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# 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 (height<sup>2</sup>/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<sup>1,2</sup>. In particular, there is an increasing focus on factors that influence early proportions of fat-free mass (FFM) and fat mass (FM)<sup>3,4</sup>. 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<sup>5</sup>. 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<sup>3,6,7</sup>. 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<sup>5,6</sup>. Bioelectrical impedance analysis (BIA) is a recognized method to estimate total body water (TBW) in epidemiologic studies<sup>5,8</sup>, although some limitations have been identified<sup>9,10</sup>. 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<sup>8</sup>. 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<sup>11</sup>. However, the hydration levels of FFM vary according to growth, maturity, ethnicity, disease, and obesity<sup>5,8,11</sup> 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)<sup>12–16</sup>. 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<sup>2</sup>, 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



Table 1 | Characteristics of the population - anthropometry, BIA and DXA

	n	Girls	n	Boys	P-value
Age (months)	49	36.2 [35.7;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 <sub>adj</sub> (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/m <sup>2</sup> )	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
SFt (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 (Ω)	49	765.0 (65.9)	50	734.5 (64.4)	0.02
Xc (Ω)	49	58.3 [56.4;62.1]	50	55.4 [52.5;57.4]	<0.001
RI (cm <sup>2</sup> /Ω)	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 <sub>DXA</sub> (g)	49	11,600 (852)	50	12,427 (1,138)	<0.001
FM <sub>DXA</sub> (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; SFt, skinfold thickness triceps; SFs, skinfold thickness subscapularis; Wc, waist circumference; Wt, digital weight; Wt<sub>adj</sub>, weight adjusted to agree with DXA weight; Xc, reactance. BMI calculated as Wt/Ht<sup>2</sup>. Xc and R measured by bioelectrical impedance. FFM<sub>DXA</sub> calculated as lean tissue mass + BMC. FM<sub>DXA</sub>, BMC and lean tissue mass was measured by DXA. RI calculated as Ht<sup>2</sup>/R.

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 from 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<sup>17</sup>) 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<sub>DXA</sub>, FM<sub>DXA</sub> 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, weight<sub>adj</sub>, was derived and used in the subsequent regression analyses. FM<sub>cal</sub> was obtained from the following equations:

$$Weight_{adj}(kg) = 0.981 \text{ digital weight}(kg) + 0.374$$

$$FM_{cal}(g) = Weight_{adj}(kg) \times 1000 - FFM_{pred}(g)$$

The final full model included RI, height, weight<sub>adj</sub>, sum of subscapular 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 weight<sub>adj</sub> 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<sub>DXA</sub> \* 100%) in the full model were 2.9% for girls and 3.1% for boys, and 2.8% for girls and 3.1% for boys in the simple model. The mean difference between FFM<sub>pred</sub> and FFM<sub>DXA</sub> was -4 g in the full model (95% limits of agreement -816;808) and 2 g (-852;856) in the simple model (Figure 1 a & b). Both models showed bias across the range of FM indicating that FFM was underestimated in leaner children and overestimated among children with higher FM<sub>DXA</sub> (magnitude of bias: full model: β = 0.12 g (0.05), P = 0.026; simple model: β = 0.16 g (0.06), P = 0.004).

Table 2 | Predictive equations for FFM (g) developed by 10-fold cross validation based on 3-year-old Danish children

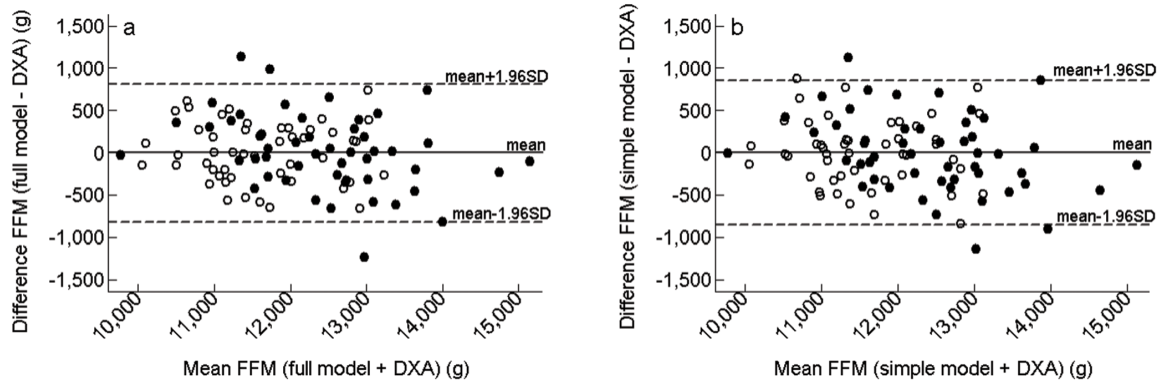
Equation	Predictor variables <sup>a</sup>											
	n	RI	Wt	Ht	Sex <sup>b</sup>	Sum SF	Constant	Adj R <sup>2</sup>	RMSE	RMSE <sup>c</sup>	PE	PE <sup>c</sup>
Full	98	297.3	354.3	43.5	331.7	-64.7	-62.7	0.85	360.4	321.1	3.0	2.7
Simple	99	327.2	223.8	76.8	417.6	-	-2784.4	0.84	355.8	333.3	3.0	2.8

