UNIVERSITÀ DEGLI STUDI DI MILANO

DOCTORAL PROGRAMME IN NUTRITIONAL SCIENCE

NUTRITIONAL FINDINGS IN NAÏVE CHILDREN AFFECTED BY SPINAL MUSCULAR ATROPHY TYPE 1 AND TYPE 2

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"What matters is the mortar, not just the bricks. [...]
Because it is only when we accept that everybody has value
that we'll liberate the Energy, and imagination, and
Momentum we need to create ***the Best beyond Measure****".*

Margaret Heffernan,
Beyond Measure
TED Books (2015)

Abstract

Spinal muscular atrophies (SMA) include a group of neuromuscular disorders characterized by degeneration of alpha motor neurons in the spinal cord with progressive muscle atrophy, weakness and paralysis. Patients are affected by the highest burden of co-morbidities that deteriorate nutritional status. Respiratory and gastrointestinal problems increase the risk of underweight, whereas the decrement in physical activity can lead to overweight and obesity. Alterations of fatty acid metabolism and hyperglycaemia have been reported. These nutritional and metabolic abnormalities have been poorly investigated by means of systematic approaches so far, but in the last decade these have become even more important thanks to the increasing evidence of improvements in survival and quality of life and the availability of the first approved treatment and other therapeutic options at an advanced phase.

Aims of this study were to investigate growth patterns, body composition, energy expenditure and dietary intake in a large sample of Italian infants and children with SMA1 and SMA2. Secondary objectives were to evaluate the accuracy of field methods on a reference one to assess body fat mass in SMA patients; to develop predictive energy formula; to investigate glucose and lipid metabolism; to evaluate feeding and dietary habits, and to investigate association of body composition with motor function.

An observational study was conducted in order to investigate nutritional and metabolic status in a large sample of Italian SMA1 and SMA2 children applying gold standard methods for body composition, energy expenditure, dietary intake, glucose and lipid metabolism.

This study provided a map of naïve SMA1 and SMA2 children in terms of growth pattern, body composition and resting energy expenditure. Anthropometry has proven to be a useful tool for nutritional assessment in SMA and the state of ventilation, besides demographic and anthropometric characteristics, determines the energy needs of SMA patients. Defects in glycaemic and fatty acid metabolism can lead to additional comorbidities, further emphasizing the importance of nutritional management, especially if several dietary deficiencies must be corrected after established checks by the specialist.

These data on natural history provide a consistent reference by better understanding the natural progression of SMA and laying the foundations for the development of national, and therefore international, references to the nutritional management of this disease, even more necessary in the new therapeutic era of SMA.

Riassunto

L’atrofia muscolare spinale (SMA) comprende un gruppo di malattie neuromuscolari caratterizzate dalla degenerazione dei motoneuroni alfa del midollo spinale con progressiva atrofia muscolare, debolezza e paralisi. I pazienti sono affetti da diverse comorbidità che deteriorano lo stato nutrizionale. I problemi respiratori e gastrointestinali aumentano il rischio di sottopeso, mentre la diminuzione dell'attività fisica può portare a sovrappeso e obesità. Alterazioni del metabolismo degli acidi grassi e iperglicemia sono riportate. Queste anomalie nutrizionali e metaboliche sono state finora poco studiate con approcci sistematici, ma nell'ultimo decennio sono diventate ancora più importanti grazie alla crescente evidenza di miglioramenti nella sopravvivenza e nella qualità della vita e alla disponibilità del primo trattamento approvato e di altre opzioni terapeutiche in fase avanzata.

Lo scopo di questo studio è stato studiare la crescita, la composizione corporea, il dispendio energetico e le abitudini alimentari di un ampio campione di bambini italiani affetti da SMA1 e SMA2. Gli obiettivi secondari sono stati: valutare l'accuratezza dell’antropometria per l’assessment della massa grassa; sviluppare una formula predittiva per il calcolo dei consumi energetici a riposo; studiare il metabolismo glicemico e lipidico; valutare le abitudini alimentari e dietetiche, e studiare l'associazione della composizione corporea con la funzionalità motoria.

È stato condotto uno studio osservazionale applicando metodi gold standard per la composizione corporea, il dispendio energetico, i consumi alimentari, il metabolismo glucidico e lipidico.

Questo studio ha fornito una mappa dei bambini SMA1 e SMA2 in termini di stato di accrescimento, composizione corporea e dispendio energetico a riposo. L'antropometria si è dimostrata uno strumento utile per la valutazione nutrizionale nella SMA, e lo stato di ventilazione, oltre alle caratteristiche demografiche e antropometriche, determina il fabbisogno energetico dei pazienti SMA. Difetti nel metabolismo glicemico e degli acidi grassi possono portare ad ulteriori comorbidità, sottolineando ulteriormente l'importanza della gestione nutrizionale, soprattutto per la presenza di diverse carenze alimentari da correggere dopo controlli da parte dello specialista.

Questi dati di storia naturale forniscono riferimenti utili a comprendere meglio la progressione naturale della SMA e pongono le basi per lo sviluppo di riferimenti nazionali, e quindi internazionali, per la gestione nutrizionale di questa malattia, ancora più necessaria nella nuova era terapeutica.

Contents

CHAPTER 1

Introduction

1.1 Spinal Muscular Atrophy

Definition of Spinal Muscular Atrophy
Spinal muscular atrophies (SMA) include a group of neuromuscular disorders characterized by degeneration of alpha motor neurons in the spinal cord with progressive muscle atrophy, weakness and paralysis [1]. The condition is medically serious, associated with significant motor disability, respiratory insufficiency, and death in infancy or childhood in more than 50% of affected children [2].

SMA is the second most common fatal autosomal recessive disorder after cystic fibrosis, with an estimated incidence of 1 in 6,000 to 1 in 10,000 live births, with a carrier frequency of 1/40 of 1/40 - 1/60 [3]. Homozygous deletion and/or mutation of the survival motor neuron 1 (SMN1) gene on 5q13 is disease-causing, and the severity inversely correlates with SMN2 copy number [3].
The diagnosis of SMA is based on a molecular genetic testing. Genetic testing of SMN1/SMN2 is highly reliable and it is first line investigation when the condition is suspected in a typical case. There is no need for muscle biopsy in a typical presentation [1].

Classification

SMA includes a wide range of phenotypes that are classified into clinical groups based on age of onset and maximum motor function achieved: very weak infants unable to sit unsupported (type 1, SMA1), non-ambulant patients able to sit independently (type 2, SMA2), up to ambulant patients with childhood (type 3) and adult onset SMA (type 4) [1].

SMA1, also known as Werdnig- Hoffman disease, is the most common and severe form of the disorder. Muscle weakness is typically evident by six months of age, and affected infants never achieve the ability to sit unsupported. Historically, the majority of children diagnosed with SMA1 did not survive past their second birthday due to the development of severe, progressive respiratory insufficiency [1]. However, due to increasingly proactive intervention in clinical care in recent years, survival rates in children with SMA1 have improved, with an increasing percentage surviving beyond 2 years of age [1,4].

For those children whose age at onset is older than 6 months, the classification should be based on maximum function achieved and not necessarily by age at onset. Children who received a diagnosis of spinal muscular atrophy during the sitting stage, could in fact advance to the walking stage. Dubowitz proposed a classification system of 2.0, 2.5 and 2.9 for weak and strong sitters (SMA2) and 3.0, 3.5 and 3.9 for weak and strong walkers (SMA3) [5].

Table 1: Classification of SMA [5]

Type	Onset	Maximum Function Achieved	Life Expectancy
0	Prenatal	Need respiratory support at birth	Fatal at birth without respirator support
1	<6 mo	Sits with support only	<2y
2	6-18 mo	Sits independently when placed	10-40 y
3	>18 mo	Walks independently 25 steps	Indefinite
4	>5 y	Walks normally	Indefinite

Respiratory and motor dysfunctions

Patients with SMA, particularly SMA1 and SMA2, are affected by the highest burden of comorbidities, especially in the field of neuromuscular and respiratory problems.

Clinically, these infants present with hypotonia, progressive symmetric and proximal weakness affecting the legs more than the arms, sparing of the facial muscles but often with bulbar muscles weakness. There is also weakness of the intercostal muscles with relative sparing of the diaphragm, which results in the typical "bell-shaped" chest and paradoxical breathing pattern [1]. Respiratory problems, including weak cough, increased work of breathing, dyspnoea, pneumonia, cyanosis and desaturation during meals, and bulbar dysfunction can result in aspiration and pulmonary infections [1].

Scoliosis is still highly prevalent in children with SMA 1 and 2, with incidence of 60-90% and initial presentation in early childhood. The hypotonic spinal curves continuously progress through childhood. Collapse of the ribs (similar to closing an umbrella) contributes to "parasol rib" deformity. Hip instability is common. Contractures are common as a result of decreased range of motion, prolonged static positioning, and agonist-antagonist muscle imbalance. Functionally and symptomatically, contractures can lead to pain and inhibit function in patients with SMA. Because of immobility, osteoporosis and low vitamin D levels, fragility fractures are common in children with SMA 1 and 2 [1].

1.2 Nutritional impairments

Nutritional impairments refer to gastrointestinal dysfunction, including constipation, delayed gastric emptying and gastroesophageal reflux (GOR), are an important determinant of mortality and morbidity, and swallowing dysfunction and reflux are important contributors to pulmonary disease [6].

However, nutritional disorders result from a severe decrement in physical activity as well that reduces energy needs and increases the risk of overweight and obesity.

On the other hand, SMA patients are at risk for underweight secondary to respiratory and gastrointestinal problems.

Respiratory problems may increase energy expenditure, while gastrointestinal problems may reduce food intake as well.

Nutritional status may remain impaired and results in growth failure [7]. Each study showed nutritional derangements, varying from overnutrition to undernutrition, and our previous results also suggested a different nutritional status and body composition in SMA1 children in relation to the magnitude of neurofunctional impairment [8]. Most commonly, SMA1 children show reduced linear growth and later weight gain [2], whereas SMA2 children tend to become overweight or obese [9,10], although this does not always hold true in individual cases.

The comparison of SMA1 children with SMA2 showed similar body weight, supine length and segmental lengths and double severe malnutrition prevalence [8]. More than 50% of SMA children showed an above average supine length, and none were stunted (chronic under-nutrition due to inadequate energy and protein intake) [8]. No SMA children had a fat mass percentage lower than the reference values [11]. SMA1 children had total and regional fat masses, similar to SMA2, whereas total fat free mass and lean body mass were lower in SMA1, particularly at the trunk level, probably related to the magnitude of neurofunctional impairment, rather than to nutritional derangement [8].

In addition, bone mineral density (BMD) is decreased in SMA patients. In a recent study on Italian children with SMA2 and SMA3, 50% of them had low BMD with asymptomatic vertebral fractures [12].

SMA has traditionally been considered a motor neuron disease. However, this view has evolved as defects in multiple non-neuronal cell types have been identified. It is unclear whether extra-neuronal components are related to the underlying genetic abnormality, and therefore to an effect of the reduction of the SMN protein also on other organs other than the neuromuscular system, or are they thought to be secondary to other factors [13–15]. Alterations of fatty acid metabolism, hyperlipidaemia and hyperglycaemia have been reported in SMA patients. These anomalies are more evident after the infusion of intravenous

glucose during illness or after prolonged fasting. A progressive shift of the composition of pancreatic islets from insulin-producing β cells to glucagon-producing α cells is a possible explanation. Histopathological evidence for such hypothesis was reported both in SMA murine models and SMA1 patients [13–15].

The above-mentioned nutritional and metabolic abnormalities have been poorly investigated by means of systematic approaches so far. Most importantly, it has become more and more obvious that they should be managed with appropriate nutritional strategies to improve quality of life for these children and their families [1]. For example, undernutrition and overnutrition should be counteracted by an appropriate intake of energy and nutrients and BMD reduction should be partly resolved by a proper introduction of calcium, vitamin D and magnesium [16,17]. Lastly, as diet, in particular the amount and quality of carbohydrates, has a high influence on glucose and lipid metabolism, a nutritional approach aimed to balance insulin and glucagon secretion in SMA patients may be valuable to counteract the metabolic abnormalities of SMA patients.

1.3 Current management

Current management of SMA is based on supportive and multidisciplinary care with a focus on reducing complications and improving quality of life [1].

Pulmonary disease is the major cause of mortality in SMA1 and SMA2 [7]. Management strategies include airway clearance, cough assistance, nocturnal non-invasive ventilatory support and continuous non-invasive ventilation [7].

Medical management of gastrointestinal dysfunction includes the use of prokinetic agents and acid neutralisers. In severe cases, severe underweight and gastrointestinal problems per se require enteral therapy in early childhood in many SMA1 and, less frequently, SMA2 patients as well. A percutaneous endoscopic gastrostomy (PEG) is the most common recommended option to feed appropriately such patients and laparoscopic Nissen fundoplication and

gastrostomy tube placement are performed in order to reduce GOR [7].

Orthopaedic care and rehabilitation strategies aim to address functional impairment caused by muscle weakness. Nutrition support, posture management, assistive equipment, physical therapy, occupational therapy, limb orthotics and pain management are key interventions used to improve the functional level and quality of life for patients [1,7,18].

The 2017 Standards of Care for Spinal Muscular Atrophy (SoC) [1,18] note that there is evidence of improvements in prognosis and an increase in survival. Indeed, over the last ten years SMA disease has been studied in many ways as a result of a more proactive approach in the management of this disorder [1], also thanks to the new available therapies will likely change the survival and natural history of the disease [18]. However, only few studies have been conducted on nutritional aspects [2,9,19–21], all of them on small samples and only few of them used gold standard methods of nutritional status, body composition and energy expenditure [6].

In addition, the recent advent of new drugs and clinical trials for SMA is changing the course of the disease, making the clinical and nutritional aspects even more important in order to improve the quality of life.

Nutritional management

With clinical trials of pharmaceutical agents and new drugs now under way [4,18], preserving optimal nutrition – especially for children – is an important priority in SMA [6]. Nutritional considerations for children with SMA are complex and poorly understood. Altered body composition and decreased fat free mass have been identified [10], but the impact of muscle wasting on growth and nutritional requirements is unclear.

There are few reports that specifically address the anthropometry, body composition and growth expectations of SMA patients. The Consensus Statement for Standard of Care in Spinal Muscular Atrophy [1,7] recommends to

measure regularly weight, height/length, weight-for-height/length and growth velocity to detect signs of growth failure or altered nutritional status. The use of reference data [22,23] developed for healthy children is suggested but the limitations of this approach are recognized because of the peculiar nature of SMA. Considering their reduced muscle mass and mobility, it can be reasonably assumed that body composition in SMA1 and SMA2 children is quite different from that of healthy children [6]. Our previously data suggested that anthropometry measurements may be misleading in assessing nutritional status, particularly in SMA1 and SMA2 with "normal" or "increased" fat mass may be classified as underweight because of their decreased fat free mass, and could lead to erroneous undernutrition diagnoses, and, in turn, to overfeeding dietary intervention [8]. Other findings demonstrating that fat mass – but not fat-free mass – increases with age suggest that "growth" may in fact be driven by an increase in body fat rather than growth and maturation of the skeletal muscles. Mehta et al. investigated weight and BMI Z-scores across a three-year time period, revealing a decline in both parameters [19].

