate.

The aim of this research was to study the accuracy of body composition measurements taken by skinfold measurements and bioimpedance in 61 children with severe neurological impairment and intellectual disability. Using established equations measurement outcomes were compared with those of a reference method: the DLW method. The second aim was to develop easy-to-use population-specific prediction equations for skinfolds and BIA in order to calculate body composition.

MATERIALS & METHODS

Subjects and design

A sample consisting of 61 children with severe neurological impairment and ID (32 males and 29 females) was entered into the study. The parents or legal guardians of the children provided written informed consent. The Dutch Central Committee on Research Involving Human Subjects approved the study protocol (P05.0102C).

To be eligible for inclusion, children had to be between 2 and 19 years of age, have a proven or estimated IQ under 55 and a motor impairment, defined as hypertonic or hypotonic generalized CP, or a motor developmental delay to such an extent that the child could at best crawl. The motor impairment had to correspond with the Gross Motor Function Classification System (GMFCS) levels four and five.[13] Children that had contracted an active infection or had an altered water balance (edema or dehydration) at the time of the study were excluded.

The doubly labeled water technique, anthropometric measurements and bioelectrical impedance analysis were performed on-site at the children's day care centers on the same day.

Doubly labeled water technique

Subjects were studied at least 3 h after their regular morning feeding. They received a weighted dose of $^{2}H_{2}{}^{18}O$ ($^{2}H_{2}O$: 10%, $H_{2}{}^{18}O$: 5%, Cambridge Isotope Laboratories, Woburn, MA, distributed by Buchem BV, Apeldoorn, The Netherlands) of 3 g kg^{-1} body weight [14] orally or via a gastrostomy tube. The DLW container was washed out with 50cc of plain tap water and this was also administered to the child. Children with severe oral-motor impairment were given the DLW in a sipping cup with a lid or through a plastic syringe in the corner of the mouth to minimize spillage. If spillage did occur, the fluid was caught using the child's own bib. It was then weighed to determine the amount of spillage.

Baseline saliva and urine samples were taken just before administration of the DLW. In the subsequent two-week period, five additional samples were collected on days 1, 5, 8, 11 and 15. Diapers with cotton batting pads were used for collection of urine samples. Saliva was sampled by swabbing the mouth of the child with a cotton mouth swab for 1 to 2 minutes and then putting it in a plastic container (Salivette, Sarstedt, Nümbrecht, Germany). Children were not allowed to drink any liquids 30 minutes prior to the saliva sampling to avoid dilution of the sample. Enrichment of the isotopes in the samples was measured in quintuplet in a high-temperature conversion elemental analyzer (TC-EA) coupled with a Delta XP isotope ratio mass spectrometer via a Conflo-III Interface (Thermo Fisher, Bremen, Germany). TBW was estimated from

Table 1. Equations for estimating percentage of body fat from skinfold measurements

Generic equations				
Slaughter[11] (sum of 2SF ≤ 35mm)				
	Males	prepubescent	white	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 1.7
			black	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 3.2
		pubescent	white	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 3.4
			black	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 5.2
		postpubescent	white	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 5.5
			black	%BF = 1.21*(sum of 2SF) - 0.008*(sum of 2SF)2 – 6.8
	Females	all		%BF = 1.33*(sum of 2SF) - 0.013*(sum of 2SF)2 – 2.5
Slaughter (sum of 2SF> 35mm)				
	Males	all		%BF = 0.783*(sum of 2SF) + 1.6
	Females	all		%BF = 0.546*(sum of 2SF) + 9.7
Durnin[10]				
	Males	prepubescent		density = 1.1690 - 0.0788*log(sum of 4SF)
		(post)pubescent		density = 1.1533 - 0.0643*log(sum of 4SF)
	Females	prepubescent		density = 1.2063 - 0.0999*log(sum of 4SF)
		(post)pubescent		density = 1.1369 - 0.0598*log(sum of 4SF)
Conversion to body fat				%BF = ((4.95/density) – 4.5)*100
Population-specific equation				
Gurka[18], correction factors to Slaughter's equation:				
	Overall			+ 12.2%
	Additional corrections:			
	More severe GMFCS			+ 5.1%
	Males			- 5.0%
	Black race			- 3.1%
	Pubescent			+ 2.0%
	Postpubescent			- 4.6%
	Sum of 2SF > 35 mm			- 3.2%

Sum of 2SF = sum of triceps and subscapular skinfold, Sum of 4 SF = sum of biceps, triceps, subscapular and suprailiacal skinfold, %BF = percentage body fat, prepubescent = tanner stages 1 and 2, pubescent = tanner stage 3, postpubescent = tanner stages 4 and 5, (post)pubescent = tanner stages 3 to 5, GMFCS = gross motor function classification system.

the ^{18}O and ^{2}H dilution spaces.[15] Saliva samples were used if they were sufficient to provide at least three successful measurements. In all other cases, urine samples were used. FFM was derived using age-related proportions of water in FFM as suggested by Fomon et al.[16] and Boileau et al.[17] After FFM was subtracted from body weight, the resulting FM was expressed as a percentage of total body weight, i.e. %BF.

Anthropometry

Tibia length was measured to the nearest 0.1 cm with a flexible tape and weight was measured to the nearest 0.1 kg using an electronic wheelchair scale (Universal PM 7050, Lopital), or a digital sling scale. Height was measured in triplicate using a flexible tape measure from crown to heel while the child was in the recumbent position.

Skinfold thickness was measured at four sites (triceps, biceps, subscapular, suprailiacal) with Harpenden skinfold calipers (John Bull, England). All measurements were made in triplicate to the nearest 0.1 mm by two investigators (RR and an experienced anthropometrist) and the mean of the measurements was used for analysis. Interobserver error for skinfold measurements was tested in a separate sample of 12 children (TEM<1 mm). Measurements were taken on the left side of the body. %BF was calculated from two skinfolds (triceps and subscapular) using the equations by Slaughter et al.[11] and Gurka et al.[18] and from four skinfolds (biceps, triceps, subscapular and suprailiacal) using the equation by Durnin et al.[10] These equations are shown in Table 1. Because pubertal state is a component of the equation by Slaughter et al.[11], secondary sex characteristics (breasts in females; pubic hair in males) were assessed using the methods of Tanner.[19]

Bioelectrical impedance analysis

Bio-impedance was measured at least four hours after the last meal using a single-frequency Bio-impedance Analyser (Akern SRL, Florence, Italy), which uses the tetrapolar technique. Two electrodes (BIAmed® electrodes, Zwaag, The Netherlands) were placed on the dorsal side of the hand and two on the foot. The active electrode gives a constant electrical current of 800 microampere at a constant frequency of 50 kHz. The child was positioned using the methods previously described by Veugelers et al.[20] The children were in a resting supine position for 10 minutes prior to starting the recording. The child was gently put in the prescribed position, with arms and legs stretched and 30° abducted from the trunk. If necessary, the investigator fixed the limbs with a flannel blanket during the recording. If the legs could not be separated due to contractures, a pillow was put between the legs to avoid skin contact. During the recording, resistance (Rz) and reactance (Xc) values were recorded in triplicate and mean values were calculated.

TBW was calculated using the equation by Pencharz et al.[21], which is based on height (H) in cm and resistance (Rz) in Ohm:

$$TBW(kg) = 2.99 \times 0.649 \frac{H^2}{Rz}$$

Statistical analysis

Percentage of body fat according to the DLW method (%BF-DLW) was used as the reference value to compare the same outcomes using the Slaughter equation (%BF-SLAUGHT) and the Gurka equation (%BF-GURKA), based on two skinfolds, and the Durnin equation (%BF-DURNIN), based on four skinfolds. TBW determined using the DLW method (TBW-DLW) was used as the reference value to be compared with TBW obtained from the Pencharz equation (TBW-PENCHARZ), based on bioimpedance values using BIA.

The strength of the relation between the outcomes of these equations and the corresponding outcome of the DLW method was evaluated using intraclass correlation coefficients (ICCs). Agreement was evaluated using the Bland and Altman limit of agreement analysis. Explained variance was expressed as R^2.

Forward-stepwise-multiple-correlation-regression analysis was used to determine the best predictors of %BF-DLW for a skinfold equation and of TBW-DLW for a BIA equation. An F-test was used for the selection of variables. An alpha of 0.05 was used as a cutoff point for selection. In order to create an equation that uses the skinfold measurements, the sum of four skinfolds, weight, age, sex and tibia length were entered into the analysis. In order to create an equation that uses the bioimpedance measurements from BIA, tibia length squared divided by the resistance (Rz), reactance (Xc), weight, age and sex were entered. In developing an equation for BIA most authors have included some variable of height squared divided by resistance.[22] Since height is not easily measured in these children, we chose to use a segmental measure, i.e. tibia length. After selection of the best predictors, they were entered into a linear regression analysis in order to formulate the equation. A bootstrap analysis was performed in order to provide cross-validation. After cross-calibration, the root mean squared error (RMSE) and ICC of the predicted value versus the value of the reference method were calculated. All analyses were performed using the SPSS software, version 18.0 (SPSS Inc, SPSS for Windows, Chicago, Illinois, United States) and R project software, version 2.10.1 (R foundation, Vienna, Austria).

RESULTS

Subject characteristics

The characteristics of the study group are summarized in Table 2. Six out of 61 children were ambulatory (GMFCS 4), the other children were all non-ambulatory (GMFCS 5). Twenty-eight out of 61 children were fed through a gastrostomy tube, either exclusively or as an adjuvant to oral feedings. Thirty-four children were prepu-

bescent (Tanner stages 1 or 2), twenty-seven children were either pubescent (Tanner 3) or postpubescent (Tanner 4 or 5). Most children, 41 out of 61, lived at home. Mean percentage body fat, TBW and tibia length are also described in Table 2.

Table 2. General characteristics

Total number (n)	61
Mean (± SD) age (y)	10.1 ± 4.3
Gender	32 m, 29 f
Mean (± SD) weight (kg)	30.4 ± 14.9
Mean (± SD) length (cm)	132.2 ± 21.1
Mean (± SD) tibia length (cm)	29.9 ± 6.4
Aetiology (n)	
Congenital	14 (23%)
Perinatal	21 (34%)
Acquired	4 (7%)
Combination	4 (7%)
Idiopathic / unknown	18 (30%)
Mean (± SD) %BF	38.1 ± 10.6
Mean (± SD) TBW (kg)	13.6 ± 5.9

SD = standard deviation, %BF = percentage of body fat, TBW = total body water.

Comparison of outcomes using previously published generic pediatric prediction equations

Measuring bio-impedance using BIA was successful in all children. In seven out of 61 children measuring either the subscapular or the triceps skinfold failed. It was not possible to measure all four skinfolds in an additional five children. Most children in whom skinfold measurement failed had a gastrostomy tube. During the measurements their skinfolds could not be easily separated from the underlying muscle tissue.

Table 3. Comparison of agreement of outcomes of skinfold measurements and of the reference method (%BF) according to previously established generic or specific equations and the newly-developed group-specific equation from this paper

Number of skinfolds	Equation	ICC	Mean difference ± 2 SD	R square
Generic equations				
Two (N=54)	Slaughter[11]	0.47	23.5 ± 16.9	0.35
Four (N=49)	Durnin[10]	0.56	18.5 ± 15.1	0.46
Group-specific equations				
Two (N=54)	Gurka[18]	0.51	9.2 ± 16.7	0.35
Four (N=49)	This paper	0.61	---	0.44

Table 4. Comparison of agreement of outcomes of bioelectrical impedance analysis and of the reference method (TBW) according to a previously established generic equation and the newly-developed equation from this paper

Equation	Measure of height used in equation	ICC	Limits of agreement	R square
Pencharz[21] (N=61)	Flexible tape measure	0.94	-2.6 ± 4.4	0.88
This paper (N=61)	Tibia length	0.96	---	0.92

The Bland and Altman limit of agreement analyses between %BF obtained from skinfold measurements using either the Slaughter, Durnin or Gurka equation and %BF of the reference method are presented in Table 3. The Bland and Altman limit of agreement analyses between TBW of BIA using the Pencharz equation and TBW obtained from the reference method are presented in Table 4. The corresponding Bland and Altman plots for %BF, measured with skinfolds, and TBW, obtained from BIA, versus the equivalent outcomes of the reference method are presented in Figure

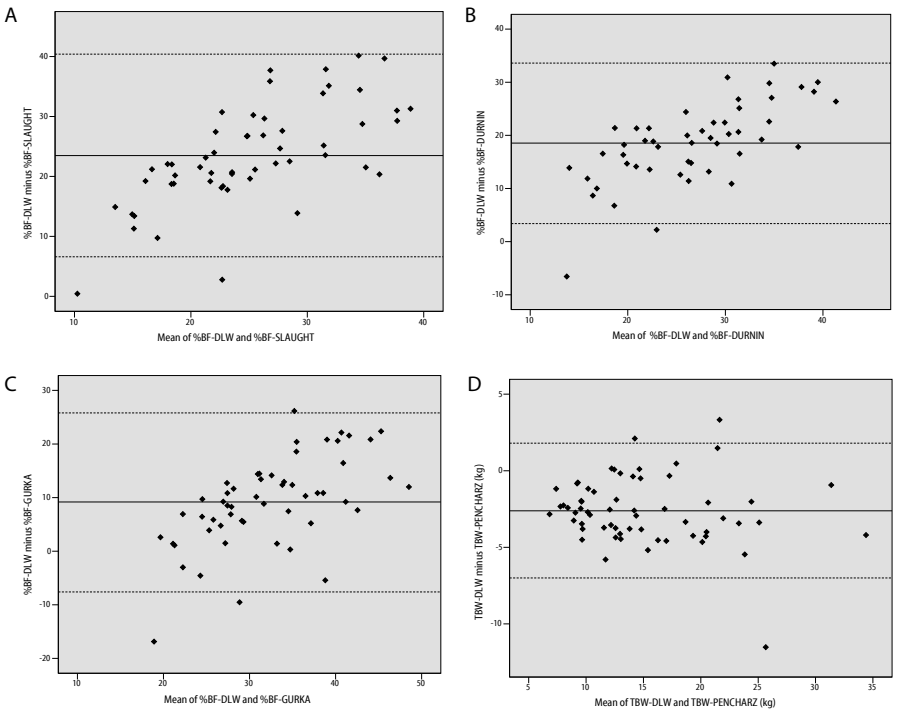


Figure 1. Bland and Altman plots of the differences of percentage body fat from (a) two skinfolds, calculated by the Slaughter equation (%BF-SLAUGHT) (n=54) and (b) after applying Gurka's corrections (%BF-GURKA) (n=54), (c) four skinfolds, calculated by the Durnin equation (%BF-DURNIN) (n=49) and (d) bio-impedance, calculated by the Pencharz equation (TBW-PENCHARZ) (n=61) compared with their corresponding outcome of the doubly labeled water method.

1. The Bland and Altman plots show that %BF determined by two or four skinfolds underestimated %BF-DLW more as the %BF increased. In BIA, using Pencharz's equation TBW was overestimated when compared with the reference value from the DLW method.

The individual variance explained (R^2) by the Slaughter and the Durnin equations for estimating %BF from two and four skinfolds in this sample was 35% and 46%, respectively. On average, calculation of %BF from two skinfolds using the Slaughter equation and from four skinfolds using the Durnin equation, underestimated %BF by 23.5% and 18.5% with limits of agreement ranging from 6.6% to 40.4% and 3.4% to 33.6%, respectively. Determining %BF from two skinfolds using the corrections on the Slaughter equation by Gurka still underestimated %BF by 9.2%, on average, with limits of agreement ranging from –7.5% to 25.9%. Individual variance explained by the Gurka equation was the same as Slaughter's, i.e. 35%. Intraclass correlation coefficients (ICCs) between the outcomes of these three equations and the outcome of the reference method varied between 0.47 and 0.56.

