

## Title page

**Title:** Predictive fat mass equations for spinal muscular atrophy type I children:  
development and internal validation

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## Abstract

**Background:** Body composition assessment is paramount for spinal muscular atrophy type I (SMA I) patients, as weight and BMI have proven to be misleading for these patients. Despite its importance, no disease-specific field method is currently available, and the assessment of body composition of SMA I patients requires reference methods available only in specialized settings.

**Objective:** To develop predictive fat mass equations for SMA I children based on simple measurements, and compare existing equations to the new disease-specific equations.

**Design:** Demographic, clinical and anthropometric data were examined as potential predictors of the best candidate response variable and non-linear relations were taken into account by transforming continuous predictors with restricted cubic splines. Alternative models were fitted including all the dimensions revealed by cluster analysis of the predictors. The best models were then internally validated, quantifying optimism of the obtained performance measures. The contribution of nusinersen treatment to the unexplained variability of the final models was also tested.

**Results:** A total of 153 SMA I patients were included in the study, as part of a longitudinal observational study in SMA children conducted at the International Center for the Assessment of Nutritional Status (ICANS), University of Milan. The sample equally represented both sexes (56% females) and a wide age range (from 3 months to 12 years, median 1.2 years). Four alternative models performed equally in predicting fat mass fraction (fat mass/body weight). The most convenient was selected and further presented.

The selected model uses as predictors sex, age, calf circumference and the sum of triceps, suprailiac and calf skinfold thicknesses. The model showed high predictive ability (optimism corrected coefficient of determination,  $R^2 = 0.72$ ) and internal validation indicated little optimism both in performance measures and model calibration. The addition of nusinersen as a predictor variable did not improve the prediction. The disease-specific equation was more accurate than the available fat mass equations.

**Conclusions:** The developed prediction model allows the assessment of body composition in SMA I children with simple and widely available measures and with reasonable accuracy.

**Keywords:** spinal muscular atrophy type I, fat mass, predictive equation, nutritional status

**Abbreviations:**

- BMI: body mass index;
- DXA: dual-energy X-ray absorptiometry;
- FFM: fat free mass;
- FM%: fat mass percentage of whole body weight;
- FM: fat mass;
- FMI: fat mass index;
- $R^2$ : coefficient of determination;
- SMA I: spinal muscular atrophy type I.
- SMA: spinal muscular atrophy;
- $R^2_{adj}$ : coefficient of determination adjusted for the number of explanatory terms;

## Introduction

Spinal muscular atrophy (SMA) is a genetic motor neuron disease that leads to muscle weakness and wasting [1]. Skeletal and respiratory muscles are variably affected and there is a high prevalence of gastrointestinal disorders, including difficulties with feeding, swallowing, digestion and bowel movements. SMA patients are classified on the basis of age of onset and maximum motor milestone achievement, with SMA type I (SMA I) being the most severe postnatal form [2]. In SMA I, the first signs of weakness occur in the first six months of life, and affected children never acquire the ability to sit without support. With the recent availability of effective treatments, the natural history of SMA I patients is changing. Nusinersen has been the first approved disease-modifying drug showing significant improvements in motor function and event-free survival, especially when administered early [3].

Body composition of SMA patients is affected by both the pathophysiology of the disease and its complications. Several studies have shown that fat-free mass (FFM) and bone mineral content are reduced [4,5]. On the other hand, the reduced energy expenditure due to low basal metabolic rate, respiratory support [6], and low motility leads to accumulation of fat mass (FM) [7–9]. When the gastrointestinal involvement is severe, particularly because of dysphagia, energy intake can be compromised, and weight can be even more severely reduced [10]. Body composition derangements are related to SMA categories, with SMA I patients having more FM and less FFM than SMA type II patients [11].

The assessment of body composition plays several roles in SMA. Besides its use to plan nutritional interventions, it can be used to track disease progression [12], and has been

shown to potentially be a biomarker of motor function [5]. Dual-energy X-ray absorptiometry (DXA) has been used in the majority of studies that included a body composition assessment in SMA [7–9,13–19], and has become the reference method in SMA and other neuromuscular diseases. DXA is an accurate method for the assessment of body composition based on the different attenuation coefficient to X-rays of FM, lean tissue mass and bone mass [20]. Besides its accuracy, DXA provides a unique set of features that make it suitable and compelling in neuromuscular diseases: it evaluates bone mass and mineral density; it allows segmental body composition assessment; can be performed in non-sitters; is cheaper and quicker than whole-body magnetic resonance imaging and is less invasive than computed tomography. However, DXA has not been extensively validated in the pediatric population, and not at all in SMA patients; therefore, normative values are lacking for these populations. Moreover, as with all imaging techniques, severe joint contractures, severe scoliosis, and large artifacts due to growing rods or other orthopedic implants can compromise body composition estimates. Moreover, performing DXA at the frequency suggested by the standard of care [1] may cause concerns about cumulative radiation exposure, especially if other imaging procedures are required [21]. Finally, DXA may not be available in every clinical setting.