There is a strong need for a description of the growth patterns of SMA children, preliminary to a possible development of SMA-specific growth charts. As the growth patterns of affected individuals differ from those of the normal population, it is challenging to detect additional conditions that influence their growth using growth charts for the normal population. In clinical settings, it is essential for physicians to consider other possible contributory factors besides the established diagnosis. To that end, disease-specific growth charts are very useful and are necessary for several reasons. First, they are helpful for understanding the growth pattern and pathogenesis of a disease. Second, they enable the detection of deviations from typical growth patterns in affected individuals and aid in early diagnosis of additional conditions. Finally, they are very useful for evaluating the effects of growth-promoting treatment among patients [24].

Meanwhile, body composition should be considered when interpreting anthropometric measures in children with

SMA. Basic anthropometry alone may be misleading and could lead to overfeeding of patients.

Dual Energy X-ray Absorption (DEXA) is the standard method for measuring bone mineral content, segmental fat mass and free fat mass. These methods may not be available outside of advanced clinical research environments due to their cost, technical requirements and minimal radiation exposure, which limits their use only to segmental level for the diagnosis of bone disease and not to total body level for body composition analysis. In addition, the availability of body composition reference data is a large problem that limit the evaluation of body composition in paediatric age.

For the ordinary evaluation of body composition, simpler methods, such as anthropometry, are required, but no data are presently available on the ability of anthropometry to serve as surrogate measure of fat in SMA children.

Very little is known about both energy requirements in SMA [6]. Reduced energy requirements in patients with SMA may reflect their unique body composition. Resting energy expenditure (REE) appears to be lower in SMA when compared to healthy peers [8,20]. Therefore, predictive energy formulas based solely on anthropometric data may have limited value in this population, and it is likely that body composition plays a large role in defining caloric needs. The assessment of energy requirements is central to quantify to avoid growth failure and to maintain or reach an adequate nutritional status. The reference method for the assessment of REE is indirect calorimetry, usually available only in research or advanced clinical centres. For this reason, predictive equations are used in clinical practice to establish the REE of healthy and sick subjects. The response, different for REE equation, is REE measured by indirect calorimetry and predictors are generally weight, height, age and gender. It is well known that REE predictive equations are population-specific and most diseases require population-specific formulae. For example, predictive equations are

currently available for cerebral palsy and amyotrophic lateral sclerosis [25,26]. Not surprisingly, a recent report has shown that REE predictive equations developed in healthy children overestimate REE in SMA children [21]. The Consensus Statement for Consensus Statement for Standard of Care in Spinal Muscular Atrophy [1,7] has concluded that "until more specific data are available, nutrient intake should meet the daily recommended intakes for age". We expect that a detailed study of REE and its relationship with anthropometry and body composition will substantially increase the knowledge about the proper nutritional management of SMA children.

About macro- (lipids, proteins and carbohydrates) and micro- nutrient (vitamins and minerals) composition of the diet needs in relation to gender, age, weight and co-morbidities in SMA, the so-called Recommended Dietary Allowances (RDA) or Livelli di Assunzione Raccomandati di energia e Nutrienti per la popolazione Italiana [27] are used to define the energy and nutrient intake of healthy persons in relation to sex, age classes (0.5-0.99, 1-3, 4-6, 7-10, 11-14, 15-17, 18-29, 30-59, 60-74, ≥ 75 years) and special physiological conditions such as pregnancy and lactation. The RDA/LARN are based on experimental and epidemiological data collected on persons in a good state of health. The energy and nutrient requirements are known to be different in most diseases and SMA is likely to be one of them [2,21]. The Consensus Statement for Standard of Care in Spinal Muscular Atrophy [1,7] concludes that "until more specific data are available, nutrient intake should meet the daily recommended intakes for age, as proposed for healthy children. Moreover, supplements to provide more than the dietary recommended intake for vitamin, mineral, protein, or fat are discouraged". This reflects the present lack of knowledge about the nutritional requirements of SMA children. It is likely, however, that some of metabolic impairments of SMA children may respond to a specifically tailored diet. In fact, the Consensus Statement for Standard of Care in Spinal Muscular Atrophy considered priority the need to conduct studies aimed to understand nutritional

deficiency and to optimize fat and protein intake because of the anomalies of mitochondrial fatty acid oxidation abnormalities and muscle wasting.

Further significant gaps in knowledge is about the relationship between nutritional status, body composition, energy expenditure and motor function.
Preliminary studies on a small cohort of patients have shown that the reduction of lean mass reflects the loss of muscle mass, and that increased adiposity may worsen motor and breathing skills because of the mechanical effect of the increases body mass on mobility and residual muscle function in high-functioning SMA1 children. Conversely, the more severe patients (particularly SMA1 and "low functioning" SMA2) may be most at risk for malnutrition [10]. Correlation studies of nutritional status, body composition, energy expenditure and degree of motor function impairment are needed to identify the best body composition that is able to assure maximum mobility and residual motor function, especially in the actual scenery with the recent approval of nusinersen by the FDA and EMA that has dramatically changed the natural history of the disease by prolonging survival and improving motor function in treated patients [4]. Standardized motor function assessments have demonstrated to be feasible, reliable and valid in monitoring disease severity and response to treatment [28]; however, given the wide variability in natural clinical course and evolution after treatment, there is an increasing need to validate new biomarkers, which could be more objective and useful, in addition to the standardized motor function assessments, in the design of future clinical trials, and in the planning of patient-specific therapeutic programs [28].

Reviewing the evidence base for nutrition in SMA reveals consistent limitations across all areas, with little high-quality evidence to guide practice. The heterogeneity of studies with regard to outcomes measured makes it very difficult to draw definitive conclusions [6]. Interventional studies are scanty, so extrapolation from observational studies with adequate

sample sizes is necessary to comprise the strongest and most accurate evidence available to assist this fragile group.
Especially nowadays with the advent of new therapeutic drugs, the availability of natural history data during the disease is useful for understanding the effect of new treatments.

CHAPTER 2

Aims

2.1 Primary aim:

1.1. to investigate growth patterns, body composition, energy expenditure and nutrient intake in a large sample of Italian infants and children affected by SMA1 and SMA2.

2.2 Secondary aims:

2.1. to evaluate the accuracy of field methods (anthropometric measurements) on a reference method (DEXA) to assess body FM in SMA children

2.2. to develop a predictive formula in SMA children for estimating REE from sex, age, weight, and ventilation

2.3. to evaluate the glucose and lipid profile to better understand metabolic abnormalities in SMA children

2.4. to evaluate food habits and nutrient intake and their association with nutritional status

2.5. to evaluate the association between growth patterns, body composition and motor function by means of disease specific assessment scales.

CHAPTER 3

Materials and methods

3.1 Study design

Patients were recruited, between October 2016 and July 2019, from 5 clinical SMA referral centers in Italy (SAPRE-UONPIA, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; UO Neurologia dello Sviluppo, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan; Dipartimento della Salute, della Donna e del Bambino, Università di Padova; Unità di Malattie Neuromuscolari e Neurodegenerative, Laboratorio di Medicina Molecolare, Dipartimento di Neuroscienze e Neuroriabilitazione, IRCCS Ospedale Pediatrico Bambino Gesù, Rome; Dipartimento di Neuroscienze e Riabilitazione, Istituto Giannina Gaslini, Genoa), all involved in a large ongoing multicentre observational study on nutritional status in SMA children (Telethon GUP15014, 2015, Italy – Principal Investigator Prof. Dr. Simona Bertoli, DeFENS, University of Milan).

Inclusion criteria were:

- genetically confirmed diagnosis of SMA1 or SMA2;
- age 1-10,99 years;
- clinical management according to the guidelines set out in the Consensus Statement for Standard of Care in SMA [1,7];
- ability to lie on DEXA scanning table;
- absence of acute infection;

- no inclusion in ongoing experimental pharmacological trials.

Every 6 months for 2 years, each child underwent the following measurements and instrumental analysis on the same morning at the International Center for the Assessment of Nutritional Status (ICANS), University of Milan:

- General clinical data assessment;
- Anthropometric measurements [Body Weight (BW), Body Length (SL), Arm Length (AL), Arm Circumference (AC), Ulna Length (UL), Waist Circumference (WC); bicipital, tricipital (TS), subscapular and suprailiac skinfolds];
- Body composition by DEXA [Fat Mass (FM), Fat Free Mass (FFM), Lean Body Mass (LBM), bone mineral content (BMC), and bone mineral density (BMD), total and segmental masses];
- Energy requirements by indirect calorimetry [Resting Energy Expenditure (REE), Respiratory Quotient (RQ)];
- Fasting blood samples [glucose, insulin, c- peptide, triglycerides, TCho-, LDH Cho-, HDL Cho-];
- Questionnaire and 7-days weighted-diary for eating habits and nutrient intake;
- Motor function assessment [The Children's Hospital of Philadelphia Infant Test for Neuromuscular Disorders (CHOP INTEND) in SMA1 [29], Hammersmith Functional Motor Scale-Expanded (HFMSE) in SMA2 [30].

The study was approved by the Ethic Committee of University of Milan (n.7/16) and the other participating centres and complied with all tenets of the Helsinki declaration. The parents, on behalf of their children, gave their informed and written consent to the study.

3.2 General clinical data assessment

At each examination, specialists from the involved 5 clinical SMA referral centres recorded data on:

- age,
- sex,
- type (invasive and/or mechanical) and hours of

ventilation,
- type of feeding (PEG), nasogastric tube (NG), per os),
- current drugs (approved or experimental),
- presence of other co-morbidities.

3.3 Anthropometric measurements

All anthropometric measurements were collected by the same dieticians who followed a one-day training workshop by applying the "Anthropometry Procedures Manual" [31] specifically implemented for children with SMA.
The principal measurements were:

- BW, measured by a wheelchair scale to the nearest 100 g, the subject and wheelchair were weighed together, then the wheelchair was weighed alone, the difference in the two measures gave the weight of the subject;
- SL, measured by a non-elastic tape to the nearest 0.1 cm, on the child's right side. The child lay on its back on an appropriate exam table with the Frankfort plane perpendicular to the table (support), shoulders and buttocks resting against the table, arms along the trunk, palms facing up, legs as straight as possible and in contact with the table (board). In cases of scoliosis and contractures, segmental lengths were taken three times and the mean measurement recorded [9];
- Body Mass Index (BMI), calculated by the following formula: BW (Kg) / SL^2 (m^2);
- AL, approximated to the nearest 0.1 cm and taken along the child's left side by a non-elastic tape and with the child lying supine on the exam table;
- UL, measured along the child's left side by a non-elastic tape with the elbow bent at 90°, from the proximal end of the ulna until the distal end of the styloid process at the wrist;
- AC, measured at the midpoint of the upper arm marked during the measurement of AL with the tape around the arm so that it is touching the skin, but not compressing the soft tissues. The tape was positioned perpendicular to the long axis of the arm at the marked midpoint;
- WC, measured at the midpoint between the lower

margin of the last palpable rib and the mid-axillary line to the top of the iliac crest (hip bone). The tape was positioned perpendicular to the long axis of the trunk at the marked point, making sure to wrap the tape over the same spot on the opposite side, not tight enough to cause compression of the skin. The measurement should be taken at the end of a normal exhalation;

- TS, measured at the upper arm mid-point mark on the posterior surface of the left upper arm by means of a Holtain LTD caliper. Measurements were made in triplicate, and the average of the three values was calculated;
- Arm Muscular Area (AMA) and Arm Fat Area (AFA), indicators of nutritional status degree, muscle and fat mass amount respectively [32], calculated according to the following formulas:

$$AMA = [AC\ (cm) - (TS\ (mm) * 3{,}14)]2 / (4 * 3{,}14)$$

$$AFA = [AC\ (cm) / (4 * 3{,}14)] - AMA^2.$$

Brook [33] and Siri [34] equations were used to calculate body fat mass percentage (FM%) from anthropometric measurements.

BW, SL, and BMI Z-scores were derived using the WHO Growth Charts [22] and the 2000 Centers for Disease Control and Prevention (CDC) Growth Charts [23] for children younger than 2 and older than 2 years old, respectively.

Sex-specific AC, TS, AMA, and AFA-Z-scores were derived using the 2000 Centers for Disease Control and Prevention (CDC) Growth Charts [23] for AC and TS and Frisancho percentiles for AMA and AFA [32].

A Z-score <5th percentile was considered under the normal range, a score between the 5th and 85th percentile was considered normal and a score >85th percentile was considered over the normal range [22,23,32].

3.4 Body composition

DEXA scans (iDXA; General Electric, formerly Lunar Corp., Madison, WI) equipped with a pediatrics software

application were used to obtain the body composition (BC) of the children. DEXA provides measurements of soft tissue and bone for the total body and the sub-regions (arm, trunk, leg) including FM (g), LBM (g) and BMC (g). The fat-free mass (FFM) was calculated by adding BMC to LBM. FM (FMI, kg/m2) and FFM indexes (FFMI, kg/m2) were calculated by dividing FM and FFM by the squared height, respectively. The FM percentage was obtained as 100 % × [total body FM(g)]/ [total body mass (FM + LBM + BMC) (g)]. To investigate the proportion between FFM and FM, their ratio was also calculated. The total FM percentages of BW were interpreted according to the body composition of reference children [11], calculating the percentage of agreement between measured FM percentage with the respective reference values for sex and age.
The scanning of the children was done with them lying supine on the table, their feet in a neutral position and arms resting along their sides, palms facing upwards. The DEXA scans, performed by well-trained and certified research staff, were all done using the one device and the same software (enCORE, 2010), for an average measuring time of 10 min. The exposure to radiation was <7 microSV. Daily quality-assurance were tested according to manufacturer directions. The DEXA scans were analysed using a custom-made software that allows BMC measurement in close relation to metal orthopaedic implants, by the exclusion of no-osseous pixels.

3.5 Energy expenditure

To measure oxygen consumption (VO2) and carbon dioxide production (VCO2) we used the indirect calorimetry, specifically an open-circuit ventilated-hood system (Sensor Medics 29, Anaheim, CA, USA) in both spontaneous breathing and ventilated patients. We took the measurements in a thermoneutral environment (ambient temperature 24-26 °C) devoid of external stimuli. At the beginning of each test, the calorimeter was calibrated: there were two reference gas mixtures (26% O2 and 74% N2; 16% O2, 4.09% CO2 and 79.91% N2, respectively). Children were fasted for at least 6 h. Data collection time was at least 20 min,

with a 5 min run-in time for stabilization and time to allow the children to get used to the canopy and instrument noise. Steady state was determined by five consecutive minutes in which VO2 and VCO2 variations were less than 10%. Children were not tested unless they had a stable respiratory function for at least 1 h. Averaging the steady state values allowed the determination of 24 h REE, done by using the abbreviated Weir equation [35]: REE Kcal/day = (3.941 VO2 mL/min + 1.106 VCO2 mL/min) x 1.44. The ratio VCO2/VO2 gave the RQ.

