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Body composition reference data for simple and reference techniques and a fourcomponent model: a new UK reference child

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Running head: Reference data for children's body composition

Abbreviations

- 4C-4-component
- BIA bioelectrical impedance analysis
- BMC bone mineral content
- BMI body mass index
- BV body volume
- DXA dual energy X-ray absorptiometry
- FFM fat-free mass
- FM fat mass
- LMS lambda sigma mu
- SDS standard deviation score
- SEE standard error of the estimate
- TBW total body water
- WT body weight

1	Abstract		
2	Background: Routine pediatric clinical assessment of body composition is increasingly		
3	recommended, but has long been hampered by two factors: a lack of appropriate techniques,		
4	and a lack of reference data with which to interpret individual measurements. Several		
5	techniques have become available, but reference data are needed.		
6	Design : We aimed to measure body composition using a gold standard four-component		
7	model, along with various widely used reference and bedside methods, in a large		
8	representative sample of British children aged 4-20+ years, to provide body composition		
9	reference data for use in clinical practice and research.		
10	Methods: Measurements were made of anthropometry (weight, height, four skinfold		
11	thicknesses, waist girth), dual-energy X-ray absorptiometry (Lunar Prodigy), body density		
12	(Bodpod \), bioelectrical impedance (Tanita BC418MA) and total body water, and four-		
13	component fat and fat-free masses were calculated. Reference charts and standard deviation		
14	scores (SDS) were constructed for each outcome using the LMS method. The same outcomes		
15	were generated for fat-free mass index and fat mass index.		
16	Results: Body composition growth charts and SDS for 5-20 years were based on a final		
17	sample of 533 individuals. Correlations between SDS by different techniques were ≥ 0.68 for		
18	adiposity outcomes and ≥ 0.80 for fat-free mass outcomes.		
19	Conclusions : These comprehensive reference data for pediatric body composition can be used		
20	across a variety of techniques. Together with advances in measurement technologies, they		
21	should greatly enhance the ability of clinicians to assess and monitor body composition in		
22	routine clinical practice, and should facilitate the use of body composition measurements in		
23	research studies.		

1 Introduction

2	Growth charts for weight and height have been the backbone of pediatric clinical	
3	assessment of nutritional status for decades (1-4). However, efforts to obtain more detailed	
4	information on body composition have long been hampered by two challenges. First, methods	
5	for the measurement of pediatric body composition have taken time to develop. Only within	
6	the past decade have techniques such as DXA, air displacement plethysmography,	
7	bioelectrical impedance analysis and isotope dilution become widely applied in the pediatric	
8	population (5). Second, even where such techniques are available, interpretation is severely	
9	hindered by the lack of appropriate reference data.	
10	Clinical practice has thus been strongly influenced by the nature of the available data.	
11	Reference data for British children's skinfold thickness measurements were provided in the	
12	1970s (1). More recently, reference data for UK children's BMI were published in the 1990s	
13	(6), using Cole's LMS method to take into account age changes in the variability and	
14	skewness of the data (7). These BMI charts have become the primary UK reference for	
15	interpreting nutritional status in the clinic, and have been replicated in many other populations	
16	(8-11). To aid convergence between these approaches, the skinfold data were also converted	
17	to LMS format (12).	
18	International BMI cut-offs for categorising overweight/obesity and underweight have	
19	also been published (13, 14). Such BMI data have been widely adopted in part because of	
20	their value in predicting clinical outcome. Nevertheless, they suffer from limitations when	
21	more detailed information about fat mass or fat-free mass is required. Historically, fat-free	
22	tissue has been considered the functional and dynamic component of weight, with fat mass	

- 23 conceptualised as a relatively inert energy store. Recent studies identifying numerous
- 24 hormonal products of adipose tissue challenge this view, and adipose tissue is now understood
- 25 to play a complex regulatory role, exerting many of its effects on fat-free tissue (15). There is

therefore increasing interest in the ability to categorise fat-free mass and fat mass, and
 monitor their changes over time.

3 Recently, we summarised a number of contexts in which information about body 4 composition could be of value to the pediatrician (16), and also described the methodologies 5 available (5). However, until reference data for children's body composition are available, 6 measurements of individual patients will remain difficult to interpret (17). Reference data for 7 individual techniques (eg skinfold thicknesses, BIA, DXA), have been reported in the 8 literature (18-25), but no study has yet provided comprehensive reference data on a range of 9 techniques in any single population. Here, we describe reference data for a number of 10 different measures of body composition, allowing our reference dataset to be used across a 11 variety of techniques.

12

13 Methods

14 A total of 565 normal healthy children and adolescents aged 4 to 23 years were 15 recruited using flyers and newspaper adverts in London and the south-east of England, 16 starting in 2001. There were no exclusion criteria for BMI, hence some individuals were 17 categorised as overweight or obese, but they were not recruited directly from obesity weight-18 loss clinics, and had no disease that might have adversely affected growth and development. 19 The lower age limit of 4 years was chosen based on our previous work, in that younger 20 children are unlikely to satisfy the protocol for air displacement plethysmography. Data 21 collection was extended to young adults in order to cover the entire pediatric age range. 22 Ethical approval was granted by the Ethical Committee of UCL Institute of Child Health and 23 Great Ormond Street Hospital. All individuals attended our body composition investigation 24 suite located at Great Ormond Street Hospital for a 2-h measurement session.

