VALIDITY OF SIX FIELD AND LABORATORY METHODS FOR MEASUREMENT OF BODY COMPOSITION IN 10 – 14 YEAR OLD BOYS.

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Declaration

I hereby declare that this thesis has been composed by myself, that the work of which it is a record has been done by myself except where assistance has been acknowledged, that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of references.

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Lisa Parker (nee Dunn)

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Abstract

OBJECTIVE: The primary objective of this study was to determine the validity of the following six body composition measurement methods against a 3 - component reference method: total body water (TBW) by isotope dilution; air displacement plethysmography (BODPOD®); estimation from body density using BODPOD®; skinfold thickness using Slaughter equations; whole body bioelectrical impedance (Bodystat); and leg to leg bioelectrical impedance (TANITA).

SUBJECTS: Forty-two healthy Caucasian 10 – 14 year old boys (mean age 12.9 years SD 1.0) recruited from local schools and youth football teams.

PROCEDURES: The body composition of each subject was measured using all 7 methods (including the reference). All measurements were carried out by the same trained observer (the author) and the same criterion for assessment was used for each child in an attempt to minimise any error. Measures of body fat mass (kg) and body fat percentage from the 6 simpler methods were compared and validated against the measures derived from the reference by calculation of biases and 95% limits of agreement.

RESULTS: Mean body fatness by the reference method was 16.4% (SD 11.6) and 8.7kg (SD 7.0). Estimates of fatness from TBW had the smallest bias relative to the reference (+0.9 $\pm$ 5.0% body fat; +0.5 $\pm$ 2.9kg fat mass). For all the other 5 methods tested, large biases and very wide limits of agreement were observed.

CONCLUSIONS: The present study suggests that the validity of newer field and laboratory methods for estimation of body composition is poor in adolescent boys. For applications where high accuracy of estimation at the individual level is essential, only reference methods would be acceptable

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Abbreviations

ABV	-	actual body volume
ADP	-	air displacement plethysmography
%BF	-	percent body fat
BIA	-	bioelectrical impedance analysis
BMI	-	body mass index
BV	-	body volume
BWt	-	body weight
Db	-	body density
DXA	-	dual-energy x-ray absorptiometry
FFM	-	fat free mass
FRC	-	functional residual capacity
$^{2}H_2O$	-	deuterium oxide
Ht	-	height
LV	-	lung volume
SAA	-	surface area artefact
SD	-	standard deviation
SFT	-	skinfold thickness
TBW	-	total body water
TGV	-	thoracic gas volume
TV	-	tidal volume

1.0 Introduction

1.1 The need for valid, simple methods of body composition measurement.

The developed world is becoming more aware than ever before of the problems and health risks associated with increased body fatness. With the prevalence of eating disorders and weight related issues being highlighted in today's media, the epidemic of childhood and adolescent obesity (Troiano *et al,* 1995), and the fact that adolescent cases of anorexia nervosa (Kerruish *et al,* 2002) are on the rise, there is an increased awareness of the need for accurate measurement of body composition (fatness and fat-free mass - FFM) in a range of fields from epidemiology to clinical research (Reilly, 1998).

Accurate measurement of body composition in the past has required sophisticated apparatus and techniques beyond the scope of most clinical practice and research, so workers have generally used simpler methods such as anthropometry and impedance measurements. Many of these simpler methods are becoming more widely available not only to the researchers and practitioners, but also to the general public. However, a recent study (Wells *et al,* 1999) has cast doubt on the accuracy of two of the most popular simpler methods, bio-electrical impedance analysis (BIA) and skinfold thickness (SFT). Wells *et al* (1999) and Reilly (1998) concluded that no simple methods of measuring body composition in children/adolescents had been validated and that more research was essential. Simple methods are potentially suitable for use in the field or at the bedside due to their ease of use, low cost and portability. This therefore makes these methods extremely desirable as a means of measuring body composition, however it is essential that the validity of these methods are assessed for use with children and adolescents.

1.2 3 Component reference method for use with children and adolescents.