Anthropometry is a cheap, widely available technique for the assessment of body composition. It involves the measurement of weight, stature/recumbent length, segmental lengths, body breadths, circumferences, and skinfold thickness [22]. Estimates of total body FM are based on population-specific prediction models developed using regression analysis [23]. Available prediction models are not suitable to assess body composition in special populations, such as patients with neuromuscular disease [19,24]. Moreover, no

anthropometric models have been developed so far in SMA patients and no field methods are available for their assessment of body composition.

The primary aim of this study was to develop and internally validate predictive FM equations for SMA I children based on demographic, clinical and anthropometric data. The secondary aim was to compare the resulting equations with available FM equations for the general population.

## Materials & Methods

### Source of data

From April 2015 to January 2020, a longitudinal observational study in SMA children was conducted at the International Center for the Assessment of Nutritional Status (ICANS, University of Milan, Milan, Italy). At the end of the study, 165 patients with a clinical and genetic diagnosis of SMA I were consecutively enrolled.

Before the body composition assessment, the patients underwent a clinical evaluation at their neurological center. Anthropometry and DXA were performed on the same morning for each patient at ICANS.

The study protocol was approved by the Ethics Committees of the University of Milan (n.7/16) and Carlo Besta Neurological Institute Foundation n.37/2016) and complied with the Helsinki Declaration. The parents, on behalf of their children, gave their informed and written consent to the study.

## Participants

Patients were recruited from 5 clinical referral centers for SMA in Italy: Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan; SAPRE UONPIA, Fondazione IRCCS Cà Granda, Policlinico di Milano, Milan; Department of Neurosciences, Neuromuscular, and Neurodegenerative Disorders Unit, Laboratory of Molecular Medicine, Bambino Gesù Children's Research Hospital, IRCCS, Rome; Italian Department of Neurosciences and Rehabilitation, Institute "G. Gaslini," Genoa; and Department of Women's and Children's Health, University of Padua, Padua.

Inclusion criteria were:

- genetically confirmed diagnosis of SMA;
- clinical diagnosis of SMA1[1];
- age 0-11,99 years;
- clinical management according to the best supportive care based on the Consensus Statement for Standard of Care in SMA[1];
- absence of acute medical conditions in the 15 days before the assessment.

DXA images with missing or overlapping portions of the body, or with large artifacts, were excluded. Excluded DXA images were identified through operator notes and by comparing DXA mass to scale weight (absolute differences greater than 1 kg), and visually inspected before exclusion.

Patients participating in experimental pharmacological trials were also excluded. Patients under nusinersen treatment (the only approved pharmacological treatment at the time of

study) were included and considered as treated patients if they had received at least 4 loading doses.

## Outcome

Three dependent variables were evaluated for the prediction of FM: FM measured by DXA (kg); FM fraction of whole body weight (FM%, expressed in percentage); FM index (FMI, kg/m<sup>2</sup>), defined as FM divided by the square of recumbent length.

All possible outcomes were obtained from DXA imaging performed as part of the main longitudinal study, from a single center, using the same device, and under strictly controlled conditions.

## Dual-energy X-ray absorptiometry

DXA was performed on the whole body with narrow fan-beam densitometer (GE Lunar iDXA, Boston, USA). Daily and yearly quality controls were performed. During measurement, all patients wore minimal clothing and kept only strictly necessary medical devices. Images were analysed with the manufacturer software (enCORE), artifact from orthopedic implants or other medical devices were manually removed. Data on bone mass, lean tissue mass and FM were recorded.

FFM was defined as the sum of soft-lean mass and bone mass. Bone, soft-lean, FFM and FM indexes were defined as the body composition compartment divided by the square of recumbent length in meters.

## Predictors

Three types of independent variables were considered: demographic, clinical and anthropometric.

The demographic variables included sex (categorical, female/male) and age (in months of years, continuous).