1	Weight and height were measured using standard protocols. WT was measured in	
2	duplicate as part of the air displacement plethysmography protocol (see below). Height was	
3	measured using a wall-mounted stadiometer (Holtain, Dyfed, UK). BMI was calculated as	
4	weight (kg) divided by the square of height (m). Data on weight, height and BMI were	
5	converted to SDS format using UK reference data (6, 26). Obesity was defined as BMI >95th	
6	centile (SDS >1.64), and overweight as BMI >85th centile (SDS >1.04) (6). Pubertal	
7	development was assessed by Tanner staging, using self-assessment based on line drawings.	
8	Skinfold thickness measurements were performed in triplicate at the biceps, triceps,	
9	subscapular and supra-iliac sites, and the mean of the three values used. Waist girth was	
10	measured using a non-stretchable fibreglass tape. BIA was conducted using Tanita BC418MA	
11	instrumentation (Tanita Corporation, Tokyo, Japan), however this instrument was only	
12	available from 2004 onwards, hence the sample size was 451 (83% of the total) for this	
13	outcome. Using whole-body values for impedance (Z, in ohms) at 50 kHz, the impedance	
14	index height ² /Z (cm^2 /ohms) was calculated. Numerous pediatric equations have been	
15	published for BIA, leaving users uncertain as to which equation to select for any given	
16	population. The BIA output was therefore analysed in raw cm ² /ohm units, avoiding	
17	influencing this outcome by the choice of one or another equation. This approach prevents use	
18	of BIA data as an index of adiposity, hence skinfolds were the primary bedside approach	
19	tested for adiposity. Given that absolute body composition values obtained from using	
20	predictive equations in combination with such bedside techniques have high SEE, our	
21	combined 'skinfolds + BIA' bedside approach has the added advantage that fat-free and fat	
22	tissues are assessed with independent techniques, and error on the adiposity index should be	
23	independent from error on the fat-free mass index.	
24	Measurements of TBW (litres) by deuterium dilution, BMC (kg) by DXA (Lunar	
25	Prodigy software version 6.7: GF Medical Systems Madison WI) and BV (litres) in	

25 Prodigy, software version 6.7; GE Medical Systems, Madison, WI) and BV (litres) in

1	duplicate by air displacement plethysmography (Bodpod \; Life Measurements, Concord, CA)		
2	were obtained as described previously, with post-dose saliva collected after 4-5 hours (27).		
3	The deuterium dilution space was converted to TBW assuming the degree of over-estimation		
4	attributable to proton exchange to be 1.044 (28). Lung volume was predicted rather than		
5	measured in the plethysmography measurements, because we have found a large proportion of		
6	children are unable to complete the lung volume measurement protocol satisfactorily. Values		
7	for fat-free mass from DXA were the sum of lean soft tissue and bone mineral mass.		
8	The 4C model is considered the most accurate in vivo approach for the differentiation		
9	of fat and fat-free masses, and is particularly valuable in patients in whom assumptions of		
10	constant fat-free tissue composition are not valid (29, 30). The 4C model used here to		
11	calculate LM and FM has been described previously (31, 32), and uses the following		
12	equations:		
13			
14	FM = (2.747 * BV) - (0.710*TBW) + (1.460*BMC) - (2.050*WT) (Equation 1)		
15			
16	LM = WT - FM (Equation 2)		
17			
18	The proportion of fat in weight (% fat) was calculated as (FM/WT)*100. In our laboratory,		
19	precision is 1% for TBW (32) and 0.24 l for BV (33). Precision of BMC is 1.1% (34).		
20			
21	<u>Statistics</u>		
22	Analyses were conducted for a range of adiposity and fat-free mass outcomes. For		
23	adiposity, the outcomes were (a) each of the four skinfolds, (b) waist girth, (c) whole-body,		
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24	adiposity, the outcomes were (a) each of the four skinfolds, (b) waist girth, (c) whole-body, arm, leg and trunk fat mass from DXA, (d) body density by air-displacement		

1	height ² /Z, (c) whole body, arm, leg and trunk fat-free mass by DXA, and (d) 4C fat-free mass.
2	For the 4C data, the fat-free mass index (fat-free mass/height ²) and fat mass index (fat
3	mass/height ²) were also calculated, as described and recommended previously (17, 35). Each
4	of these variables was converted to standard deviation scores as described below.
5	Sex-specific values by month of age were obtained for all body composition outcomes
6	using the LMS method (LMS Chart Maker, Medical Research Council, UK) (7). This
7	statistical approach, widely used to construct reference data for traits which incorporate the
8	effects of growth, provides three outputs: (a) a smoothed median (M or mu) curve which
9	represents how the outcome varies in relation to age; (b) the coefficient of variation (S or
10	sigma), which models the scatter of values around the mean and adjusts for any non-uniform
11	dispersion; and (c) the skewness (L or lambda) which is addressed using age-specific Box-
12	Cox transformation to achieve a normal distribution. Adiposity indices were fitted using
13	original age, and fat-free mass outcomes using re-scaled age, which improves the goodness of
14	fit for monotonic data by fitting the M curve twice. Goodness-of-fit was assessed with the
15	Bayesian Information Criterion, adding an extra unit of complexity to the model only if it
16	reduced the deviance by more than $\log_e(N)$ units, where N is the sample size. As the precision
17	of the M curve at any age depends on data points at younger and older ages, precision is lower
18	at the extremes of the age range. We therefore fitted the data for all ages (4-23 years) and
19	derived LMS values for the age range 5-20 years.
20	These data represent new body composition growth charts, available for both the 4C
21	model and individual techniques such as DXA, TBW, body density, BIA and skinfolds. Such
22	charts will allow the monitoring of adiposity and fat-free mass over time, to improve
23	understanding of the effects of disease and treatments. We also calculated values for fat-free
24	mass, fat mass, fat-free mass index and fat mass index by the 4C model for each sex for the

following z-score cut-offs: -2, -1.67, -1.33, 0, 1.33, 1.67 and 2, equivalent to percentiles of 2.3%, 9.2%, 25.2%, 50%, 74.8%, 90.8% and 97.7% respectively.