<sup>a</sup>Results presented as regression coefficients.

<sup>b</sup>Female = 0, male = 1.

<sup>c</sup>Based on the complete dataset.

Adj R<sup>2</sup>, adjusted R<sup>2</sup>; FFM, Fat-free Mass (g); Ht, height (cm); PE, Prediction error; RI, Resistance Index (cm<sup>2</sup>/Ω); RMSE, Root mean square error (g); Sum SF, Sum of skinfold thickness triceps and subscapularis (mm); Wt, digital weight (kg). Adj R<sup>2</sup> is the mean of the adj. R<sup>2</sup> based on the 10 training sets. RMSE is the mean of the individual RSME in the test sets. PE was calculated by dividing mean RMSE from the test sets with mean FFM<sub>DXA</sub> and multiply by 100%.



**Figure 1** | The Bland-Altman plots show the difference between predicted values of fat-free mass (FFM) and FFM measured by DXA plotted against their mean result for girls (○) and boys (●) in (a), full model and (b), simple model. The predictive equations for the full and simple model are presented in Table 2.

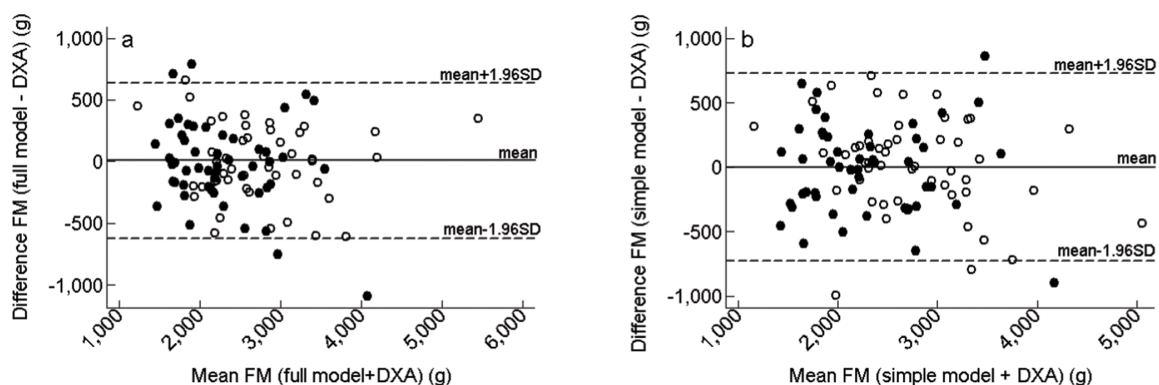
The values of RMSE for  $FM_{cal}$  were 264.3 g (girls 250.7 g; boys 277.3 g) in the full model and 303.4 g (girls 307.1 g; boys 299.9 g) in the simple model. Due to the lower FM-total body weight ratio prediction errors for  $FM_{cal}$  were 10.5% (girls 9.1%; boys 12.1%) in the full model and 12.0% (girls 11.2%; boys: 13.0%) in the simple model. Analysis of the level of agreement showed that the mean difference between  $FM_{cal}$  and  $FM_{DXA}$  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  $FM_{cal}$  ( $FM_{cal} - FM_{DXA}$ ) was depended on FM indicating an overestimated of FM among the leaner children and underestimated among children with higher  $FM_{DXA}$  (full model:  $\beta = -0.14$  g (0.04),  $P = 0.001$ ; simple model:  $\beta = -0.18$  g (0.05),  $P < 0.001$ ).

