Prediction of fat-free body mass from bioelectrical impedance and anthropometry among 3-year-old children using DXA

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For 3-year-old children suitable methods to estimate body composition are sparse. We aimed to develop predictive equations for estimating fat-free mass (FFM) from bioelectrical impedance (BIA) and anthropometry using dual-energy X-ray absorptiometry (DXA) as reference method using data from 99 healthy 3-year-old Danish children. Predictive equations were derived from two multiple linear regression models, a comprehensive model (height²/resistance (RI), six anthropometric measurements) and a simple model (RI, height, weight). Their uncertainty was quantified by means of 10-fold cross-validation approach. Prediction error of FFM was 3.0% for both equations (root mean square error: 360 and 356 g, respectively). The derived equations produced BIA-based prediction of FFM and FM near DXA scan results. We suggest that the predictive equations can be applied in similar population samples aged 2–4 years. The derived equations may prove useful for studies linking body composition to early risk factors and early onset of obesity.

An association between body composition in early childhood and risk of overweight and non-communicable diseases later in life has been found in many studies. In particular, there is an increasing focus on factors that influence early proportions of fat-free mass (FFM) and fat mass (FM). However, practical and technical limitations, such as problems lying completely still during a DXA scan make precise predictions of body composition in young children difficult to obtain. It is difficult to obtain reliable predictions of total FFM and total FM from simpler techniques. In young children weight-for-height standard deviation scores and body mass index (BMI) are frequently used as indirect estimates of total adiposity even though both are of limited use as measures of adiposity in early childhood. Anthropometric measurements like skin folds, waist- and arm circumference provide information on regional fat stores but become inaccurate when converted to full body FM and FFM in individuals. Bioelectrical impedance analysis (BIA) is a recognized method to estimate total body water (TBW) in epidemiologic studies, although some limitations have been identified. BIA estimates TBW by sending an electrical impulse through the body and measuring the resistance which depends on the amount and distribution of FM and FFM. The BIA method is quick, inexpensive, portable and easy to use in young children. In children, FFM can be calculated from TBW using age- and gender specific hydration factors. However, the hydration levels of FFM vary according to growth, maturity, ethnicity, disease, and obesity and therefore require population and age-specific interpretation of the output. Only few predictive equations for TBW or FFM are available for children in early childhood (2–4 years). None of these studies validates how accurate FM is estimated from the predicted FFM values as it is the underlying assumption that FM and FFM sum up to the total body weight of the child.

It has been recommended to begin prevention of obesity at the age of 2–4 years, and the age of the children in the present analysis (3 years) is relevant in the search for possible determinants for early body composition but also for studying the relationship between early body composition and adiposity later in life. Three-year-old children can be difficult to examine using DXA but BIA and anthropometry are measurements that are easily obtained. The aim of this study was to develop predictive equations for estimating FFM in 3-year-old children from BIA and anthropometry using DXA as reference measurement. We were mostly interested in establishing a...
comprehensive predictive equation for FFM that could explain as much variation as possible but we also considered a simpler version with BIA (resistance), height and weight only to increase the general applicability and suitability for clinical and epidemiological work.

Results

Anthropometric measurements (height, weight, sum of triceps and subscapularis skinfolds, waist circumference, and mid-upper-arm circumference) were available for 263 children; BIA data were obtained from 250 children; and 189 children completed the DXA scan. Only perfect scans and scans with minor irregularities (described in details in a previous paper) were included in this analysis, giving a total of 101 scans. In total, we had complete data from 98 children for the full model and 99 children for the simple model (Table 1). Except for three mixed couples with one parent of Danish origin and one parent of Asian origin, all other couples were Caucasian. These children were included in the analyses.

No differences were found between children who were and were not DXA scanned in weight, height and BMI at 9 months (n = 310) and 36 months (n = 263) (data not shown). Characteristics of the included population divided by gender are shown in Table 1.

