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Original Article

Comparison of methods to assess body fat in non-obese six to seven-year-old children

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SUMMARY

Background & aim: Different non-invasive methods exist to evaluate total body fat in children. Most methods have shown to be able to confirm a high fat percentage in children with overweight and obesity. No data are available on the estimation of total body fat in non-obese children. The aim of this study is to compare total body fat, assessed by different methods in non-obese children.
Methods: We compared total body fat, assessed by isotope dilution, dual energy X-ray, skinfold thickness, bioelectrical impedance analysis, combination of these methods as well as BMI in 30 six to seven-year-old children.
Results: The children had a mean BMI of 16.01 kg/m^2 (range 13.51–20.32) and five children were overweight according to international criteria. Different methods showed rather different absolute values for total body fat. Bland–Altman analysis showed that the difference between the DEXA method and isotope dilution was dependent on the fat percentage. Children with the same BMI show a marked variation in total body fat ranging from 8% to 22% as estimated from the isotope dilution method.
Conclusion: Non-invasive methods are presently not suited to assess the absolute amount of total body fat in 6–7 years old children.

1. Introduction

Worldwide, the incidence of obesity and overweight is rapidly increasing, also in young children and adolescents.[1] In the Netherlands, overweight at present is seen in approximately 12% of 4–6-year-old children, and obesity in 4%.[2] This is a marked increase compared to 1980, when in respectively 6% and 1% of 4–6-year-old children overweight and obesity was found.[2] This increase in overweight causes great concern as overweight at a young age has a high risk to persist into adulthood.[3]

Overweight in itself might not be a risk factor for diseases in later life. The real risk factor is an excess of fat. Excess fat is strongly associated with adverse health outcome, including diabetes mellitus, dyslipidemia, coronary heart disease, renal disease, cancer, musculoskeletal consequences, asthma and other respiratory problems, and finally decreased fertility.[4] This clustering of metabolic and cardiovascular abnormalities originally seen in late adulthood, is increasingly seen in children and adolescents.

In the Netherlands the national guideline to screen young children for overweight is based on the BMI. The BMI, however, is not a very reliable method to distinguish between a normal and high amount of body fat in children.[5,6] A number of other methods, like isotope dilution, dual energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), skinfolds as well as a combination of these, have been used to estimate the fat percentage in children. All these methods show different results for the amount of total body fat.[7] Only a few studies compared the results of all these different methods within the same child. Moreover, in these studies children from a rather wide age range were included, while it is known that for instance puberty is influencing fat mass.

Non-standard abbreviations: BIA, Bioelectrical impedance analysis; DEXA, Dual energy X-ray absorptiometry; LBM, Lean Body Mass; %BF, Percentage body fat; ST, Skinfold thicknesses; TBW, Total body water.

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In very overweight children there will be no doubt about the presence of excess fat.[8] The real problem is to distinguish those children with an increased total body fat within the group with a normal weight or slight overweight. Studies have shown that it is not possible to distinguish these children on clinical examination.[6] Moreover, the presence or absence of excess fat cannot reliably be estimated from the BMI. Therefore other, non-invasive methods must be used to estimate total body fat in these children. Limited data are available regarding the comparison between different methods in healthy 6–7-year-old children. Therefore, we conducted a study in healthy 6–7-year-old children comparing the results of the estimate of total body fat as assessed by isotope dilution, DEXA, BIA, skinfolds and combinations of these methods.

2. Methods

2.1. Subjects

The study was conducted in 31 subjects (14 boys and 17 girls between six and seven years of age), who were free of acute or chronic diseases. These children were recruited through advertisements in a local newspaper and on the hospital information site, and by word of mouth. The data were collected between October 11th and October 25th, 2006.

In one boy the isotope dilution could not be measured, and therefore 30 children were included in the final analyses. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the research. Written informed consent was obtained from the parents and verbal assent was provided by the children. This study was approved by the Ethics Committee of the University Medical Center Groningen and performed in accordance with the Declaration of Helsinki.

2.2. Protocol

Parents were instructed to serve their children a light lunch, consisting of a sandwich and a drink, before arriving at the University Medical Center Groningen at 14:00 hours. Upon arrival, children were requested to urinate after which they were weighed and all other measurements were done. Until all measurements had been obtained, the children were requested to refrain from drinking, eating, and urinating.