In BIA, the individual variance of TBW explained by the Pencharz formula in this sample was 87%. On average, BIA overestimated TBW by 2.6 kg with limits of agreement ranging from –7.0 to 1.8 kg. The ICC between the outcome of the Pencharz equation and the reference method was 0.94.

Development of prediction equations based on current data

To predict %BF from skinfolds, the sum of four skinfolds was the only variable selected in the stepwise-multiple-correlation- regression analysis. Weight, age, sex and tibia length did not add to the amount of variation explained in %BF determined using the DLW method. This generated the following equation:

%BF (skinfolds)= 18.9 + (0.63 x sum of four skinfolds)

where the sum of four skinfolds is expressed in millimeters. The individual variance of %BF explained in this equation is 45% and the standard error of the estimate (SEE) is 7.6%.The ICC between the outcome of the new equation and the reference method is 0.61.

To predict TBW using bio-impedance, tibia length squared divided by the resistance (Rz) and weight were selected by the stepwise-multiple-correlation-regression analysis and entered into the linear regression analysis. These variables were both significant predictors of TBW. Reactance (Xc), age, sex and pubertal status did not reach statistical significance. The resulting equation was as follows:

$$\text{TBW (BIA)} = \frac{2.09 + (5.44 \times \text{tibia length}^2)}{\text{Rz}} + (0.19 \times \text{weight})$$

where tibia length is in cm, resistance (Rz) is in Ω and weight is in kg. The individual variance of TBW explained in this new equation is 92% and the SEE is 1.7 kg. The ICC between the outcome of the new equation and the outcome of the reference method is 0.96. After bootstrap analysis, the ICC is 0.95. The root mean squared error (RMSE) is 1.8 kg.

When our newly-developed multiple regression equation for skinfold measurements was applied to the present sample, the explained variance of skinfold thicknesses for estimating %BF (R^2 = 0.44) did not increase compared with that of the Slaughter equation based on two skinfolds (R^2 = 0.35), the Gurka equation based on two skinfolds (R^2 = 0.35) or the Durnin equation based on four skinfolds (R^2 = 0.46). The newly-developed BIA equation increased explained variance of predicting TBW (R^2 = 0.92) compared with the Pencharz formula (R^2 = 0.88). The scatter plots outlining the relation of predicted %BF by the new equation using skinfolds (%BF-NEW EQ) and predicted TBW from the new equation using bio-impedance (TBW-NEW EQ) with their equivalent outcome using the reference method are shown in Figure 2.

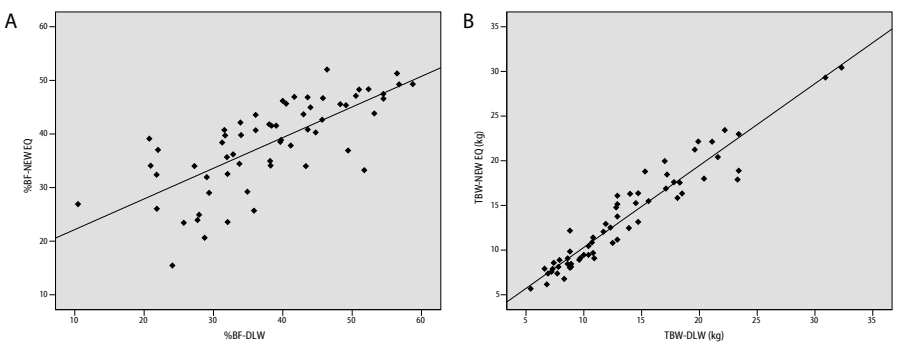


Figure 2. Scatter plots of (a) the predicted outcome of the new equation based on skinfolds (%BF-NEW EQ) and (b) of the new equation based on bio-impedance (TBW-NEW EQ) compared with their corresponding outcome of the doubly labeled water method.

DISCUSSION

This study evaluated the accuracy of measuring body composition using skinfold measurements and bioelectrical impedance analysis (BIA). Using previously developed prediction equations, percentage of body fat was calculated by using outcomes

of skinfold measurements and total body water by using outcomes of BIA. These were then compared with the equivalent outcomes of the reference method: the doubly labeled water (DLW) method. The results show that in children with severe neurological impairment and ID, the current prediction equations for skinfold measurements underestimate %BF and are of limited predictive value, rendering them of little use in clinical practice. A new equation based on our current sample did not improve the predictability of the outcome. The Pencharz equation[21] based on bioimpedance measurements overestimated total body water with reasonable limits of agreement. Based on our sample of 61 children with severe neurological impairment and ID, the newly-developed equation for BIA has very acceptable limits of agreement and, in addition, it incorporates a segmental measure (tibia length) rather than standing height, which is not readily measurable in this population due to contractures and scoliosis.

The DLW method

The doubly labeled water method is an established method of reference to study body composition.[15] It has been used on several occasions in studies on children and adults with CP, in which outcomes of either skinfold measurements or BIA were compared with those of this method of reference.[4-7, 23] One possible limitation of the DLW method is that it relies on accurate estimates of the water content of fat free mass of the subject: i.e. the hydration factor. While the hydration factor of adults is constant at 0.732, in children this factor is higher at a young age and decreases as the child grows older. The reported hydration factors in children and adolescents without disabilities by Fomon et al.[16] and Boileau et al.[17] were used in this research. Although the applicability of these hydration factors in children with CP has been debated by others[6, 7], these observations remain to be fully verified and since children with overt dehydration and edema were excluded from our study, we believe that the outcomes of the DLW method in the present study accurately reflect true body composition.

Bioelectrical impedance analysis

In our study population, the formula by Pencharz et al.[21] to calculate TBW explained around 87% of the individual variance. The limits of agreement were reasonable. The equation we developed in our sample improved explained variance by another 5%, reaching a total of 92% with acceptable limits of agreement. This outcome is surprising, since reliability of bio-impedance measured by BIA is influenced by many factors: symmetrical body position, hydration status, consumption of food and beverages, ambient air and skin temperature, recent physical activity, muscle tone and conductance of the examination table.[20, 24] As described above, we took these factors into

account to the best of our ability. However, having to take these factors into account might well impact on its suitability in clinical practice.

We decided to enter tibia length instead of recumbent length into the multiple regression model. The main principle underlying the BIA method is that bio-impedance of the geometrical system (i.e. the body) is dependent on the length of the conductor (i.e. body height) and its configuration.[24] As explained in the introduction, recumbent length cannot be measured reliably in children with severe CP because of contractures and scoliosis. Accurate measurement of body height is important because an over- or underestimation of height by 2.5 cm can result in a 1.0-L error in the estimation of TBW.[24] This could explain why the equation by Pencharz et al.[21] that includes recumbent length, predicted TBW less well than the equation using tibia length from the current data. Including tibia length in the equation rather than recumbent height increases feasibility and based on our results, also leads to a more accurate outcome.

Skinfold anthropometry

Of the previously-developed equations, Gurka's equation[18] which is based on an equation by Slaughter that adds correction factors specifically for children with CP, approximated the outcome of the DLW method best with the mean difference being 8.9 percent, but still with fairly wide limits of agreement. The population studied by Gurka et al.[18] was very homogenous with ages ranging from 8 to 18 years and GMFCS levels ranging from one to five. Children with a history of genetic, metabolic or neurodegenerative disease and children with medical conditions affecting growth, were excluded. While from a purely scientific stance this approach is certainly commendable, it does negatively impact the generalizability of their results. The population of the present sample represents more severe disability with GMFCS levels not lower than level four.

The newly-developed equation for %BF from skinfold measurements performed equally as poorly as existing equations. Imprecision in skinfold measurements is largely due to interobserver error and is measured by evaluating technical error (TE).[25] However, interobserver error can be kept to a minimum by using trained anthropometrists. Technical errors in measuring skinfolds in our sample were comparable to those reported in similar samples.[4, 26]

The observation that skinfold thicknesses predict %BF poorly must rather lie in other factors. Van den Berg – Emons et al.[7] suggested that failure to predict %BF from skinfold measurements might be due to the fact that fat is distributed differently in these children. It has been suggested that children with CP have relatively more intra-abdominal fat rather than subcutaneous fat compared with their able-bodied peers. In addition, it was also the observation of Gurka et al.[18] that their equation

performed less well in children with a higher percentage body fat. Since our sample consisted mostly of children with very high percentages of body fat, this most likely explains the poor performance of predicting %BF with skinfold measurements. The reason that BIA performs better is probably due to the fact that it provides accurate estimates of body composition irrespective of where the fat is located.

Conclusion

This study represents a new step in providing evidence of a valid and easily applied nutritional assessment technique, i.e. BIA. Based on our results, we advise against using skinfolds to measure body composition in children with severe neurological impairment and ID. While a statistical cross-validation using bootstrapping did not affect the accurateness of the new equation for BIA, cross-validation in a different group of similar children might further strengthen its validity.

Future research should focus on applying this equation in a large sample of children with severe neurological impairment to establish normative values for body composition in these children. Cut off values for undernutrition and overnutrition should be developed in children that are relatively healthy as suggested earlier by Stevenson et al.[27] in his plea for normative growth curves in these children. Having normative values and criteria for under- and overnutrition will greatly enhance the clinical utility of measuring body composition in children with severe neurological impairment and ID.

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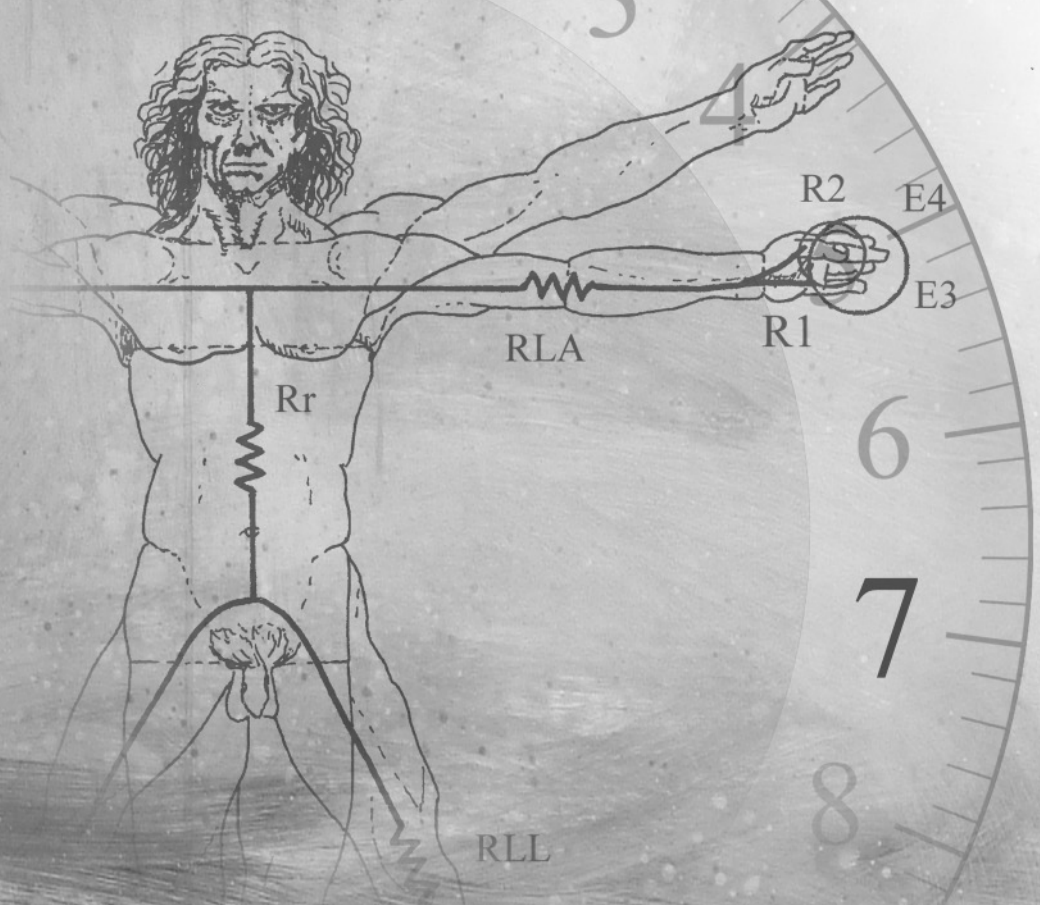
Chapter 7

Estimating total energy expenditure in children with severe neurological impairment and intellectual disability

Submitted



ABSTRACT

Background Correct estimation of energy expenditure (EE) in children with severe neurological impairment and intellectual disability (ID) is important in order to prevent over- or undernutrition. Generic pediatric equations may overestimate EE in these children.

Objective The aim of this study was to develop a new group-specific regression equation for predicting total energy expenditure using the doubly labeled water (DLW) method as reference.

Design A group of 61 children with severe neurological impairment and ID (age: 10.1 ± 4.3 yrs; 32 m) was studied. Accuracy of established equations was determined by comparing their outcome with that of the DLW method. Agreement was evaluated using Bland and Altman analyses and intraclass correlation coefficients (ICCs). Data on degree of movement, mobility, muscle tone and presence of epilepsy were collected and used to create a group-specific equation for predicting EE.

Results Indirect calorimetry failed in 6 children and provided invalid results in 21 out of 61 children. Established equations overestimated EE by at least 119 kcal with fairly wide limits of agreement. The outcome of the newly developed equation agreed well with the outcome of the DLW method (ICC=0.82, SEE=207, R^2=0.69), even after cross validation using bootstrap analysis (ICC=0.79).

Conclusions Previously available equations overestimate EE in children with severe neurological impairment and ID. For objective measurements of EE, indirect calorimetry can not be used reliably in these children. While still having some variability, the newly-developed equation provides a reasonable estimate of EE in children with severe neurological impairment and is easy to apply.

INTRODUCTION

One of the major challenges in the care for children with severe neurological impairment and intellectual disability (ID) is calculating metabolic need in order to provide optimal nutritional care. Caloric intake should be based upon a reliable estimate of energy expenditure. The most reliable estimates are made using objective measuring techniques such as indirect calorimetry, which measures resting energy expenditure (REE) and the doubly labeled water (DLW) method, which measures total energy expenditure (TEE). However, these methods require specialized equipment or analysis with mass spectrometry neither of which is readily available in every hospital and certainly not in specialized day care centers where these children often reside. Additionally, indirect calorimetry requires steady state measurement to be accurate.[1] Therefore, the feasibility of this method in children who cannot be told to remain calm during the measurement might be challenging. Alternatively, prediction equations using the aforementioned techniques that were developed in children without disabilities can be used. In 1985 the World Health Organization (WHO) Committee published revised energy and protein requirements, including prediction equations for children.[2] These WHO recommendations were incorporated into the 1989 revision of the recommended dietary allowances for the United States.[3] Schofield et al.[4] published a different set of prediction equations based on the data of 114 previous REE studies performed over the past 80 years.

However, it has been shown that these formulas, generally based on gender, age and weight, tend to overestimate energy expenditure in children with severe CP.[5-7] In these children energy requirements have been demonstrated to be lower at around 78 percent of the recommended daily allowances (RDA) for children without disabilities.[8] The main cause for the lower energy expenditure is thought to be their limited activity.[7, 8] While immobility is probably the biggest factor influencing energy expenditure in children with severe neurological impairments, in earlier research other factors have been considered such as the effect of spasticity[5], muscle tone[9] and epileptic activity[10.] Although a CP-specific prediction equation has been proposed by Krick et al.[9], as yet no study has attempted to develop a group-specific equation by using a method of reference.

The purpose of this study was to provide more insight into energy expenditure in children with severe neurological impairment by measuring its components, i.e. REE and TEE, using the reference methods indirect calorimetry and the doubly labeled water method respectively. Furthermore, we wanted to evaluate the accuracy of previously developed prediction equations for estimating energy needs in these children by comparing their outcomes with those of a reference method: the DLW method. We

hypothesized that current equations will overestimate their energy needs. Lastly, we aimed to predict energy expenditure by developing a group-specific equation for these children using variables that are believed to influence energy expenditure in these children, namely mobility, degree of movement, muscle tone and presence of epilepsy.