In spontaneous and non-invasive ventilation (NIV)-supported breathing patients, gas exchange was measured by transparent ventilated canopy. To avoid gas leakages, the subject's head was carefully wrapped with a plastic sleeve. Following the protocol validated in NIV-supported breathing patients [36], the expiration valve of the ventilation mask was placed near the canopy's aperture from which the mixed gases were suctioned into the IC. In addition, the plastic sleeve of the canopy was tucked carefully under the pillow and wrapped around the inspiration tubing and along the body of the child to minimize any leakage into the measurement circuit.

Predictors of measured resting energy expenditure by indirect calorimetry (mREE) among a sample of SMA1, to generate a predictive energy equation and to compare such models to that suggested by General Nutrition Guidelines for Spinal Muscular Atrophy (SMAGNG) [37].

Demographic [sex and age (months)], anthropometric [BW,kg, and SL,cm], body composition [total FFM,kg], ventilation status [spontaneous vs non-invasive mechanical ventilation or mechanical ventilation via tracheostomy] data were examined as potential predictors of measured REE.

3.6 Fasting blood samples

Fasting blood samples were taken by venepuncture of the antecubital vein in either the sitting or lying position, using vacuum tubes. After centrifugation (800g x 10 min at 5°C), aliquots of samples were stored at -80°C until further analysis.

Glucose, triglycerides, TCho-, LDH Cho-, HDL Cho- were

determined by electrochemical method (Cobas Integra 400 plus, Roche Diagnostics, Mannheim). Circulating insulin was measured in duplicate by immunoenzymatical method (Cobas e411 Hitachi, Roche Diagnostics, Mannheim).
Homeostasis model assessment-estimated Insulin resistance (HOMA-IR) and quantitative insulin sensitivity index (QUICKI) were estimated as [fasting glucose (mg/dL) X fasting insulin (mU/L)/405] [38] and 1/(log fasting insulin þ log fasting glucose) [39], respectively.

3.7 Questionnaire and 7-days weighted-diary

Eating habits have been collected by a structured questionnaire based on ICANS experience in children with neurological diseases *(Attachment 1: "Feeding and Gastrointestinal Survey).*
7-days weighted-diaries have been used to collect food intake. Parents or primary caregivers were trained by dietician in food recording procedures and was selected a week closely resembling child's usual dietary habits; the records were mailed and were assessed for completeness, coded and analysed using the WinFood® 3.0 software.
The intake of energy, proteins, lipids, carbohydrates, minerals and vitamins have been compared with LARN [27] and the adequacy indexes have been calculated.

3.8 Motor function

The CHOP-INTEND is a validated 16-item, 64-point scale that shown to be reliable and sensitive to change over time for SMA1. Each item constructed to capture the movement of one body segment against another or against gravity [29]. The HFMSE assesses motor function (e.g. lying, rolling, sitting, crawling, attaining standing, walking, running and jumping) in order of progressive difficulty, with higher values showing higher function abilities. The total score can range from 0 (all activities failed) to 66 (all activities achieved unaided). The HFMSE shows good test–retest reliability and correlation with other clinical measures [30].

3.9 Statistical analysis

All statistical analyses were performed using Stata 12.1 (Stata Corporation, College Station, TX). A p value <0.05 has been considered statistically significant.
Descriptive statistics of continuous variables are reported as percentiles because most of them had non-Gaussian distributions. Discrete variables are reported as count and percentage.

Between-age classes comparisons of continuous variables of interest were performed using univariable regression of the 25^{th}, 50^{th} and 75^{th} percentile of the outcome with age classes (0 = 0-11 months; 1 = 12-36 months; 2 = >36 months) as predictor. Between-age classes distribution of discrete variables were performed using a Fisher's exact test.

Concerning comparison of estimated and measured variables, Pearson correlation was performed, and Bland and Altman plots were used to compare predicted values with measured ones. Limits of agreement (LOA) were computed and a priori limits of maximum acceptable difference were set at ±5%.
Bias was calculated as (estimated - measured) and percent bias as [(estimated - measured) / measured] *100 [40].

A two-sided Student's t-test was performed using IBM SPSS Statistics program version 25 for Windows (SPSS, Inc., Chicago, IL, USA) to compare the means of data when only two groups were compared (i.e. foods habits of SMA1 vs. SMA2).

CHAPTER 4

Results

4.1 Patients

188 SMA children (106 SMA1 and 82 SMA2) were recruited in the study. Of these, 61 were aged 0-11 months, 78 were aged 12-36 months and 49 were older than 36 months. The distributions of sex and types of ventilation and feeding according to age classes are reported in *Table 2*.

Table 2: Characteristics of the recruited patients

	Age categories								
	0-1 year		1-3 years		4-10 years		Total		P value
	N	%	N	%	N	%	N	%	
SMA types									
SMA1	61	100	33	42,3	12	24,5	106	56,4	
SMA2	0	0	45	57,7	37	75,5	82	43,6	
Total	61	100,0	78	100,0	49	100,0	188	100,0	
Sex									
Females	36	59,0	41	52,6	23	46,9	100	53,2	
Males	25	41,0	37	47,4	26	53,1	88	46,8	
Total	61	100,0	78	100,0	49	100,0	188	100,0	0.601
Ventilation									
None	51	83,6	53	67,9	20	40,8	124	66,0	
NIV	6	9,8	19	24,4	25	51,0	50	26,6	
Tracheostomy	4	6,6	6	7,7	4	8,2	14	7,4	<0.001
Total	61	100,0	78	100,0	49	100,0	188	100,0	
Feeding									
per os	57	93,4	65	83,3	40	81,6	162	86,2	
NG/PEG	4	6,6	13	16,7	9	18,4	26	13,8	
Total	61	100,0	78	100,0	49	100,0	188,0	100,0	<0.001

The sex distribution was similar among the age classes. 7.4% was tracheostomized and 13.8% was fed by NG or PEG, with increasing prevalence in older children.

No one was on drug trials.

4.2 Growth pattern

Anthropometric measurements are reported in *Table 3*.

Table 3: Anthropometric measurements of the recruited patients

SMA1													
	0-1 year (n=61)			1-3 years (n=33)			4-10 years (n=12)			Total (n=106)			*P* value
	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	
Age (months)	4	6[a]	7	14	19[b]	31	64	82[c]	112	6	8	24	<0.001
ANTHROPOMETRY													
Length (cm)	65.0	68.0	72.0	79.0	84.0	96.5	106.8	124.1	137.0	67.0	73.0	87.0	<0.001
Lenght (SDS)	0.120	1.090	1.940	0.370	0.650	1.560	0.965	0.040	0.785	0.270	0.875	1.720	0.041
Weight (kg)	6.2	6.8	7.5	8.0	9.2	10.6	13.9	16.6	23.1	6.6	7.5	9.5	<0.001
Weight (SDS)	-1.440	0.810	0.170	-2.410	-2.160	0.450	-2.510	-1.675	0.795	-2.160	-1.110	0.260	0.001
BMI (kg/m2)	13.5	14.3	15.5	11.5	12.3	13.5	11.8	12.4	13.1	12.2	13.6	14.9	<0.001
BMI (SDS)	-2.520	-1.990	0.860	-4.310	-3.310	-2.340	-2.930	-2.485,00	-1.780	-3.310	-2.300	-1.250	<0.001
Arm circumference (cm)	13.0	13.5	15.0	12.6	13.5	14.5	14.0	16.8	19.6	13.0	13.8	15.0	0.01
Arm circumference (SDS)	-4010	-3.630	-3240	-3820	-3.280	-2560	-1165	0.315	0.295	-3855	-3455	-2690	<0.001
Waist circumference (cm)	38.5	40.0	42.5	40.5	43.9	47.5	44.5	50.8	57.0	38.9	41.9	45.0	<0.001
Tricipital skinfold (mm)	10.2	12.0	13.8	10.5	12.4	14.6	11.6	13.4	25.6	10.5	12.2	14.6	0.07
Tricipital skinfold (SDS)	0.070	0.430	0.860	0.370	0.730	1010	0.190	0.500	0.910	0.100	0.550	0.990	0.129
Arm muscular area	6.7	7.8	8.5	6.2	7.3	9.3	7.7	9.3	12.1	6.6	7.8	9.2	0.092
Arm muscular area (SDS)	.	.	.	-2810	-2310	-1610	-3050	-2500	-1660	-2860	-2395	-1635	0.655
Arm fat area	5.8	6.8	8.7	5.9	7.1	8.8	7.2	9.5	18.2	6.0	7.2	8.8	0.039
Arm fat area (SDS)	.	.	.	0.650	0.140	0.570	0.310	0.580	1440	0.610	0.080	0.860	0.244

SMA2										
	1-3 years (n=45)			4-10 years (n=47)			Total (n=82)			*P* value
	P25	P50	P75	P25	P50	P75	P25	P50	P75	
Age (months)	17	27	41	58	72	90	25	45	69	<0.001
ANTHROPOMETRY										
Length (cm)	80.0	89.0	97.0	105.0	111.0	119.2	87.0	99.5	110.0	<0.001
Length (SDS)	0.840	0.020	0.890	-1.830	0.930	0.090	-1.570	0.385	0.620	0.039
Weight (kg)	9.9	11.1	12.2	14.6	17.4	20.8	10.8	13.5	17.4	<0.001
Weight (SDS)	-1.890	0.950	0.220	-2.300	-1.010	0.130	-1.920	-1.005	0.190	0.001
BMI (kg/m2)	13.0	14.1	15.7	12.5	14.1	16.0	12.8	14.1	15.8	<0.001
BMI (SDS)	-2.500	-1.690	0.350	-2.520	-1.120	0.440	-2.520	-1.200	0.150	<0.001
Arm circumference (cm)	14.0	15.0	16.4	15.2	16.7	18.5	14.5	15.5	17.5	0.009
Arm circumference (SDS)	-3040	-2350	-1920	-1050	0.220	0.540	-2460	-1410	0.200	<0.001
Waist circumference (cm)	43.4	45.0	47.5	46.3	50.3	56.8	44.4	46.5	50.6	<0.001
Tricipital skinfold (mm)	8.0	10.2	12.2	10.6	13.1	17.0	8.5	11.3	14.2	0.085
Tricipital skinfold (SDS)	0.340	0.170	0.780	0.070	0.480	1150	0.170	0.335	0.980	0.154
Arm muscular area	9.3	11.1	12.9	10.5	12.5	15.1	9.6	11.3	13.9	0.112
Arm muscular area (SDS)	-1650	-1090	0.170	-2190	-1540	0.980	-1830	-1260	0.410	0.453
Arm fat area	5.0	6.9	8.7	7.5	10.1	12.1	5.4	8.0	10.9	0.028
Arm fat area (SDS)	-1.090	0.170	0.450	0.700	0.380	1320	-1.000	0.020	0.760	0.357

Percentiles of BL, BW, BMI, AC, TS, AMA and AFA are reported in *Table 4*.

Table 4. Percentiles of the principal anthropometric measurements

	SMA1								
	0-1 year		1-3 years		4-10 years		Total		P value
	N	%	N	%	N	%	N	%	
Length classes									
<=5th percentile	2	3.3	4	12.1	2	16.7	8	7.5	0.181
5-85th percentile	28	45.9	17	51.5	8	66.7	53	50.0	
85-95th percentile	13	21.3	5	15.2	0	0.0	18	17.0	
>95th percentile	18	29.5	7	21.2	2	16.7	27	25.5	
Total	61	100.0	33	100.0	12	100.0	106	100.0	
Weight classes									
<=5th percentile	13	21.3	19	57.6	6	50.0	38	35.8	0.013
5-85th percentile	43	70.5	13	39.4	6	50.0	62	58.5	
85-95th percentile	3	4.9	1	3.0	0	0.0	4	3.8	
>95th percentile	2	3.3	0	0.0	0	0.0	2	1.9	
Total	61	100.0	33	100.0	12	100.0	106	100.0	
BMI classes									
<=5th percentile	37	60.7	27	81.8	9	75.0	73	68.9	0.394
5-85th percentile	22	36.1	6	18.2	3	25.0	31	29.2	
85-95th percentile	1	1.6	0	0.0	0	0.0	1	0.9	
>95th percentile	1	1.6	0	0.0	0	0.0	1	0.9	
Total	61	100.0	33	100.0	12	100.0	106	100.0	
Arm circumference classes									
<=5th percentile	59	100.0	29	87.9	1	8.3	89	85.6	<0.001
5-85th percentile	0	0.0	4	12.1	10	83.3	14	13.5	
85-95th percentile	0	0.0	0	0.0	0	0.0	0	0.0	
>95th percentile	0	0.0	0	0.0	1	8.3	1	1.0	
Total	59	100.0	33	100.0	12	100.0	104	100.0	
Tricipital skinfold classes									
5-85th percentile	46	78.0	25	75.8	9	81.8	80	77.7	0.023
85-95th percentile	12	20.3	2	6.1	1	9.1	15	14.6	
>95th percentile	1	1.7	6	18.2	1	9.1	8	7.8	
Total	59	100.0	33	100.0	11	100.0	103	100.0	
Arm muscular area classes									
<=5th percentile	.	.	24	72.7	9	81.8	33	75.0	1,000
5-85th percentile	.	.	8	24.2	2	18.2	10	22.7	
85-95th percentile	.	.							
>95th percentile	.	.	1	3.0	0	0.0	1	2.3	
Total	.	.	33	100.0	11	100.0	44	100.0	
Arm fat area classes									
<=5th percentile	.	.	2	6.1	1	9.1	3	6.8	0.458
5-85th percentile	.	.	24	72.7	6	54.5	30	68.2	
85-95th percentile	.	.	2	6.1	2	18.2	4	9.1	
>95th percentile	.	.	5	15.2	2	18.2	7	15.9	
Total	.	.	33	100.0	11	100.0	44	100.0	

	SMA2						
	1-3 years		4-10 years		Total		P value
	N	%	N	%	N	%	
Length classes							
<=5th percentile	9	20	11	29,7	20	24,4	0.192
5-85th percentile	26	57,8	21	56,8	47	57,3	
85-95th percentile	2	4,4	4	10,8	6	7,3	
>95th percentile	8	17,8	1	2,7	9	11	
Total	45	100	37	100	82	100	
Weight classes							
<=5th percentile	15	33,3	14	37,8	29	35,4	0.028
5-85th percentile	26	57,8	20	54,1	46	56,1	
85-95th percentile	2	4,4	1	2,7	3	3,7	
>95th percentile	2	4,4	2	5,4	4	4,9	
Total	45	100	37	100	82	100	
BMI classes							
<=5th percentile	23	51,1	14	37,8	37	45,1	0.424
5-85th percentile	19	42,2	15	40,5	34	41,5	
85-95th percentile	1	2,2	2	5,4	3	3,7	
>95th percentile	2	4,4	6	16,2	8	9,8	
Total	45	100	37	100	82	100	
Arm circumference classes							
<=5th percentile	35	77,8	4	10,8	39	47,6	<0.001
5-85th percentile	9	20	25	67,6	34	41,5	
85-95th percentile	1	2,2	3	8,1	4	4,9	
>95th percentile	0	0	5	13,5	5	6,1	
Total	45	100	37	100	82	100	
Tricipital skinfold classes							
5-85th percentile	37	82,2	25	67,6	62	75,6	0.039
85-95th percentile	4	8,9	6	16,2	10	12,2	
>95th percentile	4	8,9	6	16,2	10	12,2	
Total	45	100	37	100	82	100	
Arm muscular area classes							
<=5th percentile	12	26,7	17	45,9	29	35,4	0,918
5-85th percentile	27	60	19	51,4	46	56,1	
85-95th percentile	4	8,9	1	2,7	5	6,1	
>95th percentile	2	4,4	0	0	2	2,4	
Total	45	100	37	100	82	100	
Arm fat area classes							
<=5th percentile	2	4,4	3	8,1	5	6,1	0.359
5-85th percentile	36	80	24	64,9	60	73,2	
85-95th percentile	1	2,2	3	8,1	4	4,9	
>95th percentile	6	13,3	7	18,9	13	15,9	
Total	45	100	37	100	82	100	

43% of SMA1 and 18% of SMA2 had a BL above the 85th percentile of the reference group. The 25th, 50th and 75th BL percentile were higher compared to the correspondent ones of the healthy children in all age classes, especially in younger children. Such discrepancy decreased with increasing age, but only at 50th percentile level this decrement was statistically significant.

