Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models[1,2]

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ABSTRACT

Background: Body composition in children is generally measured by 2-component (2C) models, which are subject to error arising from variation in fat-free mass (FFM) composition. The 4-component (4C) model, which divides body weight into fat, water, mineral, and protein, can overcome these limitations.

Objective: The aims of our study were to 1) describe 4C model data for children aged 8–12 y; 2) evaluate interindividual variability in the hydration, bone mineral content, and density of FFM; 3) evaluate the success with which 2C models and bedside techniques measure body composition in this age group with use of the 4C model as a reference.

Design: Dual-energy X-ray absorptiometry, underwater weighing, deuterium dilution, bioelectrical impedance analysis, and anthropometry were used to determine body composition in 30 children. The contribution of methodologic error to the observed variability in the hydration and density of FFM was evaluated by using propagation of error.

Results: Mean (±SD) FFM density and hydration were 1.0864 ± 0.0074 kg/L and 75.3 ± 2.2%, respectively, and were significantly different from adult values ($P < 0.02$). Relative to the 4C model, deuterium dilution and dual-energy X-ray absorptiometry showed no mean bias for fatness, whereas underwater weighing underestimated fatness ($P < 0.025$). Fatness determined by using skinfold-thickness and bioelectrical impedance analysis measurements along with published equations showed poor agreement with 4C model data.

Conclusions: Biological variability and methodologic error contribute equally to the variability of FFM composition. Our findings have major implications for bedside prediction methods used for children, traditionally developed in relation to underwater weighing. Am J Clin Nutr 1999;69:904–12.

KEY WORDS Body fat, dual-energy X-ray absorptiometry, total body water, 4-component model, bioelectrical impedance analysis, underwater weighing, skinfold thickness, fat-free mass, children

INTRODUCTION

There is growing recognition of the need to measure body composition in children. First, the rise in the prevalence of childhood obesity (1) has increased the demand for accurate methods for determining body fatness in younger age groups. Second, measurement of body composition is important for optimum clinical care during hospitalization because the size of the fat-free mass (FFM) is an important index of energy and fluid requirements during artificial nutrition. Third, measurements of body composition aid in the assessment and treatment of childhood growth disorders.

Despite these recognized needs, body composition in children remains difficult to measure with accuracy and precision. Most simple methods, which use a 2-component (2C) model dividing body weight into fat mass (FM) and FFM, use assumptions that ignore interindividual variability in the composition of FFM. Consequently, measured values of FM and FFM are method dependent (2), making accuracy difficult to assess and hindering the comparison of different methods and studies. The lack of accurate data on body composition further hinders the evaluation of simple bedside techniques such as skinfold-thickness measurements and bioelectrical impedance analysis (BIA).

The 4-component (4C) model of body composition (3), obtained by combining several measurement techniques, is more robust to interindividual variability in the composition of FFM. The model divides body weight into fat, water, mineral, and protein, and allows evaluation of several assumed constant relations that are central to 2C models. These assumed constants include the water content, bone mineral content (BMC), and density of FFM. Although reference data exist for these constants in children from birth to 10 y of age (4), most values were predicted by extrapolating data between infants aged 6 mo (4) and the 9-y-old reference child (4, 5). Furthermore, only mean values are given for all variables and interindividual variability is not known. Studies using these reference data are thus unable to consider the potential error of using assumed constants.

The aims of the present study were to 1) describe 4C model data for children aged 8–12 y, 2) evaluate interindividual variability in the composition and density of FFM, and 3) evaluate

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the success with which a variety of 2C and 3C models and bedside techniques measure body composition in this age group, using the 4C model as a reference.

SUBJECTS AND METHODS

Subjects

A sample of 41 children aged 8–12 y was recruited from local schools and swimming clubs; recruitment continued until 30 subjects (16 boys, 14 girls) had fully satisfied the protocol for all measurements. The sample size of 30 was determined as described in the Statistics section below. The 11 subjects who did not satisfy the protocol failed to complete either the underwater weighing (UWW) or deuterium (D_2O) dilution measurements. All subjects were healthy at the time of the study. Measurements were conducted during a visit to the study laboratory over a 2-h period ≥2 h after a light meal. Ethical permission was obtained from the ethical committees of the MRC Dunn Nutrition Unit and the Cambridge Health Authority. Written and verbal consent were obtained from the parents and children, respectively. All measurement data described below, including precision data, pertain to the 30 children who completed the protocol.

Anthropometry

Body weight was measured with electronic scales (Sauter type E1210; Todd Scales, Newmarket, United Kingdom) while subjects were wearing swimwear. The accuracy of the scales was confirmed by using solid weights of known mass. Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer (Holtain, Dyfed, United Kingdom). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Skinfold thicknesses were measured at the biceps, triceps, subscapular, and suprailiac sites with Holtain calipers. The mean of 3 measurements was used at each site. Waist and hip circumferences were measured with a metal tape (CMS Weighing Ltd, London). All measurements were made on the left side of the body.