SUBJECTS AND METHODS

Subjects

Children with severe neurological impairment and ID, aged 2-19 y, were recruited from a number of children's care centers in the Netherlands where they receive day-care or live permanently.

To be eligible for inclusion, the children had to be between 2 and 19 years of age, have an estimated or proven IQ below 55 and a motor impairment, defined as hypertonic or hypotonic generalized CP, or a motor developmental delay to such an extent that a child could at best crawl, corresponding to Gross Motor Function Classification System (GMFCS) levels of four or five.[11] To ensure that the outcomes of the doubly labeled water (DLW) method were reliable, children who had contracted an active infection or had an altered water balance (edema or dehydration) during the measurements were excluded.

Written informed consent was provided by the parents or legal guardians of the children. The study protocol was approved by the Dutch Central Committee on Research Involving Human Subjects (P05.0102C). All measurements were performed at the individual care centers within a timeframe of two weeks.

Variables influencing energy expenditure

A number of variables influencing energy expenditure were quantified: movement, mobility, presence of epilepsy and muscle tone. Degree of movement was measured by attaching accelerometers (Actigraph model GT1M, Actigraph, Florida, USA) around the wrist and ankle of the child. Accelerometry has been validated for measuring degree of movement and estimating energy expenditure in children without disabilities[12, 13], but to our knowledge, has not previously been applied in children with severe neurological impairment. The accelerometers were worn for four days: two days on the left side of the body and two days on the right in order to account for any asymmetry. The resulting data from the accelerometer were imported into Actilife Software (version 2.1.8, Actigraph, Florida, USA) as rough counts. In the absence of normative values for this population, degree of movement of the children

was roughly divided into two categories: little and high degree of movement with a cut off at the mean if distribution was normal and at the median if distribution was skewed. Mobility was assessed using the Gross Motor Function Classification System (GFMCS) developed by Palisano et al.[11], which could be either level four or five. Parents were asked whether or not their child had epilepsy in a yes or no question. The treating physiotherapist or rehabilitation physician was asked to quantify muscle tone by providing them with a standardized, but unvalidated questionnaire. This questionnaire consisted of questions regarding muscle tone of the trunk and the extremities. Muscle tone was scored in five categories ranging from completely hypotonic to completely hypertonic. These data were stratified into the following outcomes: normotonia, hypotonia, hypertonia, or hypotonic trunk and hypertonic extremities.

Resting energy expenditure

Resting energy expenditure (REE) was measured by indirect calorimetry (Deltatrac II MBM-200, Datex Division Instrumentarium, Helsinki, Finland). This measurement was carried out in a quiet room familiar to the child, in the care center.

First the child was laid down in its preferred position (either on its back or side) and familiarized with the ventilation hood. After starting some soothing music or other distraction, the hood was placed over the head. The first five minutes were reserved for environmental adjustment and were excluded from analysis. Oxygen consumption and carbon dioxide production were recorded each minute for a minimum of 20 minutes and used to calculate the mean REE (kcal) using the modified Weir formula.[14] The following observations and the extent in which they occured were recorded during the measurement: heavy movement, talking and sleeping.

Total energy expenditure and total body water

Subjects were studied at least 3 h after their regular morning feeding. They received a weighted dose of DLW (^2H_2O: 10%, $H_2{}^{18}O$: 5%, Cambridge Isotope Laboratories, Woburn, MA) of 3 g kg^{-1} body weight orally or via gastrostomy. The DLW container was washed out with 50cc of plain tap water and this was also administered to the child. In those children with severe oral-motor impairment, the DLW was given in a sipping cup with lid or through a plastic syringe in the corner of the mouth to minimize spillage. If spillage did occur, the fluid was caught using the child's own bib. It was then weighed to determine the amount of spillage.

Baseline saliva and urine samples were taken just before administration of the DLW. Urine samples from incontinent children were collected with cotton batting in the diaper and saliva by swabbing a cotton rod (Salivattes, Sarstedt, Nümbrecht,

Germany) in the mouth. Another five saliva and urine samples were obtained on days 1, 5, 8, 11 and 15, preferably in the morning. Children were not allowed to drink any liquids 30 minutes prior to the saliva sampling to avoid dilution of the sample. Urine and saliva samples were stored in glass bottles or vials, flushed with nitrogen to prevent gas exchange and then frozen at minus 21° C until analysis.

Enrichments of the isotopes from urine and saliva samples were analyzed in quintuplet by a high-temperature conversion elemental analyzer (TC-EA) coupled with a Delta XP isotope ratio mass spectrometer (IRMS) via a Conflo-III Interface (Thermo Fisher, Bremen, Germany). The dosage used for DLW led to enrichments of 0.05 atom percent excess (APE) for ^{18}O and 0.025 APE for deuterium, which is adequately sensitive for IRMS analysis. The enrichments of the two-week period were used to calculate mean daily carbon dioxide production rate (rCO_2 in mol/d) by using equation A6 as modified by Racette et al.[15] At least three of the five samples obtained over the two-week period had to be successful in order to calculate valid results. TBW was calculated by estimating isotope dilution spaces (IDS) at time zero by back extrapolation.[16] Energy expenditure was calculated from the modified Weir's equation.[14] This equation requires measuring Respiratory quotient (RQ). FQ most closely approximates RQ.[17] In all children an individual Food Quotient (FQ) was calculated on the basis of a three-day food questionnaire. In those children for whom a food questionnaire was unavailable, the mean FQ of the study population was used, which was 0.84.

Previously established prediction equations

The outcomes of previously developed prediction equations were compared with that of the DLW method. The equations of Schofield including weight only (SCHO-WT) or including weight and height (SCHO-WTHT)[4], the WHO[2] and Harris-Benedict (HARRIS)[18] equations all calculate basal metabolic rate (BMR). Weight and height were variables in most of these equations. Therefore, weight was measured to the nearest 0.1 kg with an electronic wheelchair scale (Universal PM 7050, Lopital) or a digital sling scale. Stature from crown to heel was measured in triplicate using a flexible tape measure while the child was in the recumbent position.

The outcomes of the equations were compared with the outcome of indirect calorimetry, since its outcome (REE) is a closely related measure to BMR. In order to calculate total energy expenditure (TEE) using these equations, physical activity levels (PALs) have to be assumed. Based on the literature, children with GMFCS level four were assumed to have a PAL of 1.5 and children with GMFCS level five a PAL of 1.1.[5-7] TEE is then calculated by multiplying REE with PAL.

The formula by Krick et al.[9] estimates TEE and therefore its outcome was directly compared with that of the DLW method.

Statistical analysis

Results are presented as means ± standard deviations (SDs). The outcome of the previously established equations was compared with their corresponding outcome from the reference standard (either indirect calorimetry or the DLW method) using intraclass correlation coefficients (ICCs). Agreement was evaluated using Bland and Altman limits of agreement analysis and expressed as means ± 2 SDs.

In order to create a new prediction equation for estimating energy expenditure based on this sample, a stepwise-multiple-correlation-regression analysis was used to determine the best predictors of TEE. TEE was chosen since this is clinically the most interesting outcome and since it does not require the assumption of PAL factors. Because of the relatively small sample size, the first variable entered into the analysis was the outcome of the Schofield equation with only weight (SCHO-WT) since this is based on a proven model using a much larger sample size. The Schofield equation with weight and height was purposely not chosen, because height cannot be reliably measured in children with scoliosis and contractures.[19] In a stepwise fashion, the variables mobility (GMFCS level), low or high degree of movement, muscle tone and presence of epilepsy were entered into the analysis. A bootstrap analysis was performed in order to provide cross-validation. After cross-calibration, the root mean squared error (RMSE) and ICC of the predicted value versus the value of the reference method were calculated. Multivariate analyses were performed using SPSS (SPSS for Windows 18.0, SPSS Inc.) and R project software, version 2.10.1 (R foundation, Vienna, Austria). The level of significance for all analyses was specified at $P < 0.05$.

RESULTS

Population characteristics

Characteristics of the study population are described in Table 1. Most children were non-ambulant (55 of 61) and had a severe intellectual disability (54 out of 61). Almost half of the children (28 of 61) were tube-fed, either exclusively or combined with oral feedings.

Indirect calorimetry

Indirect calorimetry failed in six children because they did not tolerate the ventilation hood. REE was higher than TEE in 21 of the 55 remaining cases and therefore these

Table 1. Population characteristics

Sample size (n)	61
Gender	32 male, 29 female
Age	10.1 ± 4.3
Weight (kg)	30.4 ± 14.9
Length (cm)	132.2 ± 21.1
GMFCS score (n)	
4	6 (9.8%)
5	55 (91.2%)
Gastrostomy tube (n)	28 (45.9%)
Level of intellectual disability (n)	
Moderate	7 (11.5%)
Severe	54 (88.5%)
Aetiology (n)	
Congenital	14 (23.0%)
Perinatal	21 (34.4%)
Acquired	4 (6.6%)
Combination	4 (6.6%)
Idiopathisch / unknown	18 (28.3%)

Average values presented as mean ± SD, GMFCS = Gross Motor Functioning Classification System.

measurements had failed as well, since REE cannot exceed TEE. Failed indirect calorimetry was not associated with the amount of movement while the child was under the ventilation hood, whether or not the child slept during the measurement or the presence of epilepsy in general (data not shown). Many children talked, laughed, moved around or had an epileptic event during the measurement. Therefore, the outcomes of indirect calorimetry were not used in further analyses.

Comparing measured TEE with estimated TEE

In all children, the DLW method was successful. The limits of agreement (mean ± 2 SD) of the Bland and Altman analyses and ICC values of the comparisons between measured TEE and outcome of the equations by Schofield for weight (SCHO-WT), Schofield for weight and height (SCHO-WTHT), WHO equation, Harris Benedict equa-

Table 2. Comparison of measured TEE with estabished equations

Equation	Limits of agreement (mean ± 2 SD)	ICC
SCHO-WT (kcal)	-127 ± 524	0.77
SCHO-WTHT (kcal)	-127 ± 512	0.78
FAO/WHO/UNU (kcal)	-406 ± 776	0.47
Harris Benedict (kcal)	-119 ± 558	0.71
Krick (kcal)	-179 ± 596	0.66

Difference = outcome of the DLW method minus the outcome of the equation.

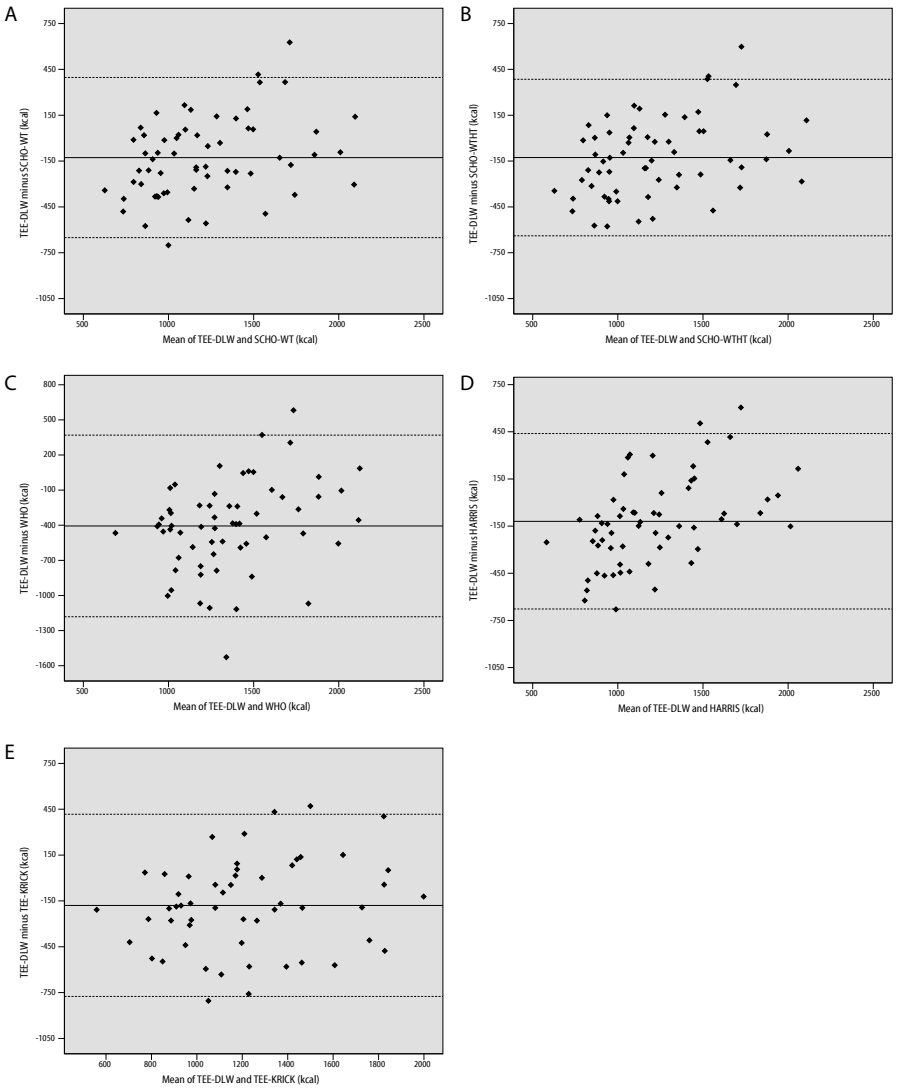


Figure 1. Bland and Altman plots of the differences of calculated total energy expenditure from (a) the Schofield equation with weight alone (SCHO-WT) (n=61), (b) the Schofield equation with weight and height (SCHO-WTHT) (n=61), (c) the WHO equation (n=61), (d) the Harris Benedict equation (HARRIS) (n=61) and (e) the equation by Krick et al. (KRICK) (n=54) compared with the corresponding outcome of the doubly labeled water method.

tion and the equation by Krick are described in Table 2. The corresponding Bland and Altman plots are shown in Figure 1. On average, all equations overestimated energy expenditure when compared with measured TEE by the DLW method.

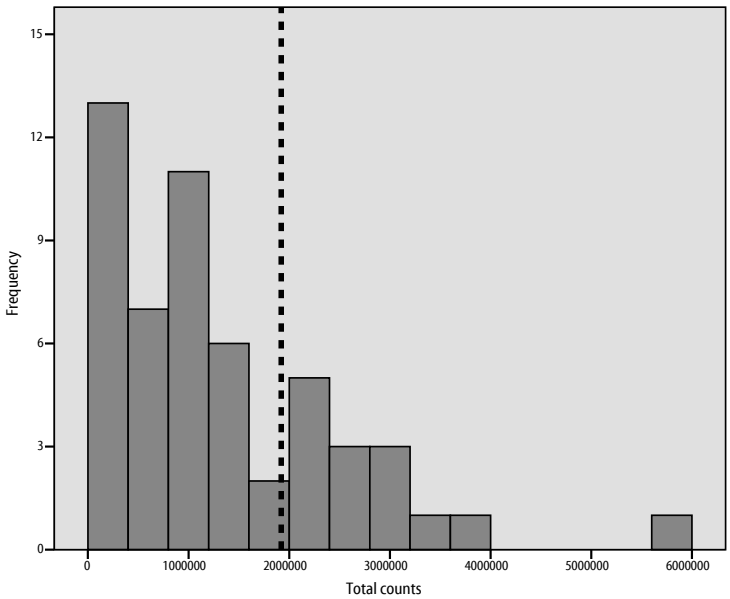


Figure 2. Distribution of data on total counts of the accelerometer in a histogram. (n=52) The dotted line signifies the median value that was used to create two categories of degree of movement: low degree of movement and high degree of movement.