36% of SMA1 and 35% of SMA2 had a BW below the 5th percentile of the reference group. BW values corresponding to the 25th, 50th and 75th percentiles were, in fact, lower than the reference standards in all age classes. Furthermore, the distance from the reference standards tended to increase significantly with increasing age at the 25th and 50th percentiles of BW.

69% of SMA1 and 45% of SMA2 were underweight. The BMI values corresponding to the 25th, 50th and 75th percentiles were lower than the reference standards in all age classes. Moreover, the 25th and the 50th percentile of the BMI standard deviation score significantly decreased with increasing age.

Almost all children, up to 36 months, both SMA1 and SMA2, had an AC below the 5th percentile of the reference standards. The values of the AC corresponding to the 25th, 50th and 75th percentiles were lower than the reference standards in all age classes. This discrepancy with respect to healthy children tended to decrease with increasing age.

8% of SMA1 and 12% of SMA2 had a TS above the 85th percentile of the reference group. The TS values corresponding to the 25th, 50th and 75th percentiles were, in fact, higher than the reference standards in all age classes. This discrepancy did not change with age.

The AMA was below the 5th percentile of the reference group in 75% of SMA1 and 35% of SMA2. The values of the AMA corresponding to the 25th, 50th and 75th percentiles were lower than the reference standards in all age classes. 25% of SMA1 and 20% of SMA2 had an AFA above the 85th percentile of the reference group. The values of the AFA corresponding to the 25th, 50th and 75th percentiles were quite in line with those of the healthy children for the age group 12-36 months, but higher in children older than 36 months. It should be noted that the sex-and-age standardized values of AMA and AFA have not been reported in children aged 0-11 months, as there are no reference values for this age group.

4.3 Body composition

Percentiles of total and segmental body composition are reported in *Table 5*.

Table 5. Percentiles of total and segmental body composition

	SMA1											
	0-1 year (n=61)			1-3 years (n=33)			4-10 years (n=12)			Total (n=106)		
	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75
BODY COMPOSITION (DEXA)												
Body Weight (kg)	6.0	6.6	7.3	7.7	8.9	10.6	13.5	16.5	22.2	6.4	7.4	9.3
Body Fat mass (kg)	2.2	2.5	2.9	2.5	3.5	4.0	4.6	7.9	11.3	2.4	2.8	3.6
Body Fat mass (%)	35.4	38.5	41.8	31.5	35.6	38.9	32.3	47.0	50.2	33.0	37.7	42.2
Body Fat free mass (kg)	3.7	4.1	4.4	5.2	5.5	6.7	8.7	10.0	11.0	4.0	4.6	5.7
Body Fat free mass (%)	58.2	61.5	64.6	61.1	64.4	68.5	49.8	53.0	67.8	57.8	62.3	67.0
Body Lean mass (kg)	3.6	4.0	4.3	5.0	5.3	6.5	8.3	9.5	10.3	3.9	4.4	5.4
Body Lean mass (%)	56.5	59.2	62.8	58.7	61.5	65.9	47.8	50.2	64.6	56.1	60.2	64.6
Body Bone mineral content (g)	116	126	138	189	214	278	409	452	603	120	141	215
Body Bone mineral content (%)	1.8	1.9	2.0	2.3	2.5	2.7	2.5	2.8	2.9	1.8	2.0	2.4
Arms Weight (kg)	0.4	0.5	0.6	0.5	0.7	0.8	1.2	1.5	2.2	0.5	0.6	0.7
Arms Fat mass (kg)	0.2	0.3	0.4	0.3	0.4	0.5	0.5	1.0	1.3	0.2	0.3	0.4
Arms Fat mass (%)	56.1	59.3	63.7	51.5	55.9	59.1	44.4	53.3	62.1	52.9	57.7	62.2
Arms Fat free mass (kg)	0.2	0.2	0.2	0.2	0.3	0.4	0.6	0.7	0.8	0.2	0.2	0.3
Arms Fat free mass (%)	36.3	40.7	43.9	40.9	44.1	48.5	37.9	46.7	55.6	37.8	42.3	47.1
Arms Lean mass (kg)	0.2	0.2	0.2	0.2	0.3	0.3	0.6	0.7	0.8	0.2	0.2	0.3
Arms Lean mass (%)	34.8	39.2	42.7	39.4	42.4	47.6	36.2	44.9,	53.8	36.1	40.8	45.5
Arms Bone mineral content (g)	5	7	8	7	9	14	23	27	36	6	8	10
Arms Bone mineral content (%)	1.2	1.4	1.5	1.3	1.6	1.9	1.6	1.7	2.0	1.3	1.5	1.7
Trunk Weight (kg)	2.5	2.8	3.1	3.2	3.8	4.8	5.5	7.0	9.1	2.7	3.2	4.1
Trunk Fat mass (kg)	0.7	0.9	1.2	0.8	1.3	1.5	1.3	3.2	4.6	0.8	1.1	1.3
Trunk Fat mass (%)	27.2	31.8	37.5	21.6	30.5	35.3	24.1	43.1	50.5	26.0	31.8	37.5
Trunk Fat free mass (kg)	1.7	1.9	2.0	2.4	2.6	3.0	3.9	4.4	4.6	1.9	2.1	2.7
Trunk Fat free mass (%)	62.5	68.2	72.8	64.7	69.5	78.4	49.5	56.9	75.9	62.5	68.2	74.0
Trunk Lean mass (kg)	1.7	1.8	2.0	2.4	2.6	2.9	3.9	4.3	4.4	1.8	2.1	2.7
Trunk Lean mass (%)	61.1	66.9	71.8	63.4	68.1	77.2	48.1	55.0	74.1	61.1	66.8	72.5
Trunk Bone mineral content (g)	27	35	42	43	51	70	98	114	162	30	42	52
Trunk Bone mineral content (%)	1.0	1.2	1.4	1.2	1.4	1.6	1.4	1.7	1.9	1.1	1.3	1.5
Legs Weight (kg)	1.3	1.5	1.8	1.7	2.3	2.5	3.8	5.0	7.1	1.4	1.8	2.4
Legs Fat mass (kg)	0.8	0.9	1.1	0.9	1.4	1.6	2.0	3.2	4.6	0.8	1.1	1.4
Legs Fat mass (%)	56.6	61.0	64.1	55.1	58.3	60.9	52.9	57.9	63.9	55.4	59.6	63.2
Legs Fat free mass (kg)	0.5	0.6	0.7	0.8	0.8	1.1	1.7	2.1	2.6	0.6	0.7	0.9
Legs Fat free mass (%)	35.9	39.0	43.4	39.1	41.7	44.9	36.1	42.1	47.1	36.8	40.4	44.6
Legs Lean mass (kg)	0.5	0.6	0.7	0.7	0.8	1.0	1.7	2.0	2.6	0.6	0.7	0.9
Legs Lean mass (%)	35.0	38.2	42.6	38.3	40.6	44.9	35.0	40.9	46.1	36.0	39.5	43.8
Legs Bone mineral content (g)	11	13	14	15	20	25	42	54	75	12	14	21
Legs Bone mineral content (%)	0.7	0.8	0.9	0.8	1.0	1.2	1.0	1.0	1.1	0.7	0.8	1.0

	SMA2								
	1-3 years (n=45)			4-10 years (n=47)			Total (n=82)		
	P25	P50	P75	P25	P50	P75	P25	P50	P75
BODY COMPOSITION (DEXA)									
Body weight (kg)	9.6	10.8	12.8	14.4	17.0	20.6	10.7	13.3	17.0
Body Fat mass (kg)	3.0	3.9	4.7	5.2	6.8	9.9	3.4	4.6	7.1
Body Fat mass (%)	30.8	36.4	38.9	35.5	39.5	47.3	32.0	37.2	42.8
Body Fat free mass (kg)	6.0	7.1	8.6	9.3	10.4	11.7	6.9	8.8	10.3
Body Fat free mass (%)	61.1	63.6	69.2	52.7	60.5	64.5	57.2	62.8	68.0
Body Lean mass (kg)	5.8	6.8	8.1	8.9	9.9	11.2	6.6	8.3	9.7
Body Lean mass (%)	58.7	60.8	66.0	49.7	57.2	61.2	54.9	60.2	65.5
Body Bone mineral content (kg)	227	303	377	435	494	563	291	398	488
Body Bone mineral content (%)	2.5	2.7	3.0	2.6	2.8	3.1	2.5	2.7	3.1
Arms weight (kg)	0.7	0.9	1.1	1.2	1.5	1.9	0.9	1.2	1.6
Arms Fat mass (kg)	0.3	0.5	0.6	0.5	0.8	1.1	0.4	0.6	0.8
Arms Fat mass (%)	46.7	55.4	58.4	47.1	51.7	58.0	46.7	52.5	58.4
Arms Fat free mass (kg)	0.3	0.4	0.6	0.6	0.8	0.9	0.4	0.6	0.8
Arms Fat free mass (%)	41.6	44.6	53.3	42.0	48.3	52.9	41.6	47.5	53.3
Arms Lean mass (kg)	0.3	0.4	0.6	0.6	0.7	0.9	0.4	0.6	0.7
Arms Lean mass (%)	39.9	42.5	51.3	40.6	45.8	50.0	39.9	45.3	51.3
Arms Bone mineral content (g)	14	18	27	27	33	43	17	26	35
Arms Bone mineral content (%)	1.8	2.1	2.7	1.7	2.0	2.7	1.7	2.1	2.7
Trunk weight (kg)	3.9	4.7	5.3	6.1	7.0	9.0	4.5	5.6	7.0
Trunk Fat mass (kg)	1.0	1.3	1.7	1.6	2.3	3.5	1.1	1.6	2.4
Trunk Fat mass (%)	22.8	28.4	33.0	26.1	33.2	43.2	24.2	30.4	38.7
Trunk Fat free mass (kg)	2.8	3.4	4.0	4.3	4.8	5.5	3.2	4.1	4.7
Trunk Fat free mass (%)	67.0	71.6	77.2	56.8	66.8	73.9	61.3	69.6	75.8
Trunk Lean mass (kg)	2.7	3.3	3.9	4.2	4.7	5.4	3.1	4.0	4.6
Trunk Lean mass (%)	65.3	69.8	75.4	55.0	64.9	72.5	59.5	67.9	74.2
Trunk Bone mineral content (g)	62	76	100	100	122	180	73	99	133
Trunk Bone mineral content (%)	1.5	1.7	1.8	1.6	1.8	2.0	1.6	1.8	1.9
Legs Weight (kg)	2.1	2.9	3.3	3.7	5.2	6.5	2.6	3.4	5.1
Legs Fat mass (kg)	1.2	1.5	2.0	1.9	3.1	4.2	1.4	1.8	3.1
Legs Fat mass (%)	54.6	57.1	60.6	54.4	59.2	63.1	54.4	58.0	61.1
Legs Fat free mass (kg)	0.9	1.2	1.6	1.7	2.1	2.5	1.1	1.6	2.0
Legs Fat free mass (%)	39.4	42.9	45.4	36.9	40.8	45.6	38.9	42.0	45.6
Legs Lean mass (kg)	0.9	1.2	1.5	1.7	2.0	2.4	1.1	1.6	2.0
Legs Lean mass (%)	38.3	41.8	44.3	35.9	40.0	44.0	37.8	41.1	44.3
Legs Bone mineral content (g)	20	26	36	44	56	66	26	37	56
Legs Bone mineral content (%)	0.8	1.0	1.2	0.8	1.0	1.2	0.8	1.0	1.2

Overall, SMA1 had a total FM of 37.7% (interquartile range: 33.0-42.2%), like SMA2 with a FM of 37.2% (interquartile range: 32.0-42.2%). Total FM increased with age. On the contrary, total LBM decreased with increasing age only at 25th percentile level. Concerning the arms composition, the FM decreased with increasing age, whilst LBM percent increased with age. An opposite trend was, instead, observed at the trunk level. FM percent, in fact, increased with age, whilst LBM percent decreased with age. Few significantly variations have been, instead, observed in the legs' composition in relation with age.

30 children (15 SMA1, 15 SMA2) were matched for sex distribution (M/F = 9/6 vs 9/6) and age (3.6 ± 1.9 vs 3.5 ± 1.8 years, p = 0.99) and adjusting for body size, to show the regions and body tissue compartments in which differences are found. *Table 6* shows the differences in total and segmental FM, FFM, LBM and BMC. Total and segmental FM did not differ in the two groups. Children with SMA1 had significantly low FFM and LBM compared to children with SMA2. Specifically, children with SMA1 presented a reduced FFM and LBM in the trunk and arms, although in these latter the difference in the groups was only marginally significant. Total BMC was similar between groups. However, children with SMA1 showed less BMC in the arms and tended to have lower BMD than children with SMA2 (p = 0.067).