2.3. Measurements

The isotope dilution method assessed total body water (TBW). Subsequently total body fat was calculated from TBW. TBW was determined by an orally administered dose of 99.8% ^2H_2O. All children received 0.15 g ^2H_2O per kg body weight. Saliva samples were collected at baseline, as well as at 3 h after ingesting ^2H (endpoint).[9] ^2H level in the saliva samples was measured by gas-isotope ratio mass spectrometry.[10,11] TBW was calculated from the saliva sample by the plateau method, assuming a plateau was reached by 3 h[9] Total body water was calculated using the following equation.[10]

$$\mathrm{TBW} = \frac{[\mathrm{W} \times \mathrm{A}/1000 \times \mathrm{a}] \times [\text{volume of } {}^2\mathrm{H} \text{ administered}/{}^2\mathrm{H} \text{ saliva (endpoint} - \text{baseline)}]}{1.04}$$

in which W is the amount of water (g) used to dilute the dose; A is the dose (g) administered to the participant; a is the dose (g) used in the dilution. To calculate TBW it is necessary to correct for in vivo isotope exchange by dividing by 1.04.[12] For calculating body fat percentage (%BF) from TBW the following equations were used:

$$\mathrm{LBM} = \mathrm{TBW}/0.72, \quad \text{Fat Mass} = \text{Weight} - \mathrm{LBM},$$
$$\%\mathrm{BF} = \text{Fat Mass}/\text{Weight} \times 100$$

(LBM = Lean Body Mass, %BF = percentage body fat).

DEXA measurements were obtained using a fan beam scanner (Hologic Discovery A, S/N 81161 Hologic Inc., Bedford, MA, USA). The equipment is calibrated regularly using the Step Phantom supplied by the manufacturer. Children were measured in their underwear, in supine position. Overall body fat was derived using the Hologic software version 12.3. All scans and analyses were performed by a trained technician.

Children were barefoot and dressed in light underwear for anthropometric measurements. All anthropometric measurements were carried out in duplicate by one trained observer. Weight was measured using a calibrated digital scale (Seca 770, Hamburg, Germany) and recorded to the nearest 0.1 kg. Height was assessed using a digital stadiometer (digital pole measure PM-5016, KDS, Kyoto, Japan) and recorded to the nearest 0.1 cm. If the duplicate measurements differed by more than 0.5 kg and 0.2 cm respectively, a third measurement was obtained. Afterwards the mean of height and weight were calculated by taking the mean of two measurements or the mean of the two nearest readings. BMI was calculated by dividing the mean weight by the mean height squared (kg/m^2).

Skinfold thicknesses (ST) were measured in duplicate at the right side of the body using a Harpenden skinfold caliper (CMS instruments, London, UK). During the assessment, a standard pressure of 10 g/mm^2 was applied and a reading was taken after 2 s. Triceps and biceps ST were measured at the point midway between the acromion and the olecranon. Subscapular ST was measured just below the scapula. Supra-iliac ST was measured just above the iliac crest and thigh ST was measured on the anterior side of the lower extremity, at the point midway between the inguinal region and the midpoint of the patella. If the measurements differed by more than 0.2 cm, a third measurement was performed. The mean of the ST was calculated using the mean of two measurements or the mean of the nearest two readings.

To calculate the percentage body fat from the ST, three equations were used, namely Slaughter, Deurenberg and Dezenberg (Appendix 1).[1–3] In addition, the sum of the skinfold measurements was calculated and was also compared to the reference methods.

For measurement of whole body reactance and resistance, a 50 kHz frequency BIA, using a BIA 101 (Akern®, Florence, Italy) was performed. The accuracy of the equipment was checked with a 500 ohm resistor supplied by the manufacturer before the measurements were performed. Electrodes were placed on the dorsal surfaces of the right hand and the right foot. Source electrodes were placed at the distal end of the third metacarpal and sensor electrodes 5.5 cm proximally to the source electrodes. Participants were asked to remove all metal jewellery. After lying supine for 10 min, with arms and legs abducted from the body, three measurements were taken. The measurements were performed by one observer.

The equation described in the article of Horlick et al. was used, which compared 13 published pediatric BIA based predictive equations for total body fat and fat free mass and afterwards

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refitted the best performing model in their study population of 1291 children (Appendix 1).[4] Although all measurements were done in the afternoon after a light lunch, results could be influenced by the meal taken and the time interval between the lunch and measurements. We are convinced, however, that these factors did not have a real influence on our measurements, as it was shown before that measurements taken between 2 and 4 h after a meal, represent an error of less than 3%.[13,14]

Goran et al. proposed an equation combining two ST measures with the resistance measurement of the BIA (Appendix 1).[5] This equation was also evaluated in our study.