Variables influencing energy expenditure

Data on muscle tone were collected from 54 children. Fifteen children had hypotonia, 20 hypertonia, and 19 a hypotonic trunk and hypertonic extremities. Epilepsy was present in 41 of 61 children. Collecting data on movement using actigraphy succeeded in 52 of 61 children. Because these data were skewed with more children having lower movement scores, the median was used to divide children in a group with a low degree of movement and a group with a high degree of movement. The histogram is shown in Figure 2. Data on mobility, muscle tone, epilepsy and movement were available from 52 children.

Development of a new equation based on the current sample

To predict total energy expenditure, the outcome of the Schofield equation with weight (SCHO-WT), movement category and GMFCS level were selected in the stepwise-multiple-correlation-regression analysis. Presence of epilepsy and muscle tone did not add to the amount of variation explained in energy expenditure measured by the DLW method.

In Table 3 the original Schofield equation that includes only weight (SCHO-WT) is presented. To calculate TEE for a child that has a GMFCS score of five and moves little

Table 3. Schofield's equation with corrections for children with severe neurological impairment and intellectual disability (n=52)

ORIGINAL SCHOFIELD EQUATION WITH WEIGHT (SCHO-WT) IN MJ		
	Under 3 years	
m	BMR = (0.249 * weight) – 0.127	
f	BMR = (0.244 * weight) – 0.130	
	3 to 10 years	
m	BMR = (0.095 * weight) + 2.110	
f	BMR = (0.085 * weight) + 2.033	
	10 to 18 years	
m	BMR = (0.074 * weight) + 2.754	
f	BMR = (0.056 * weight) + 2.898	
EQUATION FOR TEE		
(1.1 * BMR SCHO-WT * 238.8) – 280 kcal		
ADDITIONAL CORRECTIONS		
High degree of movement		+ 222 kcal
GMFCS = 4		+ 431 kcal

MJ = macrojoule, BMR = basal metabolic rate, GMFCS = gross motor functioning classification system.

(low degree of movement), the basic equation in Table 3 can be used. If a child moves a lot (high degree of movement), 222 kcal need to be added to the outcome. If the GMFCS score is four, 431 kcal should be added.

The residual standard error of the resulting equation and corrections is 207 kcal. Individual variance of energy expenditure explained by this equation is 69%. The ICC of the outcome of the equation and the reference value, obtained by the DLW method, was 0.82. After cross validation, the ICC was 0.79 and the RMSE was 213 kcal.

DISCUSSION

Over the course of this research we collected data on energy expenditure in 61 children with severe neurological impairment and ID. Previously established equations to estimate energy expenditure, such as the Schofield equation, the WHO equation and the Harris Benedict equation, overestimated TEE measured with DLW method with wide limits of agreement limiting their usefulness in children with severe neurological impairment. A newly-developed equation based on current data that builds upon the Schofield equation provides very good agreement with outcome of the DLW method (ICC=0.82), even after cross validation (ICC=0.79). Although the standard error of the new equation is still considerable (207 kcal), it is still more favorable than the standard deviation scores of the existing equations.

Measuring REE and TEE

We compared REE data measured with indirect calorimetry with TEE data obtained with the doubly labeled water method. In 21 out of 55 children measured REE was higher than TEE, indicating that these measurements failed in these children.

The key assumption of indirect calorimetry is that respiratory gas exchange is in equilibrium with gas exchange in the mitochondria[20], which requires a measurement in a steady state. In reality, this is difficult to accomplish in a group of children who cannot comprehend instructions nor be told what the measurement will entail. During our experiments children often moved a lot, talked or made other sounds, tried to remove the canopy or had an epileptic seizure during measurement. This behavior was also observed during indirect calorimetry measurements in adults with neurological impairment.[6] Consequently, because this behavior impeded steady state and presumably increased respiratory gas exchange, in about a third of the children REE measured with indirect calorimetry was higher than TEE, obtained with the DLW method. While the DLW method is also based on an indirect measurement of gas exchange, it is unaffected by temporary increases in gas exchange since its measurement is spread out of a two-week period. Since it is theoretically impossible for REE to be higher than TEE, we cannot recommend using measurements of energy expenditure using indirect calorimetry in children with severe neurological impairment and ID.

Previously established equations

As has already been established[5-7], previously available equations overestimate total energy expenditure in children with severe neurological impairment. These equations all estimate resting energy expenditure (REE) as a basis and provide corrections for activity. Since we concluded that indirect calorimetry does not lead to valid outcomes of resting energy expenditure (REE) in these children, we could not use this method to validate the established equations. Therefore, we assumed a Physical Activity Level (PAL) of 1.1 for children with GMFCS level five and a PAL of 1.5 for children with GMFCS level four. A PAL of 1.1 for non-ambulatory children was first found in the study by Bandini et al.[21] concerning 19 adolescents with CP using indirect calorimetry and doubly labeled water data, and later corroborated by a study by Aczue et al.[7] using data on nutritional intake and doubly labeled water data. In the study by Bandini et al.[21] PALs in ambulatory children with CP varied from 1.6 to 2.1. Even when assuming realistic PALs for non-ambulatory and ambulatory children, the established equations from Schofield, WHO and Harris Benedict still overestimate total energy expenditure in children with severe neurological impairment. If true PALs were lower, the overestimation of established equations might be more limited.

Krick et al.[9] proposed a new formula for estimating energy needs in children with cerebral palsy (CP) that contains corrections for muscle tone and activity level. They compared the outcome of their formula in 30 children with CP and the recommended daily allowance (RDA) for age with the diet prescription for these children on discharge. The authors conclude that their own formula performed better than RDA for age because their formula predicted the diet prescription on discharge better. However, the authors did not compare the outcome of their formula to any objective measurement of energy expenditure. Even though the outcome of the Krick equation represents total energy needs without needing to assume physical activity levels, in our group of children the outcomes of the equation by Krick et al. [9] also overestimated energy expenditure with wide limits of agreement.

New equation based on data from this research

We developed a new equation which was based on Schofield's equation using weight agreed well with the outcomes of the reference standard (ICC = 0.82), even after cross validation using bootstrapping analysis (ICC = 0.79). The amount of variability explained by the new equation is acceptable ($R^2 = 0.69$) with a residual standard error reaching 207 kcal. In contrast, Schofield et al.[4] found residual standard errors ranging from 67 kcal in young children, to 111 kcal in adolescents. The higher standard error of our data can be explained by the heterogeneity of our population with its wide age range and relatively small sample size compared with that of Schofield. Therefore the outcome of our newly-developed equation needs to be interpreted with caution in individual cases.

The new equation is based on weight, age, sex, GMFCS level and a choice for low or high degree of movement. In this study movement was measured objectively using accelerometers, yet its use in the equation should be estimated subjectively by the professional. Since the data on movement was skewed, the median value was used to discriminate between little and much movement. It is our assumption that the objectively measured amount of movement will translate reasonably well to the measure that has to be subjectively established. Assigning a child to the wrong category could result in an error of 222 kcal.

The variable muscle tone was not entered into the current equation using multivariate analyses. Although a counterintuitive finding, it is corroborated by Stallings et al.[5] who also found that spasticity - which is an entity highly related to tone - had no influence on energy expenditure in 61 children with CP. Also, the presence of epilepsy was not entered into the equation. We suspected that the presence of epilepsy would increase the measured energy expenditure. Recent evidence even suggests that resting energy expenditure is lower in children with severe epilepsy[10], although this

excludes the influence of epilepsy on activity levels. In our study there seems to be no influence of epilepsy in either direction.

Conclusion

To the best of our knowledge, this is the first study to develop an equation specifically engineered for children with severe neurological impairment and ID by basing it on an objective measurement of energy expenditure. Our newly-developed equation predicts total energy expenditure well, even after cross validation using bootstrapping. Although the residual error is quite large, this new equation is the first step to an alternative way of estimating energy expenditure. As has been reported by others and was further confirmed by our data, the existing equations all overestimate total energy expenditure.

While the agreement of the newly developed equation is very reasonable, it would be beneficial to cross validate it in a comparable group of children with severe neurological impairment. It would also be interesting to apply the currently developed equation in a group of children that are considered malnourished, then prescribe a diet based on its outcome and in a longitudinal design evaluate whether this diet improves weight and health status of these children.

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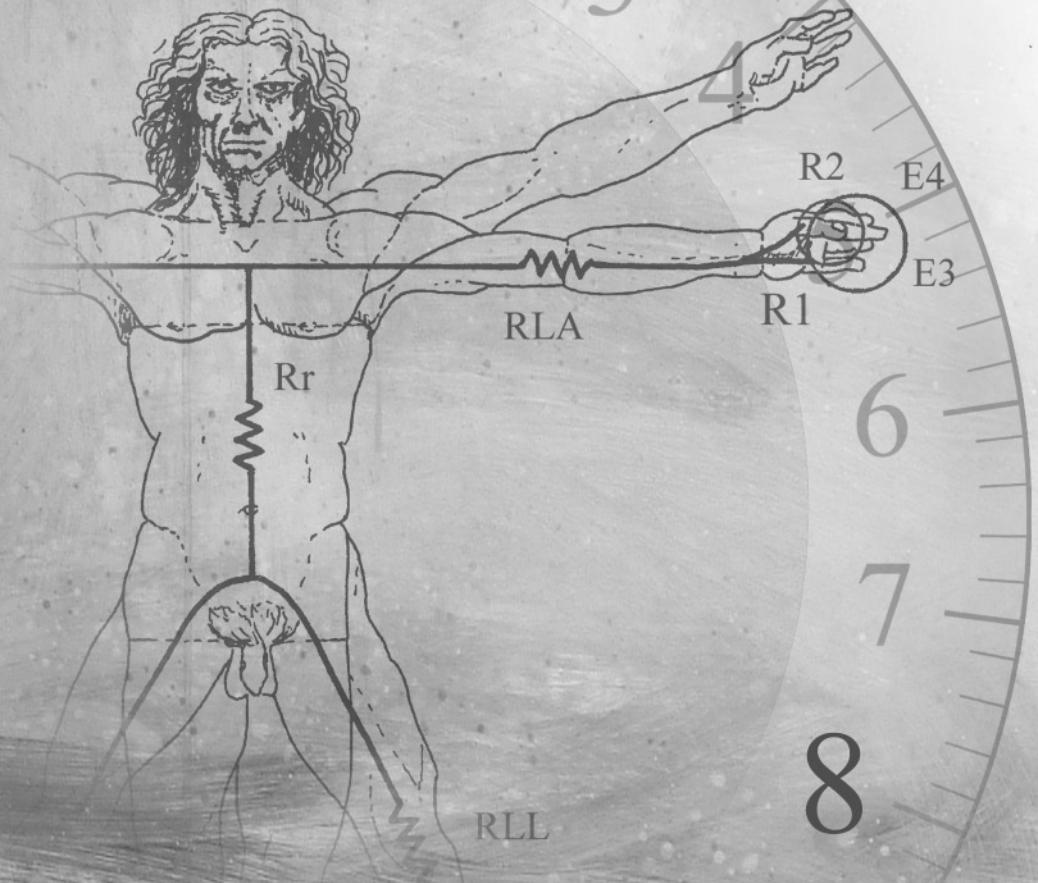
Chapter 8

General Discussion



INTRODUCTION

This thesis set out to add to the body of evidence on clinical diagnostics of nutritional status in children with severe neurological impairment and intellectual disability (ID). There is a great need for practical tools to evaluate these children's nutritional status, since they run the risk to develop undernutrition or overnutrition. The focus of this thesis was on the accurate assessment of body composition and energy expenditure.

Measurements of skinfolds proved not reliable in predicting body composition, whereas bioimpedance values were found suitable for this purpose. One of the three most important products of this thesis is a new, validated equation for calculating total body water from bioimpedance values. This new equation is displayed in Table 1. The second is an equation for estimating total energy expenditure in these children. It is based on a basic equation to which several correction factors for child-specific characteristics have been added: mobility and degree of movement. This equation is shown in Table 2. These two equations are part of a preliminary clinical guideline on diagnosing undernutrition and overnutrition in children with severe neurological impairment and ID. This guideline is the third product of this thesis.

Table 1. New equation for estimating total body water using bioimpedance in children with severe neurological impairment and intellectual disability

Total body water (kg) =
$2.09 + (5.44 * (\text{tibia length}^2 / Rz)) + (0.19 * \text{weight})$

Rz = resistance measured using single-frequency bioimpedance analysis, tibia length in cm, weight in kg. Statistics on equation: $R^2 = 0.92$, Standard Error of the Estimate = 1.7 kg, Root Mean Squared Error = 1.8 kg.

Chronologically, in the following I will focus on the absence of a unified definition of the group studied, the possible limitations of the nutritional assessment methods used in this thesis and conclude with detailing the preliminary clinical guideline.

CHILDREN WITH SEVERE NEUROLOGICAL IMPAIRMENT AND INTELLECTUAL DISABILITIES

There is no international consensus on the definition of the group of children we studied. Children with physical disabilities are commonly referred to as having cerebral palsy (CP). In 2007, Rosenbaum et al.[1] described CP as a group of permanent disorders of the development of movement and posture, causing activity limitation, which are attributed to non-progressive disturbances that occurred in the developing

Table 2. New equation for estimating total energy expenditure in children with severe neurological impairment and intellectual disability

ORIGINAL SCHOFIELD EQUATION WITH WEIGHT (SCHO-WT) IN MJ		
	Under 3 years	
m	BMR = (0.249 * weight) – 0.127	
f	BMR = (0.244 * weight) – 0.130	
	3 to 10 years	
m	BMR = (0.095 * weight) + 2.110	
f	BMR = (0.085 * weight) + 2.033	
	10 to 18 years	
m	BMR = (0.074 * weight) + 2.754	
f	BMR = (0.056 * weight) + 2.898	
EQUATION FOR TEE		
(1.1 * BMR SCHO-WT * 238.8) – 280 kcal		
ADDITIONAL CORRECTIONS		
High degree of movement		+ 222 kcal
GMFCS = 4		+ 431 kcal

BMR = basal metabolic rate (in MJ), MJ = macrojoule, GMFCS = gross motor function classification scale[1]. No correction factors are required for children with low degree of movement and GMFCS level five. Statistics on equation: $R^2 = 0.69$, Standard Error of the Estimate (SEE) = 207 kcal, Root Mean Squared Error (RMSE) = 213 kcal.

fetal or infant brain. However, this definition does not cover progressive disturbances such as metabolic diseases or neurodegenerative abnormalities. The above definition of CP[1] also describes co-morbidity, i.e. disturbances of sensation, perception, cognition, communication and behaviour, by epilepsy and by secondary musculoskeletal problems, but does not mention intellectual disability (ID). The description of children with profound intellectual and motor disabilities (PIMD) does include intellectual disability, but only gives room to children with an IQ under 20. Since the definition of CP excludes progressive diseases of motor function and the definition of PIMD does not cover less severe ID, we chose to define our study population as children with severe neurological impairment and ID. This includes children with non-progressive and progressive disturbances as well as children with moderate to severe intellectual disability.

Our definition encompasses children exhibiting a wide variety of aetiology of their physical and intellectual disabilities including pre-, peri- and postnatal causes. Most of this aetiology fits into the definition of CP. The major exceptions are metabolic diseases and acquired brain damage through trauma, infections such as bacterial meningitis or encephalitis, and near drowning with posthypoxic encephalopathy.

However, studying a group with such a broad definition comes at a cost. It also means that this group is very heterogeneous, which might have consequences for the methodological quality of the study. From a purely scientific position, homogeneous populations are to be preferred. Homogeneity can be enhanced by

limiting the age range, diversity of aetiology and severity of physical and intellectual disability. Indeed, growth charts have been developed for Cri-du-chat syndrome[2], Down syndrome[3], Turner syndrome[4], Williams syndrome[5] and Rett syndrome[6]. On the other hand, studying a homogeneous group carries the risk that research outcomes will only be applicable to a relatively select population with a particular disease or syndrome. Results in these small populations are not applicable to children with severe neurological impairment as a whole. Choosing such a narrow focus therefore does not match with current clinical needs, seeing that other children may require monitoring of their nutritional status as well.