Table 6. Total and regional fat, lean and mineral masses in SMA1 and SMA2 children [8]

	SMAI (N = 15)		SMAII (N = 15)		p Value
	Mean	sd	Mean	Sd	
Total and segmental fat mass					
FM (%)	36.9	7.0	34.2	6.6	0.277
FM (g)	4502.3	1965.6	4553.8	1504.6	0.936
FM arms (g)	543.1	287.1	561.7	185.7	0.834
FM legs (g)	1763.9	827.1	1971.5	695.2	0.463
FM trunk (g)	1642.3	846.2	1451.6	665.8	0.498
FMI	4.4	1.4	4.9	1.3	0.354
Total and segmental fat free mass					
FFM (%)	63.1	7.0	64.7	7.6	0.547
FFM (g)	7290.0	1729.1	8410.1	1508.4	**0.039**
FFM arms (g)	461.8	187.5	587.3	165.8	0.063
FFM legs (g)	1285.7	499.7	2130.8	2212.9	0.160
FFM trunk (g)	3342.4	686.7	3969.3	803.0	**0.029**
FFMI	7.4	1.2	9.1	1.1	**<0.001**
Total and segmental lean body mass					
LBM (%)	60.4	6.9	61.9	7.5	0.564
LBM (g)	6971.8	1637.1	8041.7	1427.7	**0.037**
LBM arms (g)	443.9	180.7	562.0	158.0	0.067
LBM legs (g)	1254.3	488.1	2094.5	2205.2	0.161
LBM trunk (g)	3261.6	670.1	3870.7	789.9	**0.031**
Total and segmental bone mineral content					
BMC (%)	2.7	0.3	2.8	0.3	0.450
BMC (g)	318.2	97.5	368.3	92.6	0.160
BMC arms (g)	17.8	7.4	25.2	9.6	**0.025**
BMC legs (g)	31.4	12.7	36.3	12.4	0.295
BMC trunk (g)	80.7	28.8	98.6	31.3	0.115
BMD	0.477	0.089	0.535	0.077	0.067

As shown by the *Figure 1*, all the children had a high FM% compared to the respective reference values for sex and age (mean percentage of overfat: +19,9 ± 8,0%, min +2,5% max + 34%). Conversely, the BMC percentages did not differ.

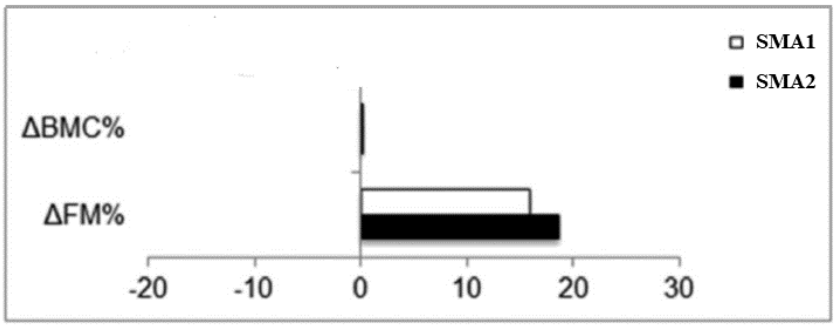


Fig. 1. Comparison of bone mineral content and total fat mass percentages between SMA1 and SMA2 patients.

Comparison of FM by skinfolds and DEXA

Pearson correlation coefficients between body fat mass (FM) by DEXA and skinfolds and skinfold derived measures are reported in *Table 7*.

Table 7. Correlations between FM by skinfolds vs DEXA

	SMA1		SMA2	
	r	p	r	p
Biceps skinfold	0,59	<0.001	0.46	<0.001
Triceps skinfold	0,55	<0.001	0.54	<0.001
Subscapular skinfold	0,56	<0.001	0.37	0.005
Suprailiac skinfold	0,61	<0.001	0.56	<0.001
Thigh skinfold	0,57	<0.001	0.70	<0.001
Calf skinfold	0,55	<0.001	0.49	0.001
Skinfold sum	0,7	<0.001	0.64	<0.001
Arm fat area	0,75	<0.001	0.69	<0.001
Thigh fat area	0,74	<0.001	0.78	<0.001
Calf fat area	0,71	<0.001	0.69	<0.001

All measures displayed a positive and significant correlation with FM. Single skinfold measure correlation coefficients were generally lower (mean r=0.52) than skinfold sum and

limb fat areas coefficients. Thigh fat area was the best performer, with a coefficient of 0.74 in SMA1 and 0.78 in SMA2.

Brook equation generally reported lower values than BF% by DXA, with a mean bias of -14.6% and upper and lower LOA of -6.18% and -23.0% in SMA1, and -16.2% ranging between -7.80% and -24.6% in SMA2, respectively. Bias was below acceptance limits for all patient, but relative to mean bias 76.4% of patients were into the acceptance limits (*Figure 2*).

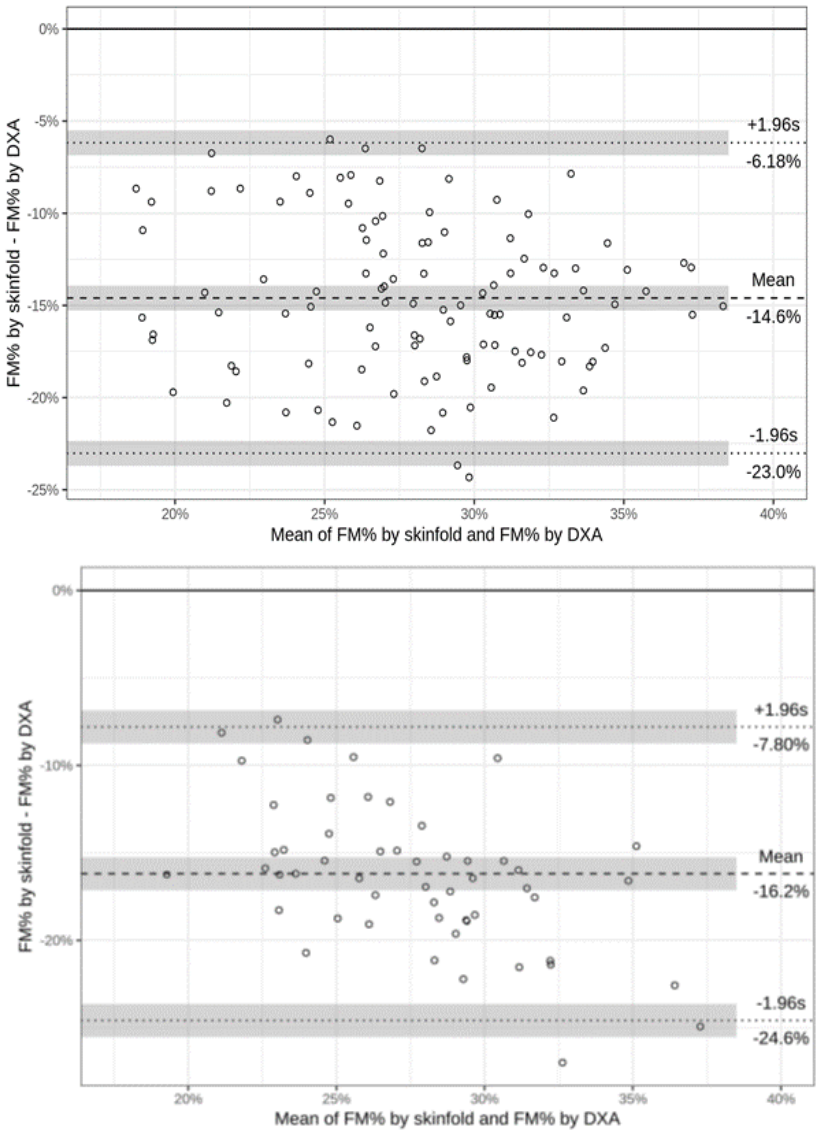


Fig.2 Bland Altman plots on FM% by skinfolds vs DXA

4.4 Energy expenditure

Total REE measured by indirect calorimetry increased with increasing age. However, the energy required for kg of body weight and fat free mass decreased with increasing age both in SMA1 and in SMA2 as shown in the *Table 8*.

Table 8. Percentiles of REE

SMA1	0-1 year (n=61)			1-3 years (n=33)			4-10 years (n=12)			Total (n=106)			*P* value
	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	
Age (months)	4	6	7	14	19	31	64	82	112	6	8	24	<0.001
RESTING ENERGY EXPENDITURE													
REE (kcal)	392	440	479	451	568	646	392	750	816	393	463	578	<0.001
REE/body weight (kcal/kg)	56	64	70	52	62	69	15	47	58	54	62	70	0.003
REE/FFM (kcal/kg)	99	105	113	85	96	111	31	83	94	89	101	113	<0.001

SMA2	1-3 years (n=45)			4-10 years (n=47)			Total (n=82)			*P* value
	P25	P50	P75	P25	P50	P75	P25	P50	P75	
Age (months)	17	27	41	58	72	90	25	45	69	<0.001
RESTING ENERGY EXPENDITURE										
REE (kcal)	531	668	828	532	703	835	532	686	832	<0.001
REE/body weight (kcal/kg)	52	62	69	32	54	83	42	58	76	0.039
REE/FFM (kcal/kg)	85	96	111	73	84	95	79	90	103	<0.001

Energy requirements with non-invasive ventilation

In SMA children with chronic respiratory failure treated with NIV for 6-16 hours/day, we measured REE during spontaneous breathing (REESB) and NIV (REENIV). Values were also normalized for BW. Differences between REESB and REENIV were evaluated and compared to REE prediction (REEpred) by the SMAGNG [37].

As shown by *Figure 3*, mean REENIV was 278±113 kcal/24h, resulting significantly lower than REESB (750±245 kcal/24 h; mean difference: 61,8%; $p<0.01$, Wilcoxon test).

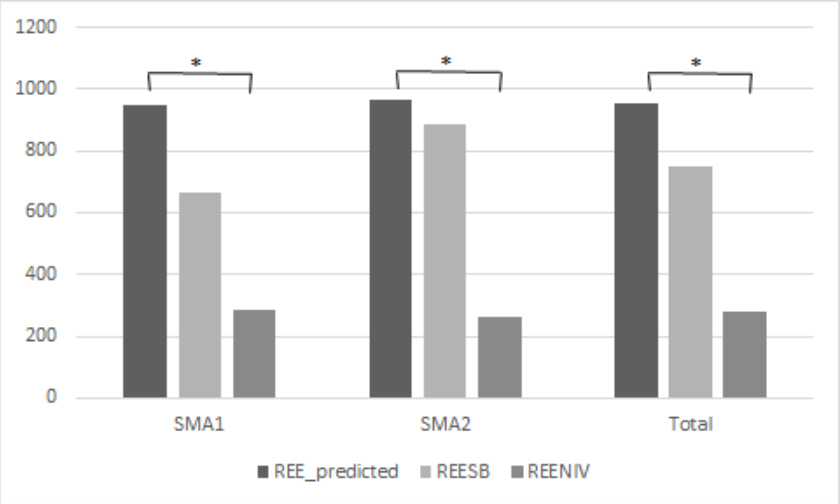


Fig.3 Comparison of REEpred, REESB and REENIV in SMA1 and SMA2

Similarly, REENIV normalized to BW and FFM compared to REESB/BW and REESB/FFM were reduced. Both REESB and REENIV were lower than REEpred *(Table 9)*.

Table 9. REE in spontaneous breathing and non-invasive ventilation

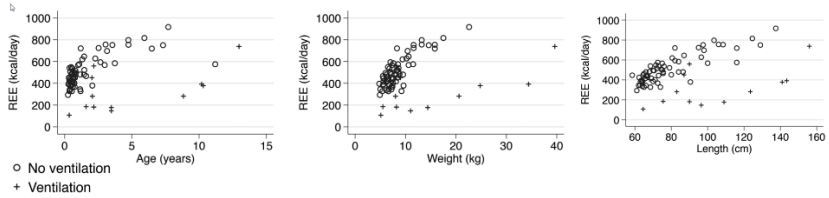
	Spontaneous breathing (REESB)		Non invasive ventilation (REENIV)		P value
	Mean	SD	Mean	SD	
VO_2	0.107	0.036	0.039	0.01	0.0003
VCO_2	0.089	0.027	0.033	0.836	0.0001
RQ	0.836	0.036	0.868	0.111	0.4623
REE measured (kcal)die)	749	246	277	113	0.0002
ΔREE %predicted	-21	26	-71	12	0.0002
REE/BW (kcal/kgBW)	53	11	20	8	0.0000
REE/FFM (kcal/kgFFM)	87	13	33	12	0.0000

Development of a predictive energy equation in SMA1

In a group of SMA1 children (n = 79), a cross sectional study was conducted to develop a predictive energy equation. The median age of the sample was 10 (6;30) months. 36 children (45,5%) were males. Age, BW, SL, FFM and ventilation status

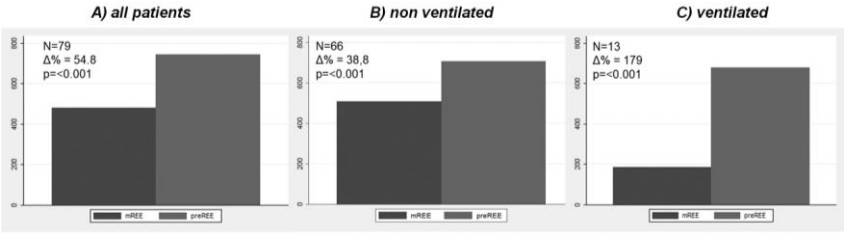
were all significantly correlated with mREE ($p<0.05$) *(Graph 1)*.

Graph 1. Correlations of age, BW and SL with REE.



mREE compared to preREE resulting significantly lower ($p<0.001$), especially in ventilated patients (*Graph 2*).

Graph 2. Comparison between measured and predicted in



After screening for multi-collinearity, the best predictive model (SMAICANS) of mREE included SL and ventilation status ($R (2) = 0.66$, $p<0.01$) is the following:

$$\text{SMAICANS REE} = -307{,}5 \times \text{VS} + 5{,}4 \times \text{SL}$$

where:

- VS=Ventilation Status
  [0=no ventilation, 1=ventilated]
- SL=Supine Length (cm)

Using Bland-Altman plots, the SMAICANS model over- and underpredicted mREE less often than the SMAGNG model and explained more than 50% variance of mREE, having a better precision in determining energy requirements for SMA1 children when compared to SMAGNG.

4.5 Metabolic profiles

Glucose profile

In 42 SMA1 and SMA2 children (2-10 yrs) we measured glucose and insulin plasma level, calculating HOMA-IR.

Hypoglycaemia (blood glucose <60 mg/dL) occurred in one patient respectively, whereas HOMA-IR above cut-off value (> 2.67) were found in 2 SMA1 patients.

SMA1 compared to SMA2 children showed both a worse nutritional status with lower LBM, and glucose profile with higher insulin and HOMA-IR values (*Table 10* and *Figure 4*).