Reilly (1998) reviewed the various methods of body composition analysis for use with children and infants and the criteria for determining the choice of method. The review outlined the necessity for the use of multi -component reference methods (fat mass, protein, mineral and total body water - TBW) when validating methods in children. The explanation given by Reilly (1998) is based on the assumptions made when using a 2-component model (fat mass and FFM) reference method, which assumes that the composition of FFM in the subject is constant for any given age/sex (Lohman, 1989). While this can be assumed for adults, the same can not be said for children since their chemical composition changes from childhood through to adulthood. Therefore, any reference model used with children should take these factors into account: it is preferable not to assume constancy of FFM composition in children but to include TBW and density [Db = body weight (BWt) / body volume (BV)] to give 3 Component reference method, or ideally to measure TBW and whole body mineral to give estimates of total body mineral for use in a 4 Component model. Furthermore, Reilly (1998) reported on the influence of the choice of prediction equations used with the simpler methods of body composition analysis. With many equations being population specific or inappropriate for use with children the choice of equation can have a large bearing on the results obtained, often leading to misinterpretation and inaccurate estimation of body composition. Finally, the review provided a set of criteria to assist the researcher or practitioner when choosing a body composition method to use for the measurement of children (Table 1).

CRITERIA FOR CHOICE OF BODY COMPOSITION METHOD

Practical Issues
Is the method acceptable to the child?
Is the cost acceptable?
Training needs: quality control/standardisation of technique.
Is the method safe?
Validity
Is the method valid?
What are the underlying assumptions of the method?
Are these assumptions valid in the children being studied? (Are the assumptions appropriate for measuring body composition of children or adults?)
Is there a published validation study?
Was validity assessed appropriately in that study?
What is the error in the method and does it meet criteria for acceptability?
Is the method valid for individuals as well as groups?
Is the particular variant of the method used valid in the population of children being studied (i.e. does it have cross-validity)?
Precision
What is the precision of the method?
Is it adequate for detection of changes in body composition?
Adequate for individuals or groups?

Table 1 – Reilly, J.J. Assessment of Body Composition in Infants and Children. Nutrition 1998; 14:824. Permission to reproduce this table authorised by Dr J.J. Reilly .

Wells *et al* (1999) also recommended that any methods be validated against a multi-component model of body composition consisting of minimum measurements of BWt, BV and TBW. They then described this 3 Component reference method for use with children (Dewit *et al*, 2000). This reference method, which will be adopted for the present study, is based on the 3 Component model as recommended by Reilly (1998). However, in this thesis, BV and Db were estimated by the new (and more acceptable to children) method of air displacement plethysmography (ADP) as opposed to hydrodensitometry. TBW was measured by deuterium oxide (^2H_2O) dilution.

1.3 Review of Individual Methods - Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) has quickly become a preferred tool for body composition analysis. It is cheap, non-invasive, easily portable, easy to use, quick and suitable for use with many subject groups that can be problematic with other methods, such as children, the sick and the elderly (Houtkooper *et al*, 1989, Houtkooper *et al*, 1992, Fuller, 1993, Reilly, 1998, Abbott and Davies, 2001). There are 2 main types of BIA in general use; the conventional whole body, hand to foot method (eg. Bodystat 1500MDD) and the newer leg to leg method (eg. Tanita TBF-521). Both methods work on the principle that the length^2/impedance of any conducting medium is proportional to the volume of that conducting medium. In the case of body composition, stature (height – Ht) is used as a proxy for the length of the conducting medium. The volume of the conducting medium within the body is essentially TBW (Abbott and Davies, 2001).

Whole body BIA uses 4 surface electrodes attached to the wrist, hand, ankle and foot on the right side of the body. The subject is required to lie down for approximately 5 minutes before the measurements are taken to allow body fluid compartments to equilibrate and then the tests carried out. With the leg to leg system, the subject stands on a specially adapted set of scales, placing their heels and toes on the four specially designed metallic plates. The measurements can be taken immediately and with no discomfort to the subject. The leg to leg BIA method is quicker and even more acceptable to most subjects than even the readily accepted whole body method. If valid, leg to leg impedance is therefore a particularly desirable tool for practitioners of body composition assessment. However, doubts regarding BIA have been raised in respect of the body composition results given directly by the impedance equipment. Since the manufacturer's equations, built into the software of the machine, are generally a commercial secret, practitioners cannot be sure that these equations are relevant to their subject population and could therefore result in inaccurate measurement and interpretation of results (Fuller, 1993, Abbott and Davies, 2001). Both methods of BIA are readily available to the public and in light of these findings, the interpretation of the results may be misleading to the general user as well as the researcher.

1.4 Whole Body Impedance: Review of use in Adults.

The whole body BIA method has been compared and validated against methods for use with both adults and children (Lukaski *et al*, 1985, Kushner & Schoeller, 1986, Segal *et al*, 1988, Houtkooper *et al*, 1989, Reilly *et al*, 1996). Lukaski *et al* (1985) were among the first to report on the assessment of FFM using BIA measurements. Tetrapolar whole body impedance measurement was compared with hydrodensitometry and TBW derived from ^2H_2O dilution and total body potassium from whole body counting in 37 adult male subjects. Through use of regression equation analysis and correlation techniques they concluded that BIA was a reliable and valid approach for estimating body composition.