2.4. Statistical analyses

Since the methods investigated in this study are expressed in different units, a sample size calculation was performed based on a maximal accepted percentage difference using Lin's Concordance Correlation Coefficient.[15] According to the table in their paper, 30 children are needed when a precision of the measurements of 96% and accepted difference of 12.5% (alpha 0.05, power 90%) is assumed. The same sample is needed when a difference of 10% and precision of 95% is assumed. A precision higher than 95% is described for both deuterium and DEXA. To compare the results of the different methods, the Bland–Altman method was used. Pearson correlations were used to assess associations between the different methods which give similar results as the concordance correlations but are easier to interpret. All statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago, IL, USA).

3. Results

In total 17 girls and 13 boys participated in the study. Three girls and two boys were overweight and none of the children were obese according to BMI criteria.[3] Apart from one African-American girl all children were Caucasian. All measurements were performed in all 30 children. The baseline characteristics of the study population are given in Table 1.

The results for the total amount of body fat as calculated by the different methods are given in Table 2. There is a rather wide difference between the estimates of total body fat obtained with the different methods. The highest amount of fat is found with the DEXA method, the lowest amount with the BIA method. In Fig. 1, the Bland–Altman plots for the comparison between the isotope dilution method with all other methods is shown. The DEXA method showed on average a 4% higher fat percentage. However, this amount was higher at a low fat percentage and lower at a high fat percentage (Fig. 1a). The BIA method (Fig. 1b) showed on average a 10% lower fat percentage compared to the total body water results, not related to the total amount of fat. The comparisons with the calculations based on skinfold measurements are shown in Fig. 1c–e. The Deurenberg method showed the least difference with the isotope dilution method. The Goran method, a combination of two skinfolds and BIA results did not show a different result compared to the skinfold methods alone (Fig. 1f). In Table 3 the correlations between different estimates for total body fat with both the isotope dilution and DEXA methods are shown. All methods showed a significant correlation. This might indicate that all methods detect the difference between a low and high fat percentage. However, the Bland–Altman analysis indicates that absolute values found with the different methods vary considerably.

The correlation between BMI and total fat as estimated from the isotope dilution is shown in Fig. 2. The five children with overweight, based on BMI and according to international criteria,[3] all show a fat percentage above 20%. The variation in the children with a normal weight is high and does not show a correlation with BMI. These results suggest that a normal BMI does not exclude an excess fat mass.

Table 1
Baseline characteristics of the study population.

	Mean	Range
Age (years)		
Boys	6.7	[6.3; 7.5]
Girls	6.8	[6.0; 7.9]
Total	6.8	[6.0; 7.9]
Weight (kg)		
Boys	25.3	[22.0; 33.4]
Girls	25.1	[17.5; 31.7]
Total	25.2	[17.5; 33.4]
Height (cm)		
Boys	124.1	[118.4; 135.8]
Girls	125.9	[113.8; 136.9]
Total	125.1	[113.8; 136.9]
BMI (kg/m^2)		
Boys	16.34	[14.67; 19.47]
Girls	15.76	[13.51; 20.32]
Total	16.01	[13.51; 20.32]

Table 2
Total amount of body fat estimated by the different methods.

	Mean	Range
Total fat isotope dilution (%)		
Boys	15.9	[8.3; 26.5]
Girls	18.8	[11.8; 26.5]
Total	17.5	[8.3; 26.5]
Total fat DEXA (%)		
Boys	21.0	[15.0; 27.5]
Girls	22.2	[16.5; 29.3]
Total	21.7	[15.0; 29.3]
Total fat ST Slaughter (%)		
Boys	17.3	[10.6; 28.5]
Girls	16.3	[12.1; 27.2]
Total	16.8	[10.6; 28.5]
Total fat ST Deurenberg (%)		
Boys	16.0	[11.1; 22.4]
Girls	17.0	[13.3; 25.4]
Total	16.5	[11.1; 25.4]
Total fat ST Dezenberg (%)		
Boys	19.5	[13.3; 32.6]
Girls	21.9	[12.5; 31.5]
Total	20.9	[12.5; 32.6]
Total fat BIA (%)		
Boys	5.1	[−7.0; 16.10]
Girls	8.9	[−4.2; 21.1]
Total	7.3	[−7.0; 21.1]
Total fat Goran (%)		
Boys	14.8	[10.3; 21.8]
Girls	16.7	[13.2; 25.3]
Total	15.9	[10.3; 25.3]

BIA, bioelectrical impedance analysis; DEXA, dual energy X-ray absorptiometry; ST, skinfold thicknesses.