Apart from this matter of applicability of the results, we had to deal with some practical considerations. In the year 2000 the Netherlands Health Inspectorate estimated the number of children with neurological impairment and ID residing in the Netherlands at about 2000.[7] To the best of our knowledge, maybe 300 to 400 of these children would be living in the region covered by our academic hospital. To answer the study question, it was necessary to recruit 60 children, representing 15 to 20 percent of the available regional population. Inclusion took us two and a half years. Then there is the matter of research satiety. Several research groups and individual researchers draw from this population, which means that parents, understandably, are very critical before they agree to let their children participate in a new study. This trend also limited inclusion rate.

It was our goal to recruit a population of children with neurological impairment whose results would be broadly applicable, but whose characteristics would be varied, too. As a result, we were facing considerable standard errors of the estimates (SEE) in the equations of BIA and energy expenditure. However, taking into account that this population is heterogeneous, it is satisfying that SEE values were within acceptable limits. Furthermore, this population is representative of children living both inside and outside of specialized care facilities.

REFERENCE METHODS FOR MEASURING BODY COMPOSITION

The doubly labeled water method has been applied several times in children with CP[8-11], most recently by Van den Berg-Emons et al.[12] Dual energy x-ray absorptiometry (DEXA)[13, 14] and the DLW method have been applied as reference methods in studies of body composition in the general population as well as in children with neurological impairments. Other reference methods, such as underwater weighing, magnetic resonance imaging (MRI) and whole body potassium counting, are considered too invasive for these children. Underwater weighing requires submersion into water,

MRI requires children to lie motionless for long periods in a confined space to a frightening noise volume, and whole body potassium counting involves potentially damaging gamma rays.[15] Despite the fact that the DLW test and DEXA are considered reference methods, these methods are also seen as indirect methods of measuring body composition.

The DLW method requires oral administration of a dose of stable isotopes (^2H_2O and $H_2{}^{18}O$) and measuring its enrichment in either urine or saliva on six occasions in a two-week period. As shown in chapters 3 and 4, most children produced sufficient numbers of samples to allow accurate calculation of body composition. The DLW method is therefore a feasible method in children with neurological impairment.

The outcome of the DLW method is total body water (TBW). In order to calculate other measures of body composition, such as fat free mass (FFM), assumptions have to be made for the water content of FFM, i.e. the hydration factor. In our calculations of FFM, we assumed that the hydration factors provided by Fomon et al.[16] and Boileau et al.[17] for healthy children aged 2 to 18 years were also applicable to children with neurological impairment. Besides the fact that the hydration factors are highly dynamic in children as they grow older, there is also conflicting evidence regarding the water balance in children with CP, suggesting either higher or lower hydration factors.[11, 12] Hydration factors in these children require more research as they are used to calculate FFM, and in turn, body fat.

An alternative reference method for measuring body composition is a full body scan using DEXA. However, as shown in chapter 5, several disrupting factors limit its application in children with neurological impairment. For full body scans, the most important limiting factor is movement during the measurement. In addition, the algorithms for our device (LUNAR-PXL) are not supplied by the manufacturer and therefore inaccessible for the scientific community. It is unknown whether they are applicable in a population of children with contractures, scoliosis and aberrant body composition. Indeed, Koo et al.[18] describe that especially in small infants measurements using DEXA should be standardized. The wearing of a diaper, for example, can alter the bone mass content (BMC) and body composition outcomes. This was especially true for body fat outcomes.

As stated above, both the DLW method and DEXA may be used to indirectly measure body composition. Since it is not known which method is most accurate, we have considered to settle on the mean value of the outcomes of both methods. However, in our opinion there are currently more limitations to the interpretation of DEXA outcomes compared to DLW outcomes. Therefore, we have opted to use the DLW method as the single method of reference, also since the latter has a larger empirical basis as a reference method.

SKINFOLD MEASUREMENTS

A new equation to predict body fat from skinfold measurements, developed with the use of our own data, performed poorly. Possible explanations are the following: intra- and interobserver error[19]; thicker skinfolds in children receiving tube feeding that are not readily separated from the underlying muscle layer; and potential differences in the distribution of fat in these children[12, 20]. Therefore, body fat estimated by skinfolds might not correlate well with "true" body fat obtained from a reference standard like the DLW method. However, if fat were to be distributed in a more homogeneous manner in all children with neurological impairment, one would expect that the new equation we formulated would not perform so poorly. Rather, there must be some heterogeneity in the way in which fat is located more subcutaneously in some children and more around the viscera in others. Indeed, Gurka et al.[14] in their study in children with CP found that their corrections on the Slaughter equation performed more poorly in children with higher percentages of body fat. The children studied in this thesis have a relatively high amount of body fat, mostly because of reduced mobility. Furthermore, a proportion of children receiving tube feeding might have been overfed. Lack of mobility also affects fat free mass (FFM). Because these children do not move around a lot, their muscles start to waste upon which bone mineral content (BMC) will decrease. Since both muscle mass and BMC are components of FFM, FFM will decrease as well, causing a relative increase of body fat when expressed in percentages. So, the poor performance of an equation to predict body fat from skinfold measurements might be the result of difficulty in measuring skinfolds in children receiving tube feeding combined with a potentially varying distribution of fat in the body.

Because of these issues we advise against using skinfold measurements to evaluate body composition in children with neurological impairment. In addition, raw measurements of skinfolds in millimetres are not recommended. Since we have established that the amount of subcutaneous fat, measured using skinfolds, does not accurately reflect body composition, the change in skinfold thickness is likely to correlate only slightly with increases or decreases of the body's fat reserves of children with severe neurological impairment and ID.

On the other hand, we do not dismiss the notion that measuring skinfolds may have more value in more homogeneous populations of children with a singular disease or disability and who may have a more homogeneous fat distribution. Gurka et al.[14] for example developed an equation based on Slaughter's equation[21] to calculate body fat from two skinfolds in children with CP. Children with a history of genetic, metabolic or neurodegenerative disease, or children with medical conditions affecting growth,

were excluded. While this equation might have performed very well in a CP population with limited co-morbidity, it may not do so in children with severe neurological impairment and ID who usually have significant multi-organ comorbidity.

BIOELECTRICAL IMPEDANCE ANALYSIS

The new equation to predict total body water based on bioimpedance values and weight performed well in our study. Compared with the equation by Pencharz et al.[22], our equation showed less variability. This was somewhat surprising since BIA values are highly dependent on maintaining standardised measurement conditions. These conditions have been described in detail by Kyle et al.[23] and include body position, previous exercise, dietary intake and skin temperature. The single frequency (SF) type of BIA we used could have influenced the outcome as well. It is considered less accurate in subjects with altered hydration status.[24] As applies to most equations based on BIA, the direct outcome of our equation is total body water (TBW). Indeed, in the validation studies reviewed in chapter 2 the TBW outcome agreed better with the reference method outcome than did body fat. Other authors have also suggested, therefore, that the most relevant outcome of BIA is TBW.[25] However from a clinical standpoint, TBW is less useful than body fat or percentage of body fat. More reference values are available for percentage of body fat than for total body water.[26] One could accept the TBW outcome of BIA and use the reference values available for children, e.g. those from the study by Horlick et al.[27] Another approach would be to calculate percentage of body fat from TBW values. However, that would require the assumption of hydration factors. As mentioned above, it is not known whether the hydration factors for healthy children are applicable to children with neurological impairment.

At the moment, BIA equipment is mostly present in academic hospitals for the purpose of diagnosing undernutrition or overnutrition, and is used in research. It is also encountered in sport facilities where it is used to measure percentage of body fat. Clinicians would do well to accept the BIA as a body composition measuring instrument outside of the realm of academic research and sports facilities. The results of the present research would certainly warrant the purchasing of at least one BIA machine by care organisations so that they can more accurately assess nutritional status in children with severe neurological impairment and ID.

Now that a precise group-specific BIA equation has been developed for children with neurological impairment and ID, the next step in research would be to collect longitudinal data in this population. First, this would produce a wealth of normative data on the development of total body water and lean body mass with growth. In

addition, it would be interesting to study the potentially beneficial effects of a nutritional intervention by performing measurements before and after the intervention.

ENERGY EXPENDITURE

In a questionnaire sent out to Dutch dieticians specialized in the care for children with neurological impairment and ID, many professionals indicated that they use Schofield's equation to estimate these children's energy needs, but did not trust its outcome (unpublished data). It was our intention to collect data on resting energy expenditure (REE) using indirect calorimetry and on total energy expenditure (TEE) using the DLW method. However, we found that indirect calorimetry did not suffice because the children suffered from stress caused by the measurement itself and by excessive movement and production of sounds during the measurement. The children also showed epileptic activity. A new equation to predict energy expenditure, based on our data and the Schofield formula, including correction factors for degree of movement and mobility using GMFCS level, performs reasonably well. While there is still quite some variability in the estimation of energy expenditure using this equation, amounting to around 200 kcal, it represents a more valid estimation than estimations using other existing equations. In order to study the efficacy of the equation developed, a longitudinal study could be set up to monitor whether children, fed according to the newly developed equation, grow better and maintain a healthier weight compared to control children fed according to current practices.

In everyday clinical practice the equation will also be helpful to prescribe a suitable diet for the individual underweight or overweight child with severe neurological impairment and ID. Combining it with dietary food intake data will enable to compare intake and energy expenditure and to compose a balanced diet to counter any caloric deficiencies or overfeeding. Note that amounts of spilled or vomited foods and consumed drinks and food between meals should also be recorded.

PRELIMINARY GUIDELINE FOR THE CLINICAL DIAGNOSIS AND TREATMENT OF UNDERNUTRITION AND OVERNUTRITION

Combining the currently available and newly developed evidence presented in this thesis, a preliminary guideline was made for the diagnosis and treatment of undernutrition and overnutrition. The flow diagram in Figure 1 shows how we would envision diagnostic assessments and referrals to take form. We see distinct roles for profes-

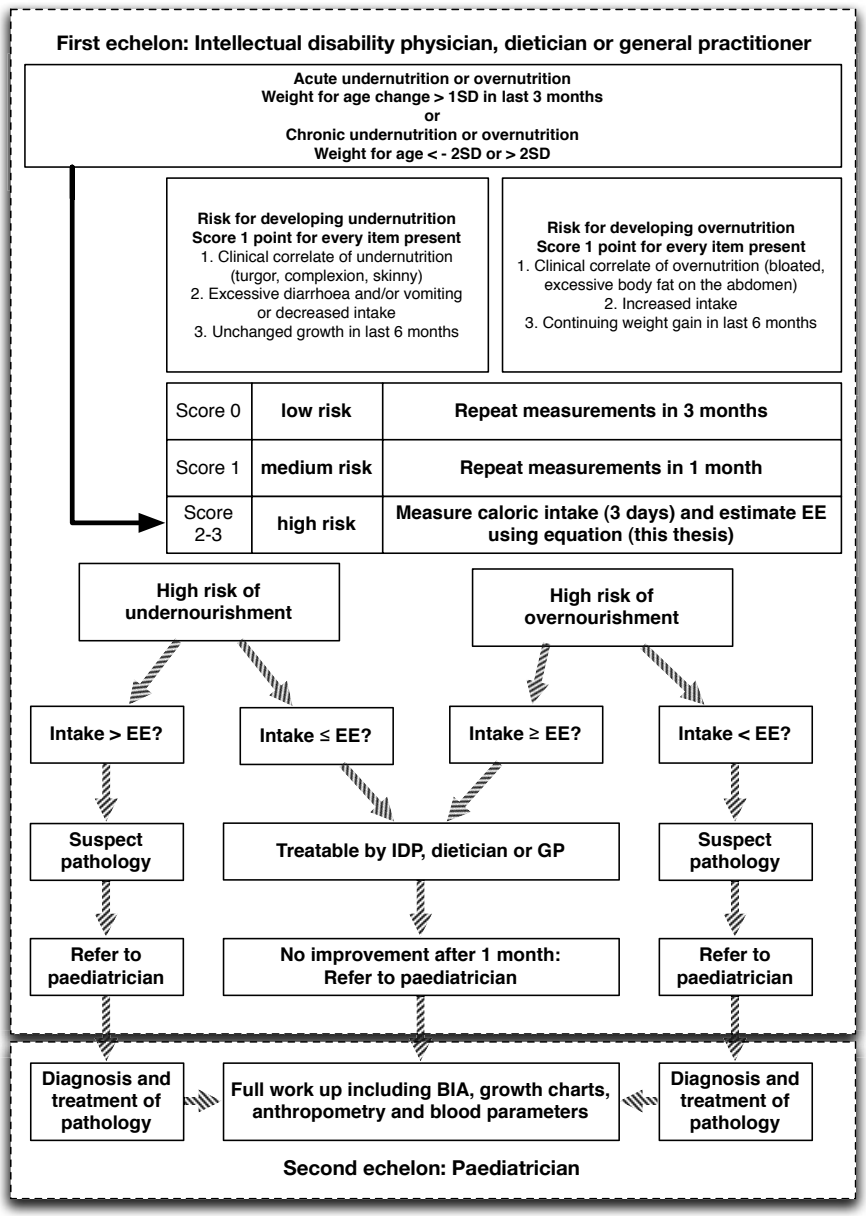


Figure 1. Diagnostic guideline
SD = standard deviation, EE = energy expenditure, IDP = intellectual disability physician, GP = general practitioner, BIA = bioelectrical impedance analysis.

sionals (physician for people with intellectual disability and dietician) at the children's care provider, the general practitioner (GP) in the first echelon and the paediatrician in the second echelon. The professionals at the children's care provider or the GP, in case children live at home, screen for malnutrition by measuring weight and length at regular intervals and have a role in the treatment of uncomplicated cases. The paediatrician will take over treatment if the child's nutritional status does not improve or if pathology is suspected. Let us focus in more detail on the responsibilities in these two settings.

Screening for undernutrition and overnutrition

Since most children with severe neurological impairment and ID are shorter than their non disabled peers[28], generic paediatric weight-for-age growth charts are not an appropriate measure of nutritional status. Instead, generic paediatric weight for height growth charts are used most often. However, Samson-Fang et al.[29] demonstrated that depleted fat stores were missed in 45% of 276 children with CP when using a generic paediatric weight-for-height percentile under a tenth. Ideally, growth charts geared to children with neurologic impairment should be used, like the one developed by Day et al.[30] for children with CP in general. However, these authors did not distinguish between different health statuses of the children included in the study. As indicated by Stevenson et al.[31], the resulting growth charts are therefore descriptive of how the CP population as a whole grows, but are not prescriptive of the ideal weight and height gains for these children. These growth charts have low sensitivity to identify underweight and overweight since a substantial proportion of children is not labelled undernourished or overnourished, because the children with aberrant nutritional status were part of the descriptive growth charts. However, the specificity is probably high, because there are fewer false positives. There is a need, therefore, to develop growth charts in children that have a relatively favorable health status. This could mean that growth characteristics of a child with relatively mild dysphagia receiving tube feeding would be incorporated, whereas those of a child with severe gastro-oesophageal reflux just treated with a fundoplication after severe postoperative complications would not be included in these charts.

Alternatively, Samson-Fang et al.[29] suggested using the triceps skinfold with the cut-off point at the tenth percentile for age and sex instead of weight for height percentiles. However in this thesis we have found that skinfold measurements do not correspond with fat stores in children with severe neurological impairment and ID. Additionally, measuring skinfolds is subject to large intraobserver and interobserver variability.[32] We will therefore not recommend the triceps skinfold as a screening tool.