The clinical variables included treatment with nusinersen (dichotomous, false/true) and the number of infusions administered to the patient (count).

The anthropometric variables included body weight (kg, continuous), recumbent length (cm, continuous), segmental lengths (arm, ulna, femur, tibia; cm, continuous), circumferences (waist, arm, thigh, calf; cm, continuous), skinfold thickness (biceps, triceps, subscapular, suprailiac, anterior thigh, calf; mm, continuous), and derived measures (calculation details below) such as body mass index (BMI;  $\text{kg}/\text{m}^2$ , continuous), arm fat area and thigh fat area ( $\text{cm}^2$ , continuous).

## Clinical evaluation

During the clinical evaluation, the caring neurologist collected a medical history, and performed a physical and neurological examination. Data on diagnosis, pharmacological trials performed, and nusinersen treatment (date of first infusion and number of infusions performed) were recorded.

## Anthropometry

The anthropometric methods are detailed in our previous publication which includes an anthropometric manual specific for neuromuscular patients [25], and inter-observer reliability data.

All anthropometric measurements were collected by 3 well-trained operators. Body weight was collected to the nearest 0.1 kg with an electronic wheelchair scale accurate to 0.1 kg (Seca 664, Seca GmbH, Hamburg, Germany). Recumbent length, segmental lengths and circumferences were collected to the nearest 0.1 mm with an inextensible metric tape, wide 0.5 cm, and graduated to 1 mm (Gima 27341, Gima S.p.A., Gessate, Italy). Skinfold thickness were measured to the nearest 0.1 mm using a skinfold caliper with a 35 mm<sup>2</sup> jaw face area, exerting a 10±2 g/mm<sup>2</sup> pressure between the jaws, with a range of 0-40 mm, calibrated to 0.2 mm (Holtain Tanner/Whitehouse Skinfold Caliper, Crosswell, UK).

BMI was defined as body weight divided by the square of recumbent length in meters. Arm and thigh fat areas were calculated assuming a cylindrical shape for the limb and its constituents, as the difference of limb cross-sectional area and limb cross-sectional muscle area [26]. Sex-specific weight, length, and BMI-Z-scores were derived using the 2006 World Health Organization growth charts [27] for patient <2 years old and the 2000 Center for Disease Control and Prevention growth charts [28] for older patients. Classes of BMI-for-age and stature-for-age were computed as per WHO or CDC guidelines [29].

FM% was calculated from body density prediction using the Brook (1971) [30] and Siri (1961) [31] equation, and directly using the Slaughter (1988) [32] equation.

## Sample size

Few similar studies matching methods and age range used in this study are available in the pediatric population, and even fewer in neuromuscular diseases and none in SMA. So, prespecification of the model's anticipated  $R^2$  was not possible and we did not perform any formal sample size calculation. Instead, we pre-specified model complexity (i.e. allowed degrees of freedom) based on the available sample size, employing several data reduction technique to arrive to a suitable set of predictors.

Adequacy of sample size was tested a posteriori using criteria identified by Riley (2018) [33].

## Missing data

As recommended [34], variables missing at random or completely at random were imputed to avoid discarding incomplete observations. A number of imputation equal to the percentage of incomplete cases was computed. A different bootstrap re-sample was drawn from complete cases for each of the multiple imputation dataset. Flexible additive models were fitted on the bootstrap samples and used to predict the variable missing in the original sample. Missing values were imputed from donor observations (complete cases) through predictive mean matching (ie. the actual observation whose predicted value was closest to the predicted missing value) [35].

## Statistical analysis

Continuous variables are reported as 50<sup>th</sup> (25<sup>th</sup>, 75<sup>th</sup> percentile), categorical variables are reported as count (fraction). Hypothesis testing between naive and nusinersen patients was performed using: 1. the Wilcoxon rank sum test with continuity correction for age and z-score of growth variables, 2. the Kruskal-Wallis rank sum test for sex categories, 3. proportional odds ordinal logistic regression controlling for sex and age, transformed with a restricted cubic spline with 3 knots, for all other variables.

Normality and constant variance assumptions were tested for the three candidate response variables. Linearity between the response and continuous predictors was not assumed by using restricted cubic spline, with degree of non-linearity pre-specified using prior knowledge of the response-predictor relationship [35]. Hierarchical cluster analysis, collinearity test and combination of multiple variables were used as data reduction strategies to achieve adequate model complexity (15 observation per degree of freedom were considered adequate) [35].