4. Discussion

Our investigations show that different non-invasive methods to estimate total body fat in healthy normal or slightly overweight 6–7-year-old children give rather different results. In order to use either of these methods to detect the children with excess fat, reference data for each separate method are needed. The BMI seems not suitable to identify normal weight children with a high percentage body fat.

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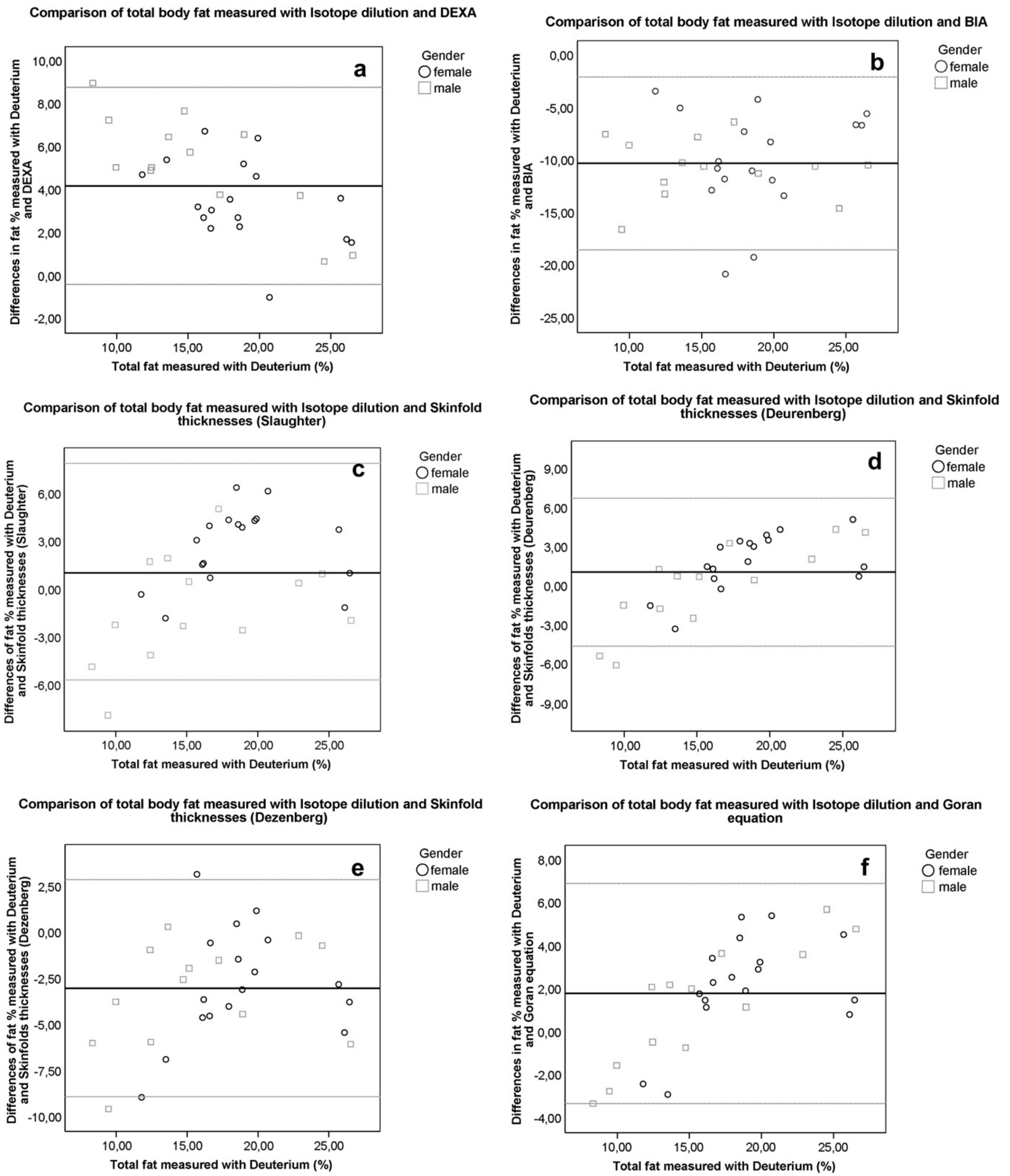

Fig. 1. (a–f) The Bland–Altman representation of the difference of total body fat estimated by different methods (DEXA, BIA and skinfold equations) and deuterium. The solid line represents the mean and the broken line the ±SD for the whole sample. Each dot represents an individual. DEXA, dual energy X-ray absorptiometry; BIA, bioelectrical impedance analysis. Symbols used are ○ – female and □ – male.