Average subscapularis skinfolds, resistance, reactance, FFM, and bone mineral content were significantly different between boys and girls (Table 1). Boys had more muscle mass and bone mass compared to girls, while girls had thicker subscapularis skinfolds and higher FM. Triceps skinfolds tended to be thicker for girls (P = 0.06). BIA data showed that girls had higher resistance, reactance, and lower RI compared to the boys. No gender differences were seen in age, weight, height, BMI, mid-upper-arm circumference and waist circumference.

Weight estimated by DXA was slightly but significantly higher than the digital weight (mean difference 99 g (95% CI: 27–171 g), P = 0.008) and an adjusted weight variable, weightadj, was derived and used in the subsequent regression analyses. FFMadj was obtained from the following equations:

\[
\text{Weight}_{\text{adj}}(kg) = 0.981\ \text{digital weight}(kg) + 0.374
\]

\[
\text{FFM}_{\text{adj}}(g) = \text{Weight}_{\text{adj}}(kg) \times 1000 - \text{FFM}_{\text{pred}}(g)
\]

The final full model included RI, height, weightadj, sum of subcapular and triceps skinfolds and gender (Table 2) and explained 85% of the variance in the training sets while the simple model including RI, height and weightadj explained 84% of the variance.

Validation of the predictive equations. RMSE for the test sets and complete dataset are found in Table 2. Prediction errors (mean RMSE/mean FFM, BMC and lean tissue mass was measured by DXA. RI calculated as Ht2/R.

| Table 1 | Characteristics of the population - anthropometry, BIA and DXA |
|---------|---------------------------------------------------------------|
|          | n | Girls |          | n | Boys |          | P-value |
| Age (months) | 49 | 36.2 [25.7,36.9] | 50 | 36.1 [35.5,37.6] | 0.92 |
| Wt (kg) | 49 | 14.26 [1.30] | 50 | 14.61 [1.46] | 0.21 |
| Wt_{adj} (kg) | 49 | 14.36 [1.28] | 50 | 14.71 [1.43] | 0.21 |
| Ht (cm) | 49 | 95.0 [3.0] | 50 | 96.0 [3.5] | 0.13 |
| BMI (kg/m2) | 49 | 15.8 [1.3] | 50 | 15.8 [1.1] | 0.77 |
| BMI z-score | 49 | 0.24 [0.92] | 50 | 0.15 [0.82] | 0.58 |
| Wc (cm) | 49 | 50.3 [2.4] | 50 | 50.1 [2.9] | 0.67 |
| SF (mm) | 48 | 9.5 [8.2;10.5] | 50 | 8.8 [7.6;9.9] | 0.06 |
| SFs (mm) | 49 | 6.6 [5.9;7.9] | 50 | 6.0 [5.4;6.9] | 0.01 |
| MUAC (cm) | 49 | 16.6 [1.1] | 50 | 16.4 [1.1] | 0.45 |
| R (cm2) | 49 | 765.0 [65.9] | 50 | 734.5 [64.4] | 0.02 |
| Xc (cm2) | 49 | 58.3 [56.4;62.1] | 50 | 55.4 [52.5;57.4] | 0.001 |
| RI (cm2 /cm) | 49 | 11.9 [1.3] | 50 | 12.7 [1.5] | 0.006 |
| BMC (g) | 49 | 428.9 [49.7] | 50 | 452.6 [52.0] | 0.02 |
| FFM_{DXA} (g) | 49 | 11,600 (852) | 50 | 12,427 (1,138) | 0.001 |
| FFM_{DXA} (g) | 49 | 2743 (771) | 50 | 2299 (662) | 0.003 |

Data presented as mean (SD) or median (25;75 percentile). Tested for statistical significance by two-sample t-test or Wilcoxon rank test.