3 Using this statistical approach, all data were converted to SDS format, and subsequent 4 analyses on those aged 5-20 years were undertaken using Datadesk ® 6.1 (Data Description 5 Inc., Ithaca, New York). The mean of the four individual skinfold SD scores was also 6 calculated. We then quantified the agreement between the individual SDS with the reference 7 4C SDS. If growth charts are to be adopted by clinicians, they will need to know if rankings 8 from one technique (eg skinfolds) are consistent with ranking by another (eg DXA, or the 4C 9 model). Expressing the data in SDS format aids such a comparison, first because it enables 10 the chart rankings to be compared, rather than the raw data, and second because body 11 composition techniques produce outputs in different units (eg kg for DXA, kg/m³ for density, 12 mm for skinfolds, $cm^2/ohms$ for BIA), preventing direct comparisons. 13 Correlation coefficients were calculated for all the adiposity and fat-free mass 14 outcomes in each sex. Pearson correlation coefficients were calculated, on the assumption that 15 associations between different z-scores were expected to be linear. For central adiposity, we 16 also calculated correlations of SDS for DXA trunk fat and waist girth. Sex-specific regression 17 analysis was undertaken, predicting 4C fat mass SDS from each individual adiposity SDS, and 4C fat-free mass SDS from individual fat-free mass, TBW or HT²/Z SDS. The slopes and 18 19 intercepts were assessed for difference from 1 and 0 respectively, and the SEE calculated. 20 Bland-Altman analysis (36) was used to illustrate agreement with 4C SDS values for DXA 21 whole body SDS, BIA SDS and average skinfold SDS. A minority of the subjects (27 boys, 22 23 girls, ie 9.3% of the sample) were of non-European ethnicity, however this sample size 23 was considered too small to allow ethnic variability in body composition to be addressed. 24

25 **Results**

1	Valid data were obtained on 533 individuals. Data on 32 other individuals were	
2	discarded because either one or more of the basic measurements was unsuccessful ($n = 16$;	
3	mostly very young children), or the modelling was unsuccessful $(n = 16)$ as indicated by	
4	spurious body composition data. As indicated in Figure 1, a wide range of BMI SDS was	
5	apparent at all ages. There was no significant correlation between BMI SDS and age in either	
6	sex.	
7	Table 1 provides data on anthropometry SDS values, and the range of % fat by sex.	
8	On average, our sample was heavier and taller than the UK reference data of the early 1990s	
9	(p<0.005 in all cases). Females unsurprisingly had significantly greater % fat than males	
10	(p<0.0001, adjusted for age). The prevalence of obesity was 11.5% and 14.7% in males and	
11	females respectively, and was uncorrelated with age. The numbers by pubertal stage 1-5 were	
12	as follows: male 98; 60; 28; 24; 50, female 87; 48; 34; 22; 80, and two others not recorded.	
13	Figure 2 shows LMS centiles for 4C fat-free mass, 4C fat mass, 4C fat-free mass	
13 14	Figure 2 shows LMS centiles for 4C fat-free mass, 4C fat mass, 4C fat-free mass index and 4C fat mass index respectively against age for each sex. Fat-free mass increased	
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values, together with SEE values. No intercept differed significantly from 0, however most

1	slopes were significantly lower than 1, the exception being DXA fat mass SDS in females.	
2	For DXA fat mass in males, the upper 95% confidence interval of the slope was just below 1	
3	(0.983). The smallest SEE values were obtained from DXA fat mass SDS (0.33 SDS in males,	
4	0.21 SDS in females) whereas values for skinfolds were ~0.5 to ~0.6 SDS. Thus, in most	
5	cases individual SDS underestimated 4C fat SDS in those with higher adiposity, with this	
6	effect being minimal for DXA whole-body data. Figure 3 illustrates Bland-Altman analysis	
7	of agreement between 4C and DXA values for fat mass SDS, showing no systematic trend in	
8	bias across the range of adiposity, but greater random inconsistency in those of low adiposity.	
9	Table 8 shows correlation coefficients for SDS for indices of fat-free mass by sex. All	
10	correlations were ≥ 0.80 (p<0.0001). Table 9 shows coefficients and standard errors for	
11	intercepts and slopes for the regression of 4C fat-free mass SDS on each individual proxy	
12	SDS value, together with SEE values. No intercept differed significantly from 0, however	
13	most slopes were significantly lower than 1, the exceptions being DXA fat-free mass SDS in	
14	both sexes, and TBW SDS in both sexes. SEE values were ~ 0.2 for DXA whole body SDS,	
15	~0.2 for TBW SDS, and ~0.44 for BIA SDS. Figure 3 illustrates Bland-Altman analysis of	
16	agreement between 4C and DXA values for fat-free mass SDS in males and females, showing	
17	no variability in bias across the range of fat-free mass SDS.	
18	The r^2 values calculated from Tables 6 and 8 indicate that DXA fat SDS accounts for	
19	88% and 96% of the variance in 4C fat SDS in males and females respectively, while DXA	
20	fat-free SDS accounts for 96% and 94% of the variance in 4C fat-free SDS in males and	
21	females respectively. In both sexes, agreement is better for fat-free SDS than fat SDS (Figure	
22	3), which shows poorer consistency between methods in those with low adiposity. For central	
23	fat, the correlation of DXA trunk fat SDS and waist SDS was 0.81 in males and 0.83 in	
24	females. Waist SDS therefore explains 66% and 69% of the variance in trunk fat SDS in	
25	males and females respectively.	