Multiple linear models fitted on the imputed samples were compared on the basis of overall performance (coefficient of determination,  $R^2$ ), discrimination ability (g-index), and calibration plots (slope and intercept) [33,35,36]. Results from our previous inter-observer reliability study [25] were considered in choosing alternative models. Such results are reported in Appendix B. The final models were also evaluated graphically using partial effects plots, using both case-wise deletion of missing variables and pooling from the imputed datasets.

To quantify the optimism of the final models, internal validation was performed on each imputed dataset using 1000 bootstrap resamples, with further pooling of results. Optimism was estimated for  $R^2$ , g index and calibration slope and intercept. The bootstrap re-samples were also used to estimate the distribution of regression coefficient and quantile-based knot locations in each imputed dataset and pooled mean and 95% confidence intervals (CIs) were computed. Regression coefficients were optimism-corrected in the final models using the pooled calibration slope from bootstrap internal validation as a uniform shrinkage factor, and adjusting for the pooled calibration intercept. The internal validation was also performed with limited backward step-down variable selection on a stacked and weighted dataset of all the imputation datasets, to tentatively develop a more parsimonious model.

The potential contribution of nusinersen treatment to the unexplained variability of the outcomes was evaluated as follows. Nusinersen treatment was added to the full final models either as a categorical variable (naive/treated patient) or as a continuous variable (days from first injection). The regression coefficient of the nusinersen variable was used to assess the clinical importance of the addition of a nusinersen variable, while the predictive ability of the nusinersen variable was assessed comparing the  $R^2$  of the models with and without the nusinersen variable.

The final model is presented as an R function suitable for computerized implementation. For field use, an approximated version of the final model was computed to allow calculations with simple calculators.

To compare the final models with available predictive FM equations, Bland and Altman plots [37] were drawn.

The statistical procedures used in the development of the equations are described in detail by Harrell (2015) [35] and Steyerberg (2019) [38]. Statistical analyses were performed in R 4.0.2 [39], with the addition of the *rms* package [40] for imputation, model fitting and model validation.

## Results

### Participants

Of the 165 enrolled SMA I, 12 (7.3%) didn't meet the inclusion criteria and were excluded from the analysis. The 12 excluded patients displayed absolute differences between scale and DXA weight greater than 1 kg. Visual inspection of DXA images and operator notes showed overlapping body parts, missing body parts, portion of the body scanned multiple times or big artifacts area due to manual elimination of extensive orthopedic implants (mainly growing rods). A flow diagram is available in Figure B1 in Appendix B.

The characteristics of the study population (N = 153) are summarised in Table 1. There was a slight prevalence of girls (56% females) and, while most patients were < 2.6 years of age (75<sup>th</sup> percentile), age ranged from 3.0 months to 12 years.

As shown by our previous study [11], both weight and BMI z-scores distribution were biased towards lower values; in detail, they were centered around -1.4 and -2.6 z-scores respectively. The median recumbent length was higher than the 50<sup>th</sup> percentile, being

approximately 0.3 z-score. Almost all patients (93%) displayed normal recumbent length, while only 34% had normal weight, with the remaining being underweight. When compared to reference values estimated by Fomon (1982) [41], all but 2 patients had higher FM% than healthy peers and the FM% difference between SMA I patients and healthy peers was 15.4% (11.0%, 21.2%). A more comprehensive comparison with Fomon data is included in Appendix A.

Considering treatment, the nusinersen patients were older, and had lower weight, recumbent length, and BMI z-scores than the naive patients, but recumbent length and BMI categories were not significantly different. Controlling for age and sex, all other variables were not significantly different (except recumbent length, as already noted by the differences in recumbent length z-score).

### **Missing variables and imputation**

Not all patients in our sample completed the whole protocol: ulna length was missing in 16% of patients, and calf circumference and calf skinfold were both missing in 10% of patients. Analysis of the period in which examinations of incomplete cases was conducted confirmed that those measurements were missing because they were not part of the original data collection procedure. Missing measurements were considered missing completely at random (their absence was proved to be unrelated to any characteristics or the candidate response variables).

Since the fraction of incomplete cases was 16%, 16 imputation datasets were computed using demographic, body composition and anthropometric data. Due to the high collinearity of missing measurements with other measurements, the  $R^2$ s with which each

missing variable could be predicted were generally high (ulna length  $R^2 = 0.87$ , calf circumference  $R^2 = 0.85$ , calf skinfold  $R^2 = 0.76$ ). Moreover, the empirical cumulative distribution functions of missing variables drawn from complete observations and the imputed datasets were remarkably similar (Figure B2 in Appendix B).