Table 10. Glucose profile of SMA children

	Total (N=42)		SMAI (N=9)		SMAII (N=33)	
	Mean	SD	Mean	SD	Mean	SD
LBM	8.47	2.02	6.48	1.22	**9,03***	1.84
GLYC	84.0	9.0	80.0	21.0	83	9.0
INS	4.92	3.8	8.51	6.76	**4,24***	2.58
HOMA-IR	1.07	0.88	1.81	1.45	**0,81***	0.57
QUICKI	0.41	0.07	0.39	0.09	0.42	0.07

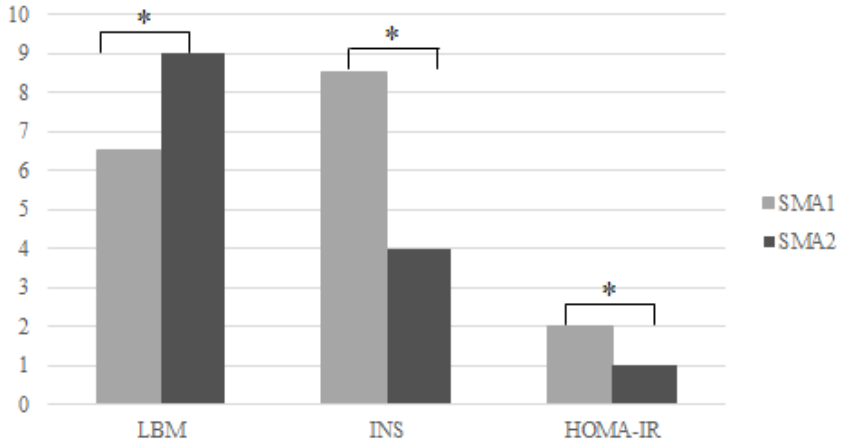
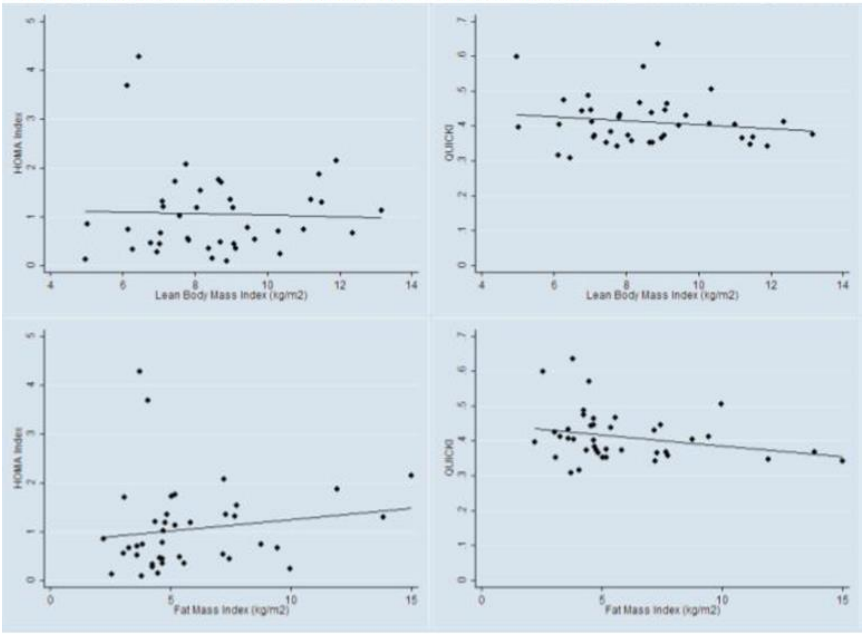


Figure 4. Nutritional status and glucose profile

Glucose, insulin plasma level and HOMA-IR were not related to BC (*Graph 3*).

Graph 3. Correlations between HOMA and QUICKI with LBM and FM.



Lipid profile

We performed lipid profiling on 72 paediatric SMA patients (14 SMA1, 52 SMA2, 6 SMA3 – demographics in *Table 11*). Only data on SMA1 and SMA2, object of this thesis, are reported.

Table 11. SMA patients' cohort demographics [15]

Pediatric cohort	Number	Percentage	Median age (years)	Time before last meal (hours)
Total	72	100	3.8	5
Male	39	54.17	3.7	5
Female	33	45.83	4	5
Type I	14	19.44	3.1	5
Male	5	6.94	2	5
Female	9	12.5	3.2	5
Type II	52	72.22	3.8	5
Male	31	43.05	3.7	5
Female	21	29.17	4.2	5

The median time before their last meal was 5 h. Note that fasting has minimal effect on lipid levels in comparison to non-fasting [41]. We focused on TCho-, LDH Cho-, HDL Cho-, non-HDL, and triglycerides to assess abnormalities in fatty acid metabolism in a minimally invasive manner. Over a third (37.5%) of SMA patients had at least one positive readout out of the five indicative tests for laboratory-defined dyslipidaemia (*Table 11*), in comparison to less than a quarter (20–24%) of the general population in published datasets [42]. Furthermore, close to 20% and 13% of SMA patients had more than two or three positive readouts out of the five tests of laboratory-defined dyslipidaemia respectively (*Table 12*). LDL prevalence was doubled in comparison to the general paediatric population [42–44].

Patients with borderline values made up 61% of SMA patients with at least one indicative lipid result, and 40% would have 3 or more (*Table 12*).

Table 12. SMA children dyslipidaemia [15]

	Criteria	All SMA patients	Type I	Type II
Abnormal	TC > 200 mg/dL	10/72 (13.89%)	1/14 (7.14%)	9/52 (17.31%)
	LDL > 130 mg/dL	9/72 (12.5%)	1/14 (7.14%)	7/52 (13.46%)
	HDL < 40 mg/dL	12/72 (16.67%)	1/14 (7.14%)	10/52 (19.23%)
	TG > 100 mg/dL‡	15/72 (20.83%)	5/14 (35.71%)	7/52 (13.46%)
	Non HDL-cholesterol > 145 mg/dL	10/72 (13.89%)	1/14 (7.14%)	8/52 (15.38%)
	1/5 abnormal dyslipidemia reading	27/72 (37.5%)	6/14 (42.85%)	18/52 (34.62%)
	2/5 < abnormal dyslipidemia reading	14/72 (19.44%)	2/14 (14.29%)	11/52 (21.15%)
	3/5 < abnormal dyslipidemia reading	10/72 (13.89%)	1/14 (7.14%)	8/52 (15.38%)
Borderline	TC > 170 mg/dL	30/72 (41.67%)	5/14 (35.71%)	23/52 (44.23%)
	LDL > 110 mg/dL	21/72 (29.17%)	2/14 (14.29%)	18/52 (34.62%)
	HDL < 45 mg/dL	20/72 (27.78%)	5/14 (35.71%)	13/52 (25%)
	TG > 75 mg/dL§	23/72 (31.94%)	7/14 (50%)	13/52 (25%)
	Non HDL-cholesterol > 120 mg/dL	32/72 (44.44%)	6/14 (42.86%)	23/52 (44.23%)
	1/5 < borderline dyslipidemia reading	44/72 (61.1%)	11/14 (78.57%)	30/52 (57.69%)
	2/5 < borderline dyslipidemia reading	35/72 (48.61%)	6/14 (42.86%)	26/52 (50%)
	3/5 < borderline dyslipidemia reading	29/72 (40.28%)	6/14 (42.86%)	20/52 (38.46%)

4.6 Feeding, eating habits and nutrient intake

Feeding and eating habits

48% of SMA1 and 21% of SMA2 answered to the survey.

Table 13 show the prevalence of gastrointestinal, swallowing, constipation, feeding and supplements intake.

Table 13. Prevalence of feeding and eating habits

	SMA1		SMA2		TOTAL	
	n	%	n	%	n	%
Assesment of gastrointestinal function						
Gastroesophageal (acid) reflux	22	44%	3	20%	25	33%
Delayed gastric emptying	21	42%	3	20%	24	31%
Vomit/regurge	23	46%	3	20%	26	33%
Swallowing problems						
Choking	21	42%	3	20%	24	32%
Chewing problems	24	48%	4	27%	28	36%
Swallowing difficulties	28	56%	3	20%	31	40%
Dysphagia	22	44%	3	20%	25	32%
Constipation						
Constipation	29	58%	4	27%	33	42%
Straining / Bowel-emptying difficulties	26	52%	4	27%	30	38%
Extra firm/hard stools	27	54%	4	27%	31	40%
Feeding						
Nasogastric tube	20	40%	3	20%	23	31%
Gastrostomy button	24	48%	3	20%	27	36%
Fundoplication	23	46%	3	20%	26	33%
Feeding time:						
<15 min	16	32%	2	13%	18	26%
15<20 min	10	20%	7	47%	17	26%
30/35 min	0	0%	0	-	0	0%
45<50 min	2	4%	0	-	2	3%
>60 min	5	10%	0	-	5	6%
Supplements						
Multivitamin / mineral supplements	19	38%	6	40%	25	36%
Herbal preparations	18	36%	2	13%	20	27%
Commercial formula preparation	12	24%	1	7%	13	18%
Antiacids/Inhibitors of gastric acid secretions	24	48%	3	20%	27	36%
Laxatives	28	56%	2	13%	30	38%
Fiber's supplements	20	40%	3	20%	23	29%

Overall, SMA1 patients have a higher prevalence of gastrointestinal, swallowing and have a higher consumption of supplements as multivitamin and herbal preparations, as well as drugs as antiacids and laxatives, as expected.

Nutrient intake

Table 14 shows mean values and standard deviation of macronutrients both in SMA1 and SMA2.

Table 14. Macronutrients' intake

	SMA1		SMA2		P-value
	n	sd	n	sd	
Energy (kcal)	756	257	996	240	0.000
Energy % adherence REE	161%	83%	134%	36%	0.005
Carbohydrate	46.46	7.67	47.76	6.13	0.081
Carbohydrate % adherence PRI	-6%	17%	-7%	12%	0.972
Protein (g)	23.15	13.26	39.91	10.4	0.000
Protein/Weight (g/Kg)	2.51	1.2	2.94	1.02	0.025
Protein ratio % adherence PRI	185%	125%	200%	102%	0.641
Lipid (g)	32.08	11.07	39.91	13.3	0.000
Lipid % adherence PRI	-7.33%	19.8%	-6.43%	15.97%	0.846
SFA %	8.42	5.31	12.89	5.76	0.000
SFA % adherence PRI	69%	58%	129%	58%	0.000
PUFA%	4.32	3.01	4.12	2.17	0.523
MUFA%	12.37	8.32	16.88	7.44	0.001
Water (ml/die)	858.3	340.14	887.74	348.15	0.706
Water % adherence AI	-25.37%	23.94%	-35.19%	25.78%	0.011

The value described as "% adherence PRI" means the adherence of the value to the population reference intake (PRI), i.e. the level of nutrient intake sufficient to satisfy the needs of almost all (97,5%) healthy subjects in the Italian Population [27]. If not possible to measure the percentage of adherence to PRI, it could be measured the adherence to the adequate intake (AI). The Adequate Intake is the level of nutrient intake that should be adequate to fulfil the needs of the population. Normally, it is gained from the average intake of a healthy population [27].

Concerning macronutrients, the adherence to the LARN (AI and PRI) is high for energy and protein and low for water and lipids. SMA2 children consume a high proportion of saturated fatty acid (SFA) adherence and twice as much protein as is recommended for a healthy population (*Figure 5*).

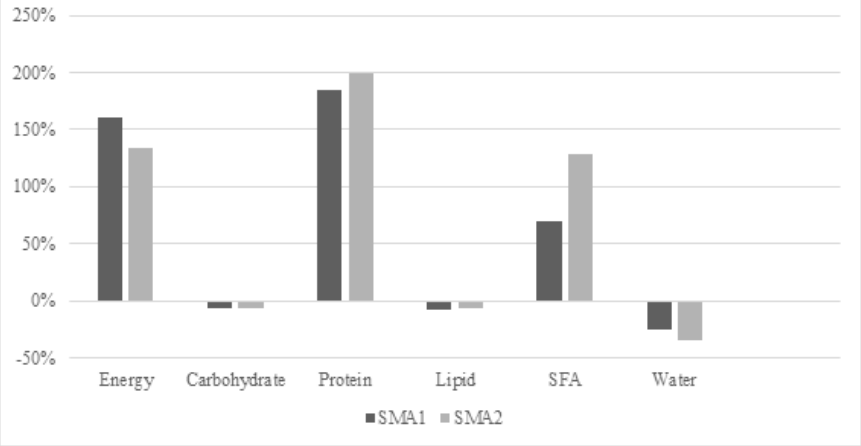


Fig. 5. Macronutrients adequacy indexes.

About minerals, calcium, iron, magnesium, selenium, iodine and molybdenum were below the recommendations. On the other hand, the consumption of natrium, potassium and phosphorus were twice as high as the recommendations (*Figure 6*).

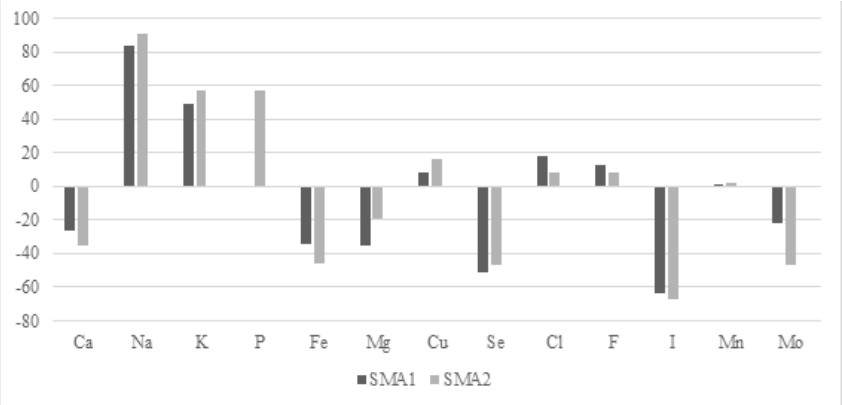


Fig. 6. Minerals adequacy indexes.

As regards vitamins, consumption of vitamins B8, B9, D, E and K were 40% lower than the recommendations. On the other hand, especially SMA1 reported a higher consumption of vitamins A, B2, B1, B1, B6 and C, about 50% more the recommendations (*Figure 7*).

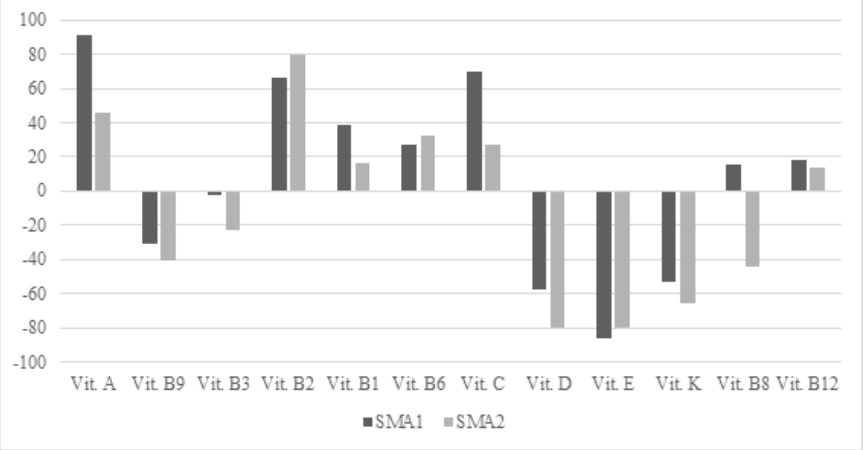


Fig. 7. Vitamins' adequacy indexes.