Underwater weighing

Body volume, and hence density, was measured by weighing the subject underwater while simultaneously determining lung volume by helium dilution. Body weight was recorded immediately before the measurement. The procedure was practiced in stages until completed successfully to ensure the comfort of the subjects during the actual measurement. Duplicate measurements were obtained in 24 subjects to assess precision; the mean value was used when appropriate in subsequent analyses.

Deuterium dilution

Total body water (TBW) was determined by D_2O dilution with a dose equivalent to 0.4 g D_2O/kg body wt (99.9 atom percent excess; Europa Scientific, Crewe, United Kingdom). After providing a predose saliva sample, subjects consumed the dose made up as fruit juice and then provided further saliva samples 5 and 6 h postdose. The subjects rinsed out their mouths 30 min before taking a sample and refrained from introducing any food or fluid into the mouth during this 30-min period. Saliva samples were analyzed in duplicate by using an infrared spectroscopy technique (6). D_2O dilution space was assumed to overestimate TBW by a factor of 1.044 (7). Correction was made for dilution of the dose by water intake during the 5-h equilibration period as described previously (8) by using an assumed k_d value of 0.1/d (9). Agreement between the 5- and 6-h samples (each corrected for k_d) was used to assess the precision of TBW measurements in a subsample of 23 children.

Dual-energy X-ray absorptiometry

BMC, FFM, and FM were determined by using a Hologic QDR 1000W whole-body scanner (Hologic Inc, Waltham, MA) in conjunction with enhanced CHILDREN'S WHOLE BODY software (version 5.61; Vertec Scientific Ltd, Reading, United Kingdom). Scans were performed while the subjects were wearing light indoor clothing (typically T-shirts and shorts) and no metal objects. The typical scan duration was 10–12 min, depending on the height of the subject. The radiation exposure per scan was estimated to be 5 mSv, which was lower than the daily background radiation level in the Cambridge area. The software package was used by only one member of the investigative team (MSF). For ethical reasons, duplicate scans were not performed and data from a whole-body phantom (10) were used to estimate precision.

Bioelectrical impedance analysis

Whole-body impedance (Z; in Ω) at 50 kHz was measured by using a SEAC impedance machine (SEAC, Brisbane, Australia). Electrodes were placed on the left side of the body and duplicate measurements were obtained in all subjects to assess precision. The mean impedance value was used in subsequent analyses.

Two-component models

Two-component models of body composition, distinguishing FM and FFM, were calculated as follows.

Anthropometry

Skinfold-thickness measurements were converted into percentage body fat by using a selection of published equations for children in the same age range (11–14). When required, predicted body density was used to derive percentage body fat by using a modified version of Siri's equation (15) as described below for underwater weighing. FFM and FM were then calculated from percentage body fat and body weight.

Underwater weighing

The density of fat was assumed to be 0.9007 kg/L, whereas that of FFM (D_{FFM}) was assumed to change with age as described in the reference child (4) and modeled previously (15, 16). Note that the equations of Weststrate and Deurenberg (15) contain a small (<1%) error due to the incorrect adjustment of D_2O for proton exchange in Fomon's data (17). Gastrointestinal volume was assumed to be 100 mL (18). For estimation of the fraction of body weight that is fat (F_f) from body density (D), the following equations were used, the second being equivalent to Siri's equation (19) without assuming constant values for D_{FFM}:

$$1/D = (F_f/0.9007) + [(1 - F_f)/D_{FFM}] \quad (1)$$

$$F_f = [(D_{FFM}/D) - 1]/[(D_{FFM}/0.9007) - 1] \quad (2)$$

Predicted values for D_{FFM} from 2 sources (15, 16) were used for comparison. FM and FFM were then calculated as the anthropometric indexes were.

D_2O dilution

TBW was divided by the water content of lean tissue, with use of the age- and sex-specific reference values of Fomon et al (4)

as revised by Schoeller (17) to take into account proton exchange, to give FFM. Hydration values for 11- and 12-y-olds were predicted by extrapolating the curves of hydration against age for each sex, giving values of 76.2% and 76.0% for girls and 74.3% and 73.9% for boys, respectively. FM was calculated as the difference between FFM and weight.

DXA

Values for FM and FFM (lean tissue + BMC) were obtained directly by using Hologic's software.

Bioelectrical impedance analysis

TBW or FFM was predicted as appropriate by using published equations for the same age range (20–23). When necessary, TBW was converted to FFM as D_2O dilution was above and FM was calculated as the difference from weight.

Three-component model

The 3C model divides the body into fat, water, and the remaining fat-free dry mass, which is assumed to have a constant ratio of protein to mineral. The advantage of this model over the 2C model is that it avoids the assumption that the water content of FFM is constant between individuals of a given age and sex, and it can also provide an estimate of the hydration and density of FFM. The model used data on body weight, body volume, and TBW, and was calculated as described previously in detail for adults (3, 19).

Taking into account the various assumed densities of the 3 components and the assumed constant ratio of protein to mineral, FM was calculated from the basic measurements as follows:

$$\text{FM (kg)} = [(2.220 \times \text{BV}) - (0.764 \times \text{TBW})] - (1.465 \times \text{BW}) \quad (3)$$

where BV is body volume in liters, TBW is in liters, and BW is body weight in kilograms.