1	Figure 4 shows the association between 4C fat-free mass SDS and BIA SDS in each	
2	sex. The r^2 values from Table 8 indicate that height ² /Z accounts for 83 and 81% of the	
3	variance in 4C fat-free SDS in males and females respectively. Figure 4 also shows the	
4	association between 4C fat mass SDS and mean of 4 skinfold SDS in each sex. For each of	
5	the sum of four skinfolds, density, DXA indices and 4C fat mass, the r^2 values calculated	
6	from Table 6 indicate that each individual adiposity SDS accounts for 66-96% and 61-96% of	
7	the variance in the other SDS in males and females respectively.	
8		
9	Discussion	
10	Although reference data for children's body composition have long been desired, their	
11	development is complicated by the difficulty of obtaining accurate measurements. Advances	
12	in modelling, combining several raw measurements, have allowed accurate 4C data to be	
13	obtained in children \geq 4 years (31, 32). This approach is unlikely to be widely applied in	
14	clinical practice or research studies, due to its expense and requirement for sophisticated	
15	equipment. Several techniques are used more routinely, including skinfolds, DXA and BIA,	
16	however each method uses different approaches to convert raw measurements to final body	
17	composition values (37, 38)	
18	Over the past two decades, various pediatric body composition reference data have	
19	been reported, including skinfold data in Spain (19) and the US (18), BIA data for the US	
20	(20), Turkey (21) and Japan (22), and DXA data for Sweden (23), Holland (24) and the US	
21	(25, 39). These represent an advance over BMI, which can assess nutritional status but not fat	
22	and fat-free masses, or their regional distribution. However, because of the different	
23	theoretical assumptions and population variability in body size and nutritional status, these	
24	heterogeneous datasets cannot easily be compared. No study has previously reported	

reference data for a wide range of outcomes, which would allow future studies to benefit from
 converging on a common dataset, regardless of which technique was used.

3 We have attempted to resolve this problem, by developing reference charts and SDS 4 for both the accurate 4C model and a number of simpler techniques, across the age range 5-20 5 years. We have further described correlations between SDS calculated using the different 6 techniques, and have shown medium-to-high agreement in all cases. Thus, whether 7 measurements are made using skinfold calipers, DXA, BIA, densitometry, isotopes or the 4C 8 model, there is relatively good ranking consistency, although different techniques cannot be 9 used interchangeably when monitoring individuals over time. These new data will aid both 10 single assessments of children, and also longitudinal monitoring over time. They are suitable 11 for use in conditions in which there is no acute perturbation of water distribution (oedema).

12 From our clinical experience, children with specific diseases are often able to undergo 13 only a subset of body composition measurements. For example, many patients are too sick to 14 undergo plethysmography or DXA, but can have BIA or TBW measured at the bedside (40, 15 41). Some obese children are too large to be successfully scanned by DXA, and are difficult 16 to measure using skinfold calipers, but can undergo plethysmography (42). Where hydration 17 varies beyond the normal range, and where patients are able to undergo a wider range of 18 measurements, the 4C model is ideal, as we have demonstrated for obesity (29), acute 19 lymphoblastic leukaemia (30) and cystic fibrosis (43). Thus, our reference data should 20 substantially increase the capacity of clinicians to acquire and interpret data in a wide range of 21 diseases, contributing to a range of components of clinical management. For more general 22 community studies of nutritional status, TBW is the most accurate field method (32, 44), and 23 can be applied in combination with our published reference data for hydration (38). 24 Comparing between techniques for adiposity, the highest correlations with 4C fat mass

25 SDS were found for whole-body DXA fat mass SDS, with coefficients of 0.98 in females and

1	0.94 in males, and SEE of ~0.2 SDS. The next best technique was density SDS, while the	
2	individual skinfolds performed slightly less well (correlations ranging from 0.78 to 0.84 and	
3	SEE of ~0.6 SDS), but the average of the four skinfold SDS values had a correlation very	
4	similar to that of density in both sexes and SEE of ~0.4 SDS. For 4C fat-free mass SDS, DXA	
5	whole-body fat-free mass SDS likewise showed the highest correlations in both sexes, 0.98 in	
6	males and 0.97 in females, and SEE of ~0.2 SDS. Other outcomes also showed high ranking	
7	consistency, with the least successful being DXA arm fat-free mass SDS (correlations of 0.86	
8	in males and 0.88 in females and SEE of ~0.5 SDS). For both primary outcomes, therefore,	
9	DXA whole-body SDS proved most consistent for accurate ranking individuals against the	
10	reference method, explaining 88% to 96% of the variance in 4C SDS values.	
11	While absolute accuracy of DXA remains imperfect (42, 45, 46), its use for	
12	categorising relative fat and fat-free masses on the basis of whole body measurement	
13	therefore appears the best simpler option, if the 4C model is not available. Nevertheless,	
14	caution is required before extrapolating our findings to other DXA instrumentation. Pediatric	
15	cross-calibration studies have shown relatively good agreement between different machines	
16	from a single manufacturer (47, 48), but poorer agreement between different manufacturers'	
17	machines (49), and further research is required using other DXA instrumentation.	
18	Furthermore, for both DXA and other techniques, consistency between 4C SDS and other	
19	SDS was poorer for adiposity at the lower end of the scale, especially in males, whereas for	
20	fat-free mass, techniques ranked with consistency across the whole range of the outcome	
21	(Figures 3 and 4). Thus, even DXA is a poor option compared to the 4C model when	
22	attempting to rank adiposity in leaner individuals.	
23	Ideally, interpretation of body composition data requires adjustment for body size.	
24	This is particularly evident when children grow between two measurement occasions, but is	
25	also important if making a baseline assessment of patients who may have abnormal weight or	