**Comparison of FFM predicted from published equations and  $FFM_{DXA}$ .** The agreement between  $FFM_{DXA}$  and  $FFM_{pred}$  based on previously published predictive equations is shown in Table 3. The newest predictive equation by Rush *et al.*<sup>16</sup>, which is also using DXA as the reference method, showed the best accordance with  $FFM_{DXA}$  with a mean over-estimation of 0.21 kg (95% CI: 0.13–0.30 kg) and mean underestimation of FM of 0.31 kg ( $-0.39$ – $-0.24$  kg). The other equations were based on other reference methods to predict TBW and underestimated FFM by 0.87–2.68 kg. In most of the predictive equations the magnitude of bias was not independent of FM indicating that FFM was underestimated in leaner subjects and overestimated in fatter subjects. For all predictive equations considered differences between predicted values and DXA values were significant (Table 3).

## 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 equations. 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<sup>12–16</sup>. Rush *et al.*<sup>16</sup> 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<sup>16</sup>. 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



**Figure 2** | The Bland-Altman plots show the difference between calculated values of fat mass (FM) and FM measured by DXA plotted against their mean result for girls (○) and boys (●) in (a), full model and (b), simple model.



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 <sub>pred</sub> (kg)	Rho <sup>a</sup>	Bias <sup>b</sup>	LOA (kg)	Magnitude of bias <sup>c</sup>				
					β (SE)	FM <sub>cal</sub> (kg)	Rho <sup>a</sup>	Bias <sup>b</sup>	LOA (kg)
Fjeld <i>et al.</i> <sup>d</sup>	11.07	0.87	-0.94*	-2.03;0.14	0.48 (0.05)*	3.36	0.82	0.85*	-0.06;1.75
Kushner <i>et al.</i> <sup>d</sup>	10.64	0.88	-1.38*	-2.53;-0.22	0.20 (0.08) <sup>†</sup>	3.80	0.75	1.23*	0.18;2.37
Bedogni <i>et al.</i> <sup>d</sup>	9.34	0.88	-2.68*	-3.95;-1.41	0.12 (0.09)	5.09	0.73	2.58*	1.33;3.83
Masuda & Komiya <sup>d</sup>	11.15	0.88	-0.87*	-1.91;0.18	0.10 (0.07)	3.29	0.83	0.77*	-0.14;1.68
Rush <i>et al.</i>	12.23	0.91	0.21*	-0.65;1.08	0.16 (0.06) <sup>†</sup>	2.21	0.87	-0.31*	-1.06;0.43

<sup>a</sup>Pearson's correlation coefficient.  
<sup>b</sup>Predicted - DXA value. Tested for significance by paired *t* test that bias = 0.  
<sup>c</sup>Linear regression model relating differences (FFM<sub>pred</sub> - FFM<sub>DXA</sub>) to mean-centered FM<sub>DXA</sub>.  
<sup>d</sup>Predicted TBW was converted to FFM by the assumption that the age standardized hydration levels of FFM in 3-year-old children is 77.9% for girls and 77.5% for boys<sup>11</sup>.  
\*P ≤ 0.001; †P ≤ 0.01.  
FFM, Fat-free mass; FM, Fat mass; LOA, Limits of agreement by Blandt Altman.

by deuterium dilution led to higher estimation of FM than DXA and the four-compartment model in children<sup>18</sup>. The magnitude of bias of FFM<sub>pred</sub> increased with increasing FM<sub>DXA</sub> for prediction equations by Fjeld *et al.*<sup>13</sup>, Kushner *et al.*<sup>14</sup> and Rush *et al.*<sup>16</sup>. 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 subjects<sup>18</sup>. Thus, albeit being a valued technique for measuring body composition, DXA has its own limitations<sup>19</sup>. 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 have other limitations and has also been shown to overestimate FM in children compared to the four-compartment model<sup>18</sup>. 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<sup>5,8,11</sup>. The equations by Fjeld *et al.*<sup>13</sup> and Kushner *et al.*<sup>14</sup> were developed on children from Peru, while the equation by Masuda & Komiya<sup>15</sup> 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<sup>20</sup> with 17.1% having BMI z-scores above 1, 2% above 2, and none exceeding 3 z-scores according to the WHO growth standards<sup>21</sup>). 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<sup>8,10</sup> but also positioning of the body and electrode placements affect impedance measures<sup>8</sup>. 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<sup>8</sup>. However, this seemed not to be the case, since FFM estimated by the BIA software did not differ from FFM<sub>DXA</sub> for the boys and was significantly overestimated in girls. We used the BIA instrument from RJL systems in this study. It is a risk that different BIA machines measure resistance slightly different<sup>8,10</sup>. However, RJL models are some of the most frequently used BIA instruments in epidemiologic studies<sup>10</sup>.