Kushner and Schoeller (1986) compared results of BIA with ^2H_2O dilution for measuring TBW in a total of 58 obese and non-obese adult subjects. After developing sex-specific equations by multi-regression analysis they also found that BIA could predict TBW results that correlated highly ($r = 0.96$ in males and 0.93 in females) with their ^2H_2O dilution results, and certainly more accurately than Wt, Ht, and/or age alone. Jackson *et al* (1988), examined the reliability and validity of BIA for measuring body composition and compared its accuracy with the results obtained by standard anthropometric methods. The BIA method in 68 adult subjects was compared with % body fat (%BF) results derived from hydrodensitometry and SFT measurements. However, they could not confirm from their findings that BIA predicted %BF accurately enough to replace either hydrodensitometry or SFT measurement and concluded that further research was needed to firmly establish the validity of BIA.

Segal *et al* (1988) produced research evidence which they felt validated further the BIA method for body composition estimation in adults. This large study over four laboratories compared BIA with hydrodensitometry in 1567 adults. With their large sample group, and the use of regression analysis for Ht^2 and resistance individually, they felt that their study provided evidence that BIA again was a useful and accurate indicator of body composition in adults. Ross *et al* (1989) took a slightly different approach when they looked at the sensitivity of BIA to detect changes in body composition rather than using BIA to directly measure body composition. They compared estimates of FFM and %BF from BIA and SFT with hydrodensitometry in 17 adult males before and after a 10-week diet and exercise regime. Using the equations derived for BIA (Lukaski, 1988 and Segal, 1988) they found no significant differences between the FFM and %BF results from the BIA and the hydrodensitometry. Furthermore, they concluded that BIA was a valid method for detecting and tracking changes in body composition over a period of time and in particular after a diet and exercise intervention programme.

1.5 Leg to Leg Impedance: Review of use in Adults

Nunez *et al* (1997) evaluated the leg to leg BIA system in adults based on pressure contact foot electrodes. Their study had two aims: firstly, they evaluated the validity of the footpad electrodes in comparison to the gel electrodes of the whole body BIA system. Secondly, they assessed the potential for body composition assessment by comparing the leg to leg system results against TBW by ^2H_2O dilution and FFM by hydrodensitometry and dual-energy X-ray absorptiometry (DXA). Regression analysis and paired 't' tests were performed to examine the correlation and statistical significance of mean differences observed in leg to leg impedance measured with pressure contact and gel electrodes. The differences between the two electrode types were plotted against mean impedance and assessed for 95% limits of agreement (Bland and Altman, 1986). They concluded that the newer leg to leg system could be interchanged with the conventional whole body BIA system for the measurement of body composition in healthy adults. Concerns were expressed, however, over the use of the footpads for patients or subjects who have foot calluses or other foot problems that could limit electrode reliability. Jebb *et al* (2000) evaluated the leg to leg impedance system (Tanita) for measuring body composition in 205 adults in comparison with a 4 - component model (fat, water, mineral and protein). Comparisons were also made with the individual methods of hydrodensitometry, DXA, and TBW by ^2H_2O dilution. Jebb *et al* (2000) found, using correlation and 95% limits of agreement between methods (Bland and Altman, 1986), that the Tanita leg to leg system gave poorer accuracy in comparison to the reference when compared with the 4 - component model, but accuracy was similar to the reputed conventional tetrapolar whole body BIA. Given the practical advantage of the leg to leg system over the whole body BIA system, Jebb *et al* (2000) suggested that it should be the method of choice in adults if impedance has to be used. Whether this is true for children is unclear.

1.6 Whole Body Impedance: Review of use in Children and Adolescents

Houtkooper *et al* (1989) reported on the validity of BIA for body composition assessment in children. 94 children aged 10 – 14, were measured using whole body bioelectrical impedance to estimate FFM in comparison to hydrodensitometry combined with TBW analysis. Regression equations were derived and the researchers concluded that the BIA method was a valid predictor of body composition in this population. Lohman (1989) reviewed the various methods for measuring body composition in children. With the inclusion of BIA in his review, he acknowledged the need for multi-component models to compare the newer methods to, particularly for use with children because of the changes in water and mineral content of the FFM throughout childhood and youth. Referring to the aforementioned research by Houtkooper *et al* (1989) he concluded that BIA should be a valuable tool for use in childhood body composition research and due to its apparent consistency and ease of use, could be used to track levels of body composition in children through to adult life.