Four-component model

The 4C model divides the body into fat, water, protein, and mineral, thereby further avoiding the assumption that the ratio between mineral and protein in FFM is constant. However, the ratio of bone mineral to total body mineral is still assumed to be constant. The ability of the 4C model to adjust for body mineral mass may result in improved accuracy in the estimation of the hydration and density of FFM, compared with the 3C model. The 4C model used body weight, body volume, TBW, and total BMC, and was again calculated as described in detail previously for adults (3). The various assumed densities of the 4 components were taken into account when calculating FM from the basic measurements as follows:

$$\text{FM (kg)} = [(2.747 \times \text{BV}) - (0.710 \times \text{TBW})] + [(1.460 \times A) - (2.050 \times \text{BW})] \quad (4)$$

where A is BMC determined by DXA (in kg). Total-body mineral mass was calculated as BMC × 1.2741 (24).

Hydration and density of FFM

FFM was calculated in each model as the difference between body weight and FM; the hydration fraction of FFM was calculated as TBW/FFM. D_{FFM} was calculated as follows:

$$D_{FFM} = (\text{mass of water + protein + mineral}) / (\text{volume of water + protein + mineral}) \quad (5)$$

where the mass and volume of protein and mineral were calculated separately in the 4C model and as a single component in the 3C model.

Statistics

On the basis of theoretical data from the reference child (4) and measured data from adults (3), the sample size of 30 was chosen to be able to detect a difference from adults of 2% in FFM hydration with 80% power. Agreement between 2C, 3C, and 4C models was assessed by using the method of Bland and Altman (25). Assessment was made of mean differences between techniques, termed bias, and its 95% limits of agreement, defined as ±2 SDs of the difference between techniques. Assessment was also made of the extent to which the magnitude of the difference was related to the magnitude of the variable. This relation was termed correlation and was described by the correlation coefficient between the difference and the mean of the measured values.

Different approaches were adopted for comparisons of FFM and FM because FFM is usually measured as an absolute index of body size, whereas FM is usually expressed as a proportion of body weight, ie, fatness. Hence, differences in FFM were investigated by using natural logarithmic differences and means, in which between-technique differences in log FFM are equivalent to a fraction of the mean FFM value and can be expressed as a percentage of the mean measured value. Differences in log FFM are therefore equivalent to (difference/average) and are multiplied by 100 to give a percentage value. For fat, values were expressed as a percentage of body weight, and no further adjustment was made. Between-technique differences are therefore expressed as percentage body fat.

To determine the extent to which measurement error accounted for observed variability, error was propagated for FM and FFM—calculated by D_2O, UWW, and the 3C and 4C models—by using the precision values for each measurement given above. Error was propagated by the delta method, with use of Fieller's theorem to take into account covariance in ratios (26). DXA was excluded from this analysis because of the lack of suitable data. For the 2C models, the hydration and density of FFM were assumed to be 75.0% and 1.086 kg/L, respectively.

Error was also propagated for the hydration and density of FFM in both the 3C and 4C models and for the ratio of mineral to protein and the components of FFM in the 4C model. Biological variation (V_b), methodologic variation (V_m), and total observed variation (V_t) were then differentiated by using the following equation:

$$V_t^2 = V_m^2 + V_b^2 \quad (6)$$

where V_t is the observed SD of a given measurement, and V_m is the SD of the propagated methodologic error in the same units, assumed to be uncorrelated with biological variation. All statistics were carried out by using the MINITAB software release 6.2 (1990; Minitab Inc, State College, PA).

RESULTS

The sex distribution of boys and girls, respectively, by age was as follows: 8 y, 6 and 4; 9 y, 5 and 4; 10 y, 1 and 1; 11 y, 3 and 3; 12 y, 1 and 2. Anthropometric characteristics are given in **Table 1**. Girls tended to be moderately fatter, as evidenced by higher values for most indexes, but significant sex differences were only observed for midupper arm circumference and hip circumference. Mean (±SD) scores relative to UK reference data (27, 28) were 0.20 ± 0.98 for weight, 0.27 ± 1.04 for height, and 0.09 ± 0.98 for

TABLE 1
Characteristics of the sample: age, anthropometry, and impedance[1]

	Boys (n = 16)	Girls (n = 14)
Age (y)	9.7 ± 1.3	10.1 ± 1.4
Weight (kg)	31.6 ± 7.6	36.4 ± 9.0
Height (m)	1.36 ± 0.1	1.40 ± 0.1
BMI (kg/m^2)	16.8 ± 2.0	17.5 ± 2.2
Skinfold thickness (mm)		
Biceps	7.1 ± 3.4	7.0 ± 2.6
Triceps	10.0 ± 3.3	12.3 ± 4.3
Subscapular	6.1 ± 2.3	8.0 ± 2.8
Suprailiac	9.3 ± 5.0	11.7 ± 6.5
MUAC (cm)	20.3 ± 2.6	22.6 ± 2.6[2]
Waist circumference (cm)	59.4 ± 5.7	60.8 ± 5.2
Hip circumference (cm)	65.1 ± 7.3	73.5 ± 6.1[3]
Impedance at 50 kHz (Ω)	691 ± 42	688 ± 72

[1] $\bar{x}$ ± SD. MUAC, midupper arm circumference.
[2,3] Significantly different from boys: [2] $P < 0.025$, [3] $P < 0.0025$.

