

Evaluation of lean tissue density for use in air displacement plethysmography in obese children and adolescents

Jonathan CK Wells [1], Dalia Haroun [1], Jane E Williams [1], Tegan Darch [1], Simon Eaton [2], Russell Viner [3], Mary S Fewtrell [1].

[1] Childhood Nutrition Research Centre, [2] Department of Surgery and [3] [2] General and Adolescent Paediatrics Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

Author and address for correspondence

Jonathan Wells [1]

J.Wells@ich.ucl.ac.uk

Tel +44 207 905 2104

Fax + 44 207 831 9903

Running title: **Air displacement plethysmography in obese children**

Funding: This study used core funding from the UK Medical Research Council. Dalia Haroun was funded by the Child Growth Foundation and the British Heart Foundation

1 **Abstract**

2 **Background:** Body composition techniques are required for monitoring response to treatment
3 in individual obese children, and assessing the efficacy of weight loss programmes.
4 Densitometry is readily undertaken, using air-displacement plethysmography (ADP), but
5 requires appropriate information on the density of lean tissue (D_{LT}).

6 **Aims:** To develop predictive equations for D_{LT} in obese children and adolescents, and to test
7 the accuracy of ADP when using such predicted D_{LT} values in an independent longitudinal
8 sample, using the four-component model as the reference method.

9 **Methods:** Equations for the prediction of D_{LT} from age, gender and body mass index standard
10 deviation score were developed in 105 children (39 boys). Accuracy of ADP, when
11 incorporating predicted D_{LT} values, was tested for baseline body composition and its change
12 over time in a separate sample of 51 children (20 boys).

13 **Results:** The predictive equation explained 33% of the variance in D_{LT} . Fat mass obtained
14 from ADP using such predicted values had a mean (SD) bias of 0.32 (1.39) kg, ns, while
15 change in fat mass had an error of -0.25 (1.38) kg, ns. Hydration was strongly correlated with
16 D_{LT} .

17 **Conclusions:** Use of ADP with predicted D_{LT} values was associated with non-significant bias
18 when estimating fat mass and its change over time. This study aids the application of ADP in
19 childhood obesity research and clinical practice. The limits of agreement (± 2.8 kg) relative to
20 4C values are moderately better than those for DXA (± 3.2 kg). Further improvement to
21 accuracy would require assessment of lean tissue hydration, by bioelectrical impedance.

22

23 **Keywords:** Body composition; densitometry; fat mass; air-displacement plethysmography;
24 childhood obesity

25

26

1 **Introduction**

2 There is growing interest in the measurement of body composition in obese children
3 and adolescents (Wells and Fewtrell 2008). In clinical practice, body composition assessment
4 may aid assess excess adiposity in individual patients, and monitor their response to
5 treatment. In research studies, accurate methods are required for assessing the efficacy of
6 weight loss interventions. In both contexts, body composition may aid understand how both
7 diseases, and their management, impact on cardiovascular and metabolic risk (Wells and
8 Fewtrell 2008). Such assessment is particularly important in obese children, who are now
9 known to have increased cardiovascular risk compared to non-obese children both in
10 childhood (Reilly et al. 2003), and also in adulthood due to the tendency of their excess
11 weight to persist into later life (Dietz 1998; Lloyd et al. 2010).

12 Childhood obesity remains categorised on the basis of standard deviation scores (SDS)
13 of body mass index (BMI) (Cole et al. 2000; Cole et al. 1995). Whilst international obesity
14 cut-off values are used to monitor prevalence across populations, clinical treatment is
15 typically applied on the basis of national cut-offs, as the 95th centile for the UK 1990 data
16 identifies more children as obese than does the international cut-offs. BMI is easy, cheap and
17 quick to obtain. However, numerous research studies have identified ethnic variability in the
18 association between BMI and body fat content in both adults and children (Deurenberg et al.
19 2002; Deurenberg et al. 1998; Ehtisham et al. 2005; Haines et al. 2007). This means that BMI
20 does not index individual body components equally across ethnic groups. Furthermore, there
21 is substantial variability within populations in fat content for a given BMI value for children
22 of any given age and sex (Wells 2000; Wells et al. 2006), so that again, BMI is not
23 appropriate for comparing adiposity. Finally, BMI is particularly poor for assessing the
24 consequences of factors acting in early life, as weight gain in different periods may act on
25 either fat or lean masses (Wells et al. 2007), yet BMI cannot differentiate these effects. For

1 these reasons, accurate technologies capable of direct measurement of body fat content are
2 required.

3 A number of so-called "two component" body composition techniques differentiate
4 the fat and lean components of body weight. For example, isotope dilution quantifies total
5 body water which can be used to predict lean mass (used here synonymously with fat-free
6 mass), on the assumption of a known water content of lean tissue for any given age and sex
7 (Schoeller 1996). Densitometry quantifies the proportion of fat in weight, on the assumption
8 that fat and lean tissue likewise have known densities for age and sex (Lohman 1986; Siri
9 1961) as described in greater detail below. However, we have shown that the composition of
10 lean tissue differs between those obese versus normal weight, confounding the conversion of
11 raw data on body water or density into body composition values in those obese (Haroun et al.
12 2005; Wells et al. 2006). Dual energy X-ray absorptiometry (DXA) differentiates fat and lean
13 mass on the basis of differential x-ray attenuation (Mazess et al. 1970). However, several
14 studies have shown that the accuracy of DXA varies according to tissue depth, with fat mass
15 overestimated in fatter individuals (Sopher et al. 2004; Wells et al. 2010a; Williams et al.
16 2006), which indicates the need to improve other techniques as alternatives.

17 In practical terms, densitometry is relatively easy to apply to those obese (Ginde et al.
18 2005; Haroun et al. 2005; Petroni et al. 2003) and this is important because some techniques
19 such as DXA or MRI are unable to accommodate children of very large body size (Wells et
20 al. 2010a). Whole body air-displacement plethysmography (ADP), in the form of the Bodpod
21 (Life Measurements Inc, Concord, CA), has now been used extensively in children and
22 adolescents and has excellent acceptability. Validation studies have also shown good accuracy
23 for measurement of body volume, and hence body density, in relation to underwater weighing
24 in children (Dewit et al. 2000; Nunez et al. 1999). In a study of obese adults, no significant
25 bias between these techniques was observed (Ginde et al. 2005).

1 However, whilst this shows that it is possible to measure body volume and body
2 density with acceptable accuracy, it does not demonstrate accuracy of either technique for
3 assessing body fatness itself. Archimedes principle, which is used to estimate body fat content
4 from whole-body density, requires information on the specific densities of fat and lean tissue.
5 If inappropriate values for lean tissue density (D_{LT}) are used, densitometry will generate
6 significant bias. Our studies reporting reduced D_{LT} in obese children compared to children of
7 normal weight (Haroun et al. 2005; Wells et al. 2006) indicate that further work is required to
8 identify appropriate D_{LT} values for obese children, in order to generate accurate data on body
9 composition from densitometry.