BMC, Bone Mineral Content; DXA, dual-energy X-ray absorptiometry; FFM, Fat-free mass; FM, Fat mass; Ht, height; MUAC, mid-upper-arm circumference; R, resistance; RI, Resistance Index; SF, skinfold thickness triceps; SFs, skinfold thickness subscapularis; Wc, waist circumference; Wt, digital weight; Wt_{adj}, weight adjusted to agree with DXA weight; Xc, reactance. BMI calculated as Wt/Ht2. Xc and R measured by bioelectrical impedance. FFM_{DXA} calculated as lean tissue mass = BMC. FM_{DXA}, BMC and lean tissue mass was measured by DXA. RI calculated as Ht2/R.

| Table 2 | Predictive equations for FFM (g) developed by 10-fold cross validation based on 3-year-old Danish children |
|---------|---------------------------------------------------------------|
|          | n | RI | Wt | Ht | Sec | Sum SF | Constant | Adj R2 | RMSE | RMSEadj | PE | PEadj |
| Full    | 98 | 297.3 | 354.3 | 43.5 | 331.7 | -64.7 | -62.7 | 0.85 | 360.4 | 321.1 | 3.0 | 2.7 |
| Simple  | 99 | 327.2 | 223.8 | 76.8 | 417.6 | . | -2784.4 | 0.84 | 355.8 | 333.3 | 3.0 | 2.8 |

*Results presented as regression coefficients.
Female = 0, male = 1
*Based on the complete dataset.
Adj R2, adjusted R2; FFM, Fatfree Mass (g); Ht, height (cm); PE, Prediction error; RI, Resistance Index (cm2/cm2); RMSE, Root mean square error (g); Sum SF, Sum of skinfold thickness triceps and subscapularis (mm); Wt, digital weight (kg); Adj R2 is the mean of the adj. R2 based on the 10 training sets. RMSE is the mean of the individual RMSE in the test sets. PE was calculated by dividing mean RMSE from the test sets with mean FFM_{DXA} and multiply by 100%.
The values of RMSE for FM\textsubscript{cal} were 264.3 g (girls 250.7 g; boys 277.3 g) in the full model and 303.4 g (girls 307.1 g; boys 299.9 g) in the simple model. Due to the lower FM-total body weight ratio prediction errors for FM\textsubscript{cal} were 10.5% (girls 9.1%; boys 12.1%) in the full model and 12.0% (girls 11.2%; boys 13.0%) in the simple model. Analysis of the level of agreement showed that the mean difference between FM\textsubscript{cal} and FM\textsubscript{DXA} was 6 g based on the full model (95% limits of agreement $-623;636$) and 0 g ($-724;725$) in the simple model (Figure 2 a & b). The magnitude of bias for FM\textsubscript{cal} (FM\textsubscript{cal} - FM\textsubscript{DXA}) was depended on FM indicating an overestimated of FM among the leaner children and underestimated among children with higher FM\textsubscript{DXA} (full model: $\beta = -0.14$ g (0.04), $P = 0.001$; simple model: $\beta = -0.18$ g (0.05), $P < 0.001$).

**Discussion**

In the present study, predictive equations for FFM have been generated and validated using data from a large sample of 99 healthy Caucasian children aged 3 years. This is one of few predictive equations covering the age group of 3-year-old children and the first equation to show how accurate FM can be calculated based on the predicted FFM. Only a small gain in explained variance was obtained by including sum of subscapularis and triceps skinfolds in the predictive model. We recommend using the simple predictive equation since the difference between the two models was shown to be negligible and the use of skinfold measurement, which can be highly dependent on the examiner, is avoided. The relatively wide 95% limits of agreement indicate that despite high agreement between predicted and DXA values on population level, there is some predictive uncertainty at the individual level. In this age group, BIA and anthropometry have practical advantages compared to DXA and other sophisticated techniques as the measurements are easily obtained.