1	height for their age. BMI represents the established index of weight adjusted for height in	
2	pediatric clinical practice. BMI may be divided into two components, the fat-free mass index	
3	and fat mass index. Each of these is adjusted for height, and unlike % fat, the fat mass index is	
4	not confounded by variability in fat-free mass and therefore represents a more objective index	
5	of adiposity (17, 50). However, it has also been shown that whilst fat-free mass scales with	
6	height ² , fat mass scales with height raised to a higher power, eg height ⁶ in 9 year-old children	
7	(50). There is currently uncertainty over how best to adjust pediatric body composition data	
8	for size (51), and our new reference data for 4C fat-free mass index and fat mass index	
9	therefore represent a pragmatic preliminary attempt, which we intend to address further in	
10	future work.	
11	A limitation of our study is that we are unable to extend the age range below 5 years.	
12	We have collected a large amount of isotope and skinfold data from 6 weeks to 4 years (52),	
13	however these were collected a decade earlier than those reported here, and there is a poor	
14	statistical fit between the two datasets, most likely due to differential exposure to obesogenic	
15	environmental factors. Many patients requiring body composition assessment are aged <5	
16	years, however further technical advances are required before our approach can be applied to	
17	this age range. A second limitation is that we were unable to include all possible techniques	
18	(eg MRI, TOBEC), or instrumentation. Our Lunar DXA data may not be appropriate for other	
19	manufacturers' instrumentation, while our BIA data were collected using standing	
20	instrumentation in combination with foot-plates and hand-grips, and hence will not be entirely	
21	consistent with data collected from supine individuals using adhesive electrodes. However,	
22	standing BIA removes a degree of inter-observer error, as it avoids the need to place	
23	electrodes on anatomical landmarks. A third limitation is that ethnic variability in our sample	
24	was not adequate to allow us to explore this issue in our analysis.	

1	In summary, we have described the measurements available in our new reference	
2	dataset and provided examples of how the data can be presented; there are many alternative	
3	formats and a large amount of additional data are available from each of the two-component	
4	techniques. We anticipate that the most appropriate use of the reference data will vary in	
5	clinical and research settings. To facilitate their use by clinicians and researchers, we intend to	
6	make the data available through an internet portal, allowing individual raw data for each	
7	technique to be entered with age and sex data, to calculate SDS (7). The graphs will also be	
8	available for download. While for some purposes (eg evaluating cardiovascular risk) BMI	
9	SDS remains adequate for differentiating clinical status (53,54), growth charts that allow	
10	partitioning of weight into its fat and fat-free components are likely to be valuable for	
11	monitoring more immediate effects of disease and response to treatment (16).	
12		
13	Acknowledgements	
14	The study was designed by JCKW, MSF and TJC. Raw data were collected by JEW, DH, SC	
15	and CGE, and modelled by JEW. All DXA scans were undertaken by CW. Mass	
16	spectrometric analysis was undertaken by TD and SE. LMS analyses were undertaken by	
17	JCKW and CGE under the supervision of TJC. Statistical analyses was conducted by JCKW.	
18	JCKW wrote the first draft of the manuscript, and all authors contributed to subsequent	
19	revisions. All authors declare no conflict of interest.	

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	Males (n = 2	61)		Females (n = 272)					
	Mean	SD	Range	Mean	SD	Range			
Weight SDS	0.31	1.08	-2.42, 3.44	0.42	1.10	-2.75, 3.46			
Height SDS	0.21	0.96	-2.09, 3.28	0.34	1.02	-2.77, 3.42			
BMI SDS	0.24	1.15	-2.99, 3.49	0.31	1.15	-3.33, 3.32			
% fat (4C model)*	19.2	8.0	4.9, 45.5	27.0	8.0	11.7, 46.9			
	Prevalence			Prevalence					
Overweight (%)	9.2			10.7					
Obese (%)	11.5			14.7					

Table 1. Summary statistics for anthropometry and weight status by sex

* % fat different between sexes p<0.0001, using multiple regression analysis to assess significance of female gender adjusting for age.
SD – standard deviation. SDS – standard deviation scores calculated using UK reference data (6,25). Overweight categorised as BMI SDS
> 1.04 (85th centile). Obese categorised as BMI SDS > 1.64 (95th centile).

Age				Males						F	emales			
(y)	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0
5.0	12.76	13.89	15.08	16.35	17.70	19.13	20.64	11.24	12.27	13.39	14.60	15.91	17.33	18.86
6.0	14.10	15.37	16.72	18.16	19.69	21.31	23.03	12.83	14.02	15.30	16.70	18.21	19.85	21.62
7.0	15.32	16.73	18.23	19.83	21.54	23.34	25.26	14.49	15.84	17.31	18.91	20.64	22.51	24.55
8.0	16.56	18.12	19.79	21.56	23.45	25.45	27.58	16.23	17.77	19.45	21.26	23.24	25.38	27.71
9.0	18.02	19.76	21.62	23.61	25.73	27.98	30.38	17.88	19.61	21.49	23.54	25.77	28.20	30.83
10.0	19.66	21.62	23.72	25.98	28.38	30.95	33.68	19.56	21.49	23.60	25.89	28.39	31.12	34.08
11.0	21.60	23.85	26.28	28.88	31.67	34.64	37.83	21.62	23.79	26.16	28.74	31.56	34.63	37.98
12.0	24.06	26.71	29.56	32.63	35.94	39.48	43.27	24.23	26.66	29.31	32.21	35.37	38.82	42.58
13.0	27.24	30.36	33.75	37.41	41.35	45.59	50.14	27.06	29.71	32.61	35.76	39.19	42.93	47.00
14.0	31.09	34.72	38.65	42.91	47.50	52.44	57.75	29.67	32.46	35.49	38.77	42.34	46.21	50.41
15.0	35.03	39.06	43.41	48.12	53.19	58.64	64.50	31.62	34.46	37.54	40.87	44.48	48.38	52.59
16.0	38.55	42.82	47.43	52.39	57.73	63.46	69.59	32.81	35.67	38.76	42.10	45.70	49.59	53.78
17.0	41.35	45.74	50.47	55.55	60.99	66.82	73.05	33.46	36.32	39.41	42.75	46.34	50.21	54.38
18.0	43.45	47.90	52.68	57.80	63.28	69.13	75.38	33.71	36.57	39.67	43.00	46.58	50.45	54.61
19.0	45.02	49.50	54.30	59.43	64.92	70.77	77.00	33.75	36.62	39.71	43.04	46.63	50.49	54.65
20.0	46.24	50.73	55.54	60.68	66.17	72.01	78.22	33.86	36.73	39.82	43.15	46.73	50.59	54.75