BMI.

Results of the 3C and 4C models are given in **Table 2**. With the 4C model, the mean (±SD) FFM was 26.6 ± 5.9 kg and FM was 7.2 ± 3.9 kg. Girls had a significantly lower whole-body density than boys, reflected in their greater FM and percentage body fat in both models. There was no significant difference in the density and hydration of FFM between the 2 models, for groups or individuals, or between the sexes (hydration: $P = 0.62$; density: $P = 0.76$). With the 4C model, the mean (±SD) FFM hydration was 75.30 ± 2.22% and FFM density was 1.0864 ± 0.0074 kg/L. Neither FFM density ($P = 0.30$) nor FFM hydration ($P = 0.95$) was significantly correlated with age. The mean (±SD) BMC of FFM was 5.1 ± 0.5% and it was not significantly different between the sexes (boys: 5.1 ± 0.6%; girls: 5.1 ± 0.3%), but was positively related to age ($r = 0.59$, $P < 0.025$). The mean (±SD) protein content was 19.6 ± 2.2% and was not significantly different between the sexes (boys: 19.8 ± 2.5%; girls: 19.3 ± 1.9%) and was unrelated to age ($P = 0.55$).

Assuming the density of fat to be 0.9007 kg/L, the value for adults (19), the mean (±SE) FFM density with our 4C model was 1.0864 ± 0.0013 kg/L and was used to derive a new equation for estimating fatness from total body density in this age group with use of the Archimedes principle (24):

$$\text{Fat (\%)} = (527/D) - 485 \quad (7)$$

Sex-specific values for percentage body fat obtained from the various models and measurement methods are given for comparison in **Table 3**. Both the 3C and 4C models showed girls to be significantly fatter than boys, as did the 2C models using D_2O and DXA. With UWW, a sex difference in the percentage of body fat was only detected when Lohman's (16) values for FFM density were used. Of the skinfold-thickness equations used, only those of Johnston et al (12) and Deurenberg et al (14) showed a sex difference. Of the BIA equations used, only those of Deurenberg et al (21) and Danford et al (23) showed a sex difference.

Regression analysis of the relation between TBW and height^2/Z gave the following equation:

$$\text{TBW (in L)} = 2.69 + 0.601\ \text{height}^2/Z \quad (8)$$
$$(\text{SEE} = 1.15\ \text{L}, R^2 = 93.2\%)$$

where height is in centimeter. Expression of this equation in logarithmic terms, to allow the SEE to be expressed as a percentage of the predicted value, gave an SEE value of 5.6% and an R^2 value of 91.3%. A similar equation, in which log weight and log height were used rather than log height^2/Z, gave an SEE value of 6.6% and an R^2 value of 88.0%.

Bland-Altman comparisons of the agreement in FFM and percentage body fat by 2C (UWW, D_2O dilution, and DXA), 3C, and 4C models are shown in **Table 4**. UWW was found to overestimate FFM and underestimate FM, regardless of which published values for FFM density were used. The other techniques showed no significant bias compared with the 4C model, despite the fact that DXA systematically underestimated total body weight compared with values determined by weighing scale ($\Delta = -0.30 \pm 0.26$ kg, $\bar{x}$ ± SD; $P < 0.0001$). No technique showed a significant correlation between the difference and the mean. For individual values of percentage body fat, 95% limits of agreement, given as ±2 SDs, ranged from ±4.9% with D_2O dilution to ±6.5% with DXA.

FFM and percentage body fat predicted by BIA against the 4C model are compared in **Table 5**. All equations showed significant bias for both FFM and percentage body fat. For each equation, between-method differences were related to either body size or body fatness. The lowest mean bias was given by the equations of Houtkooper et al (22), but 95% limits of agreement for individual values were wide for all equations, eg, > ±7% for percentage body fat.

Comparisons of FFM and percentage body fat predicted by using skinfold-thickness equations against measurements made with the 4C model are shown in **Table 6**. Three of the equations showed significant mean bias in FFM and percentage body fat. The equations of Deurenberg et al (14) showed no mean bias, but there was a significant correlation between bias and fatness. Limits of agreement for individual values were again wide, being > ±8% for percentage body fat for all equations.