Effect of PEG on nutrient intake, nutritional status and body composition in SMA1 children.

In 7 SMA1 children, we investigated the effect of PEG placement on energy and macronutrients daily intake and on anthropometrics and body composition parameters in SMAI children.

538±228 days have gone by the first to the second evaluation.

Before PEG placement, difference between daily energy intake and measured REE was 262±178 kcal/die. After PEG placement, children showed a significant increment in energy intake which consisted of an increment of both protein, fat and carbohydrates daily mean amount intake. After PEG placement, protein intake was equal to twice the Italian recommendations, while fat and carbohydrates intake remained in the reference range (*Figure 8*).

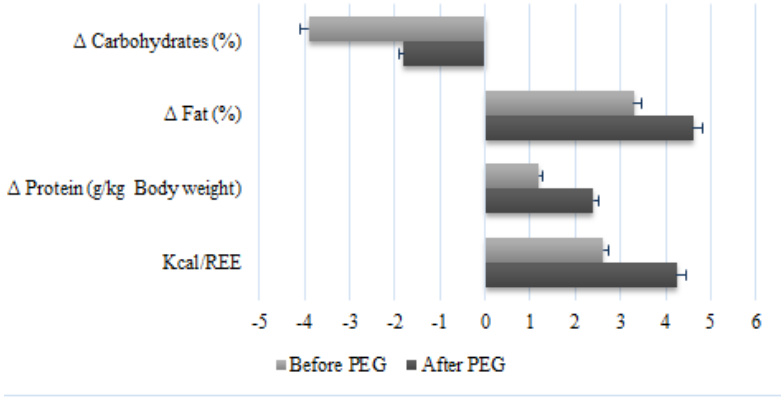


Fig. 8. Dietary intakes before and after PEG placement.

Concerning nutritional status and body composition, anthropometric measurements did not change after PEG placement compared baseline, but legs FM significantly increased with concomitant legs LM decrement (*Table 15*) (*Figure 9*).

Table 15. Effect of PEG placement on dietary intake, nutritional status and body composition parameters in SMA children

	Before PEG N=7		After PEG N=7		*P value*
	mean	sd	mean	sd	
Dietary Intake					
Daily Energy Intake (kcal/die)	764	301	993	303	**<0,001**
Δ Daily energy intake/REE (kcal/die)	262	178	424	189	**0,010**
Protein intake (g/day)	21,5	13,4	36,4	16,4	**0,006**
Protein intake (g/kg Body weight)	2,4	1,1	3,4	1,3	**0,046**
Δ Protein intake/Protein recommendation (g/kg Body weight)	1,2	1,2	2,4	1,3	**0,038**
Fat intake (g/day)	33,1	9,2	39,1	11	**0,050**
Fat intake (%)	40,8	5,9	35,9	4,1	0,077
Δ Fat intake/Fat recommendation (%)	3,3	4,6	0,9	3,2	0,357
Carbohydrates intake (g/day)	96	46	126,4	44,8	**<0,001**
Carbohydrates intake (%)	48,6	5,5	50,7	5,1	0,342
Δ Carbohydrates intake/Carbohydrates recommendation (%)	-3,9	5,5	-1,8	5,1	0,342
Nutritional status and body composition parameters					
REE (kcal/die)	502	168	569	234	0,299
Supine Lenght Z-score	0,79	0,85	0,22	1,24	0,133
Body Weight Z-score	-1,55	1,15	-2,42	0,84	0,233
BMI Z-score	-3,69	3,06	-4,67	1,82	0,478
Fat Mass Arms (%)	54,2	6,6	52,9	5,9	0,533
Lean Mass Arms (%)	44	6,3	45,5	5,6	0,486
Fat Mass Legs (%)	55,2	4,7	59,6	2,5	**0,022**
Lean Mass Legs (%)	43,8	4,6	39,4	2,4	**0,019**
Fat Mass Trunk (%)	30,9	4	29,5	8,1	0,676
Lean Mass Trunk (%)	67,6	4,3	69	8,3	0,658
Fat Mass Total Body (%)	36,1	2,8	37,1	3,4	0,514
Lean Mass Total Body (%)	61,6	2,8	60,4	3,6	0,444

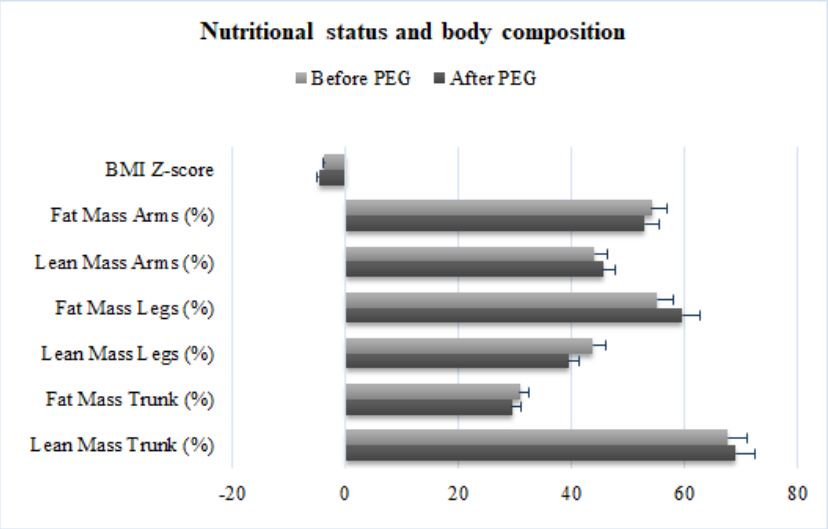


Fig. 9. Nutritional status and body composition before and after PEG placement.

Association between FFM and protein intake.

In 14 SMA1 children (57% males; age: 5.5; 4-13 months) we investigated the association between FFM and daily food protein intake (PI) in a SMA1 sample with an optimal energy intake according to sex, age and energy metabolism.

Concerning nutritional status, BMI Z-Score was -2.519 (IQR= -4.119 - -1.725). FFM was 63% (IQR= 62-69%) and FFM/length ratio was 63 g/cm (IQR=59-66 g/cm). Median energy expenditure was 450 kcal/die (IQR= 397-541 kcal/die): 66 kcal/kg BW (60-74 kcal/BW).

Concerning food intake, energy intake was 544 kcal/die (IQR= 433-619 kcal/die) equal to 76 kcal/kg BW (IQR: 68-82 kcal/kg): 100% patients had an energy intake in line with energy needs. Carbohydrates representing 47% of the diet (IQR= 42-53%) and only 43% satisfied the recommendations. Lipids intake correspond with 42% (IQR= 38-47%): 71% of children consumed more fat than recommended value. Proteins/kg BW were 1.9 g/kg (IQR= 1.4 - 2.1 g/kg), +46% of recommendation (IQR= +5 - +83 %). 64% of children has a protein intake over 10% of recommendations (*Figure 10*).

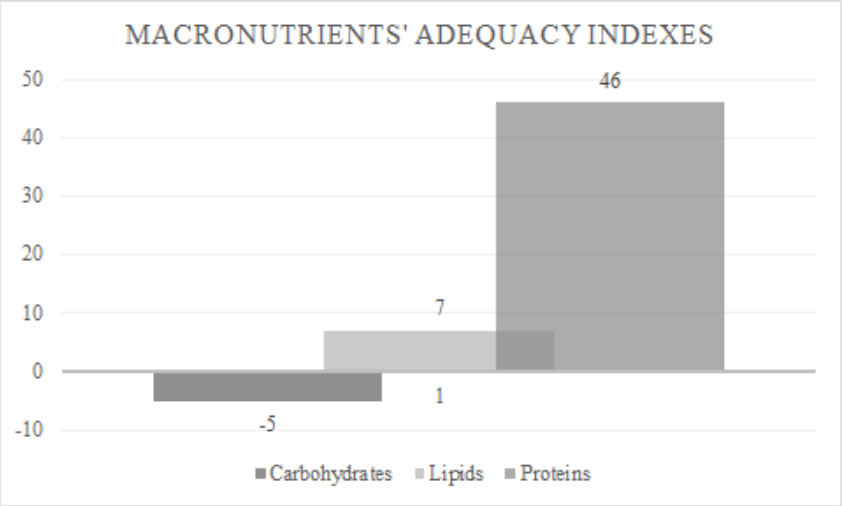
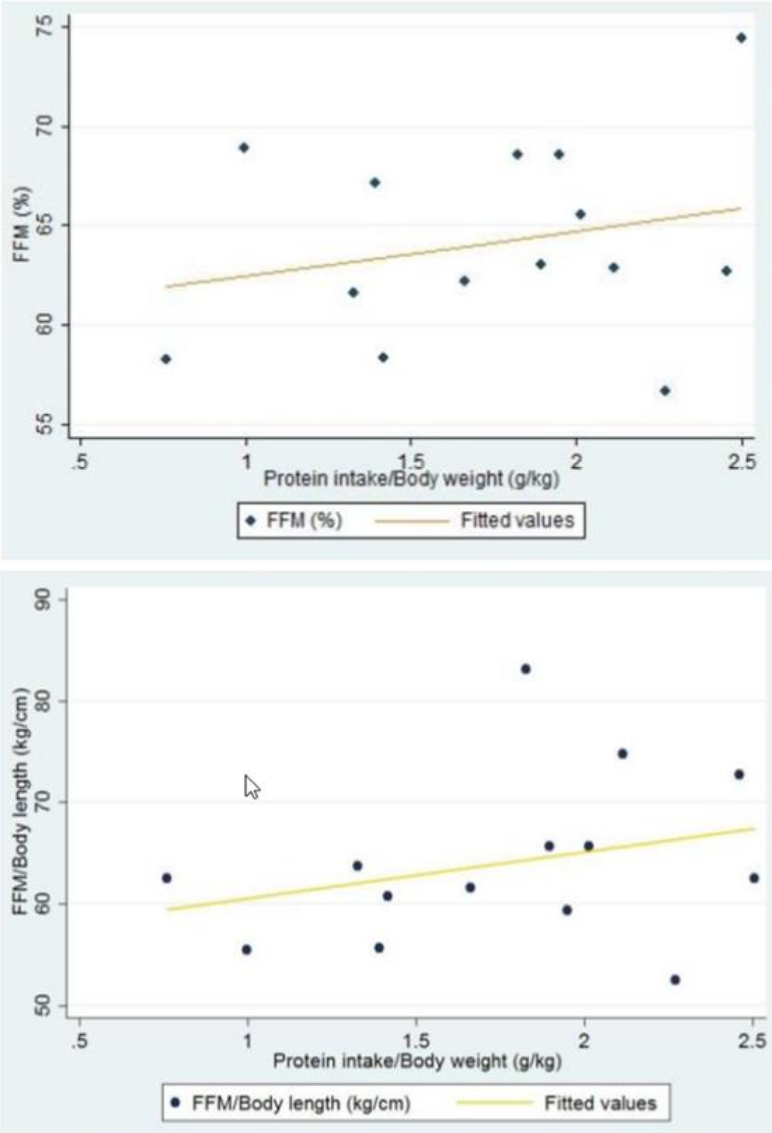


Fig. 10. Macronutrients adequacy indexes.

Protein intake/kg BW (r=0.2936, p=0.3083) and adequacy index (r=0.4863, p=0.0779) were not correlated with FFM and FFM/length ratio (*Graph 4*).

Graph 4. Correlations between FFM and protein intake.



4.7 Motor function

88 children (49 females, 39 males) were included in the analysis, 43 with SMA1 and 45 with SMA2, to investigate the relationship between the evaluation of FFM by DEXA and SMA specific motor function assessments.

Anthropometric measurements, BC and motor function scores are reported in *Table 16*.

Table. 16. Anthropometric measurements, body composition and motor function of SMA1 and SMA2 children.

	SMAI			SMAII			Total		
	P25	P50	P75	P25	P50	P75	P25	P50	P75
Age (months)	5	8	14	33	47	66	8	27	50
Anthropometric measurements									
Weight (kg)	6.4	7.5	8.5	11.6	14.0	18.6	7.5	10.1	14.3
Weight (z-score)	-1.960	-1.210	-0.240	-2.110	-0.900	-0.220	-1.980	-1.035	-0.225
Length (cm)	66.5	72.0	79.0	91.0	100.0	117.5	72.3	89.5	102.0
Length (z-score)	-0.120	0.760	1.570	-1.410	-0.130	1.040	-0.840	0.340	1.495
BMI (kg/m2)	12.3	14.1	15.4	12.7	14.1	14.7	12.6	14.1	15.2
BMI (z-score)	-3.570	-1.990	-0.860	-2.520	-1.720	-0.520	-2.805	-1.755	-0.730
Body composition									
Fat mass (kg)	2.4	2.6	3.4	3.7	4.7	7.6	2.6	3.6	5.0
Fat mass index (kg/m2)	4.0	5.3	6.0	4.0	5.2	6.1	4.0	5.3	6.0
Fat free mass (kg)	4.0	4.4	5.3	7.3	9.1	10.8	4.4	6.5	9.3
Fat free mass index (kg/m2)	7.5	8.5	9.1	7.8	8.6	9.5	7.6	8.5	9.2
Lean mass (kg)	3.8	4.3	5.1	7.0	8.7	10.3	4.3	6.2	8.9
Lean mass index (kg/m2)	7.2	8.2	8.9	7.4	8.2	9.0	7.3	8.2	8.9
Lean:Fat mass ratio	1.3	1.6	2.0	1.3	1.5	2.0	1.3	1.6	2.0
Motor function									
CHOP INTEND score	17	29	40	.	.	.	17	29	40
HFMSE score	.	.	.	6	10	16	6	10	16