## Model development

Outcome measures were available for all 153 participants. Visually testing the normality and constant variance assumption of the three candidate responses (Figure B4 in Appendix B) excluded FM from the candidate predictors. While we considered FM% and FMI to be both adequate responses, but FM% was preferred to tentatively develop a model that would not require a stature measurement, as measuring stature pose several challenges in SMA I. Also, using FM% as response variable makes the model comparable with existing equations.

With a total of 153 independent observations, we limited model parameters to  $153/15 \approx 10$ . Variable clustering (Figure B5 in Appendix B) identified four independent dimensions: sex, development stage (age, recumbent length, weight, and segmental lengths), circumferences, skinfolds. To represent the skinfold cluster, sums of different skinfold combination were computed testing for the equal weight assumption [38]. To represent all dimension in our model, alternative models were fitted for each “development stage” variable, circumference and skinfold sum combination. All continuous variables were transformed with restricted cubic splines, but more degrees of freedom were reserved to the “development stage variable” (5 knots instead of 3) [35]. All tested models had a total

of 10 degrees of freedom. Redundancy analysis was performed on the resulting models, but no variables proved to be redundant in the final models.

Fitting the models using either complete cases for the specific model variables or complete cases for all variables highlighted selection bias of subjects. Models using incomplete cases appeared indeed to perform better than models using all subjects, but the ranking of models fitted on incomplete cases was equal to that of models fitted on imputed datasets.

Among the many competing models, we excluded those including measurements that could not be reliably collected in our pilot study [25]. Four alternative models having as predictors one “development stage” variable (age, recumbent length, ulna length or tibia length), calf circumference and the sum of triceps, suprailiac and calf skinfolds, were deemed to be the best compromise between performance, discrimination, calibration and parsimony. The unadjusted association between each predictor and outcome is available in Figure B3 in Appendix B, and a visual comparison of selected tested models is available in Figure B6 in Appendix B.

### **Model specification and performance**

The four alternative model coefficients are shown in Table 2, but, as the coefficients of a variable transformed with a restricted cubic spline are hard to interpret, partial effect plots are available in Figure B7 in Appendix B. The calibration plots are shown in Figure 1 and model performance statistics in Table 3. The models performed very similarly with apparent  $R^2$  of  $\sim 0.76$  and mean squared error of  $\sim 12.4$  (rooted mean squared error  $\approx 3.5$ ). Optimism detected by bootstrap internal validation was limited to  $\sim 0.04$  of the apparent  $R^2$  and  $\sim -1.9$  of the mean squared error (rooted mean squared error  $\approx 1.4$ ). Little optimism

was also observed in discrimination ability, with g-index optimism of  $\sim 0.11$ . The shrinkage factor obtained by the bootstrapped slope of the calibration plot was  $\sim 0.98$ , very close to 1, denoting minimal over-fitting. The adjustment for the calibration intercept was  $\sim 0.90$ . The limited backward step-down variable selection did not remove any factor in all the alternative models.

### **Influence of nusinersen treatment**

The addition of nusinersen status variable as covariable to the models did not improve their prediction. The regression coefficients of nusinersen were in fact not clinically relevant and the  $R^2_{\text{adj}}$  did not change (Table 4).

### **Model presentation and simplification**

As the four alternative models provided very similar performance, the age model was picked for presentation as age should be the most convenient and reliable variable to collect of the four alternative “development stage” variables. To take advantage of the full model with spline transformation, R code is included in Appendix B for prediction of FM fraction. For field use, the regression equation of an approximated version of the age model is presented in Box 1, already split by sex and age. The approximated model was obtained with a linear age spline (with knots at 6 months, 1.5 years and 5 years) and quadratic transformation of calf circumference and skinfold sum. The approximated model was able to predict almost perfectly the fitted FM% values from the full age model ( $R^2_{\text{adj}} = 0.995$ ).

## Comparison with other equations

Figure 2 shows Bland and Altman plots of the age model and other FM equations available for the pediatric general population. Both systematic and proportional bias can be detected in previously available predictive equations.

## Discussion

We developed the first predictive equations to estimate FM% in SMA I patients. The equations are based on demographic and anthropometric data, but the influence of relevant clinical variables was also taken into account. These equations require relatively inexpensive equipment and a limited but fundamental training to assess their predictors [25]. On the other hand, they allow the assessment of body composition in virtually any setting and as often as required.