The precision (reproducibility) of the principal measurement methods was determined from duplicate or repeated measurements as described in the Methods section. The values were as follows—body weight: 0.01 kg, equivalent to 0.03%; body volume: 0.19 L, equivalent to 0.58%; TBW: 0.21 L, equivalent to 1.05%;

TABLE 2
Body composition on the basis of 3- and 4-component models[1]

	Boys	Girls
Body density (kg/L)	1.049 ± 0.010	1.035 ± 0.016[2]
Total body water (L)	19.3 ± 3.3	20.8 ± 5.3
Bone mineral content by DXA (kg)	1.04 ± 0.30	1.11 ± 0.31
3-Component model		
FFM (kg)	25.7 ± 4.6	27.5 ± 7.2
FM (kg)	5.8 ± 3.4	8.9 ± 3.8[2]
FFM hydration (%)	75.1 ± 2.3	75.5 ± 1.6
FFM density (kg/L)	1.087 ± 0.009	1.085 ± 0.006
Protein + mineral (kg)	6.40 ± 1.45	6.74 ± 1.95
4-Component model		
FFM (kg)	25.7 ± 4.6	27.5 ± 7.2
FM (kg)	5.8 ± 3.5	8.9 ± 3.8[2]
FFM hydration (%)	75.1 ± 2.5	75.5 ± 1.8
FFM density (kg/L)	1.087 ± 0.009	1.086 ± 0.006
Protein (kg)	5.1 ± 1.1	5.3 ± 1.6
Total body mineral (kg)	1.33 ± 0.38	1.42 ± 0.39

[1] $\bar{x}$ ± SD. DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FM, fat mass.
[2] Significantly different from boys, $P < 0.05$.

TABLE 3
Percentage of fat in boys and girls by different measurement methods[1]

	Boys	Girls
4-Component model	17.4 ± 5.4	23.9 ± 7.8[2]
3-Component model	17.5 ± 5.2	23.9 ± 7.7[2]
2-Component models		
UWW, Weststrate and Deurenberg (15)	16.2 ± 5.2	21.0 ± 8.3
UWW, Lohman (16)	16.5 ± 5.0	22.4 ± 8.4[2]
DXA	16.6 ± 5.8	24.3 ± 7.5[3]
Deuterium	17.5 ± 6.5	25.2 ± 7.8[4]
Skinfold-thickness equations		
Slaughter et al (13)	15.6 ± 4.9	18.6 ± 5.2
Johnston et al (12)	9.9 ± 5.5	15.8 ± 4.9[4]
Deurenberg et al (14)	17.3 ± 4.1	21.1 ± 5.2[2]
Brook (11)	14.7 ± 6.2	15.9 ± 8.0
BIA equations		
Deurenberg et al (21)	24.5 ± 3.2	28.6 ± 3.9[3]
Davies et al (20)	32.7 ± 4.8	35.9 ± 7.2
Houtkooper et al (22)	16.5 ± 5.3	19.2 ± 5.9
Danford et al (23)	25.2 ± 5.0	29.4 ± 5.9[2]

[1] $\bar{x}$ ± SD. UWW, underwater weight; DXA, dual-energy X-ray absorptiometry; BIA, bioelectrical impedance analysis.
[2–4] Significantly different from boys: [2] $P < 0.05$, [3] $P < 0.01$, [4] $P < 0.005$.

mineral mass (based on values obtained by using a whole-body phantom as described above): 0.01 kg, equivalent to 1.09%. The precision of impedance was 3.5 Ω, equivalent to 0.4% of TBW. The propagated errors for FM and FFM in the 2C, 3C, and 4C models are summarized in **Table 7**. In the 4C model, the propagated error for protein mass was 0.54 kg, equivalent to 10.4%.

The biological variability of the components and properties of FFM, after taking methodologic precision into account, are shown in **Table 8**. The range of values reported in the reference child is also given for comparison. Approximately half of the variability in the water content, protein content, and ratio of mineral to protein may have been due to biological variation. Almost all the variability in the BMC was probably due to biological variation, as was more than half of the variability in FFM density.

Mean (±SD) hydration and density of FFM for children were significantly different from values for adults (3): 73.32 ± 2.13% ($P < 0.02$) and 1.1015 ± 0.0073 kg/L ($P < 0.001$), respectively. The mean (±SD) ratio of mineral to protein of 0.208 ± 0.031 was also significantly different from the reference adult value (24) of 0.262 by paired t test ($\Delta = -0.05 \pm 0.03$; $P < 0.0001$)

DISCUSSION

Although some measurement techniques are more accurate and precise than others, there is no gold standard for body-composition measurements in vivo. All methods incorporate assumptions that do not hold true in all cases and the best model is derived by using a combination of measurements, whereby the importance of such assumptions is minimized. We measured BMC and TBW with techniques specifically designed for these components. This approach leaves the UWW measurement uninfluenced by the water content and BMC of FFM, such that it distinguishes not fat and FFM, but fat and protein. The variability in the density of protein is less than that of FFM, making this part of the model more reliable. The 4C model thus allows the quantification of FFM, and by inference FM, with a degree of accuracy not achievable with 2C models.

Propagation of error indicates that the precision of the 3C and 4C models is ≈0.5 kg of FFM and FM. It might be assumed that precision should be better in 2C than in 3C and 4C models because they are influenced by fewer separate measurements. Thus, D_2O dilution has a precision of <0.3 kg for FFM and FM. Data from repeat scans of an adult (10, 29) suggest that the precision of DXA may be ≈0.2–0.4 kg of FM and FFM, although whether adult values are applicable to children is doubtful. In contrast, precision of UWW is poor because of the practical difficulties of this measurement. However, our modeling indicates that use of UWW in the 4C model transmits its error into a smaller proportion of body weight, thereby reducing the influence of its poor precision.