10 We therefore investigated this issue in obese children and adolescents. We developed
11 predictive equations for the density of lean tissue (D_{LT}) measured by the gold standard four
12 component (4C) model, using as predictors age, gender and BMI SDS. This approach was
13 intended to establish whether information readily available in the clinic could be used to
14 predict D_{LT} values tailored to individual obese children, in order to avoid the need to rely on
15 age- and sex-constant values obtained from non-obese children. We then tested these
16 equations by applying them in plethysmography as a two-component (2C) model in an
17 independent longitudinal sample, again using the 4C model as the reference against which the
18 accuracy of our new 2C approach could be assessed.

20 **Methods**

21 Data on children and adolescents were obtained from two sources: a study of reference
22 data on children's body composition, and cross-sectional and longitudinal studies of childhood
23 obesity. The cut-off for obesity was the 95th centile of UK 1990 reference data (Cole et al.
24 1995), as used in UK clinical practice, equivalent to BMI SDS of 1.64. Ethical approval was
25 granted by the Ethical Committee of Great Ormond Street Hospital.

1 Anthropometry was undertaken in duplicate using standard protocols. Weight was
2 measured using the scales integral to the ADP instrumentation. Height was measured using a
3 wall-mounted stadiometer. BMI was calculated, and converted to SDS using 1990 UK
4 reference data (Freeman et al. 1995). Fat mass and lean mass were calculated using the 4C
5 model, as described in detail previously (Wells et al. 1999; Williams et al. 2006). Briefly,
6 measurements of total body water by deuterium dilution, bone mineral content by dual energy
7 X-ray absorptiometry (DXA: Lunar Prodigy instrumentation; GE Medical Systems, Madison,
8 WI) and body volume (in duplicate) by air-displacement plethysmography (ADP: Bodpod
9 instrumentation, Life Measurements Inc., Concord, CA) were obtained as described
10 previously. For ADP, lung volume was predicted using children's equations (Dewit et al.
11 2000). Precision of total body water in our laboratory is ~1% (Wells et al. 1999). Precision of
12 body volume from duplicate measurements in obese children and adolescents was 0.24 litres.
13 Precision of bone mineral content, according to published data for Lunar instrumentation, is
14 1.1% for bone mineral content (Kiebzak et al. 2000).

15

16 Calculations

17 A schematic diagram illustrating the concept of Archimedes principle for densitometry
18 is given in **Figure 1**. The left hand panel illustrates the general principle, whereby total body
19 density is used to predict body fat content. The right hand panel illustrates the changing
20 values that are obtained for % fat, holding constant the density of fat but altering the density
21 of lean tissue. Thus, inappropriate values for lean tissue density generate systematic
22 inaccuracy in body composition outcomes. Lean tissue density must therefore be established
23 empirically, and we have recently published such reference data for normal healthy children
24 (Wells et al. 2010b), whilst also noting variability of this tissue property with nutritional
25 status (Haroun et al. 2005; Wells et al. 2006).

1 For any given pair of density values for lean tissue (d_1) and fat (d_2), it is possible to
2 revise the classic equation of Siri (Siri 1961) which states:

$$\% \text{ fat} = (k_1/D - k_2) * 100 \quad \text{Equation 1}$$

3
4
5
6 where D is whole body density obtained from densitometry, and k_1 and k_2 are age- and sex-
7 specific constants. Values for k_1 are calculated as $[(d_1 * d_2)/(d_1 - d_2)]$, while values for k_2 are
8 calculated as $[d_2/(d_1 - d_2)]$ (Lohman 1986). The value of d_2 was taken to be 0.9007 at all ages,
9 while values for d_1 can either be taken from the literature, using the age- and sex-specific
10 values of Lohman (Lohman 1986), or they can be obtained empirically from the 4-component
11 model, as in this study (see below). In each case, values for %fat obtained from equation 1 are
12 combined with weight to calculate total body fat mass and lean mass.

13 14 **Statistics**

15 Our first aim was to demonstrate any limitations of the conventional 2C approach, by
16 comparing our measured D_{LT} values, and the %fat values resulting, with corresponding values
17 from the literature published by Lohman (1986). We tested agreement between the literature
18 2C values and empirical 4C values in a cross-sectional data set of 106 children, using the
19 method of Bland and Altman (Bland and Altman 1986).

20 Having ascertained the magnitude of this 2C literature bias, our second aim was derive
21 equations whereby we might predict D_{LT} from three variables readily available in the clinic
22 (age, sex and BMI SDS). Ideally, this approach would allow us to develop a 2C densitometric
23 approach specifically for obese children. We undertook this using multiple regression analysis
24 in the same cross-sectional data set, using our measured 4C D_{LT} values as the dependent
25 variable, and the three predictors as the independent variables.

1 Our third aim was to test the agreement between our new predicted D_{LT} values and the
2 resulting %fat values, versus empirical D_{LT} and % fat values obtained from the 4C model in a
3 second independent sample of obese children, in who we had data both on baseline fatness,
4 and change in fatness following obesity treatment. Again, we used the Bland and Altman
5 method to compare agreement in actual 4C fatness, or change in 4C fatness, versus 2C
6 predicted fatness, or change in 2C fatness, respectively.

7 Finally, we investigated the association between hydration of lean tissue and D_{LT} .
8 Theoretically, there is a strong inverse correlation between these variables, as increasing the
9 water content must decrease the density. Potentially, if D_{LT} cannot be accurately predicted
10 directly from variables such as age, sex and BMI SDS, an alternative approach might be to
11 measure hydration using instrumentation such as bio-electrical impedance analysis, and then
12 predict D_{LT} from these hydration values. This approach would thus require information on the
13 empirical association between hydration and D_{LT} . We explored the empirical association
14 between D_{LT} and hydration using regression analysis, both with or without adjustment for the
15 other predictors, in order to ascertain what magnitude of variance in D_{LT} is accounted for by
16 hydration. We determined the slope of the relationship whereby changes in hydration alter
17 D_{LT} . We further considered whether this relationship differed significantly between the sexes,
18 or whether a single equation for both sexes was adequate.

20 Results

21 A total of 106 children and adolescents (39 boys and 66 girls), ranging in age from 5
22 to 21 years, were available for the cross-sectional analysis. Of these, 59 were recruited into
23 our study of reference body composition and were not being treated as patients, while the
24 remaining 46 were recruited through obesity clinics and were being treated by behavioural
25 therapy (n=36) or the drug metformin (n=10). The sample was categorised as 76 white

1 European, 7 Asian, 12 black African or Afro-Caribbean, and 10 'other'. **Table 1** presents data
2 on their anthropometric characteristics, measurements of body volume, water and mineral,
3 and values from the 4C model. Boys had significantly greater values for total body water and
4 the hydration of lean tissue, and had significantly lower values for D_{LT} . No child was too
5 large for the ADP instrumentation (the maximum body volume was 114 litres).