As we have previously shown [11], body weight and BMI measurements are misleading in SMA I, with the majority of children diagnosed as “underweight” by “reference” growth charts while having a high FM%. This is due to the concomitant slower velocity of fat-free mass gain and higher velocity of fat mass gain in comparison with healthy peers. These results underline the importance of body composition assessment in SMA I and the need of widely available tools to carry out the assessment. As we have shown, currently available equations to estimate FM in the general population are grossly inaccurate in SMA I children, and disease specific equations were needed.

The equations presented here were developed in patients aged 2 months to 12 years, with a FM% measured by DXA between 20% and 60%. All models showed high predictive ability ( $R^2 > 0.7$ ) and an error we deem acceptable in the clinical setting (root mean square error  $\approx 3.8$ ). We internally validated the model by quantifying the optimism of the obtained equations. The bootstrap internal validation indicates little optimism for the apparent performance of the models, with a global shrinkage factor  $> 0.9$  and small absolute differences ( $< 0.05$ ) in the  $R^2$ . While external validation is required to assess generalizability of our models, they currently offer the only available estimate for FM% in SMA I not requiring reference or gold-standard methods.

The inclusion of nusinersen treatment in the model did not improve the prediction of FM% in SMA I children. While it is possible that nusinersen had an effect on body composition, it was fully explained by variation of the other variables included in the models. The differences between the two groups highlighted in Table 1 are seemingly due to the age difference, and in particular the lower weight z-score could be attributed to the disease progression, but studies designed to describe the nusinersen effects on body composition are required to confirm those speculative findings.

As the four developed models had similar performance and validated equally well, the most convenient (the age model) was further simplified for field use, and a calculator is also available at <https://icans.shinyapps.io/smanutrition/>.

This study has several strengths. The sample was relatively large considering the rarity of the disease, and included both naive and nusinersen treated SMA I patients. The measurements were of high quality: the DXA data came from a single center using the same

device, and were collected under strictly controlled conditions; the anthropometric procedures were specifically designed for SMA children and included several different measurements; an inter-observer reliability study for the anthropometric procedures was specifically performed on SMA patients; all patients came from an ongoing longitudinal study on nutritional status in SMA children. The wide age range of our sample makes the equations applicable to a wide target population.

On the other hand, ethnicity is a known factor affecting body composition and this study only included Caucasian patients. While the age range is wide, most of our patients were below 3 years of age; this was the unavoidable but welcomed result of improved survival of SMA I patients recorded in the last few years. These equations may not be valid for SMA I patients with a severely stunted phenotype; it is plausible that FM may be reduced in those patients although, to our knowledge, no body composition study targeted this phenotype. On the other hand, the sample of the cited reliability study is small and may not represent all patients included in this study [25]. We still value the results of the reliability study as it is the only one ever performed on SMA patients and the exclusion of unreliable measurements impacted very little on the predictive ability of our equations. Further steps will also include external validation of the developed equations which showed promising results from internal validation.

## Conclusion

The equations described above allow the assessment of FM% in SMA I with relative ease and reasonable accuracy, and will be helpful in the nutritional management of SMA I children in many clinical settings.

**Funding statement:** This study was supported by Fondazione Telethon (Application GUP15014, 2015, Italy) and the Italian Association of Spinal Muscular Atrophy Families (Famiglie SMA, 2015–2016 contribution).

**Conflict of interest:** Some authors (EB, CB, AD) of this publication are members of the European Reference Network for Neuromuscular Diseases (ERN EURO-NMD). GiBa has received speaker and consultancy honoraria from AveXis, Inc., Roche, PTC, and Sarepta Therapeutics, but he received no funding for this specific study. All the other authors declare no conflicts of interest.

**Authors' contributions:** AF: conducted the research, performed the statistical analysis, and wrote the paper; RD: designed the research, conducted the research; AB, SB: designed the research, had primary responsibility for final content; SR: conducted the research, revised the manuscript; GiBe: validated the statistical analysis and revised the manuscript; AL, AD, EB, MP, CB, CA, CM, EG, RM, GiBa: revised the manuscript.

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## Figure legends/captions

**Figure 1:** Calibration plots for fat mass fraction (%) for all alternative models. The continuous line in each plot is the line of equality, while the dashed line is a locally estimated scatterplot smoothing line (LOESS).

**Figure 2:** Bland and Altman plots of the developed age model and available fat mass equations for the pediatric general population.