Table 2. Fat-free mass reference data for males	and females, by z-score or percentile
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Z-score equivalents in centiles as follows: -2 = 2.3%; -1.67 = 9.2%; -1.33 = 25.2%; 0 = 50%; 1.33 = 74.8%; 1.67 = 90.8%; 2 = 97.7%

<u>Age</u>				<u>Males</u>						E	emales			
(y)	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0
5.0	1.37	1.77	2.32	3.11	4.26	6.00	8.72	1.60	2.12	2.87	3.97	5.59	8.09	12.06
6.0	1.61	2.09	2.76	3.71	5.11	7.25	10.62	2.02	2.67	3.60	4.95	6.95	9.99	14.78
7.0	1.85	2.40	3.18	4.31	5.97	8.52	12.60	2.45	3.23	4.34	5.94	8.29	11.85	17.41
8.0	2.08	2.71	3.61	4.91	6.84	9.84	14.66	2.89	3.80	5.09	6.92	9.61	13.66	19.94
9.0	2.30	3.01	4.03	5.50	7.72	11.18	16.81	3.34	4.38	5.84	7.91	10.93	15.44	22.37
10.0	2.52	3.31	4.44	6.10	8.61	12.56	19.04	3.81	4.97	6.59	8.89	12.23	17.17	24.72
11.0	2.73	3.60	4.85	6.70	9.51	13.97	21.37	4.28	5.57	7.36	9.88	13.51	18.86	26.97
12.0	2.93	3.89	5.26	7.30	10.42	15.42	23.80	4.76	6.18	8.13	10.86	14.78	20.52	29.15
13.0	3.13	4.16	5.66	7.89	11.34	16.90	26.33	5.26	6.80	8.91	11.85	16.04	22.14	31.23
14.0	3.32	4.44	6.06	8.49	12.28	18.43	28.96	5.77	7.43	9.69	12.84	17.29	23.72	33.24
15.0	3.51	4.71	6.45	9.09	13.22	19.99	31.70	6.29	8.07	10.48	13.82	18.52	25.26	35.17
16.0	3.70	4.97	6.84	9.69	14.18	21.59	34.55	6.82	8.72	11.28	14.81	19.74	26.77	37.03
17.0	3.87	5.23	7.23	10.28	15.14	23.23	37.53	7.37	9.38	12.09	15.79	20.94	28.24	38.82
18.0	4.05	5.48	7.61	10.88	16.12	24.92	40.62	7.93	10.05	12.90	16.78	22.14	29.68	40.53
19.0	4.21	5.73	7.99	11.48	17.11	26.64	43.85	8.50	10.74	13.72	17.76	23.32	31.09	42.18
20.0	4.38	5.97	8.36	12.08	18.11	28.41	47.21	9.08	11.43	14.55	18.75	24.49	32.46	43.76

Table 3. Fat mass reference data for males and females, by z-score or percentile

Z-score equivalents in centiles as follows: -2 = 2.3%; -1.67 = 9.2%; -1.33 = 25.2%; 0 = 50%; 1.33 = 74.8%; 1.67 = 90.8%; 2 = 97.7%

<u>Age</u>				<u>Males</u>			<u>Females</u>								
(y)	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0	
5.0	11.59	12.13	12.68	13.25	13.84	14.43	15.04	10.75	11.16	11.61	12.11	12.68	13.33	14.07	
6.0	11.59	12.13	12.69	13.27	13.87	14.49	15.14	10.88	11.32	11.81	12.35	12.94	13.59	14.33	
7.0	11.50	12.03	12.59	13.17	13.79	14.43	15.11	10.98	11.47	12.00	12.57	13.20	13.89	14.64	
8.0	11.43	11.95	12.50	13.09	13.72	14.39	15.10	11.05	11.59	12.18	12.81	13.49	14.22	15.03	
9.0	11.48	11.99	12.55	13.15	13.79	14.49	15.25	11.10	11.69	12.33	13.01	13.76	14.56	15.43	
10.0	11.61	12.15	12.72	13.35	14.03	14.78	15.59	11.16	11.80	12.49	13.24	14.05	14.93	15.89	
11.0	11.80	12.38	13.02	13.71	14.48	15.32	16.27	11.31	12.00	12.75	13.56	14.44	15.40	16.45	
12.0	12.05	12.71	13.44	14.24	15.14	16.15	17.28	11.57	12.29	13.09	13.95	14.91	15.95	17.11	
13.0	12.45	13.19	14.02	14.95	15.98	17.15	18.49	11.89	12.64	13.47	14.39	15.40	16.52	17.77	
14.0	12.98	13.81	14.74	15.76	16.91	18.20	19.65	12.24	13.01	13.85	14.80	15.84	17.01	18.33	
15.0	13.55	14.46	15.46	16.56	17.78	19.13	20.63	12.57	13.33	14.18	15.12	16.18	17.37	18.72	
16.0	14.07	15.06	16.12	17.28	18.53	19.88	21.36	12.84	13.58	14.41	15.33	16.37	17.56	18.91	
17.0	14.51	15.57	16.69	17.87	19.13	20.45	21.86	13.03	13.75	14.54	15.43	16.44	17.60	18.93	
18.0	14.87	15.99	17.15	18.36	19.60	20.88	22.19	13.18	13.85	14.60	15.45	16.41	17.52	18.81	
19.0	15.14	16.33	17.53	18.74	19.96	21.19	22.42	13.28	13.91	14.61	15.40	16.31	17.37	18.60	
20.0	15.34	16.60	17.83	19.05	20.24	21.41	22.57	13.35	13.93	14.58	15.32	16.18	17.17	18.35	