In this study we did not aim to establish which method most reliably predicts total body fat. For that purpose the comparison with a gold standard is needed. The real gold standard, carcass analysis, clearly is impossible. The four-compartment model is seen as an alternative method that will reliably estimate total body fat.[6,16] For this method body weight, body volume, total body water and bone mineral content need to be measured separately. This method is complex and expensive. In children, body weight, total body water and bone mineral content can be measured with established methods. As yet, there is no established method to

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Table 3
Pearson correlation coefficients for all used methods.

	Total fat isotope dilution (%)	Total fat DEXA (%)
Total fat isotope dilution (%)	1.000	0.902[a]
Total fat DEXA (%)	0.902[a]	1.000
Total fat Goran equation (%)	0.859[a]	0.867[a]
Total fat ST Deurenberg (%)	0.819[a]	0.816[a]
Total fat BIA (%)	0.798[a]	0.805[a]
Total fat ST Dezenberg (%)	0.831[a]	0.771[a]
Total fat ST Slaughter (%)	0.736[a]	0.767[a]
BMI (kg/m^2)	0.650[a]	0.666[a]

BIA, bioelectrical impedance analysis; DEXA, dual energy X-ray absorptiometry; ST, skinfold thicknesses.
[a] Correlation is significant at the 0.01 level (2-tailed).

measure body volume in children. Underwater weighing is the method used in adults. This method, however, is not ethically acceptable in children. A new method, air displacement, is not yet validated for use in children. This method certainly has great potential. However, at this stage not all confounders that influence this measurement are known. For instance, the effect of the surface area on the measurements it not well understood.

The absolute amount of total body fat has recently been compared in older children as estimated from underwater weighing, air displacement (ADP), DEXA and deuterium dilution. Studies in 6–7 years old children are very limited. The first comparison between underwater weighing, DEXA and air displacement was published in 1999.[17] In that study no children below 9 years of age were included. Total body fat as estimated from ADP was not different from DEXA in children with an average age of 13 years. Field reviewed all studies done until 2003 with ADP and concluded that ADP was very comparable to DEXA in children of at least 8 years of age.[18] A study done thereafter confirmed that DEXA showed comparable results of total body fat compared to ADP. However, that study showed that DEXA is more reliable than ADP.[19] A recent study compared total body fat estimated from deuterium dilution and ADP. Despite the high correlation between ADP and deuterium dilution, a significant difference was found in percentage total body fat.[20] Based on these arguments, we compared all methods with two methods used most frequently so far, the DEXA and the isotope dilution method. These methods are regarded to give consistent results in healthy children within one

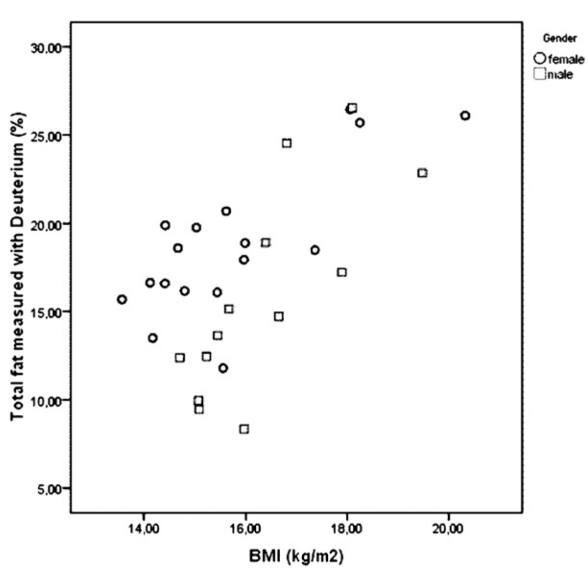

Fig. 2. Correlation between BMI and total fat estimated by the isotope dilution method. Symbols used are ○ – female and □ – male.