We found only five other published equations that included children aged 2–3 years\textsuperscript{12–16}. Rush et al.\textsuperscript{16} predicted FFM with DXA as reference method, while the other four equations predicted TBW with different methods as reference. Our study indicates that the Rush et al. equation provides good predictions for FFM in the group of 3-year-old Danish children considered in the present study. The Rush et al. prediction equation was generated in a group of 77 2-year-old children from New Zealand with mixed ethnicity born to mothers treated for gestational diabetes\textsuperscript{16}. The other validated prediction equations over-estimated FM compared to DXA and this finding is in line with an earlier study showing that TBW determined...
by deuterium dilution led to higher estimation of FM than DXA and the four-compartment model in children. The magnitude of bias of FFM\textsubscript{pred} increased with increasing FM\textsubscript{DXA} for prediction equations by Fjeld et al., Kushner et al., and Rush et al. For Rush et al. the magnitude bias was of the same size as the magnitude of bias in our predictive equation derived from the simple model. The magnitude of bias shows a discrepancy between the predictive equations in question and DXA. However, an analysis of the potential bias of FM assessed by DXA compared to the four-compartment model in 9 to 14-year-old children showed that DXA underestimated FM in leaner subjects and overestimated FM in more obese children. Thus, albeit being a valued technique for measuring body composition, DXA has its own limitations. An alternative reference method suitable for this age group could have been determination of TBW using deuterium dilution with subsequent application of age and gender specific hydration factors. However, this method has other limitations and has also been shown to overestimate FM in children compared to the four-compartment model. Besides different measurement errors by the different techniques used as reference methods in the evaluated equations, the discrepancies among the equations can be explained by large age spans, varying numbers of participants, and differences in population characteristics and settings that may influence the relative proportion of TBW and hydration level of FFM. The equations by Fjeld et al. and Kushner et al. were developed on children from Japan, while the equation by Masuda & Komiya was developed on children from Japan.

A considerable strength of our study is that we excluded the 47% of the DXA scans with low quality and still retained a large number of high-quality DXA scans for use as a reference. We see it as a great strength that the generated equations are made to predict FFM directly without requiring age and gender dependent determination of hydration factors to account for different hydration level in FFM. Cross validation was used for calculating RMSE, ensuring that the uncertainty of the prediction model when applied to new data (data not used for fitting the model) was more appropriately accounted for than would be the case if we reported the RMSE derived directly from the fitted values. However, the reported cross validation-based RMSE may still be slightly too optimistic as it is based on the same data as the prediction model. Therefore, the reported RMSE may serve as a lower bound on the uncertainty to expect when using the prediction model for new data.

The generated predictive equations are derived from a group of 3-year-old children who were homogeneous in age (3 years ± 1 month) and ethnicity. This setting should increase accuracy, also in case the predictive equations are applied in other populations that are similar to the SKOT cohort. However, in terms of generalizability this is a limitation as our equation might be less accurate for studies with a focus on obese children or very undernourished children. Only few of the SKOT children whose data were used forming the predictive equations were overweight or obese (7.8% overweight and none obese according to the IOTF cut-off values with 17.1% having BMI z-scores above 1, 2% above 2, and none exceeding 3 z-scores according to the WHO growth standards). None of the children had BMI z-scores below minus 2. Limitations of the BIA method is especially the responsiveness to variations in the hydration state seen with age, size, ethnicity, temperature, clinical conditions, fasting state, bladder voiding and exercise but also positioning of the body and electrode placements affect impedance measures. Therefore, caution should be taken before the predictive equation is applied in study settings where the children differ considerably in age, size or ethnicity or in studies with sick children if the disease is likely to affect the hydration level of FFM.

A possible source of error in this study was the BIA electrodes being placed less than 5 cm apart on the hand due to the small size of the hand. There is a risk that this placement has increased resistance, leading to a systematic underestimation of FFM. However, this seemed not to be the case, since FM\textsubscript{DXA} estimated by the BIA software did not differ from FM\textsubscript{DXA} for the boys and was significantly underestimated in girls. We used the BIA instrument from RJL systems in this study. It is a risk that different BIA machines measure resistance slightly differently. However, RJL models are some of the most frequently used BIA instruments in epidemiologic studies.

It is of interest which age span the predictive equations can be applied to. We speculate that the applicable age range for the generated predictive equations is 2–4 years of age where the hydration level of FFM only changes with approximately 1.1% in boys and 0.5% in girls. This age span has been found to be a critical period for excessive weight gain and risk of overweight in adolescence.