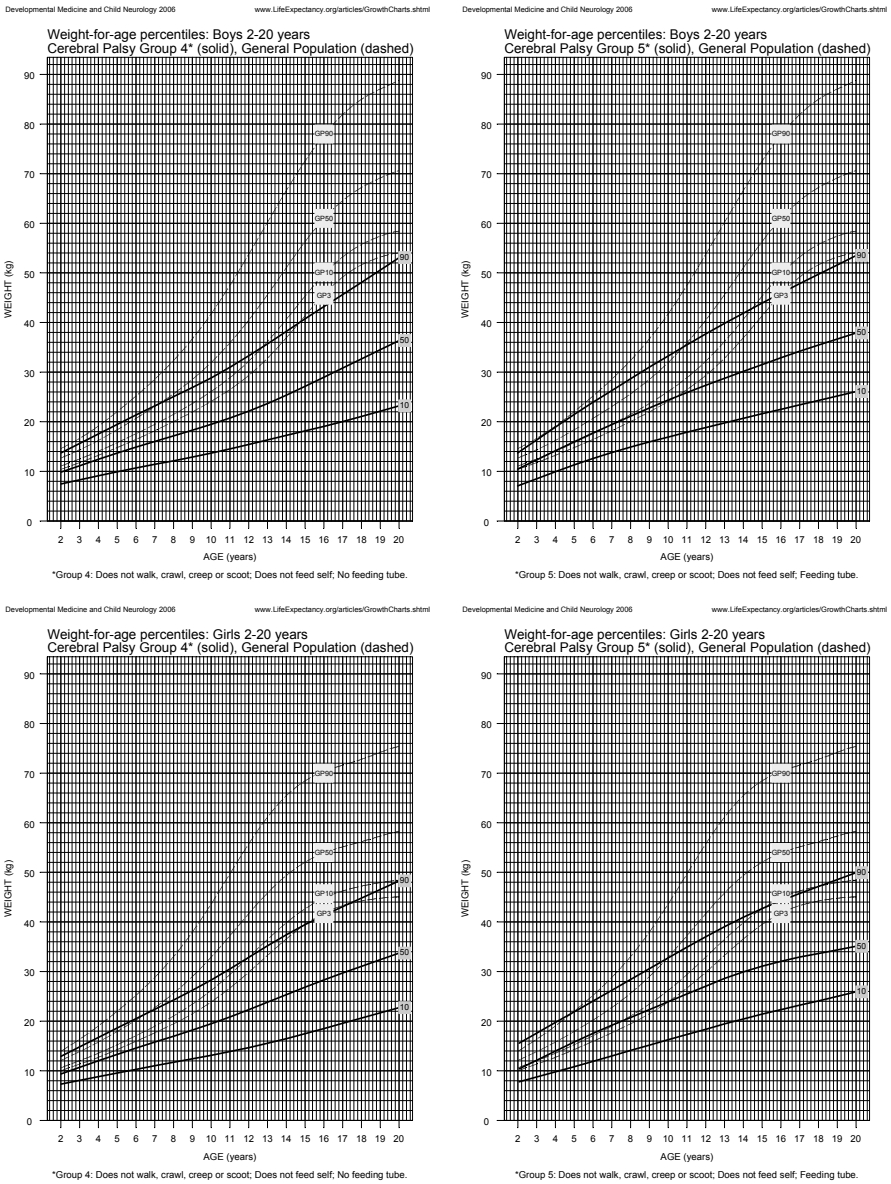


Figure 2. Weight-for-age percentiles for boys and girls with cerebral palsy by Day et al.[31] a) Boys 2 – 20 years; GMFCS level 4 b) Boys 2 – 20 years; GMFCS level 5 c) Girls 2 – 20 years; GMFCS level 4 d) Girls 2 – 20 years; GMFCS level 5.

Ideally, measuring body composition using BIA would be warranted for screening. However, from a cost perspective it would not be logical to assume that every child's day-care provider or general practitioner can afford a BIA machine.

The weight-for-age growth charts by Day et al.[30] seem to be the only viable option as a first step in diagnosing undernutrition or overnutrition, although mild cases might be missed. (Figure 2) To boost sensitivity, a screening tool for undernutrition and overnutrition should be added. To this end we used selected items from the STRONGkids screening tool by Hulst et al.[33] This screening tool is an easy-to-use tool that a physician or dietician could employ without much training. The tool is mostly directed towards detection of undernutrition. Therefore, we also added some items to identify overnutrition. In its final form it includes clinical correlates of undernutrition and overnutrition, such as excessive diarrhoea or vomiting, reduced or excessive food intake, and presence of dysphagia, absence of growth or continuing weight gain in the last six months. All items are scored on the presence or absence of the symptom described. The risk of undernutrition or overnutrition is assessed from the sum total, i.e. as either low, medium or high.

So now we have two tools for evaluating undernutrition and overnutrition (growth charts and a screening tool) by the physician for people with intellectual disability and the dietician at the children's day care centres or by the GP. We propose to weigh the children every three months. The deviation from ideal weight-to-age ratios is expressed in standard deviations. The risk of undernutrition or overnutrition is assessed from the combination of these two tools and categorized into low, medium and high risk. If the risk is considered low, measurements are repeated after the regular interval of three months. If the risk is considered medium, measurements are repeated after one month. High risk is present if weight to age ratio is lower than – 2 SD or higher than + 2 SD regardless of the score of the screening tool.

Diagnostic work-up

High risk of undernutrition or overnutrition calls for evaluation of the child's food intake and energy expenditure by the ID physician, dietician or GP using the equation developed in chapter 7. Food intake and energy expenditure are compared and intake is considered deviant if there is a difference of more than 10% in either direction. Cases when the child is undernourished according to the criteria above and intake is smaller than the child's energy expenditure or when the child is overnourished and intake is larger than the child's energy expenditure are considered uncomplicated and treatable by the dietician and physician of the day-care provider or the GP. However, if the child is undernourished and intake exceeds energy expenditure, pathology should be suspected. Then the child is not absorbing enough nutrients, for example because of a malabsorption syndrome, food loss because of emesis, or diarrhoea because of side effects of medication. If the child is overnourished and energy expenditure exceeds intake, metabolic abnormalities such as hypothyroidism

(e.g. as a side effect of anti-epileptics) should be suspected. In both cases care should be handed over to the paediatrician. If intake and energy expenditure are the same or within the 10% limit, this is also regarded as an uncomplicated case that can be treated by the ID physician, dietician or GP. However, if then the child's condition does not improve after three months of treatment, the paediatrician should also be consulted.

In addition to the data gathered by the dietician from the care organisation and outcomes of further diagnostics such as additional growth charts, anthropometry and micronutrient status, the paediatrician should perform a BIA measurement. Total body water can be calculated by using the equation described in chapter 6. Table 3 shows reference values for TBW adopted from Horlick et al.[27] If total body water deviates more than 1 standard deviation from ideal body water for age, this could further strengthen the diagnosis and the need for a nutritional intervention.

Table 3. Reference TBW values for children without disabilities aged 4 to 18 years

Age (yrs)	Mean TBW (kg)	SD (kg)	Normative values (kg)
4 – 8	15.3	3.5	11.8 – 18.8
9 – 12	23.1	5.6	17.5 – 28.7
13 – 15	32.7	6.7	26 – 39.4
16 - 18	36.6	7.8	28.8 – 44.4

Adopted from Horlick et al.[28]

TBW = total body water, SD = standard deviation, Normative values = mean ± 1 SD.

RECOMMENDATIONS FOR FUTURE RESEARCH

We have developed equations to predict total body water and energy expenditure in children with neurological impairment. Our analyses using bootstrapping to cross validate these equations would need to be validated using an objective reference method in similar samples of children with neurological impairment. The equation of BIA could then be applied in a large number of children, preferably in international context, to calculate reference values for total body water. At the moment such reference values are only known for children without disabilities. Reference values for children with neurological impairment are badly needed because there is some evidence of altered water balance in these children. A study group would have to be relatively "healthy", as suggested by Stevenson et al.[31] in a similar discussion of development of growth curves in this population. Otherwise, if undernourished and overnourished children were to be part of the "reference" group, mild nutritional problems may be missed. The definition of "healthy" poses some problems here. Where to draw the line

when faced with many co-morbid disorders? However, it will be challenging to study a group large enough to draw strong conclusions.

The equation we developed for energy expenditure would also benefit from cross validation in a separate sample. In addition, it would also be interesting to study how the equation would perform when applied in children with neurological impairment that are considered undernourished by current standards. One could then study if adjusting their caloric intake based on this equation would improve their weight, growth rate and health outcomes.

CONCLUDING REMARKS

In summary, body composition can be accurately measured using bioelectrical impedance analysis in children with neurological impairment. Furthermore, energy expenditure can be predicted with reasonable accuracy using a newly developed equation for this population. This allows for the collection of longitudinal data to describe the normative development of body composition and energy expenditure in this group of children. Furthermore, these tools can be used in intervention studies.

For clinical practice today, these tools can already be applied since no other, easy-to-use validated instrument exists for measuring body composition and energy expenditure in children with neurological impairment and ID. The treatment of nutritional problems would certainly further benefit from international consensus on the definitions of undernutrition, overnutrition, and the group itself. In addition, further research could elucidate if and how these children's extensive comorbidity affects their nutritional status. Hopefully, future research will lead to the development of reliable and prescriptive growth charts for these children.

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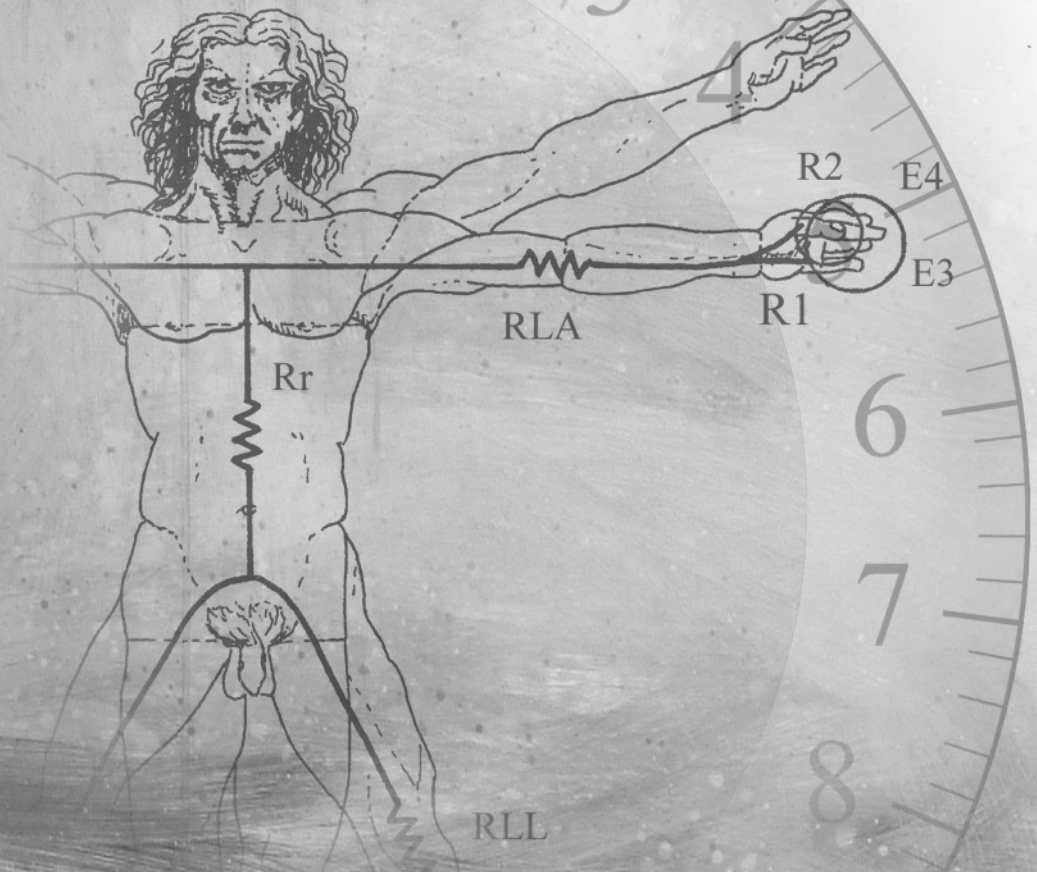
Summary



Children with severe neurological impairment and intellectual disability have many health problems and constitute a very vulnerable pediatric population. Malnutrition has always been a leading cause of morbidity and mortality in these children. This is one of the reasons why their life expectancy has been markedly reduced. Gastrostomy feeding in underfed children has turned the trend, however, although there is now increasing evidence that children receiving tube feeding run the risk of developing overnutrition.

Undernutrition and overnutrition, then, are both substantial problems that require a systematic, diagnostic approach. Regrettably, there is no evidence-based clinical guideline outlining a successful approach. Specifically, validated instruments for measuring body composition in this population are lacking. Validation is needed because children with severe neurological impairment have different growth patterns on comparison with children without neurological impairment. They also have more visceral fat, wasted muscle mass and reduced bone mass. Furthermore, there is evidence that children with severe neurological impairment may require fewer caloric intake than children without disabilities. Also lacking are equations to estimate energy expenditure in these children.

In this thesis we review current knowledge of the accuracy of easily applicable methods of evaluating body composition, i.e. skinfold measurements and bioelectrical impedance analysis (BIA). We also validated these two methods by comparing their outcomes to the equivalent outcome of the method of reference: the doubly labeled water (DLW) method. This technique uses the principle of isotope dilution and elimination to accurately measure body composition and energy expenditure. First we studied whether we could validly compare the enrichments of the isotopes used and the clinical outcomes of the DLW method in urine and saliva samples. We then descibed the occurrence of disruptive factors in an important alternative nutritional assessment technique: dual energy x-ray absorptiometry (DEXA). After having measured resting and total energy expenditure, we developed an equation to estimate energy expenditure in these children. This thesis concludes with a preliminary guideline for diagnosing undernutrition and overnutrition in children with severe neurological impairment and intellectual disability.

Chapter 1 focuses on the definition, epidemiology and aetiology of severe neurological impairment. The impact of nutritional problems on health is discussed with an emphasis on the growing practice of placing gastrostomy tubes. The instruments for measuring body composition and energy expenditure that were studied in this thesis are described in detail.

In **Chapter 2** we presented a review of available literature on the validity of easily applicable methods of evaluating body composition, i.e. skinfold measurements and

BIA, in children with severe cerebral palsy. We conducted a literature search on prior validation studies. Eligible studies had to report on a statistical comparison between these two methods and any method of reference. The QUADAS tool was used for quality assessment. The little research that has been done in this area was hampered by small sample sizes and methodological and statistical weaknesses. No solid conclusions on the validity of skinfold measurements and BIA could be drawn from these studies. There seems to be a clear need for a methodologically sound study that evaluates the validity of these two practical methods of measuring body composition in a sufficiently large group of children with severe neurological impairment and intellectual disability. Validity has to be tested by comparing the outcomes of these methods against the outcome of a 'gold standard' method of reference. The study that was set up to test the validity of skinfold measurements and BIA is discussed in chapter 6.

Chapters 3 and 4 centre on the gold standard we chose, i.e. the doubly labeled water method. They also provide a blueprint for future field research using the DLW method in children with severe neurological impairment and intellectual disability. The aim of **Chapter 3** was to compare enrichments of doubly labelled water (deuterium and oxygen^{18}) in saliva and urine samples, using automated high temperature conversion elemental analyzer isotope ratio mass spectrometry (TC-EA/IRMS). In a two-week period, urine and saliva samples of ten subjects were collected and analyzed after administering the doubly labelled water. Excellent linearity was found for measurement of enrichments of deuterium and oxygen^{18}. Good intra-assay precision and inter-assay precision was shown for both isotopes. Thus, the difference between results obtained in urine and saliva samples was small. Saliva sampling is to be preferred as time of production can be determined more precisely.