age group, as it can be assumed that the FFM hydration is not different between these healthy children of the same age and individual variability is rather low.[6]

In our study group we included a sample of the normal population of 6–7-year-old children in The Netherlands. We did not focus on overweight children, as done in other studies. Establishing a high fat percentage on physical examination is impossible in normal weight or slightly overweight children, but at the same time it is very important. Establishing which children do have excess fat might be a way to prevent the development of overweight in these children. Studies have shown that measurement of BMI or skinfolds in children with clinically significant overweight has no real advantages.[8]

A potential limitation is the small sample size. Considering the amount of measurements, the decision was made to investigate a small group of children. Our sample size does not allow to investigate subgroups like gender or overweight. Our aim was to investigate a homogeneous group of non-obese children; therefore one age group of healthy normal and slightly overweight 6–7-year-old children was examined. The conclusions should not be extrapolated to other ages or weight groups. All children ate a light lunch before arriving at the hospital. We do not consider this influenced the results since a light lunch has shown to have only minor effect on the measurements. For deuterium dilution, it is known that all subjects within one study should use the same protocol, concerning food intake.[21] TBW values do not differ for fasted versus fed state. For BIA, the consumption of food and beverage may decrease impedance by 4–15 ohm over 2–4 h after meals, representing an error smaller than 3%.[13] DEXA, skinfold thicknesses and BMI are not influence by a light lunch.

Our results show that DEXA on average gives a 4% higher total body fat percentage compared to the isotope dilution method. Secondly, the lower the fat percentage, the higher the difference. This is in accordance with the study of Sopher et al. who compared DEXA against the four-compartment model in 6–18-year-old children. DEXA overestimated total body fat at a fat percentage of 10–15%, and underestimated total body fat at 40% total body fat.[16]

Total body fat assessed by the BIA method showed the lowest of all results. Moreover, figures varied between negative to positive values. The BIA measures properties of the FFM, fat mass is calculated thereafter. We were surprised by the BIA results, as our population was rather homogenous, and we applied a formula especially designed for our age group.[22] Shaikh et al. recently compared the BIA with the DEXA method in 11-year-old obese children. The percentage fat mass measured with BIA was 7% lower compared to the DEXA method. The absolute fat mass varied between 4 kg lower and 8.3 kg higher with the BIA method compared to the DEXA method.[23] Our results as well as those of Shaikh might indicate that the BIA is not suitable to detect children with a high fat percentage.

The estimations of total body fat based on the different equations using the skinfold thickness were not very different from the results of the isotope dilution method. The Deurenberg method has special equations for different age groups.[24] This might explain why this method showed the least difference with the isotope dilution results. The Goran method, a combination of two skinfolds and BIA results, was not different from skinfolds alone. This method therefore seems not to have advantages.

In our study, we did not observe a relation between the assessment of total body fat by the isotope dilution method and the BMI. A study in 8–11-year-old children found a good correlation between BMI and fat mass as calculated by the DEXA method.[25] However, these results show that, for an equal BMI between 12 and 18, the percentage body fat varies between 8% and 20%. The correlation is due to the high percentage fat mass at a high BMI.

That the BMI does not distinguish between non-overweight children with and without a high body fat, was also found in other studies.[26,27]

Our results do not answer the question whether the BMI or any of the non-invasive methods predicts the presence of excess fat as a risk factor to the development of obesity-related diseases in later life. In a large cohort study, Baker et al. found a linear relation between cardiovascular diseases in adulthood, and BMI at 7–13 years of age.[28] BMI therefore might predict risks in large cohorts, but might not be suitable for the determination of risk factors in individual children.

In conclusion, our results indicate that different non-invasive methods to estimate total body fat including the BMI show rather variable results. In order to use any of these methods, determination of reference data for each method, compared to the four-compartment model, is needed. As long as those data do not exist, caution must be used when applying these methods.

Conflict of interest

This study is free of any conflicts of interest.

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Statement of authorship

CA was responsible for design of the experiment, recruitment of the participants, collection of data, data interpretation, and writing of the manuscript; GV gave significant advices and help concerning the isotope dilution method, collection of data and data interpretation; EL was responsible for design of the experiment, recruitment of the participants, collection of data, and content of article; DK was responsible for design of the experiment, content of the article; PS has expertise on body composition, was responsible for data interpretation, content and writing of the article; RS has expertise on ultrasound, was responsible for design of the experiment, data collection, data interpretation and content of the article. All authors read and approved the final manuscript.

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