6 **Table 2** presents correlations in this sample between D_{LT} and age, BMI SDS and
7 gender. Age was significantly correlated with D_{LT} in both sexes, whilst BMI SDS was
8 negatively correlated. The table also presents results of the regression of D_{LT} on age, gender
9 and BMI SDS. Considering the sexes together, the model explained 34% of the variance in
10 D_{LT} . In boys, the combination of age and BMI SDS explained 44% of variance, whereas in
11 girls, these two variables explained only 19% of variance, due to the lack of statistical
12 significance of the BMI SDS term. However, use of the two sex-specific equations was
13 considered preferable, as the coefficient for BMI differed between boys and girls. **Thus, these**
14 **regressions show the ability of simple variables to predict DLT in obese children, allowing**
15 **application of a 2C densitometric model.**

16 **Table 3** shows the bias in fat mass obtained when using the Lohman values for D_{LT} ,
17 relative to our own values measured by the 4C model, **in this cross-sectional sample**. In the
18 whole sample, the Lohman values resulted in an underestimation of fat mass by 0.7 kg
19 ($P=0.001$), with limits of agreement of ± 4.1 kg (**Figure 2**). Considering the sexes separately,
20 the bias was significantly worse in the girls compared to the boys ($p<0.05$). Although there
21 was one major outlier in the girls' data, removing this data point did not decrease the sex
22 difference in the bias substantially. In the boys, the bias in fat mass increased in proportion to
23 fat mass ($r = 0.44$, $p = 0.005$), but this was not the case for girls, or for either sex for D_{LT} .
24 **Thus, using literature values in a 2C densitometric model in obese children does not provide**
25 **accurate body composition values.**

1 The longitudinal sample of 20 boys and 35 girls were 11.0 (SD 2.5) years old (age
2 range 6 to 19 years), and had mean (SD) SDS of 2.9 (0.8) for weight, 1.2 (SD 1.2) for height,
3 and 3.0 (0.6) for BMI. The sample was categorised as 32 white European, 7 Asian, 8 black
4 African or Afro-Caribbean, and 8 'other'. As in the cross-sectional sample, boys had
5 significantly greater hydration of lean tissue (boys: 77.6 (SD 2.4)%; girls: 76.6 (SD 1.9)%;
6 $p < 0.05$), and lower D_{LT} (boys: 1.0845 (SD 0.0080) kg/l; girls: 1.0881 (SD 0.0121) kg/l;
7 $p < 0.05$) than girls. The average duration over which body composition change was assessed
8 was 0.56 years (SD 0.16, range 0.36 to 1.37 years). The change in weight averaged 2.3 kg
9 (SD 3.9, range -8.2 to 13.0 kg).

10 **Table 3** also presents data on the bias both in baseline body composition, and its
11 change over time, in the longitudinal sample, comparing values using our new D_{LT} prediction
12 equation against those obtained using the 4C model. There was a small but significant bias in
13 predicted baseline D_{LT} in girls ($p = 0.041$), but not in boys or in the whole sample. When
14 extrapolated to fat mass, no significant bias was detected in either sex or the whole sample,
15 though the bias in girls was almost significant ($p = 0.067$). The limits of agreement for baseline
16 fat mass were similar in both boys and girls, at ± 2.8 kg. When change over time was
17 addressed, there was no significant bias in either density of lean tissue, or fat mass (**Figure 3**),
18 either in the entire sample or in either sex. These data indicate that our new approach resolves
19 the systematic bias that occurs when literature values for D_{LT} are used. However, the limits of
20 agreement of 2.8 kg suggest accuracy is not excellent in individuals. This is consistent with
21 the findings presented in Table 2, whereby less than half the variance in D_{LT} is explained by
22 our three predictor variables. Thus, more accurate prediction of D_{LT} could still further
23 improve 2C densitometric assessment of body composition in obese children.

24 A potential way to achieve such improvement is shown by the data in **Figure 4, which**
25 plots hydration of lean tissue against D_{LT} . The correlation between D_{LT} and hydration of lean

1 tissue was **-0.83** ($p < 0.0001$), and after adjusting for the effects of age, gender and BMI SDS
2 remained **-0.81** ($p < 0.0001$). This implies that variability in hydration, even after taking into
3 account age, gender and size, accounts for the majority (65.6%) of the variance in D_{LT} , **and**
4 **this explains why age, sex and BMI SDS were themselves relatively poor predictors. In**
5 **Figure 4, the** negative regression slopes for boys and girls are almost identical, reflecting the
6 4C model assumptions. **Thus, if data on lean tissue hydration were available, the ability to**
7 **predict DLT accurately would improve substantially.**

8

9 **Discussion**

10 Despite growing interest in the body composition of obese children, and its change in
11 response to treatment, obtaining accurate measurements remains difficult. First, many
12 techniques may have poorer accuracy in those obese, due to uncertainty as to the composition
13 of lean tissue. Second, some techniques are incapable of accommodating those of large body
14 size. This results in the well-known paradox that body composition is particularly difficult to
15 measure in a group of patients who would particularly benefit from such assessments.

16 The majority of body composition techniques are 2C methodologies, involving the
17 measurement of body properties, such as body water content or body density. Data on these
18 body properties are then converted to final body composition values using theoretical
19 assumptions about the composition of lean tissue. We have recently demonstrated significant
20 differences in the properties of lean tissue in obese children and adolescents, compared to
21 those non-obese (Haroun et al. 2005; Wells et al. 2006). Hydration is increased, and D_{LT}
22 lower, in obese compared to normal weight individuals (Haroun et al. 2005). These
23 differences have been attributed in part to expansion of the extra-cellular water space
24 (Battistini et al. 1995) which in turn reflects irreversible changes in hemodynamics (Leone et
25 al. 2000; Marken Lichtenbelt and Fogelholm 1999); but also in part to reduced mineralisation

1 (Goulding et al. 2000). Thus, values for the hydration or density of lean tissue obtained from
2 individuals within the normal range of body weight will not transfer successfully to those
3 obese.

4 In this study, we therefore first quantified D_{LT} in a sample of 105 obese children, using
5 the accurate 4C model as the reference method, and used these data to develop predictive
6 equations specifically for this patient group, so that we could predict D_{LT} in individual obese
7 children with the aim being to improve accuracy of the 2C densitometric approach. We then
8 successfully tested these predicted D_{LT} values using densitometry as a 2C method in an
9 independent sample of obese children followed longitudinally. However, we also indentified
10 the limitations in accuracy of our new approach, and show how it could be improved further.