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<sup>11</sup>. This age span has been found to be a critical period for excessive weight gain and risk of overweight in adolescence<sup>2</sup>.

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: Småbørns 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<sup>22</sup>). 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 4 | Published BIA-based equations relevant for 3-year-old children

Source	Reference method	BIA instrument	Equation for FFM	n	Age (years)	Country
Fjeld <i>et al.</i> <sup>a</sup>	<sup>18</sup> O dilution	RJL	$(0.18 \text{ Ht}^2/\text{R} + 0.39 \text{ Wt} + 0.76)/\text{HF}$	30	0.3–2.5	Peru
Kushner <i>et al.</i> <sup>a</sup>	<sup>18</sup> O dilution	RJL-101	$(0.593 \text{ Ht}^2/\text{R} + 0.065 \text{ Wt} + 0.04)/\text{HF}$	29	Preschool	Peru
Bedogni <i>et al.</i> <sup>a</sup>	D <sub>2</sub> O dilution	Dietosystem Human IM scan	$(0.716 \text{ Ht}^2/\text{I} - 1.504)/\text{HF}$	23	3–19	Italy
Masuda & Komiya <sup>a</sup>	D <sub>2</sub> O dilution	TP-95 K	$(0.149 \text{ Ht}^2/\text{R} + 0.244 \text{ Wt} + 0.460 \text{ age} + 0.501 \text{ sex} + 1.628)/\text{HF}$	46	3–6	Japan
Rush <i>et al.</i>	DXA	BIM4	$0.367 \text{ Ht}^2/\text{R} + 0.188 \text{ Wt} + 0.077 \text{ Ht} + 0.273 \text{ sex} - 2.490$	77	2	New Zealand

<sup>a</sup>Original prediction of TBW - converted to FFM by dividing with the age standardized hydration levels of FFM<sup>11</sup>.  
BIA, bioimpedance analysis; D<sub>2</sub>O, deuterium dilution; DXA, Dual-Energy X-ray Analysis; FFM, fat free mass; HF, hydration factor; Ht, height (cm); I, impedance (Ω); <sup>18</sup>O dilution, Oxygen-18 dilution; R, resistance (Ω); Wt, weight (kg).

**Anthropometrics.** Weight at 36 months was measured without clothes to the nearest 0.1 kg using a yearly calibrated digital scale (Tanita WB-100MA, Tanita Corporation, 1-14-2, Maeno-chi, Itabashi-ku, Tokyo, Japan). Height was measured using a stationary digital height measurer (235 Heightronic Digital Stadiometer, Issaquah, WA, USA) to the nearest 0.01 cm. Waist circumference was measured on naked skin at the level of the umbilicus to the nearest mm with a nonflexible tape measure (Lasso-o; Child Growth Foundation, London, UK). This tape measure was also used to measure mid-upper-arm circumference at the mid-point between the olecranon process of the ulna and acromion process on the shoulder blade. The same mid-point point was used for measuring the triceps skinfold thickness while the subscapular skinfold was measured 2 cm below the inferior angle of the scapula (the lower left shoulder blade). Triceps and subscapularis skinfolds were measured using a Harpenden skinfold calliper (Chasmors Ltd, London, UK) and recorded to the nearest 0.1 mm. Except for weight, all measurements were performed in triplicates, and the mean value was used in subsequent analyses. Four well-trained observers conducted the examinations following standardized procedures. Age- and gender-specific BMI z-scores were obtained using the software WHO Anthro 2005<sup>23</sup>.

**Bioelectrical impedance analysis.** Whole body resistance, reactance and impedance were measured using a single frequency (50 kHz) tetrapolar bioelectrical impedance analyser Quantum III (RJL 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 (LMP3 Diagnostic Tab Electrodes, Kendall, Covidien, Mansfield, USA) on the foot were 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, resistance and reactance (RJL Systems, personal communication, 2010).