Table 4. Fat-free mass index reference data for males and females, by z-score or percentile

Z-score equivalents in centiles as follows: -2 = 2.3%; -1.67 = 9.2%; -1.33 = 25.2%; 0 = 50%; 1.33 = 74.8%; 1.67 = 90.8%; 2 = 97.7%

<u>Age</u>				<u>Males</u>		Females								
(y)	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0	Z=-2.0	Z=-1.33	Z=-0.67	Z=0	Z=0.67	Z=1.33	Z=2.0
5.0	1.41	1.79	2.26	2.84	3.56	4.45	5.53	1.59	1.99	2.53	3.30	4.42	6.14	8.90
6.0	1.24	1.60	2.06	2.65	3.38	4.32	5.49	1.67	2.11	2.70	3.54	4.77	6.63	9.58
7.0	1.11	1.44	1.88	2.45	3.20	4.17	5.45	1.75	2.22	2.86	3.77	5.10	7.10	10.24
8.0	1.12	1.46	1.92	2.54	3.38	4.52	6.10	1.83	2.33	3.03	4.01	5.43	7.56	10.87
9.0	1.29	1.69	2.23	2.98	4.03	5.55	7.76	1.91	2.45	3.19	4.24	5.75	8.00	11.47
10.0	1.45	1.89	2.50	3.37	4.66	6.59	9.61	1.98	2.56	3.35	4.46	6.06	8.42	12.00
11.0	1.51	1.95	2.59	3.52	4.93	7.17	10.91	2.07	2.68	3.51	4.68	6.35	8.79	12.44
12.0	1.45	1.88	2.49	3.40	4.83	7.20	11.43	2.16	2.80	3.68	4.90	6.63	9.12	12.80
13.0	1.35	1.75	2.32	3.18	4.57	6.96	11.49	2.26	2.93	3.85	5.11	6.88	9.41	13.07
14.0	1.28	1.65	2.19	3.02	4.37	6.76	11.54	2.36	3.06	4.01	5.32	7.12	9.65	13.26
15.0	1.23	1.59	2.10	2.90	4.23	6.61	11.54	2.47	3.20	4.18	5.51	7.33	9.85	13.38
16.0	1.20	1.55	2.06	2.84	4.15	6.54	11.53	2.59	3.34	4.35	5.70	7.52	10.01	13.43
17.0	1.22	1.58	2.10	2.90	4.24	6.69	11.84	2.71	3.49	4.51	5.88	7.69	10.13	13.43
18.0	1.32	1.70	2.27	3.14	4.60	7.25	12.78	2.84	3.64	4.68	6.05	7.84	10.22	13.38
19.0	1.48	1.92	2.56	3.55	5.19	8.17	14.31	2.98	3.79	4.85	6.21	7.98	10.29	13.31
20.0	1.67	2.16	2.89	4.01	5.87	9.21	16.01	3.12	3.95	5.01	6.37	8.11	10.35	13.22

Table 5. Fat mass index reference data for males and females, by z-score or percentile

Z-score equivalents in centiles as follows: -2 = 2.3%; -1.67 = 9.2%; -1.33 = 25.2%; 0 = 50%; 1.33 = 74.8%; 1.67 = 90.8%; 2 = 97.7%

Males:	Biceps	Triceps	Subscap	Suprailiac	Mean	Density	4C fat	DXA fat	DXA arm	DXA leg	DXA
Females:					skinfold		mass	mass	fat	fat	trunk fat
Biceps	-	0.86	0.78	0.76	0.92	-0.79	0.84	0.82	0.87	0.81	0.79
Triceps	0.82	-	0.74	0.82	0.93	-0.82	0.83	0.83	0.86	0.81	0.81
Subscapular	0.68	0.78	-	0.78	0.90	-0.75	0.81	0.84	0.82	0.81	0.83
Suprailiac	0.69	0.80	0.79	-	0.92	-0.82	0.83	0.84	0.83	0.81	0.85
Mean skinfold	0.88	0.94	0.90	0.91	-	-0.87	0.90	0.91	0.92	0.88	0.89
Density	-0.77	-0.82	-0.76	-0.81	-0.88	-	-0.90	-0.87	-0.86	-0.83	-0.86
4C fat mass	0.78	0.84	0.80	0.80	0.89	-0.93	-	0.94	0.92	0.93	0.94
DXA fat mass	0.77	0.84	0.81	0.79	0.89	-0.88	0.98	-	0.95	0.97	0.98
DXA arm fat	0.81	0.87	0.80	0.79	0.91	-0.88	0.95	0.97	-	0.92	0.94
DXA leg fat	0.75	0.82	0.75	0.75	0.85	-0.86	0.95	0.97	0.93	-	0.93
DXA trunk fat	0.75	0.82	0.82	0.80	0.88	-0.87	0.96	0.98	0.95	0.93	-

Table 6. Correlations for adiposity outcomes expressed in standard deviation score (SDS) format

Pearson correlation coefficients: male values above diagonal, female values below diagonal. All correlations significant p<0.0001