Early multicomponent studies of body composition, focusing on the fetus (30) and the 9-y-old reference boy (5), led to the development of data on the reference child (4). Although these data remain the most detailed to date and are widely used, they were acknowledged by the authors to be preliminary data, with most values being extrapolated from data derived only at birth, 6 mo, and 9 y. Furthermore, values for the various components were derived from separate studies of different children, all data being adjusted in relation to weight and height. Finally, the reference child represents only the population mean, and interindividual variability at any age was not reported.

Our data therefore represent the first measured values for FFM density in children, the mean value of 1.0864 kg/L being significantly lower than the value for adults of 1.1 (3, 19). In contrast with the predicted values of Fomon et al (4), our data show no sex difference in the density, protein content, and BMC of FFM. Previously, 2 approaches were adopted for predicting FFM density in children (15, 31). Our inability to reproduce the values given by Fomon et al indicates that the equations of Weststrate and Deurenberg (15) are not appropriate when body den-

TABLE 4
Bias and 95% limits of agreement for fat-free mass (FFM) and percentage body fat compared with 2-component (2C), 3C, and 4C models[1]

	Bias	Limits of agreement	Correlation
3C model			
FFM (%)	0.0	±1.1	0.20
Percentage body fat (%)	0.0	±0.9	−0.30
TBW			
FFM (%)	−0.9	±6.1	−0.08
Percentage body fat (%)	0.6	±4.9	0.30
UWW, Weststrate and Deurenberg (15)			
FFM (%)	2.5[2]	±6.8	0.26
Percentage body fat (%)	−2.0[2]	±5.6	−0.07
UWW, Lohman (16)			
FFM (%)	1.46[3]	±6.3	0.29
Percentage body fat (%)	−1.15[3]	±5.2	−0.04
DXA			
FFM (%)	−0.7	±8.2	−0.17
Percentage body fat (%)	−0.2	±6.5	0.08

[1] Bias was calculated as 2C or 3C model measurements minus 4C model measurements. Correlation calculated as the correlation between the difference and mean. 95% limits of agreement calculated as ± 2SD of the difference between techniques. FFM values were log transformed to express difference as a percentage of mean. Values for percentage body fat are expressed as a percentage of body weight. TBW, total body water; UWW, underwater weighing; DXA, dual-energy X-ray absorptiometry.
[2] $P < 0.001$.
[3] $P < 0.025$.

TABLE 5
Bias and 95% limits of agreement for fat-free mass (FFM) and percentage body fat between predictions made by using bioelectrical impedance analysis (BIA) and measurements with the 4-component (4C) model[1]

Equations	Bias	Limits of agreement	Correlation
Davies et al (20)			
FFM (%)	−19.0[2]	±11.4	0.36[3]
Percentage body fat (%)	13.7[2]	±8.5	−0.29
Deurenberg et al (21)			
FFM (%)	−7.4[2]	±11.1	0.26
Percentage body fat (%)	5.9[2]	±8.6	−0.77[2]
Houtkooper et al (22)			
FFM (%)	3.5[2]	±10.4	−0.02
Percentage body fat (%)	−2.7[2]	±7.9	0.43[3]
Danford et al (23)			
FFM (%)	−8.6[2]	±9.1	−0.05
Percentage body fat (%)	6.7[2]	±7.2	−0.45[3]

[1] Bias was calculated as BIA predictions minus 4C model measurements. Correlations were calculated as the correlation between the difference and mean. 95% limits of agreement calculated as ± 2SD of the difference between techniques. FFM values were log transformed to express the difference as a percentage of the mean. Values for percentage body fat are expressed as a percentage of body weight.
[2] $P < 0.0001$.
[3] $P < 0.05$.

sity is used to derive fatness with a 2C model. We therefore derived a new equation, which is suitable for children in the age range studied here, but which may not be applicable to other age groups. This new equation matched closely the theoretical equation derived by Lohman et al (31). In contrast with FFM density, our values for FFM hydration closely agreed with those of the reference child.

Since 1982, there have been 2 further multicomponent studies of body composition in prepubertal children. Using a 3C model identical to ours in 43 white prepubertal children (mean age: 10 y), Boileau et al (32) published values for FFM hydration (boys: 75.1 ± 2.8%; girls: 76.0 ± 3.7%) that were almost identical to those found in the present study. More recently, Hewitt et al (33) determined FFM hydration in 28 prepubescent children aged 5–10 y using a 3C model in which body density was adjusted for individual variation in bone mineral density assessed at the radius shaft. Use of the 3C model alone gave FFM hydration values similar to those obtained in other studies (boys: 75.5 ± 1.8%; girls: 74.9 ± 1.4%), but the further adjustment for bone density resulted in substantially lower values (boys: 73.1 ± 1.6%; girls: 72.2 ± 1.4%) (33). Hewitt et al admitted that their adjustment for bone density rested on the assumption that regional bone density represented total-body bone mineral. Our study indicates that this assumption may not be valid, given that when we included data on total-body bone mineral in our 4C model, FFM hydration remained the same as that with the 3C model. Another study of body-composition analysis appropriately used a combination of methods, but did not report data on FFM hydration and FFM density (34). Our study therefore is the first of a 4C model in children in which total-body bone mineral mass was measured, allowing mineral and protein to be quantified separately.