While chapter 3 focused on the technical comparability of enrichments of urine and saliva samples in children with severe neurological impairment in a more lab-oriented approach, **chapter 4** instead evaluated feasibility and comparability of the clinical outcomes of the DLW method using both sampling methods. Total energy expenditure and total body water were calculated using urine and saliva samples of thirteen children. Collection and analysis proved feasible in 88.5% of urine and 79.5% of saliva samples. Outcomes of the DLW method were comparable between both sampling methods. From a theoretical standpoint, saliva sampling is slightly preferred to urine sampling. Saliva is collected almost instantaneously after production, whereas urine can be produced earlier than its collection. However, since sampling urine and saliva is equally feasible in these children and yields comparable clinical results, applying either is justified if the other should fail.

An alternative to the DLW method as a reference method is the dual energy X-ray absorptiometry (DEXA) technique, known for its accurate bone mass density and body composition measurements. This measurement requires children to lie down in a standardized position for five to ten minutes and its outcome is sensitive to movement, scoliosis and overprojection of limbs. **Chapter 5** aimed to describe the occurrence of factors that may influence the feasibility of DEXA and the accuracy of bone density outcome in children with severe neurological impairment. Possible disrupting factors were derived from the literature and expert opinion. Occurrence of these factors was assessed in 27 children with severe neurological impairment and intellectual disability who underwent measurement of lumbar spine and total body bone mineral density with DEXA. Factors most frequently seen were movement during measurement (81.5%), aberrant body composition (66.7%), small length for age (55.6%) and scoliosis (37%). Although the amount of disrupting factors was not correlated with structural deviation of bone mineral density, physicians should be aware of the possible negative influence of these factors on the accuracy of bone density outcome in these children.

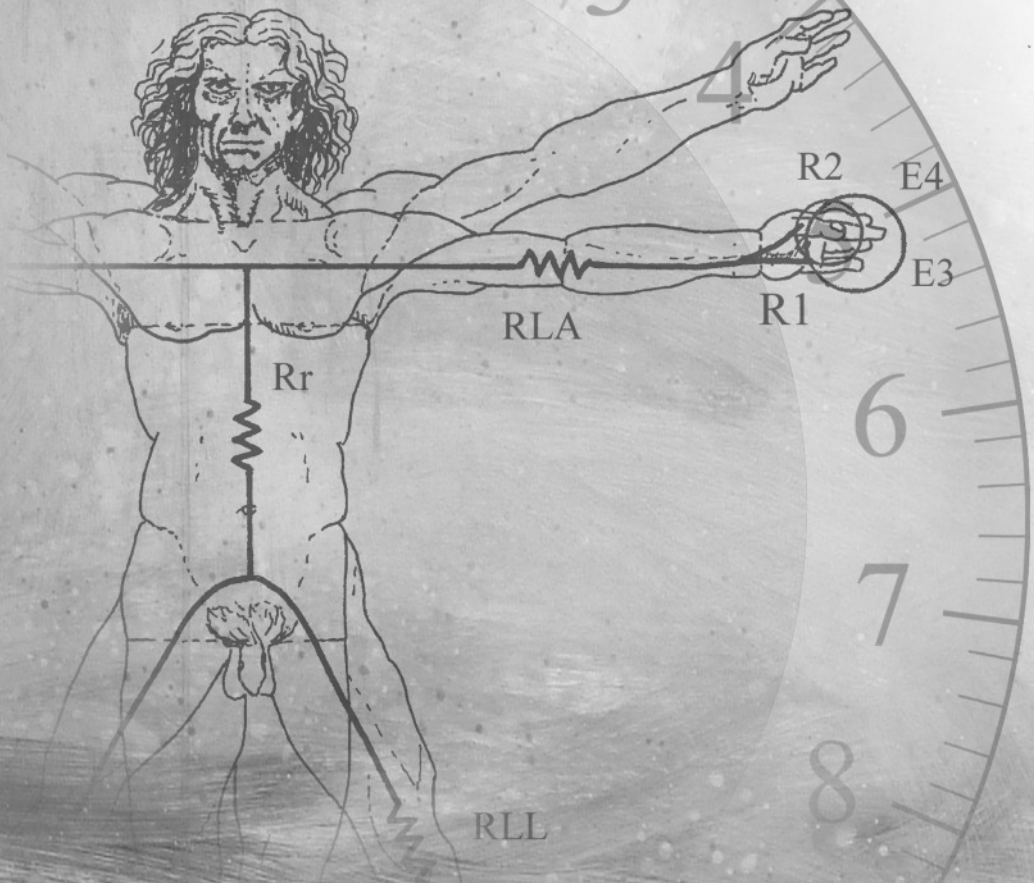
In **chapter 6** the focus was on developing new group-specific regression equations for predicting body fat based on skinfold measurements and BIA. We studied 61 children with severe neurological impairments and intellectual disability. Percentage of body fat based on skinfold measurements, calculated by previously established equations using two or four skinfolds, was underestimated in these children compared with the equivalent outcome of the DLW method. A newly-developed equation for transformation of the sum of four skinfolds into percentage of body fat, developed using the outcome of the DLW method, did not improve agreement. In BIA, the previously developed equation overestimated total body water compared with the equivalent outcome of the DLW method. In contrast with the equation developed using skinfold measurements, the newly-developed BIA equation did improve agreement considerably. The practicality of this new equation was also high because it included tibia length as an alternative to standing height, which can be difficult to measure in children with scoliosis and contractures. Measuring total body water using BIA proved more accurate than measuring percentage of body fat using skinfold measurements in children with severe neurological impairment and intellectual disability.

In **chapter 7** the aim was to develop a new group-specific regression equation for predicting total energy expenditure using the DLW method as reference. Energy expenditure was measured using two methods of reference in a group of 61 children with severe neurological impairment and intellectual disability. Indirect calorimetry was used to measure resting energy expenditure (REE); the DLW method method to

measure total energy expenditure (TEE). Indirect calorimetry failed in six children and measured REE using this method was higher than measured TEE in almost a third of children, which is theoretically impossible. We therefore discourage its use in this particular population. Previously established equations, most of which are widely used in daily practice to evaluate caloric requirement, overestimated TEE measured by the DLW method. Data on degree of movement, mobility, muscle tone and presence of epilepsy were successfully collected in 52 of 61 children. These data were used to create a group-specific equation for predicting energy expenditure. Although the standard error using this new equation is still considerable, it is a first step in the development of a group-specific equation to adequately estimate energy expenditure in children with neurological impairment and intellectual disability.

In **chapter 8** a general discussion of this thesis is presented. It discusses the choice for the definition of the children studied. The limitations of the nutritional assessment methods used in this thesis are described in the context of the results. Lastly, based upon the knowledge acquired, a preliminary guideline is introduced that aims to diagnose undernutrition and overnutrition in children with severe neurological impairment and intellectual disability.

Samenvatting



Kinderen met ernstige meervoudige beperkingen hebben veel gezondheidsproblemen en vormen een zeer kwetsbare populatie. Ondervoeding heeft altijd een belangrijke rol gespeeld in de morbiditeit en mortaliteit van deze kinderen en om deze reden is hun levensverwachting ook doorgaans zeer beperkt. Het feit dat kinderartsen tegenwoordig eerder besluiten tot plaatsing van een PEG sonde bij ondervoede kinderen heeft deze trend doorbroken. Het heeft echter ook een andere ongewenste ontwikkeling in gang gezet: er is steeds meer evidentie dat kinderen die gevoed worden via een PEG sonde juist meer risico hebben op het ontwikkelen van overvoeding.

De preventie en behandeling van onder- en overvoeding behoeven een systematische aanpak. Helaas bestaat er momenteel nog geen op evidentie gebaseerde klinische richtlijn die een dergelijke aanpak beschrijft voor deze groep kinderen. Gevalideerde instrumenten om de voedingstoestand te meten ontbreken zelfs op het moment. Het is belangrijk deze instrumenten te valideren omdat kinderen met ernstige meervoudige beperkingen een ander groeipatroon hebben dan kinderen zonder beperkingen. Ze hebben ook meer visceraal vet, minder spiermassa en een beperkte botmassa. Daarnaast is er ook steeds meer bewijs dat kinderen met ernstige meervoudige beperkingen minder calorieën verbruiken dan kinderen zonder beperkingen. Formules om het energieverbruik te schatten bij deze kinderen zijn nog niet ontwikkeld.

In dit proefschrift beschrijven we de huidige stand van zaken met betrekking tot de nauwkeurigheid van enkele eenvoudig toepasbare meetmethoden om de lichaamssamenstelling te meten, namelijk huidplooimetingen en bioelectrische impedantie analyse (BIA). We hebben deze methoden ook gevalideerd in deze groep door hun uitkomsten te vergelijken met de vergelijkbare uitkomst van een referentiemethode: de dubbel gelabeld water (DLW) methode. Deze laatstgenoemde techniek is gebaseerd op het principe van isotoopverdunning en -eliminatie om zeer nauwkeurig de lichaamssamenstelling en het energieverbruik te kunnen meten. Allereerst bestudeerden we of de verrijkingen van de gebruikte isotopen en de klinische uitkomsten van de DLW methode in urine- en speekselmonsters vergelijkbaar waren. Daarna onderzochten we de verstorende factoren, die een rol kunnen spelen bij de beoordeling van een andere techniek om de voedingsstatus te meten: dual energy x-ray absorptiometry (DEXA). Naast de vergelijking van de uitkomsten van huidplooimetingen en BIA met bestaande formules, hebben we nieuwe formules ontwikkeld waarmee de lichaamssamenstelling berekend kan worden in deze specifieke groep kinderen. Nadat we het rustverbruik en het totaal energieverbruik hadden gemeten, hebben we een formule ontwikkeld waarmee het energieverbruik van deze kinderen kan

worden geschat. Dit proefschrift besluit met een voorlopige richtlijn om onder- en overvoeding te diagnosticeren in kinderen met ernstige meervoudige beperkingen.

Hoofdstuk 1 richt zich op de definitie, epidemiologie en etiologie van een ernstige meervoudige beperking. De impact van voedingsproblemen op de gezondheid wordt besproken waarbij de nadruk wordt gelegd op de toename van het plaatsen van een PEG sonde bij deze kinderen. De instrumenten die in dit proefschrift zijn gebruikt om de voedingstoestand en het energieverbruik te meten, worden in detail beschreven.

In **hoofdstuk 2** wordt een overzicht gegeven van de beschikbare literatuur over de validiteit van huidplooimetingen en BIA bij kinderen met ernstige cerebrale parese. We hebben een uitvoerige literatuurstudie gedaan naar de validiteit van deze instrumenten bij deze kinderen. Enkel studies die een statistische vergelijking hadden gedaan tussen deze twee methoden en een referentiemethode zijn geïncludeerd. Het QUADAS instrument is gebruikt om de kwaliteit van de studies te evalueren. We concludeerden dat het weinige onderzoek dat is gedaan verder beperkt werd door kleine populaties en methodologische en statistische tekortkomingen. Harde conclusies over de validiteit van huidplooimetingen en BIA konden niet worden getrokken op basis van deze studies. Er bestaat een duidelijke behoefte aan een studie zonder methodologische tekortkomingen die de validiteit van deze twee praktische methoden om de voedingstoestand te meten evalueert in een grotere groep kinderen met ernstige meervoudige beperkingen. Het is belangrijk dat de validiteit wordt getest door de uitkomsten van deze methoden te vergelijken met de uitkomst van een 'gouden standaard'. De studie die is opgezet om de validiteit van huidplooimetingen en BIA te bepalen wordt besproken in hoofdstuk 6.

Hoofdstuk 3 en 4 richten zich op de gouden standaard die we hebben gekozen: de dubbel gelabeld water methode. Deze hoofdstukken zijn een blauwdruk voor de uitvoering van de dubbel gelabeld water methode bij kinderen met ernstige meervoudige beperkingen. Het doel van **hoofdstuk 3** was om de verrijkingen van dubbel gelabeld water (deuterium en zuurstof18) te vergelijken in speeksel- en urinemonsters. Hierbij werd gebruik gemaakt van 'automated high temperature conversion elemental analyzer isotope ratio mass spectrometry' (TC-EA/IRMS). In een periode van twee weken werden bij 10 proefpersonen urine- en speekselmonsters afgenomen en geanalyseerd na toediening van deuterium en zuurstof[18]. De betrouwbaarheid binnen een meting en tussen metingen was goed voor beide isotopen. Het verschil tussen de verrijkingen van urine- en speekselmonsters was klein. Het bemonsteren van speeksel verdient echter de voorkeur omdat het tijdstip van productie veel preciezer kan worden bepaald dan dat van urine.

Terwijl hoofdstuk 3 zich meer richtte op de vergelijkbaarheid van de verrijkingen van urine- en speekselmonsters bij kinderen met ernstige meervoudige beperkingen vanuit een labtechnische benadering, richtte **hoofdstuk 4** zich meer op de haalbaarheid van de monsterafname en de vergelijkbaarheid van de klinische uitkomsten van de DLW methode tussen beide bemonsteringsmethoden. Aan de hand van de uitkomsten van urine- en speekselmonsters van 13 kinderen werden het totaal energieverbruik en totaal lichaamswater berekend. De afname en analyse was succesvol bij 88.5% van de urinemonsters en 79.5% van de speekselmonsters. De uitkomsten van de DLW methode waren vergelijkbaar tussen beide bemonsteringsmethoden. Vanuit theoretisch oogpunt heeft het afnemen van speekselmonsters een lichte voorkeur ten opzichte van het afnemen van urinemonsters: speeksel wordt meestal direct verzameld na de productie ervan, terwijl urine veel eerder geproduceerd kan zijn dan de daadwerkelijke verzameling. Echter, aangezien het afnemen van urine- en speekselmonsters even haalbaar is bij deze kinderen en vergelijkbare klinische resultaten oplevert, kan een van beide bemonsteringsmethoden worden toegepast als de andere niet mogelijk is.

Een alternatieve referentiemethode is de dual energy x-ray absorptiometry (DEXA) techniek, die bekend staat om zijn nauwkeurige metingen van de botmassa en de lichaamssamenstelling. Om deze methode uit te voeren, is het noodzakelijk dat kinderen gedurende vijf tot tien minuten in een geprotocolleerde houding liggen. De uitkomst van de DEXA kan negatief worden beïnvloed door beweging tijdens de meting, scoliose en overprojectie van ledematen. In **hoofdstuk 5** worden de verstorende factoren beschreven die de haalbaarheid van DEXA en de nauwkeurigheid van de botmetingen negatief beïnvloeden bij kinderen met ernstige meervoudige beperkingen. Een uitgebreide literatuurstudie en een enquête onder experts op dit vakgebied leverde een aantal mogelijk verstorende factoren op. Het vóórkomen van deze factoren is onderzocht bij 27 kinderen met ernstige meervoudige beperkingen die botdichtheidmetingen ondergingen van de lumbale wervelkolom en van het gehele lichaam. Verstorende factoren die het meest werden geobserveerd tijdens deze metingen waren: beweging tijdens de meting (81,5%), een afwijkende lichaamssamenstelling (66,7%), korte lengte naar leeftijd (55,6%) en scoliose (37%). Alhoewel de hoeveelheid verstorende factoren niet gecorreleerd was met structurele afwijkingen van de botdichtheid, zouden artsen zich bewust moeten zijn van de mogelijk negatieve invloed van deze factoren op de nauwkeurigheid van botdichtheidmetingen bij deze kinderen.