**Dual-energy X-ray absorptiometry.** DXA is a non-invasive technique that estimates bone mineral content, lean mass and FM with high reproducibility<sup>24</sup> 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 fasting 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.*<sup>17</sup>.

**Ethics.** This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003). Written consent was obtained from both parents or custody holders of all participating children.

**Statistical analysis.** FFM was calculated as the sum of lean tissue mass and bone mineral content measured by DXA and will be referred to as FFM<sub>DXA</sub>. It is normal that the total body weight estimated by the DXA scanner (lean tissue mass + bone mineral content + FM) differs slightly from the measurement obtained using a digital weight. A deviation of 100–200 grams is more influential in children since their total body weight is lower and the relative error is therefore higher compared to adults. This means that a prediction of FFM (FFM<sub>pred</sub>) can show a high degree of agreement with FFM<sub>DXA</sub> but a discrepancy between the digital weight and the DXA weight will also be reflected in the calculated FM value (FM<sub>calc</sub>) when subtracting FFM<sub>pred</sub> from the digital weight. Consequently, the prediction error had to be taken into account in order to calculate FM values equivalent to DXA FM values (FM<sub>DXA</sub>). We generated an adjusted weight variable, weight<sub>adj</sub>, obtained by simple linear regression of the DXA weight and the digital weight and used the slope estimate as a correction factor in the multiple regression analysis used for generating the predictive equations.

Characteristics of anthropometry, BIA and DXA were expressed as means ± SD or, if not Normal distributed, median + interquartile range. Differences between genders were evaluated using two-sample *t*-test or, when appropriate, Wilcoxon rank test.

Height(cm)<sup>2</sup>/resistance(Ω), subsequently referred to as the resistance index (RI), was chosen as the variable representing the BIA outcome since it has proven to be an accurate measure for prediction of TBW and FFM in linear regression models<sup>8,14</sup>. FFM<sub>DXA</sub> was considered the dependent variable. For the comprehensive model the predictor variables included in the multiple linear regression were: RI, height, weight<sub>adj</sub>, sum of triceps and subscapularis skinfolds, waist circumference, mid-upper-arm circumference, and gender. Stepwise backward elimination resulted in a simplified model containing RI, height, weight<sub>adj</sub>, sum of triceps and subscapularis skinfolds, and gender. The simple model included only RI, height, weight<sub>adj</sub>, and gender as predictor variables. Age was not included in the analyses, since all children were scanned at 3.1 years (±0.1).

The prediction error of the obtained predictive equations was assessed through a 10-fold cross validation approach. Subjects were randomly placed in 10 groups, equally distributed by gender. Predictive equations were generated ten times, each time leaving out one group that subsequently served as a test data set for validation. The remaining 9 groups that were used to generate each predictive equation is called a training set. In total, we ended up with 10 training set and 10 test set. An average root mean square error (RMSE) was calculated from the predictions in all test sets and used to quantify the predictive performance of the two models for both FFM and FM. We used the adjusted R<sup>2</sup> to compare the proportion of the variability in data accounted for by the multiple regression models. Adjusted R<sup>2</sup> values for the predictive equations reported were obtained as the average of the adjusted R<sup>2</sup> values from the reduced regression models in the 10 training sets. To quantify the prediction errors in terms of percentages, we calculated prediction errors for FFM by dividing mean RMSE from the 10 test sets with mean FFM<sub>DXA</sub> and multiplying by 100. The same procedure was applied for calculating prediction errors for FM. The level of agreement between DXA and the final predictive equations applied to the whole dataset was assessed through Bland-Altman approach. Potential bias between the predicted values and DXA (magnitude of bias) was quantified by means of the estimated slope parameter obtained from a linear regression model relating differences to mean-centered FFM<sub>DXA</sub>. A significant correlation indicates a bias in the prediction equation across the range of fatness.

Five published BIA-based prediction equations covering the age group of 2–3-year-old children (Table 4) were compared with FFM<sub>DXA</sub> in this sample of children. The performance of each of these equations was assessed by means of Pearson's correlation coefficient and Bland-Altman approach. The difference between predicted and



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. analysed the data and prepared the first draft of the manuscript. S.M.J. and C.R. supervised the quality standards of the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.

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