11 The validity of ADP in obese children and adolescents depends on two independent
12 factors: first, the accuracy with which body volume itself is measured, and secondly, the
13 validity of the equations used to convert raw density values to final body composition outputs.
14 Numerous studies have shown acceptable accuracy of ADP for the measurement of body
15 volume itself in children (Dewit et al. 2000; Nunez et al. 1999), while the study of Ginde and
16 colleagues has demonstrated its accuracy in those of very large body size (Ginde et al. 2005).
17 Less attention has been given to the appropriate values for D_{LT} in obese children and
18 adolescents, hence our interest in exploring this issue in detail.

19 We showed that use of D_{LT} values from the literature resulted in a significant error in
20 fat mass in obese children and adolescents, with the difference of greater magnitude in girls
21 (1.3 kg, $p<0.001$) than in boys (0.4 kg, $p=0.066$). We used age, gender and BMI SDS as
22 predictors of D_{LT} in our cross-sectional sample, for two reasons. First, these three terms have
23 all been shown to account for variability in the outcome in children in general, in part because
24 of the process of chemical maturation that occurs during growth, at different rates in the two
25 sexes (Fomon et al. 1982; Lohman 1986; Wells et al. 2010b). Second, this information is

1 readily obtainable in the clinic. However, our analysis showed that the three predictors
2 explained only a minority (33%) of the variance in D_{LT} in obese children and adolescents.

3 The majority of the variance could be attributed to the hydration of lean tissue,
4 however **in this study** we had no independent information on this trait. **We showed that**
5 **hydration** and density of lean tissue in our dataset had a negative correlation coefficient of -
6 0.81, even after adjusting for age, gender and BMI SDS. Whilst this highly negative
7 correlation necessarily incorporates antagonistic effects of measurement error, in that any
8 underestimation of hydration automatically overestimates density, the same inverse
9 correlation must also emerge once such error is taken into account. Increasing water content
10 must reduce density, due to displacing the denser fat-free dry components of lean tissue,
11 protein and mineral. What this study demonstrated however is that the variability in hydration
12 in obese children and adolescents is to a large extent independent of age, sex and degree of
13 obesity, **which is why we were much more successful in accounting for the variance of D_{LT}**
14 **when information on hydration was included in the regression model.**

15 The poor ability to predict D_{LT} from age, sex and BMI SDS detracts from the accuracy
16 of ADP for predicting adiposity in individuals in the second group of children. Group mean
17 values had low and non-significant error, and we also showed a non-significant bias (0.3 kg;
18 $p=0.088$) in fat mass at baseline, and non-significant bias (0.2 kg; $p=0.2$) in change in fat
19 mass. However, the limits of agreement were ± 2.8 kg for fat mass at baseline, and ± 2.8 for
20 change in fat mass. These **limits of agreement of 2.8 kg are not good enough to allow small**
21 **changes in fatness to be assessed accurately when using densitometry as a 2C approach.**

22 We propose that these limits of agreement could be reduced substantially, if
23 information on the hydration of lean tissue were incorporated into the prediction of D_{LT} . An
24 index of hydration could potentially be obtained in obese children using multi frequency
25 bioelectrical impedance analysis (BIA), for example using vector analysis (Piccoli 2004).

1 Regrettably, this instrumentation was not available to us in the present study. The advantage
2 of this approach would be that both ADP and BIA are non-invasive techniques easily applied
3 in the vast majority of overweight individuals, in both cross-sectional and longitudinal
4 contexts. Thus, ADP and BIA could potentially offer a convenient and accurate protocol for
5 densitometry as a 2C body composition technique in obese children, and this approach
6 strongly merits further research given the limitations of other techniques.

7 Our data are of particular interest for comparison with our similar study of the
8 accuracy of DXA in obese children and adolescents. Comparing data from DXA against the
9 four component model, in both cross-sectional and longitudinal studies as conducted here, we
10 found bias of 0.9 kg ($p < 0.001$) and limits of agreement of 4.2 kg for cross-sectional data, and
11 limits of agreement of 3.2 kg for change in fat mass, though with no significant mean bias.
12 We will also be unable to scan or analyse a proportion of obese children and adolescents using
13 DXA, due to their exceeding the weight limits or scanning dimensions of the instrumentation
14 (Luna Prodigy). Therefore, ADP appears to have higher take-up in those of large body size,
15 as well as moderately improved accuracy over DXA for measuring fat mass and lean mass in
16 obese children and adolescents, with the potential for further improvement in accuracy if
17 hydration could be measured.

18 The strengths of this study comprise the relatively large sample size, allowing the
19 sexes to be considered separately, and our use of the 4C model as the reference method. The
20 limitations comprise the use of predicted lung volumes in the ADP measurements, as the lung
21 volume prediction equations may introduce systematic error in those obese. However, we
22 have consistently elected to predict rather than measure lung volume in our pediatric work on
23 ADP, as we have found that many children struggle with the protocol for direct lung volume
24 measurement. Furthermore, the correction of raw body volume for air in the lungs involves
25 multiplying the predicted values by 0.4, hence any error is similarly reduced.

1 In conclusion, we demonstrated that the generation of prediction equations for D_{LT} in
2 obese children and adolescents improves the accuracy of ADP in this patient population.
3 These findings are valuable due to the paucity of alternative techniques available for
4 measuring individuals of large body size. Our data indicate that further improvements in
5 accuracy could be made if information about hydration of lean tissue were collected, for
6 example using multi-frequency BIA.

7

8 **Acknowledgements**

9 The study was designed and analysed by JCKW. The subjects were recruited by DH, JEW
10 and RMV, and measured by DH and JEW under the supervision of JCKW and MSF. The
11 mass-spectrometric analyses were conducted by TD and SE. The body composition modelling
12 was undertaken by DH and JEW under the supervision of JCKW and MSF. All authors
13 critically appraised the manuscript. No author declares any conflict of interest.

14

Table 1. Characteristics of the cross-sectional sample

	Boys (n = 39)			Girls (n = 66)		
	Mean	SD	Range	Mean	SD	Range
Age (y)	12.6	3.5	5.0, 20.0	11.7	3.6	5.0, 21.7
Weight (kg)	68.4	22.1	24.3, 107.4	65.7	22.2	28.4, 112.3
Height (cm)	155.1	16.8	112.9, 182.0	150.2	14.7	111.0, 175.9
BMI (kg/m ²)	27.6	5.2	18.1, 39.8	28.3	5.9	19.8, 43.0
Weight SDS	2.3	0.8	0.7, 4.8	2.6	0.9	0.9, 4.8
Height SDS	0.5	0.9	-1.1, 2.7	0.8	1.0	-2.1, 3.8
BMI SDS	2.6	0.7	1.7, 4.7	2.6	0.8	1.6, 4.5
Body volume (l)	67.8	22.1	23.6, 109.4	65.4	22.4	28.5, 114.0
Body density (kg/l)	1.0101	0.0185	0.9732, 1.0438	1.0047	0.0140	0.9738, 1.0389
Total body water (l) *	32.8	10.7	14.5, 50.7	28.8	8.6	12.9, 46.4