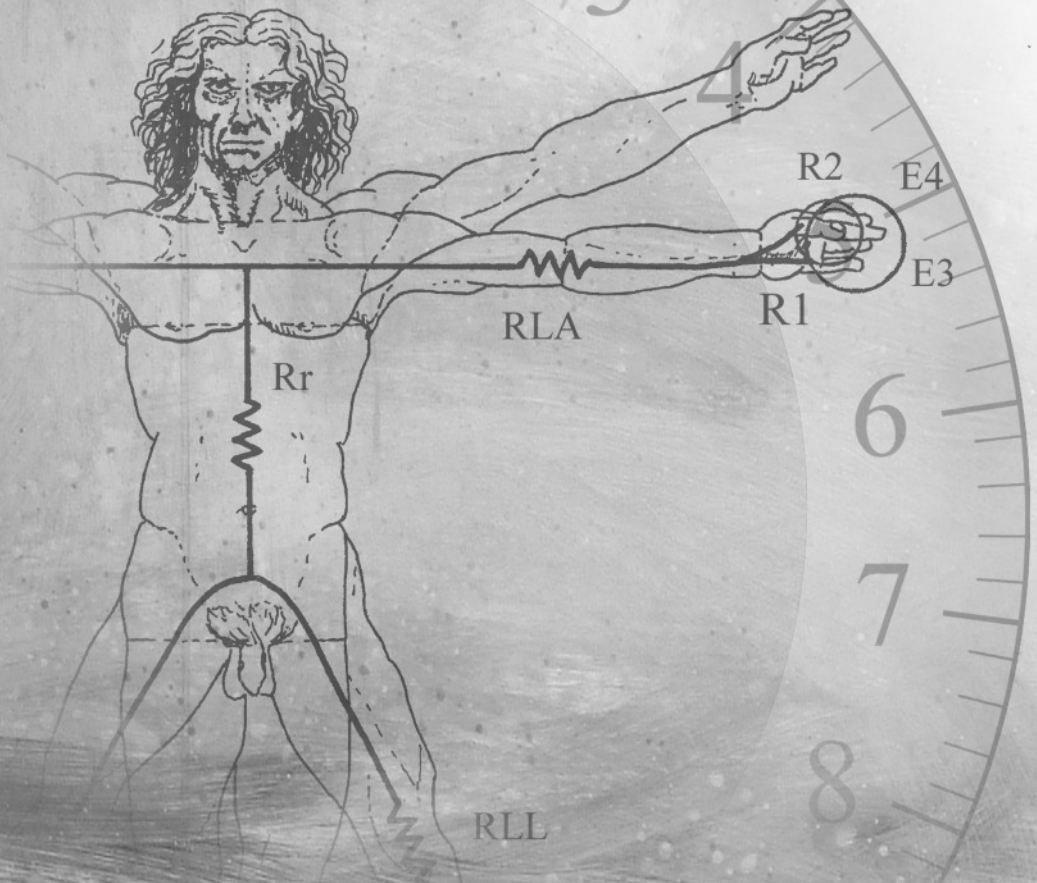
Het focus van **hoofdstuk 6** was het ontwikkelen van een formule om de lichaamssamenstelling te berekenen op basis van de resultaten van huidplooimetingen en BIA van 61 kinderen met ernstige meervoudige beperkingen. Het percentage

lichaamsvet, berekend met twee verschillende formules gebaseerd op twee of vier huidplooien, werd onderschat bij deze kinderen vergeleken met de uitkomst van de DLW methode. Een nieuw ontwikkelde formule gebaseerd op de som van vier huidplooien verbeterde de overeenkomst niet. De eerder ontwikkelde formule voor BIA uitkomsten overschatte het lichaamswater vergeleken met de uitkomst van de DLW methode. In tegenstelling tot de nieuw ontwikkelde formule voor huidplooimetingen, is de nieuwe BIA formule wel in staat het lichaamswater beter te voorspellen. Deze BIA formule is ook beter toepasbaar aangezien deze gebruik maakt van de tibialengte in plaats van de volledige lichaamslengte, die moeilijk te meten is bij kinderen met scoliose en contracturen. Het meten van het totaal lichaamswater met behulp van BIA was nauwkeuriger dan het meten van het percentage lichaamsvet met behulp van huidplooimetingen bij kinderen met ernstige meervoudige beperkingen.

Het doel van **hoofdstuk 7** was om een nieuwe formule te ontwikkelen om het totale energieverbruik te schatten van kinderen met ernstige meervoudige beperkingen met behulp van de DLW methode als referentie. Het energieverbruik was gemeten met twee referentiemethoden in een groep van 61 kinderen met ernstige meervoudige beperkingen. Het rustverbruik werd gemeten met indirecte calorimetrie; het totale energieverbruik werd bepaald met behulp van de DLW methode. Indirecte calorimetrie mislukte in zes kinderen. In een derde van de resterende groep kinderen was het gemeten rustverbruik hoger dan het gemeten totale energieverbruik, wat theoretisch onmogelijk is. We adviseren daarom deze methode niet te gebruiken bij deze populatie kinderen. Eerder ontwikkelde pediatrische formules om de calorische behoefte te bepalen overschatten het totale energieverbruik gemeten met de DLW methode. Gegevens over beweeglijkheid, mobiliteit, spiertonus en de aanwezigheid van epilepsie zijn succesvol verzameld bij 52 van de 61 kinderen. Deze gegevens zijn gebruikt om een nieuwe formule te ontwikkelen die het energieverbruik van deze kinderen voorspelt. Hoewel de standaardfout van deze formule nog aanzienlijk is, is het een eerste stap in de ontwikkeling van een formule, specifiek gericht op deze groep kinderen, om het energieverbruik in te kunnen schatten.

In **hoofdstuk 8** wordt een algemene discussie gevoerd over de inhoud van dit proefschrift. Het hoofdstuk stelt de keuze voor de definitie van de doelgroep ter discussie. Verder worden de beperkingen van de methoden, waarmee de lichaamssamenstelling en het energieverbruik gemeten zijn, besproken in het licht van de resultaten. Tenslotte wordt een voorlopige richtlijn gepresenteerd gebaseerd op de verworven kennis met als doel onder- en overvoeding te diagnosticeren bij kinderen met ernstige meervoudige beperkingen.

Dankwoord



Het is tijd om het laatste hoofdstuk van het gehele proefschrift te schrijven. Hoe makkelijk het ook is om mensen te bedanken voor hun bijdrage aan dit werk, zal ik onherroepelijk sommigen van jullie vergeten te vermelden. Weet dan toch dat ik iedereen dankbaar ben die mij in de loop der jaren gesteund heeft met een telefoontje of praatje op de gang.

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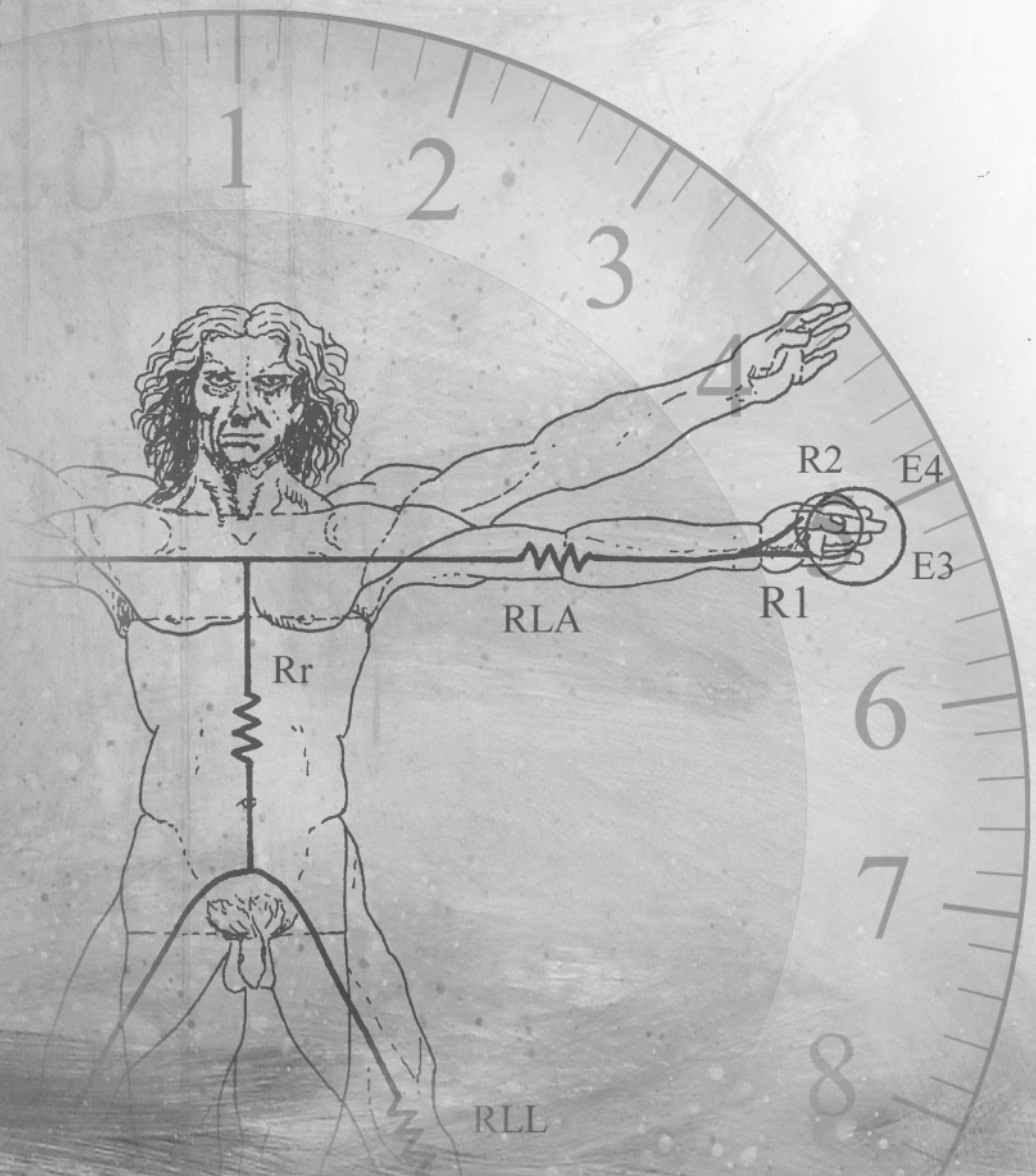
About the author

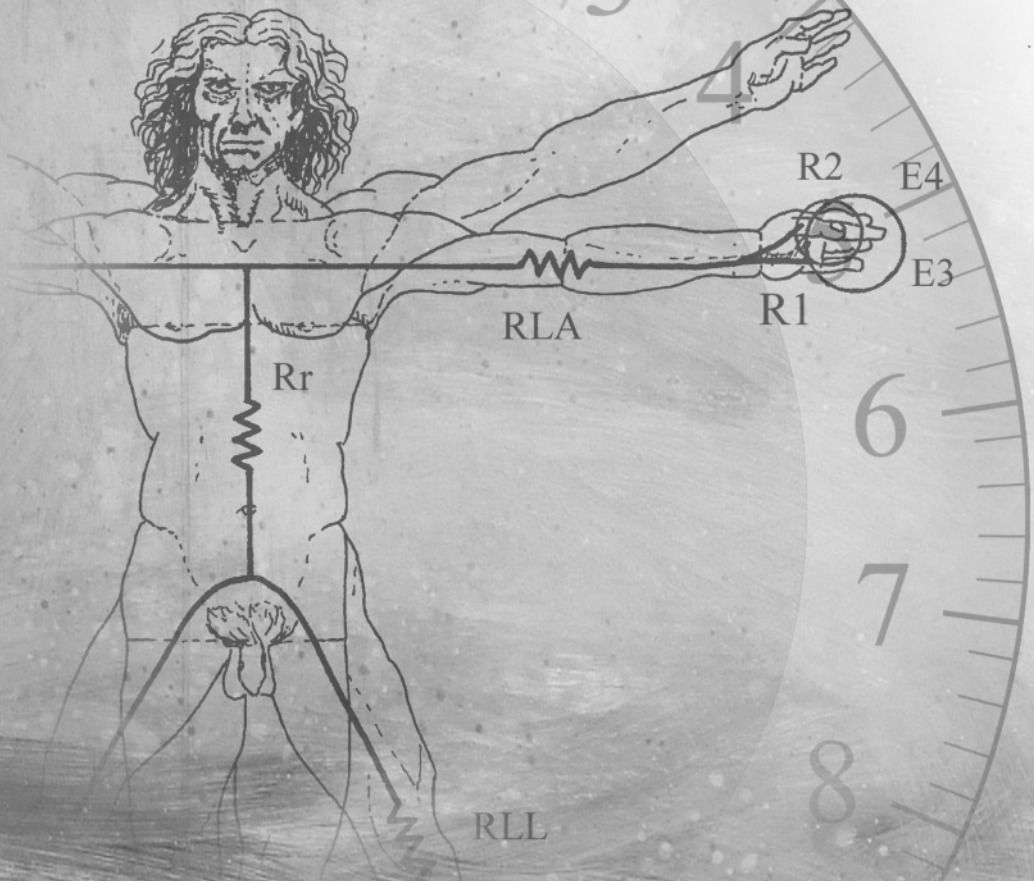


Rob Rieken was born on February 18th, 1978 in Luyksgestel, the Netherlands. He went to grammar school (VWO) at the Were Di College in Valkenswaard and graduated in 1996. In that same year, he started as a student of Psychology at Radboud University Nijmegen. In 1998 he switched studies and began his medical training at the same university. He received his medical degree in April of 2005, during which he completed his student research as part of the medical training ("The reproducibility of measurements of lung function in children with severe neurological impairment and intellectual disability") at the Chair of Intellectual Disability Medicine (head: Prof.dr. Evenhuis), department of General Practice at the Erasmus MC, Rotterdam.

In July of 2005 he started his PhD track ("Measuring body composition and energy expenditure in children with severe neurological impairment and intellectual disability"), which is a collaborative effort of the department of Intellectual Disability Medicine and the department of Paediatric Surgery (head: Prof.dr. Tibboel) in the Erasmus MC – Sophia Children's Hospital, Rotterdam. His PhD studies resulted in this doctoral thesis. Since October 2009 he is working as "ANIOS" (physician not in training) at the department of Child and Adolescent Psychiatry in the Erasmus MC – Sophia Children's Hospital.

Rob Rieken is happily married to Patrick Dorssers and they live in Delft.

PhD portfolio



Name PhD student:	Rob Rieken
Erasmus MC department:	Intellectual Disability Medicine / Dpt. of General Practice; Dpt. of Pediatric Surgery
PhD period:	2005 – 2010
Promotors:	Prof.dr. H.M. Evenhuis Prof.dr. D. Tibboel
Copromotor:	Dr. C. Penning

1. PHD TRAINING

General academic skills

Biomedical English Writing and Communication, 2009 (1.4 ECTS)
Basiscursus Regelgeving en Organisatie voor Klinische onderzoekers (BROK), 2009 (32 hrs)

Research skills

Methodology
Principles of Research in Medicine, 2008 (0.7 ECTS)
Paediatric Clinical Epidemiology (KEK), 2007 (24hrs)

Statistics
SPSS (Learnit), 2007 (16hrs)

Presentations

Oral presentation NVK Veldhoven, 2005 (40hrs)
Oral and poster presentation IASSID Maastricht, 2006 (40hrs)
Departmental presentations, 2006, 2008 en 2009 (12hrs)

International conferences

AACPDM Boston, 2006 (21hrs)
AACPDM Vancouver, 2007 (21hrs)
AACPDM Washington, 2010 (21hrs)

Seminars and workshops

Masterclass V. Moyer: "Improving your Clinical Research", 2006 (4hrs)
Dutch conference on nutrition Ede, 2008 (8hrs)

2. TEACHING ACTIVITIES

Lecturing

Guest lecturer (ID physician training), 2008 and 2009 (8hrs)

Supervising practicals and excursions

Workshop: Spoorzoekers – Platform EMG, 2007 (10hrs)

Other

Supervising student research, 2006 (80hrs)