The values for the reference child gave no indication of interindividual variability in FFM composition, making it difficult to assess the potential error arising from the use of theoretical assumptions, such as its constant density and hydration. We therefore attempted to distinguish the contribution of methodology and biology to total variability. Propagation of error showed that approximately half of the variability in FFM hydration in our sample was due to the method used, indicating that true biological variability in healthy children is relatively low, although it may vary more between disease states. Our analysis showed that mineral and protein contents rather than water are the more variable components of FFM, the CVs for mineral and protein contents being 9.1% and 11.4%, respectively, but that for water content being only 2.9%. Hence, more than half of the variability in FFM density was biological rather than methodologic in origin and was due to the cumulative effects of variation in mineral, water, and protein contents.

A comparison of models showed that, relative to the 4C model, the 3C model adequately estimated FFM hydration. This indicates that the theoretical assumption of a constant ratio of mineral to protein in FFM, on which the 3C model relies, is acceptable in this age group. Hence, FFM hydration in children aged 8–12 y can be satisfactorily estimated by measuring weight, body volume, and TBW. Recent advances in the rapidity with which measurement of TBW can be completed (6) and in the application of new techniques for the assessment of body volume (35) may soon make routine assessment of FFM hydration viable for clinicians.

Data from our 4C model support some of the theoretical differences between adults and children outlined in the reference child. The total-body mineral content was lower than the value for adults, being 5% of FFM rather than 7%, but we found no evidence of a sex difference. Mineral deposition was apparent over the age range we studied and is believed to be completed in early adulthood (31). FFM hydration was significantly higher than in adults; however, although previous work has shown similar results (32, 33), investigators continue to use adult-derived values in 2C models used to study children (36). The ratio of

TABLE 6
Bias and 95% limits of agreement for fat-free mass (FFM) and percentage body fat predicted by using skinfold-thickness equations against measurements made with the 4C model[1]

Equations	Bias	Limits of agreement	Correlation
Slaughter et al (13)			
FFM (%)	4.5[2]	±10.8	−0.02
Percentage body fat (%)	−3.5[2]	±8.0	−0.55[3]
Johnston et al (12)			
FFM (%)	9.5[2]	±11.7	0.25
Percentage body fat (%)	−7.8[2]	±8.6	−0.33
Deurenberg et al (14)			
FFM (%)	1.9	±11.2	−0.02
Percentage body fat (%)	−1.4	±8.4	−0.59[3]
Brook (11)			
FFM (%)	6.4[2]	±13.4	−0.27
Percentage body fat (%)	−5.2[2]	±10.5	−0.06

[1] Bias was calculated as skinfold-thickness values minus values from use of the 4C model. Correlations were calculated as the correlation between the difference and mean. 95% limits of agreement calculated as ±2SD of the difference between techniques. FFM values were log transformed to express the difference as a percentage of the mean. Values for percentage body fat are expressed as a percentage of body weight.
[2] $P < 0.0001$.
[3] $P < 0.005$.

TABLE 7
Propagation of methodologic error on fat mass (FM) and fat-free mass (FFM) values obtained by using different models

	Error[1]	Error	Error
	kg	% of FFM	% of FM
4C model	0.54	2.0	7.5
3C model	0.45	1.7	6.2
2C models			
Deuterium dilution	0.27	1.3	3.8
UWW[2]	1.00	3.8	13.8

[1] Error on either FM or FFM.
[2] Underwater weighing.

mineral to protein also differed significantly by ≈20% from that in adults, but the magnitude of this difference was not sufficient to cause error when the adult value was used in combination with a 3C model to estimate hydration and density of FFM.

Previous research in children has shown that 2C methods give method-dependent values for percentage fatness (2). However, although UWW has tended to be regarded as the ideal reference method against which to compare other techniques in children (15, 37), all 2C models rely on theoretical assumptions that may be inappropriate for some groups or individuals. Our study provided the first opportunity to compare 2C models with a 4C reference model in this age group. This is important given that widely used bedside prediction equations (skinfold-thickness and BIA equations) have almost invariably been developed by using other 2C models.

Our results indicate that DXA and D_2O dilution showed negligible mean error in measuring FFM relative to the 4C model. This suggests that the prototype Hologic DXA software for children (38) may have overcome artifacts of body size reported by us previously (39). Most limits of agreement for the D_2O comparison can be attributed to measurement precision, so this method is appropriate for assessing both groups and individuals. However, limits of agreement are wider for DXA than for D_2O dilution, and, in the absence of DXA precision data specific to children, it is difficult to evaluate the relative contributions of methodologic error and biological effects. If DXA precision data for adults are used (10, 29), measurement precision accounts for a minority of the limits of agreement. Given the difference in limits of agreement, D_2O dilution is preferable to DXA as a 2C method when used in individuals. In contrast, UWW showed a systematic error relative to the 4C model. This error was reduced when Lohman's (16) values for FFM density were used rather than those of Weststrate and Deurenberg (15), but it remained significant. The limits of agreement may have been due entirely to measurement error.