Bone mineral content (kg)	2.1	0.8	0.8, 3.7	1.9	0.7	0.7, 3.6
4C Fat mass (kg)	25.7	11.5	5.1, 55.5	27.6	11.9	9.4, 58.1
4C Lean mass (kg)	42.7	14.4	18.7, 68.6	37.9	11.6	16.6, 61.6
Hydration lean tissue (%) *	76.9	2.0	71.5, 81.4	75.6	2.1	68.0, 80.3
Density lean tissue (kg/l) *	1.0873	0.0071	1.0709, 1.1073	1.0919	0.0074	1.0733, 1.1123

* sex difference $p < 0.05$

Table 2. Correlation and regression analysis of D_{LT} in relation to age, gender and BMI SDS

Correlations

	<u>All (n = 105)</u>		<u>Boys (n = 39)</u>		<u>Girls (n = 66)</u>	
	r	p	r	p	r	p
Age	0.41	<0.0001	0.55	<0.0001	0.43	<0.0001
BMI SDS	-0.33	0.003	-0.48	0.002	-0.27	0.027
Gender	0.23	0.003				

Regression of D_{LT} on age, gender and BMI SDS

	B	SE	p	r ²	SEE
<u>All</u>					
Constant	1.078	0.004	<0.0001	0.34	0.0062
Age (y)	0.001	0.000	<0.0001		

BMI SDS	-0.002	0.001	0.005
Gender	0.005	0.001	<0.001

Boys

Constant	1.085	0.005	<0.0001	0.44	0.0054
Age (y)	0.001	0.000	0.001		
BMI SDS	-0.004	0.001	0.006		

Girls

Constant	1.087	0.005	<0.0001	0.19	0.0067
Age (y)	0.001	0.000	0.001		
BMI SDS	-0.002	0.001	0.11		

Table 3. Bias in lean tissue density or fat mass in the cross-sectional and longitudinal studies

Cross-sectional sample *

	<u>D_{LT} (kg/l)</u>				<u>Fat mass (kg)</u>			
	N	Mean	LOA	p	Mean	LOA	p	
All	105	-0.0025	0.0172	0.003	105	-0.68	4.11	0.001
Boys	39	0.0029	0.0149	0.019	39	-0.43	2.83	0.066
Girls	66	-0.0057	0.0152	<0.001	66	-1.33	4.20	<0.0001

*Bias calculated as values for (D_{LT}) or using (fat mass) Lohman's D_{LT} values, minus those obtained using the 4C model

Longitudinal sample **

<u>D_{LT} (kg/l)</u>	<u>Fat mass (kg)</u>
------------------------------	----------------------

	N	Mean	LOA	p	Mean	LOA	p
Baseline							
All	55	0.0023	0.0027	0.057	0.32	2.77	0.088
Boys	20	-0.0008	0.0150	0.6	0.11	2.76	0.7
Girls	35	0.0040	0.0224	0.041	0.45	2.80	0.067
Change							
All	55	0.0002	0.0159	0.8	0.24	2.77	0.2
Boys	20	0.0010	0.0129	0.5	0.14	2.29	0.6
Girls	35	-0.0003	0.0174	0.8	0.31	2.95	0.19

** Bias calculated as values for (D_{LT}), or using (fat mass) our new D_{LT} prediction equations, minus those obtained using the 4-component model

D_{LT} - density of lean tissue

Legends for illustrations

Figure 1. Schematic diagram of Archimedes principle and its limitations, using data from adults. (a) Total body density must lie between pure fat (0.9007 kg/l) and pure lean (1.1000 kg/l). According to Archimedes' principle, there is a linear relationship between total body density and % fat in body weight, hence % fat is predicted from density measurement. (b) The accuracy of this method depends on the validity of the two component density values, in this case fat density and lean density. The graph shows the % error in fat mass that will result if the actual density of lean tissue varies between values of 1.080 and 1.100 kg/l, but is assumed to be 1.090 kg/l.

Figure 2. Bland-Altman plot illustrating the comparison of fat mass values, either predicted from body density using literature values for the density of lean tissue, or measured using the four-component model, in the cross-sectional sample of 105 children. The difference in fat mass between methods is plotted against the mean fat mass. The mean bias was an under-estimation of 0.68 kg ($p < 0.001$), limits of agreement ± 4.11 kg. The bias was larger in girls (-1.33 kg) than in boys (-0.43 kg).

Figure 3. Bland-Altman plot illustrating the comparison of change in fat mass values, either predicted using our equations for the prediction of the density of lean tissue, or measured using the four-component model, in 51 children followed for an average of 7 months. The difference in fat mass change between methods is plotted against the mean fat mass change. The mean bias was an over-estimation of 0.24 kg (ns), limits of agreement ± 2.77 kg.

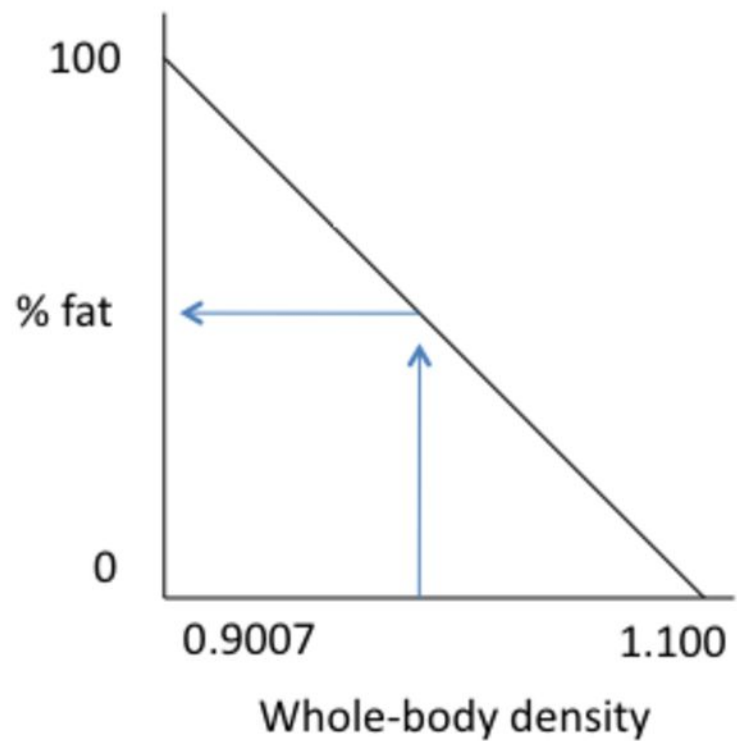
Figure 4. Density of lean tissue plotted against the hydration of lean tissue, both measured by the four-component model. The negative correlations in each sex reflect both antagonistic measurement errors, and physical effects of covariance in the water, protein and mineral components of lean tissue.