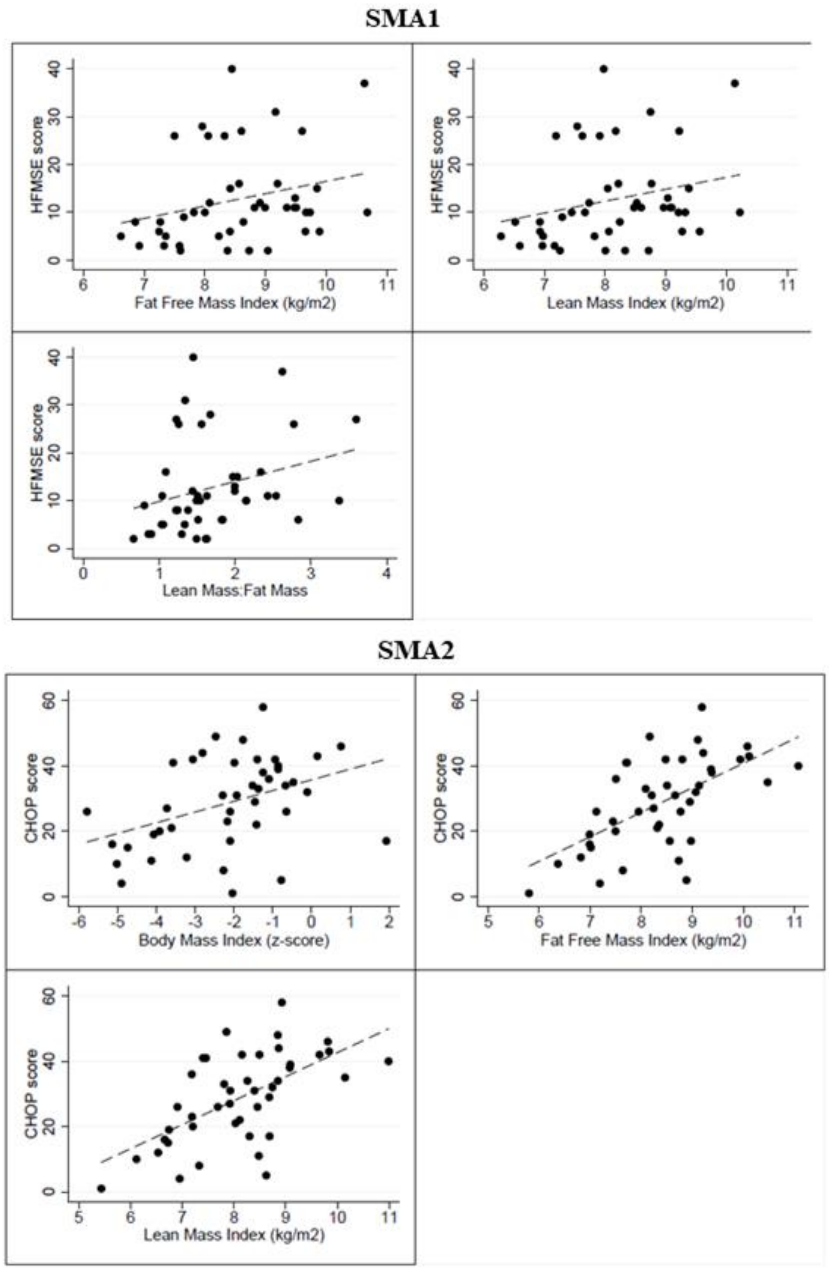
Both SMA1 and SMA2 children showed a weight reduction and a normal length growth with a median weight and height z-score respectively lower and equal to the median values of standard of growth charts. BMI z-scores showed an underweight condition, especially in SMA1 children.

All the children had a higher FM percentage compared to the respective reference values for sex and age (median percentage of overfat: 16.7 %; min 2,1%, max 45,0%).

SMA1 patients had a median CHOP INTEND score of 29; SMA2 patients had a median HFMSE score of 10 (*Table 16*).

CHOP INTEND score in SMAI was positively correlated with BMI, FFMI and LMI (ϱ=0.4153, p=0.0056; ϱ=0.5913, p=<0.001; ϱ=0.5867, p=<0.001; respectively). Similarly, HFMSE score in SMAII was correlated with FFMI, LMI and LM:FM ratio (ϱ=0.3787, p=0.0103; ϱ=0.3660, p=0.0134; respectively), but was not correlated with BMI (ϱ=-0.1274, p=0.4044) (*Graph 5*).

Graph 5. Correlations between body composition and motor function.



CHAPTER 5

General discussion

The present study provides for the first time an overview of the natural history of a large sample of naïve SMA1 and SMA2 children in terms of anthropometric measurements, body composition, resting energy expenditure, metabolic profiles and feeding and dietary habits, including in relation to motor function.

These data were collected as part of a large multicentre natural history study in SMA, in 5 Italian centres where patients are regularly monitored and followed up according to the Standard of Care (SoC) for SMA [1,18]. Over the past decade the implementation of SoC in the management of patients with SMA has strongly improved survival and quality of life for these patients. Additionally, the recent approval of nusinersen by the US Food and Drug Administration and by the European Medicines Agency, and its availability to patients in many countries, along with the current clinical investigation of other therapeutic options, are expected to change significantly the natural history of the disease over the next future. This further highlights the need to have big amount of data collected in untreated patients, in order to assess the efficacy of the available and forthcoming treatments not only on motor and bulbar functions and survival, but also on anthropometric growth, nutritional and metabolic status [45].

To date, only few studies have been conducted on nutritional aspects in SMA [2,10,19–21], all of them on small samples and only few of them used gold standard methods to assess NS, BC and REE [6]. In this scenario, our study shows the high prevalence of underweight and overfat in SMA1 children exacerbated by physical immobilization, as reported in previous studies [2,10], and highlighted the importance of a correct nutritional assessment in SMA. Indeed, we found that more than 68% of the SMA1 children showed an impaired nutritional status compared to healthy peers (BMI-z score below the 5th percentile), confirming that BMI is not a good indicator of the nutritional status in SMA1 children. However, as previously described [8], none reported stunted growth: all children had an above average BL, but with a nutritional deterioration over time, as found by Mehta et al [19]. All children showed a FM% higher than the reference values, showing an increase in overfat in the prepubertal period (4-10 years). Consequently, FFM remained lower than healthy peers, especially at the trunk level, as expected in non-sitters with higher motor and respiratory function impairments [8,46]. Concerning comparison of field and reference methods for FM, skinfolds and skinfold-based measures were highly and significantly correlated to DXA FM. Computation of limb fat area can provide a quick and non-invasive tool in the clinical setting to track FM changes. There are few FM% predictive equations available for children and Brook equation [33] was unable to correctly classify any SMA patient. This seems due to a systematic negative bias, probably caused by reduction of fat free mass and consequently an abnormal inflation of percentage values of FM. Indeed, a high percentage of patients are correctly classified when adjusting for this, seemingly systematic, bias. These results show us that anthropometry can be a useful tool in the nutritional assessment in SMA, but disease-specific equations are needed.

About REE, total REE measured by indirect calorimetry increased overtime, probably due to the high respiratory

function impairment, that requires respiratory support. However, the energy required for kg of body weight and fat free mass decreased with increasing age, especially during NIV-support, both in SMA1 and in SMA2. NIV can acutely decrease resting energy expenditure probably alleviating the ventilatory burden imposed to compensate respiratory muscle weakness. These results show that mechanical ventilation significantly reduces REE. The intense energy expenditure during REESB is probably explained by the peculiar thoracic abdominal patterns of breathing in SMA (particularly in SMA1), causing an intensive work of residual intercostal muscles [47,48]. Comparing measured REE to predicted REE [37], SMAGNG overestimated REE than measured REE by IC, while the new formula SMAICANSREE model including SL and ventilation status explained more than 50% variance of mREE and had better precision in determining energy requirements for SMA children. However, SMAICANSREE was tested only in the same group of patients from which it was derived, so validation in a larger patient group is required.

10% of SMA1 and SMA2 children showed impairment of fasting glucose, lacking association with FM and FFM. The lack of associations suggests that other mechanisms could be involved in the deregulation of glucose metabolism in SMA disease, probably due to pancreatic defects [14], and further insights could be gain by glucose tolerance by OGGT associated with insulin and C-peptide profile. A strong evidence was also provided for the susceptibility to dyslipidaemia in a large cohort of SMA patients. To our knowledge, this is the first time that such abnormalities are reported in SMA [15]. Our studies showed that 37% of SMA patients have dyslipidaemia, 17% more than healthy children [42]. More strikingly, 14% of patients had more than 3 laboratory-defined measures of dyslipidaemia, for which prevalence data is sparse in the normal paediatric population but is suspected to be quite low in the absence of familial dyslipidaemia.

Investigating feeding and dietary habits, SMA1 patients showed a higher prevalence of gastrointestinal and swallowing problems compared to SMA2, as expected [1]. Consequently, SMA1 reported a higher consumption of supplements as multivitamin and herbal preparations, as well as drugs as antiacids and laxatives. Concerning the adherence to the LARN [27] with only diet, SMA children showed adequate calorie consumption, but reported twice as much protein, especially during artificial feeding. However, greater protein intake was not related to FFM in SMA1 and would not seem to contribute to the improvement of FFM. Water and carbohydrates' consumption were lower than recommendations, especially in SMA2 children. Dietary minerals (calcium, iron, magnesium, selenium, iodine) resulted below the recommendations, as well as vitamins B9, D, E and K. Overall, these data suggest the need for continuous monitoring and proper dietary adjustment, as well as specific guidelines for SMA, as demonstrated by other previous studies [2,6,21].

However, body composition, in terms of FFM, was related to motor functional capacity in both SMA1 and SMA2 patients. No correlations between FM were found, as previously speculated [10], but the FFM/FM ratio was correlated with motor scores. Regular body composition assessment is recommended for adequate nutritional management in all non-sitting and non-sitting patients from the updated SoC [10], but body composition assessment is rarely included in routine follow-up assessments of patients with SMA. In the new drug scenario, some patients respond well to treatment with the acquisition of unexpected motor stages, while others have a more limited clinical response. There is a strong need to identify informative clinical and laboratory biomarkers that can be used to better understand the response to treatments and body composition may be one of them.

This study has several strengths. The first is the age distribution of the sample that allowed us to have cross-

sectional data in untreated patients and to potentially investigate the effect of drugs on all the collected parameters in different age groups. Secondly, this is the largest work in the literature studying a SMA sample with a confirmed diagnosis. The third strength of the study is the data quality: dispersion of patients was overcome by a strict cooperation of the involved centres that also allowed a fair distribution in the different classes of age; dieticians which performed anthropometric measurements followed the same standardized protocol; last, body composition and REE were measured with gold standard methods.

A possible limitation of the study is that all children were Caucasian and their patterns of nutritional status, body composition and energy expenditure could be not globally representative of all ethnic groups. In addition, the data presented comes only from the baseline of the multi-center longitudinal study conducted, so this study has a cross-sectional design and not longitudinal, this did not allow to construct the growth patterns of SMA children. For the development of energy equations, because of the well-established difficulties in obtaining accurate and repeatable measurements of supine length in neuromuscular diseases due to scoliosis and contractures [49], surrogate parameters of SL have to be considered. Moreover, SMA1 population is heterogeneous and these patients may have several metabolic changes, which increases the difficulty of finding one prediction equation that will be accurate for all patient types. Finally, the numerous and different reference data sets for the different measurements and the small sample size for some subgroup analyses were a limit that did not allow to draw some conclusions, for example on the association between protein intake and FFM. The reason for the low number of data for some specific points, in particular for the nutritional survey, is the low number of diaries and related questionnaires returned, probably because of the long effort required to complete them (these were 3-days diaries).

CHAPTER 6

General conclusion

Nutritional aspects play a significant role in the multidisciplinary management of children with SMA, particularly in the treatment of nutritional derangements including swallowing and gastrointestinal problems; in the last decade this has become even more important thanks to the increasing evidence of improvements in survival and quality of life in the natural history of the disease and, the availability of the first approved treatment and other therapeutic options at an advanced phase of clinical development. The increase in the knowledge on nutritional aspects of patients with SMA is paramount in the appropriate management of patients during the disease, and even more in the personalization of treatment and selection of the best therapeutic option for that specific child. However, the lack of specific, standardized and coordinated nutritional assessment for SMA patients is confirmed and the use of reference data developed for healthy children increase the risk of an inadequate appropriate nutritional support because of the peculiar nature of SMA.

So, this study fits in this evolving scenario, providing a picture of the largest sample of naïve SMA children in terms of growth pattern, body composition, resting energy

expenditure, metabolic aspects, dietary habits and motor function.

These data on natural history provide a consistent reference by better understanding the natural progression of SMA and using this information to facilitate efficient clinical development aimed to improve quality of life in SMA patients. Anthropometry has proven to be a useful tool for nutritional assessment in SMA and the state of ventilation, besides demographic and anthropometric characteristics, determines the energy needs of SMA patients. Defects in glycaemic and fatty acid metabolism can lead to additional comorbidities, further emphasizing the importance of nutritional management, especially if several dietary deficiencies must be corrected after established checks by a specialist.

This study has expanded the poor nutritional knowledge on growth pattern, body composition, energy expenditure at rest, metabolic aspects and motor function in SMA, laying the foundations for the development of national, and therefore international, references to the nutritional management of this disease, even more necessary in the new therapeutic era of SMA, where life span is extending and the nutritional status could become a potential biomarker of the disease.

CHAPTER 7

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Attachment 1. "Feeding and Gastrointestinal Survey".

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale di Cesena

Feeding and Gastrointestinal Survey

Subject Number _____

Assessment of gastrointestinal function

1) Does you son/daughter have gastroesophageal (acid) reflux?
__Yes __No __Don't know

2) Does your son/daughter have delayed gastric emptying?
__Yes __No __Don't know

3) Does your son/daughter vomit or regurgitate frequently?
__Yes __No __Don't know

Swallowing problems

4) Does your son/daughter choke frequently with feeding?
__Yes __No __Don't know

5) Does your son/daughter have chewing problems?
__Yes __No __Don't know

6) Does your son/daughter have swallowing difficulties?
__Yes __No __Don't know

7) Has dysphagia been diagnosed?
__No __Yes __Yes to liquids___Yes to solids__Yes, both liquids and solids

Constipation

8) Does your son/daughter have constipation?
__Yes __No __Don't know

9) Does your son/daughter have straining or difficulty emptying her bowels?
__Yes __No __Don't know

10) Does your son/daughter have extra firm or hard stools?
__Yes __No __Don't know



Feeding

11) Does your son/daughter have a nasogastric tube?
__Yes __No __Don't know

12) Does your son/daughter have a gastrostomy button?
__Yes __No __Don't know

13) Does your son/daughter have a fundoplication?
__Yes __No __Don't know

14) How long does it take to feed your son/daughter?
__Less than 15 minutes __15-20 minutes __ 30-35 minutes
__ 45-50 minutes __60 minutes or more

Supplements

15) Do you currently give multivitamin and/or mineral supplements to your son/daughter?
__Yes __No __Don't know

16) Do you currently give herbal preparations or health food supplements to your son/daughter?
__Yes __No __Don't know

17) Do you currently give a commercial formula preparation (e.g. Pediasure,Ensure, Peptomen) to your son/daughter?
__As a supplement__As his/her primary food source
__Currently do not use formula preparations__Don't know

18) Do you currently give antiacids/inhibitors of gastric acid secretions?
__Yes __No __Don't know

19) Do you currently give laxatives?
__Yes __No __Don't know

20) Do you currently give fiber's supplements?
__Yes __No __Don't know