N = 245 males, 259 females

Table 7. Intercepts, slopes and standard errors of the estimate for the regression of 4C fat mass SDS on individual adiposity

SDS

Predictor	Males				Females				
	Intercept	SE Slop	e SE	SEE	Intercept	SE	Slope	SE	SEE
Biceps	0.044	0.036 0.84	0 0.036	0.55	0.032	0.040	0.802	0.041	0.63
Triceps	0.027	0.036 0.83	6 0.036	0.56	0.028	0.034	0.856	0.035	0.54
Subscapular	0.046	0.038 0.82	1 0.038	0.58	0.032	0.038	0.828	0.039	0.60
Suprailiac	0.037	0.037 0.86	2 0.038	0.58	0.037	0.039	0.820	0.039	0.61
Mean skinfold	0.038	0.026 0.83	7 0.026	0.40	0.028	0.027	0.826	0.027	0.41
Density	-0.017	0.028 -0.89	2 0.027	0.43	0.021	0.024	-0.927	7 0.024	0.38
DXA fat mass	0.018	0.021 0.94	1 0.021	0.33	0.010	0.013	0.979	0.013	0.21
DXA arm fat	0.002	0.025 0.92	6 0.025	0.39	0.017	0.018	0.946	0.018	0.30
DXA leg fat	0.006	0.024 0.93	1 0.024	0.37	-0.002	0.019	0.951	0.019	0.30
DXA trunk fat	0.010	0.023 0.92	3 0.022	0.35	-0.0.004	0.017	0.962	0.017	0.27

4C fat mass SDS regressed on each individual adiposity SDS

All slopes significantly different from 1 (p<0.05) except for DXA fat mass in girls

- SE standard error; SEE standard error of the estimate
- N = 245 males, 259 females

Males:	Total body	4C FFM	DXA <mark>FFM</mark>	DXA arm <mark>FFM</mark>	DXA leg <mark>FFM</mark>	DXA trunk	Height ² /Z
Females:	water					FFM	
Total body water	-	0.98	0.95	0.84	0.92	0.91	0.90
4C FFM	0.99	-	0.98	0.86	0.95	0.94	0.91
DXA <mark>FFM</mark>	0.96	0.97	-	0.88	0.96	0.97	0.91
DXA arm <mark>FFM</mark>	0.86	0.88	0.89	-	0.83	0.80	0.86
DXA leg <mark>FFM</mark>	0.94	0.95	0.96	0.84	-	0.87	0.89
DXA trunk <mark>FFM</mark>	0.91	0.93	0.97	0.82	0.87	-	0.86
Height ² /Z	0.90	0.90	0.90	0.84	0.87	0.86	-

Table 8. Correlations for fat-free mass outcomes expressed in standard deviation score (SDS) format

 $Pears on \ correlation \ coefficients: \ male \ values \ above \ diagonal, \ female \ values \ below \ diagonal. \ All \ correlations \ significant \ p<0.0001$

N = 245 males (except BIA, n = 195), 259 females (except BIA, n = 227)

FFM – fat free mass

Table 9. Intercepts, slopes and standard errors of the estimate for the regression of 4C fat-free mass SDS on individual fat-freemass SDS

Predictor	Males				Females				
	Intercept	SE Slop	e SE	SEE	Intercept	SE	Slope	SE	SEE
Total body water	0.009	0.013 0.98	5 0.013	0.20	-0.002	0.009	0.987	0.009	0.15
DXA <mark>FFM</mark>	0.002	0.013 0.97	3 0.013	0.21	-0.006	0.014	0.977	0.014	0.23
DXA arm <mark>FFM</mark>	0.003	0.033 0.86	0.032	0.51	-0.014	0.030	0.881	0.030	0.48
DXA leg <mark>FFM</mark>	0.000	0.021 0.94	7 0.021	0.33	-0.007	0.020	0.949	0.020	0.32
DXA trunk <mark>FFM</mark>	0.002	0.022 0.92	0 0.022	0.34	-0.004	0.023	0.933	0.023	0.37
Height ² /Z	-0.012	0.030 0.92	1 0.030	0.42	-0.012	0.029	0.901	0.029	0.44

4C fat-free mass SDS regressed on each individual fat-free mass SDS

All slopes significantly different from 1 (p<0.05) except TBW in both sexes and DXA fat-free mass in both sexes

SE – standard error; SEE – standard error of the estimate; FFM – fat-free mass

N = 245 males (except BIA, n = 195), 259 females (except BIA, n = 227)

Legends for illustrations

Figure 1. Distribution of body mass index standard deviation score (BMI SDS) against age in the sample. N = 261 males and 272 females.

Figure 2. Centiles for fat-free mass, fat mass, fat-free mass index and fat mass index by the 4-component model for males (n = 261, left hand panel) and females (n = 272, right hand panel). The 2nd, 9th, 25th, 50th, 75th, 91st and 98th centiles are displayed.

Figure 3. Bland Altman plots illustrating agreement between DXA fat-free mass SDS and 4C fat-free mass SDS (upper panel), and DXA fat SDS and 4C fat mass SDS (lower panel), in males (n = 245, left hand side) and females (n = 259, right hand side). The scatter plot shows agreement between techniques in individuals, the dotted lines show mean bias and the limits of agreement (±2 standard deviations of the bias).

Figure 4. Bland Altman plots illustrating agreement between Height²/Z SDS and DXA fat-free mass SDS (upper panel), and the average of 4 skinfold SDS and 4C fat mass SDS (lower panel), in males (n = 195 for BIA and 245 for skinfolds, left hand side) and females (n = 227 for BIA and 259 for skinfolds, right hand side). The scatter plot shows agreement between techniques in individuals, the dotted lines show mean bias and the limits of agreement (±2 standard deviations of the bias).





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