References

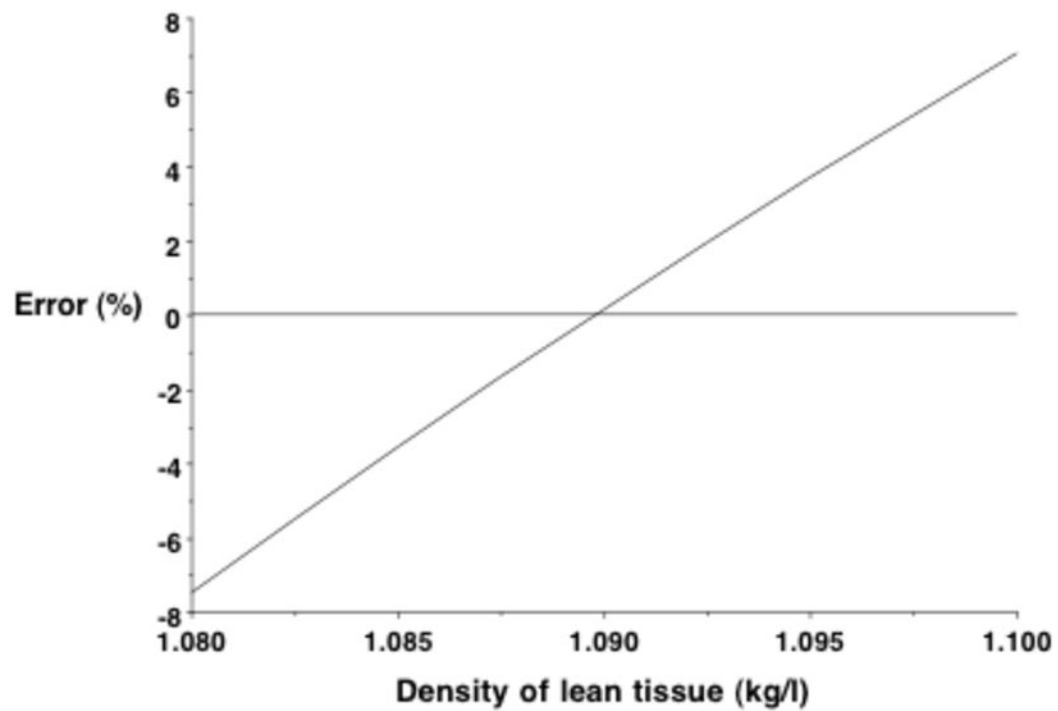
- Battistini N, Virgili F, Severi S, Brambilla P, Manzoni P, Beccaria L, and Chiumello G. 1995. Relative expansion of extracellular water in obese vs. normal children. *J Appl Physiol* 79(1):94-96.
- Bland JM, and Altman DG. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1(8476):307-310.
- Cole TJ, Bellizzi MC, Flegal KM, and Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 320(7244):1240-1243.
- Cole TJ, Freeman JV, and Preece MA. 1995. Body mass index reference curves for the UK, 1990. *Arch Dis Child* 73(1):25-29.
- Deurenberg P, Deurenberg-Yap M, and Guricci S. 2002. Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. *Obes Rev* 3(3):141-146.
- Deurenberg P, Yap M, and van Staveren WA. 1998. Body mass index and percent body fat: a meta analysis among different ethnic groups. *Int J Obes Relat Metab Disord* 22(12):1164-1171.
- Dewit O, Fuller NJ, Fewtrell MS, Elia M, and Wells JC. 2000. Whole body air displacement plethysmography compared with hydrodensitometry for body composition analysis. *Arch Dis Child* 82(2):159-164.
- Dietz WH. 1998. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics* 101(3 Pt 2):518-525.
- Ehtisham S, Crabtree N, Clark P, Shaw N, and Barrett T. 2005. Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. *J Clin Endocrinol Metab* 90(7):3963-3969.
- Fomon SJ, Haschke F, Ziegler EE, and Nelson SE. 1982. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 35(5 Suppl):1169-1175.
- Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, and Preece MA. 1995. Cross sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child* 73(1):17-24.
- Ginde SR, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S, and Heymsfield SB. 2005. Air displacement plethysmography: validation in overweight and obese subjects. *Obes Res* 13(7):1232-1237.
- Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, and Williams SM. 2000. Overweight and obese children have low bone mass and area for their weight. *Int J Obes Relat Metab Disord* 24(5):627-632.
- Haines L, Wan KC, Lynn R, Barrett TG, and Shield JP. 2007. Rising incidence of type 2 diabetes in children in the U.K. *Diabetes Care* 30(5):1097-1101.
- Haroun D, Wells JC, Williams JE, Fuller NJ, Fewtrell MS, and Lawson MS. 2005. Composition of the fat-free mass in obese and nonobese children: matched case-control analyses. *Int J Obes (Lond)* 29(1):29-36.
- Kiebzak GM, Leamy LJ, Pierson LM, Nord RH, and Zhang ZY. 2000. Measurement precision of body composition variables using the lunar DPX-L densitometer. *J Clin Densitom* 3(1):35-41.

- Leone PA, Gallagher D, Wang J, and Heymsfield SB. 2000. Relative overhydration of fat-free mass in postobese versus never-obese subjects. *Ann NY Acad Sci* 904:514-519.
- Lloyd LJ, Langley-Evans SC, and McMullen S. 2010. Childhood obesity and adult cardiovascular disease risk: a systematic review. *Int J Obes (Lond)* 34(1):18-28.
- Lohman TG. 1986. Assessment of body composition in children. *Pediatr Exerc Sci* 1:19-30.
- Marken Lichtenbelt WD, and Fogelholm M. 1999. Increased extracellular water compartment, relative to intracellular water compartment, after weight reduction. *J Appl Physiol* 87(1):294-298.
- Mazess RB, Cameron JR, and Sorenson JA. 1970. Determining body composition by radiation absorption spectrometry. *Nature* 228(5273):771-772.
- Nunez C, Kovera AJ, Pietrobelli A, Heshka S, Horlick M, Kehayias JJ, Wang Z, and Heymsfield SB. 1999. Body composition in children and adults by air displacement plethysmography. *Eur J Clin Nutr* 53(5):382-387.
- Petroni ML, Bertoli S, Maggioni M, Morini P, Battezzati A, Tagliaferri MA, Liuzzi A, and Testolin G. 2003. Feasibility of air plethysmography (BOD POD) in morbid obesity: a pilot study. *Acta Diabetol* 40 Suppl 1:S59-S62.
- Piccoli A. 2004. Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status. *Kidney Int* 65(3):1050-1063.
- Reilly JJ, Methven E, McDowell ZC, Hacking B, Alexander D, Stewart L, and Kelnar CJ. 2003. Health consequences of obesity. *Arch Dis Child* 88(9):748-752.
- Schoeller DA. 1996. Hydrometry. In: Roche AF, Heymsfield SB, and Lohman TG, editors. *Champaign, IL: Human Kinetics.* p 25-43.
- Siri WE. 1961. Body composition from fluid spaces and density. In: Brozek J, and Henschel A, editors. *Washington DC: National Academy of Sciences.* p 223-244.
- Sopher AB, Thornton JC, Wang J, Pierson RN, Jr., Heymsfield SB, and Horlick M. 2004. Measurement of percentage of body fat in 411 children and adolescents: a comparison of dual-energy X-ray absorptiometry with a four-compartment model. *Pediatrics* 113(5):1285-1290.
- Wells JC. 2000. A Hattori chart analysis of body mass index in infants and children. *Int J Obes Relat Metab Disord* 24(3):325-329.
- Wells JC, Chomtho S, and Fewtrell MS. 2007. Programming of body composition by early growth and nutrition. *Proc Nutr Soc* 66(3):423-434.
- Wells JC, and Fewtrell MS. 2008. Is body composition important for paediatricians? *Arch Dis Child* 93(2):168-172.
- Wells JC, Fewtrell MS, Williams JE, Haroun D, Lawson MS, and Cole TJ. 2006. Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. *Int J Obes (Lond)* 30(10):1506-1513.
- Wells JC, Fuller NJ, Dewit O, Fewtrell MS, Elia M, and Cole TJ. 1999. Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models. *Am J Clin Nutr* 69(5):904-912.
- Wells JC, Haroun D, Williams JE, Wilson C, Darch T, Viner RM, Eaton S, and Fewtrell MS. 2010a. Evaluation of DXA against the four-component model of