In conclusion, the derived predictive equations enable BIA-based prediction of FFM and FM close to DXA scan results in a preschool population. The equations are particularly relevant for use among healthy Caucasian children aged 2 to 4. The predicted FFM proved useful at calculating FM although researchers should be aware that the relative error is greater when using the equations to calculate FM than when calculating FFM. The generated equations can prove useful for population studies linking early risk factors to body composition and early onset of obesity.

### Methods

**Study design and participants.** Data were from the observational cohort study SKOT (in Danish: Sma˚børns Kost Og Trivsel). Mailed invitations were sent to 2211 families randomly selected from the National Danish Civil Registry, and 330 Danish children were enrolled in the study and monitored at 9, 18 and 36 months of age (described in details elsewhere). Inclusion criteria were singleton infants born ≥37 week of gestation, without diseases expected to affect growth or food intake. Eighteen children dropped out before the first examination and one child with late manifestation of a severe chronic disorder was excluded. All physiological measurements were made at the Department of Nutrition, Exercise and Sports, Copenhagen, Denmark.

A total of 263 (80%) completed the 36-months examination which was conducted from October 2009 to October 2010. As part of the 36-months examination all children were invited to a DXA scan.

### Table 3 | Comparison of DXA FFM and FFM predicted by other published BIA-based equations in this group of 3-year-old children

| Equation         | FFM\textsubscript{pred} (kg) | Rho\textsuperscript{a} | Bias\textsuperscript{b} | LOA (kg) | β (SE) | FM\textsubscript{cal} (kg) | Rho\textsuperscript{a} | Bias\textsuperscript{b} | LOA (kg) |
|------------------|-----------------------------|-------------------------|-------------------------|-----------|-------|-----------------------------|-------------------------|-------------------------|-----------|
| Fjeld et al.\textsuperscript{4} | 11.07                       | 0.87                    | -0.94\textsuperscript{*} | -2.03;0.14 | 0.48 (0.05)\textsuperscript{*} | 3.36                        | 0.82                    | 0.85\textsuperscript{*} | -0.06;1.75 |
| Kushner et al.\textsuperscript{4} | 10.64                       | 0.88                    | -1.38\textsuperscript{*} | -2.53;0.22 | 0.20 (0.08)\textsuperscript{*} | 3.80                        | 0.75                    | 1.23\textsuperscript{*} | 0.18;2.37 |
| Bedogni et al.\textsuperscript{4} | 9.34                        | 0.88                    | -2.68\textsuperscript{*} | -3.95;1.41 | 0.12 (0.09)\textsuperscript{*} | 5.09                        | 0.73                    | 2.58\textsuperscript{*} | 1.33;3.83 |
| Masuda & Komiya\textsuperscript{4} | 11.15                       | 0.88                    | -0.87\textsuperscript{*} | -1.91;0.18 | 0.10 (0.07)\textsuperscript{*} | 3.29                        | 0.83                    | 0.77\textsuperscript{*} | -0.14;1.68 |
| Rush et al.\textsuperscript{4}     | 12.23                       | 0.91                    | 0.21\textsuperscript{*}  | -0.65;1.08 | 0.10 (0.06)\textsuperscript{*} | 2.21                        | 0.87                    | -0.31\textsuperscript{*} | -1.06;0.43 |

\textsuperscript{a}Pearson’s correlation coefficient.

\textsuperscript{b}Predicted – DXA value. Tested for significance by paired t-test that bias = 0.

\textsuperscript{c}Linear regression model relating differences (FFM\textsubscript{pred} – FM\textsubscript{cal}) to mean-centered FM\textsubscript{cal}.

\textsuperscript{d}Predicted TBW was converted to FFM by the assumption than the age standardized hydration levels of FFM in 3-year-old children is 77.9% for girls and 77.5% for boys.

FFM, Fat-free mass; FM, Fat mass; LOA, Limits of agreement by Bland-Altman.