We used our FFM density values to derive a new equation, one analogous to that of Siri's (19) for adults, in which body fatness can be calculated from total body density according to the Archimedes principle (24). Use of this equation removes the systematic error described above and is supported by a similar equation derived theoretically by Lohman et al (31). Use of these equations was more successful in our sample than was Lohman's (16) age-adjusted values for individuals (16), given that we found no observable trend of FFM density with age in our sample. However, our study highlights the pitfalls of using UWW to validate bedside prediction methods. Both measurement error and the choice of values for FFM density substantially influence the relation between fatness measured by UWW and alternative techniques, thereby increasing predictive error in both groups and individuals.

Because they are easy to use, skinfold-thickness prediction equations remain the most widely used method of assessing fatness for clinical purposes. Equations have generally been derived in relation to 2C models, although Slaughter et al (13) used the 4C data of Boileau et al (32). Our study provided the first opportunity to test the validity of these equations relative to a 4C model in a separate sample of children and showed that all 4 equations underestimated fatness in this age range. A previous study of children in Glasgow, United Kingdom, compared fatness derived from several sets of skinfold equations with those by UWW as a 2C model (37). Our 4C data reproduced very closely the findings of that study, indicating that systematic biases in equations persist beyond particular populations of children. Limits of agreement for individuals were wide, indicating that even when the mean bias is small, individual values are not accurate. Furthermore, the bias was related to fatness, which may reduce the ability of skinfold-thickness measurements to evaluate changes within individuals accurately over time.

The limitations of using skinfold-thickness equations for assessing fatness have fueled interest in BIA as an alternative, rapid, noninvasive method that is easily tolerated by children whether healthy or ill. However, it is clear from the various equations published for children that different researchers have found different relations between height^2/Z and TBW or FFM. Equations have varied in both slope and intercept, which may have been due to methodologic or biological factors. Both UWW and TBW have been used as reference methods to validate BIA; therefore, height^2/Z has not been related to the same variable consistently. Different age ranges have also been studied [5–9 y (23), 5–17 y (20), 8–11 y (21), and 10–14 y (23)]. Changes in the relative lengths of limbs and trunk during growth may influence the relation between TBW and height^2/Z; therefore, the use of different age ranges may introduce incompatibility.

TABLE 8
Variability of the components of fat-free mass (FFM) and comparison with the value for Fomon et al's (4) reference child

FFM components	Mean	Total variability	Methodologic error	Biological variability	Reference child[1]	
					Males	Females
		SD	SD	SD		
Water content (%)	75.3	2.2	1.5	1.6	75.1–75.7	76.9–77.2
Mineral content (%)	4.02	0.37	0.09	0.36	4.6–4.8	3.8
Protein content (%)	19.6	2.2	1.6	1.5	19.1–19.5	18.4–18.7
Mineral:protein	0.208	0.031	0.021	0.023	0.241–0.246	0.203–0.206
Density (kg/L)	1.0864	0.0074	0.0046	0.0058	1.082–1.085	1.074–1.075

[1] Ranges include values for children aged 8–10 y only.

Poor agreement between BIA-predicted and 4C values for FFM and percentage body fat might be attributed mainly to between-study differences in the relation between height^2/Z and TBW or FFM. The slope of the line relating TBW to height^2/Z was 0.60, identical to that reported by Davies et al (20); for FFM and height^2/Z the slope was 0.82, which was similar to that reported by Houtkooper et al (22). However, in both cases, our intercept was substantially different, creating between-method bias. The equations of Danford et al (23) and Deurenberg et al (21), which incorporated even more variables, were no more successful. The smallest mean bias was shown by the equations of Houtkooper et al (22), but was significant and related to body fatness.

Our study indicated that current BIA equations do not adequately predict fatness with accuracy in individuals or groups of children. Furthermore, the relation between height^2/Z and TBW was barely better than that between weight and height and TBW, raising the possibility that biological variation between children in body proportions may limit the success with which BIA can predict TBW, even when appropriate equations are used. However, BIA may be suitable for assessing short-term changes in TBW within individuals over time, particularly because measurement precision was high (equivalent to $< 0.5\%$ of TBW).

The lack of a gold standard for measurements of body composition in children makes evaluation of simple methods difficult. We used a 4C model robust to interindividual variation in FFM composition with relatively high precision as a reference. D_2O dilution and DXA appear to be acceptable 2C models for children aged 8-12 y, but no bedside prediction method was satisfactory. UWW was found to show bias compared with the 4C data and was the technique most susceptible to imprecision. This is of concern, given that children's bedside methods have traditionally been validated against UWW data. The need to improve bedside methods remains a priority, as does the need to revise values for the reference child, particularly with regard to FFM composition. This would have important implications for the variety of 2C and bedside methods that remain widely used in pediatric research and clinical management.

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