- body composition in obese children and adolescents aged 5-21 years. *Int J Obes (Lond)* 34(4):649-655.
- Wells JC, Williams JE, Chomtho S, Darch T, Grijalva-Eternod C, Kennedy K, Haroun D, Wilson C, Cole TJ, and Fewtrell MS. 2010b. Pediatric reference data for lean tissue properties: density and hydration from age 5 to 20 y. *Am J Clin Nutr* 91(3):610-618.
- Williams JE, Wells JC, Wilson CM, Haroun D, Lucas A, and Fewtrell MS. 2006. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model. *Am J Clin Nutr* 83(5):1047-1054.



(a)



(b)

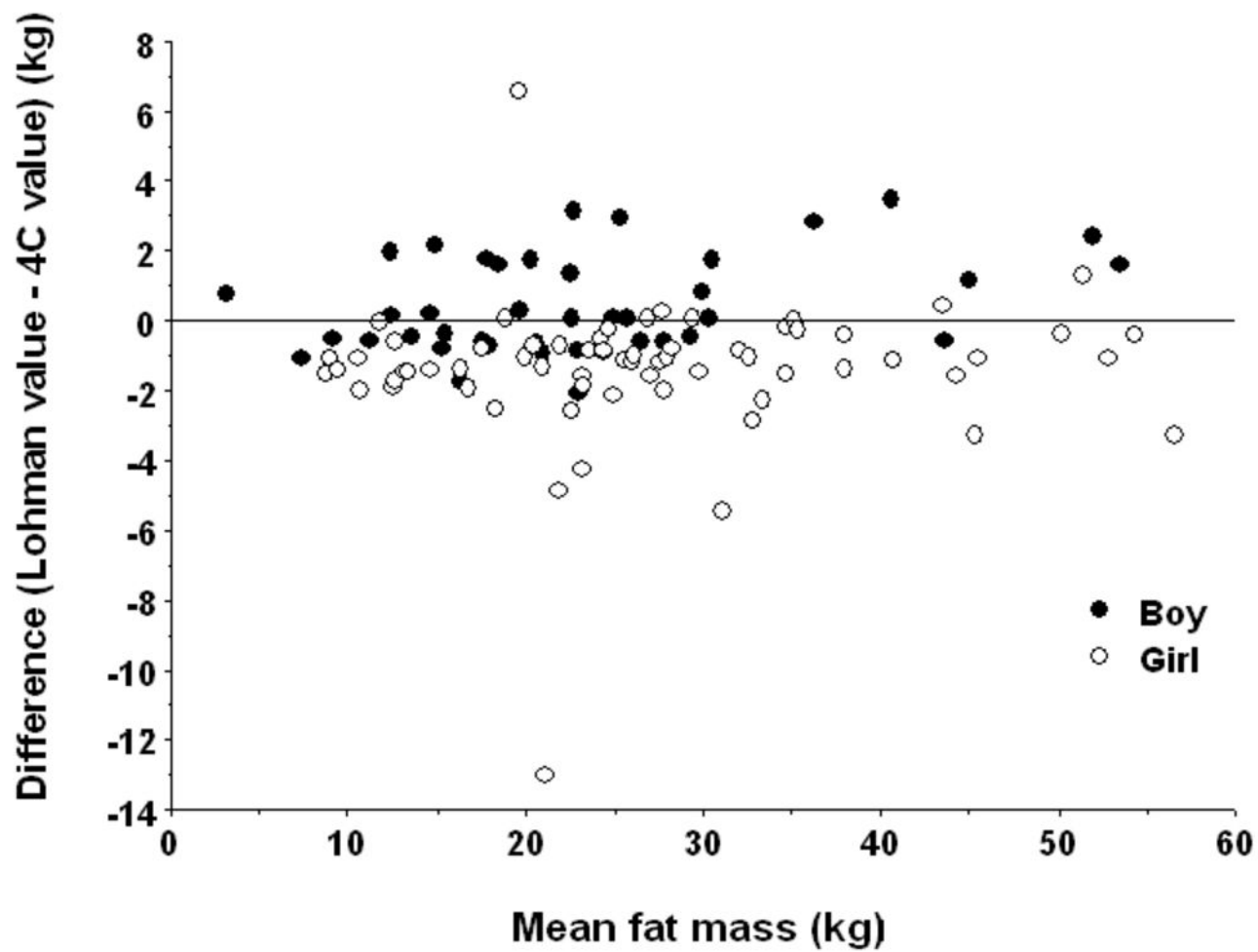


Figure 2

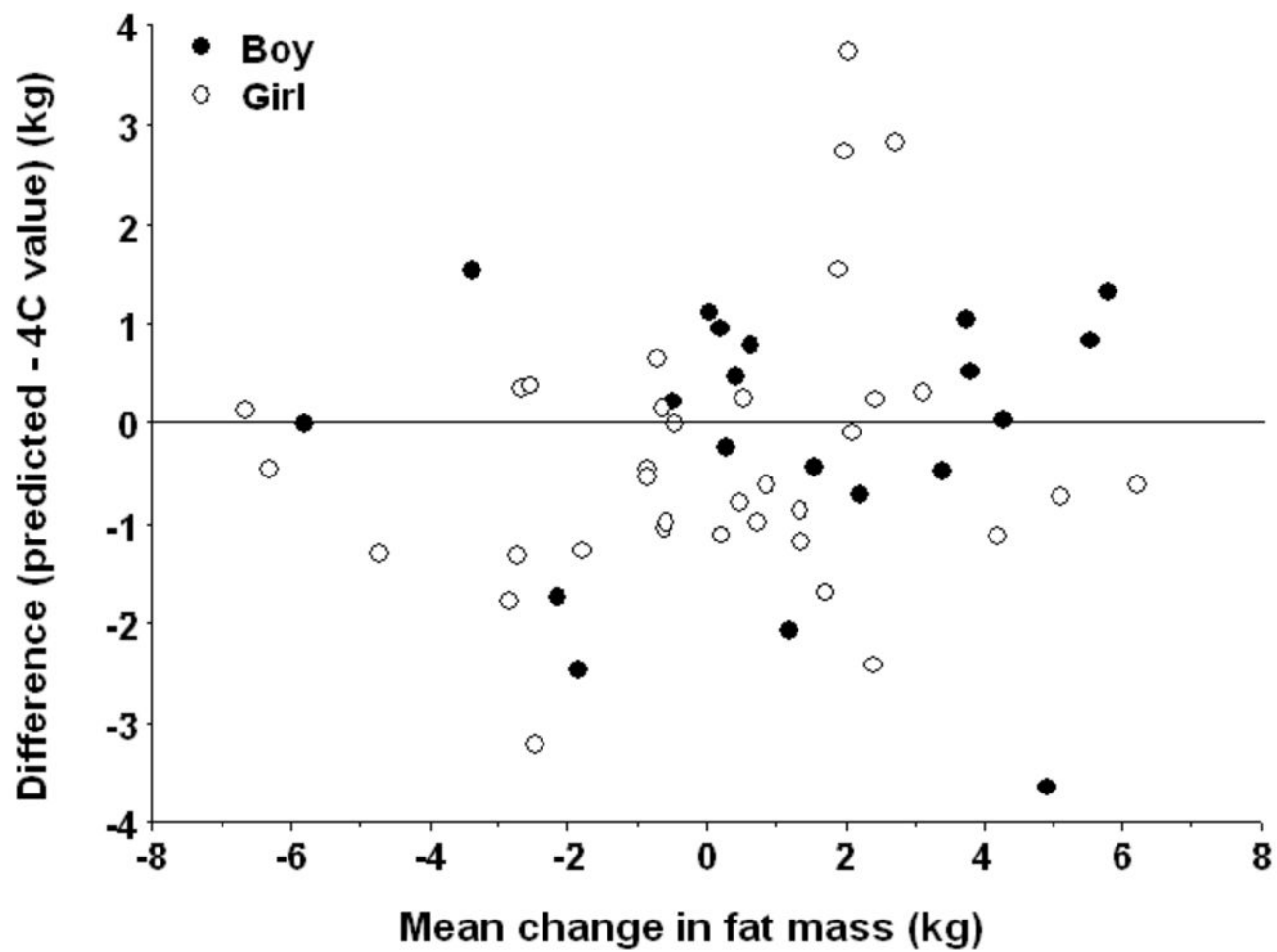


Figure 3

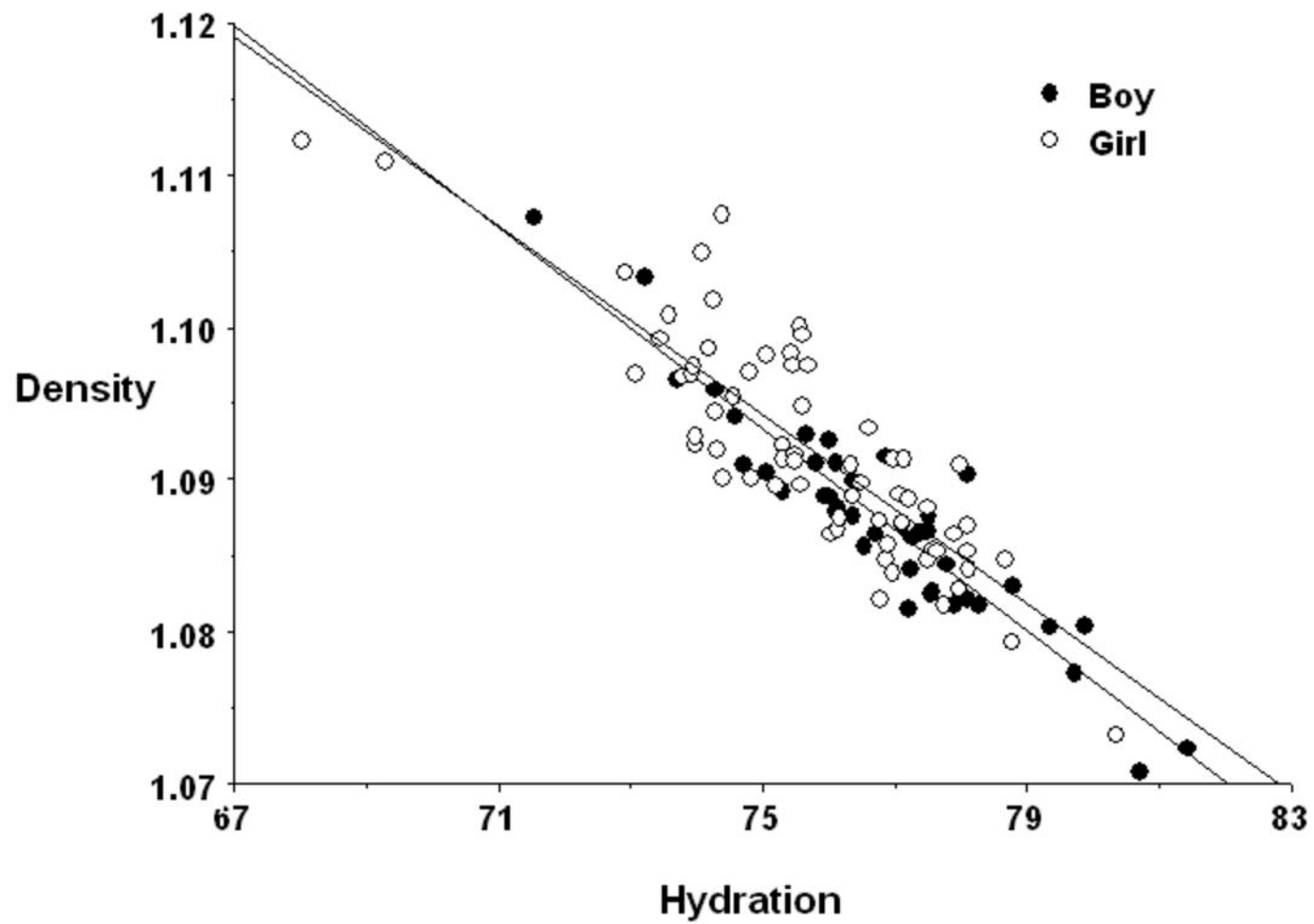


Figure 1