\textsuperscript{*}P ≤ 0.001; \textsuperscript{p} P ≤ 0.01.
conducted the examinations following standardized procedures. Age- and gender-
nearest 0.1 mm. Except for weight, all measurements were performed in triplicates,
shoulder blade). Triceps and subscapularis skinfolds were measured using a
stationary digital height measurer (235 Heightronic Digital Stadiometer, Issaquah,
USA) on the foot were placed over the distal portion of the second metatarsal (the
signal electrode (LMP3 Diagnostic Tab Electrodes, Kendall, Covidien, Mansfield,
the BIA measurement. During measurement the child was lying relaxed on an
no restrictions on physical activity. No request for bladder voiding was given before
were measured using a single frequency (50 kHz) tetrapolar bioelectrical impedance
analyser Quantum III (RIL Systems, Michigan, USA) between right hand and right
foot. The child had been fasting approximately 2 hours prior to the examination with
no restrictions on physical activity. No request for bladder voiding was given before the
BIA measurement. During measurement the child was lying relaxed on an
examination couch in light clothing, without metal or persons touching the skin. The
signal electrode (LMF3 Diagnostic Tab Electrodes, Kendall, Cridaen, Mansfield,
USA) on the foot was placed over the distal portion of the second metatarsal (the
base of the second toe). The signal electrode on the hand was placed above the
metacarpophalangeal joint of the middle finger and not wrapped around the middle
finger (proximal phalanx) as specified by the manufacturer, because the hands of the
children were too small for this placement. The detecting electrode on the foot was
placed at the anterior ankle on an imaginary line bisecting the medial malleolus and
the detecting electrode on the hand was placed on an imaginary line bisecting the
ulnar head as specified by the manufacturer. The procedure was performed twice with
approximately 60 s between. Electrodes were not replaced. Mean values of resistance,
reactance and impedance were used in the analyses. At the time the study was
conducted the Quantum III software ‘New pediatric’ was only validated for
individuals above 4 years of age. This has no influence of the physical measurements
of impedance, reactance and resistance (RIL Systems, personal communication, 2010).

Bioelectrical impedance analysis. Whole body resistance, reactance and impedance
were measured using a single frequency (50 kHz) tetrapolar bioelectrical impedance
analyser Quantum III (RIL Systems, Michigan, USA) between right hand and right
foot. The child had been fasting approximately 2 hours prior to the examination with
no restrictions on physical activity. No request for bladder voiding was given before the
BIA measurement. During measurement the child was lying relaxed on an
examination couch in light clothing, without metal or persons touching the skin. The
signal electrode (LMF3 Diagnostic Tab Electrodes, Kendall, Cridaen, Mansfield,
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Dual-energy X-ray absorptiometry. DXA is a non-invasive technique that estimates
bone mineral content, lean mass and FM with high reproducibility and was used as the
reference method for body composition measurements of FM, lean tissue mass, and
bone mineral content. Whole body DXA scans were performed with a Lunar
Prodigy Advance (GE Healthcare, Madison, WI, USA) using the software enCore,
version 12.30. Radiation dose from each scan was maximum 0.0012 mSv according to
the manufacturer. No lasting was required before the DXA measurement. The
parents were requested to take the child to the toilet before the scan if the child needed
to empty the bladder. The children were scanned lying supine in light clothing
without metal and with no or dry nappy. Due to the young age of the children, some
children found it challenging to lie still during the scanning process (approximately 5
minutes). All scans were subsequently assessed by one person to ensure consistency.
This person manually went through all scans to see if the body regions defined by the
software were correct. The cut lines were adjusted if there were disagreement between
the placement of the child and the software’s definition of regions. DXA scans were
divided into four categories (“perfect scans”, “good scans with minor irregularities”,
“scans with several irregularities” and “useless scans”) according to the quality of the
scan. The procedure for selecting usable scans was described in detail by Jensen
et al.1."
DXA values was evaluated using a paired t-test. The magnitude of bias of the predicted FFM was quantified as described above.

Data were analysed by STATA version 11.0. Statistical significance was set at P < 0.05.

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Author contributions

K.F.M. and C.M. designed the study. K.F.M. obtained the funding, K.T.E. and L.B.C. managed the data collection at the 3-year examinations. K.T.E. and L.B.C. supervised the quality standards and the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript.

Additional information

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