Houtkooper *et al* (1992) produced a cross validation study on the use of BIA for estimating FFM in children and youth. The purpose of the study was to develop and validate the best prediction equation for estimating body composition, in particular FFM, in children using BIA. For their initial validation sample they compared BIA with hydrodensitometry and total body water (TBW) by isotope dilution in 94 10 – 14 year old children (Houtkooper *et al*, 1989). Prediction equations were developed using multiple regression analyses, which were then cross-validated using the same methods with another 131 children over 3 different laboratories. The four samples were then combined to develop a recommended equation for estimating FFM. This particular equation has since been validated by other paediatric researchers against a 2 - component model (Reilly *et al*, 1996) as the reference and consequently was used in the present study.

The accuracy of 4 equations for use with tetrapolar bioelectrical impedance in children was tested in a study by Reilly *et al* (1996). They compared the effect of four different published equations on the results of 98 children between the age of 7 and 11. The results were compared against hydrodensitometry (2 - component model used as a reference) where Db was converted to FFM using the model described by Westrate and Deurenberg (1989) which assumes changing FFM density during childhood. Calculation of biases and 95% limits of agreement between methods enabled using the Bland and Altman (1986) technique. Again, this study reported problems in using hydrodensitometry as a reference method, both in practical problems due to the nature of the method for use with children, and theoretical problems with assumptions made from densitometry and suggested that a multi-component reference model may be preferable for use with paediatric studies. However, Reilly *et al* (1996) suggested that one particular equation (Houtkooper *et al*, 1992) out of the four compared had a high degree of cross validity and could be considered as an acceptable equation for estimation of body composition in children.

Houtkooper *et al* (1996) produced a review which argued that BIA should be widely used for estimating body fatness in large groups of children. They reported on the general agreement between researchers that the impedance technique, when used properly, provides a simple, non-invasive, inexpensive quick and reliable method for measuring body composition in large healthy populations. Moreover, when different investigators follow the same standard BIA procedures and use the same population criterion method, similar prediction equations and relatively small prediction errors have been reported for the measurement of FFM and TBW. Though they claimed that BIA has been reported as having limited accuracy for estimating body composition in individuals, they also suggested that BIA is more sensitive and specific for grading average body fatness in groups than some other anthropometric indices calculated on Ht and Wt relationships alone. This suggests that with the use of indices regarding levels of adiposity, this tool could be utilised as a more accurate method for national comparisons than the commonly used body mass index (BMI) in a variety of situations from the doctor's office to the home.

A further study which again questions the value of certain methods used as reference methods to compare BIA was a study by Gutin *et al* (1996) where they compared the results of BIA, SFT and DXA in 43 9 – 11 year old children in America. Using the Bland and Altman (1986) approach, intraclass correlation and Spearman rank correlation they compared all 3 methods. Since they found that the difference between the fat free soft tissue and FFM when using DXA was negligible, indicating that bone mineral content did not provide independent information, the remainder of their analyses used the 2 - component model (fat mass and FFM) for all 3 techniques. They found that the reliability of each technique was good, but found that although the three methods were highly correlated, there was a tendency for the DXA results to be consistently higher than the other 2 methods. From this they concluded, along with the large 95% limits of agreement derived from the Bland and Altman (1986) procedure that these methods should not be used interchangeably, but might be used as a reliable indicator of changes in body composition.

BIA is also readily used as a predictor of body composition in athletes. Various studies have depicted this use and formed athlete specific equations. Evetovich *et al* (1997) conducted a validity of BIA to estimate FFM in 117 young athletes aged 9 – 13. They compared 11 published equations and the manufacturer's equation (via the results obtained directly from the impedance equipment) with hydrodensitometry, a 2 - component model. In their conclusions they recommended one equation in particular (Guo *et al,* 1989) to be most useful for application with BIA for estimating FFM in young athletes. This equation requires skinfold measurements of the calf and midaxillary and therefore defeats the purpose of the highly acceptable and easy to use BIA method. The paper does go on to say however, that the Houtkooper (1989) equation is a reasonable substitute when the Guo equation is not available or perhaps the skinfold measurements are not feasible (Evetovich *et al,* 1997).

Further research into equations used with tetrapolar bioelectrical impedance was carried out in a study by Jurimae *et al* (1998). 107 boys and 105 girls between the ages of 9 and 11 had bioelectrical impedance measurements and SFT measurements taken and 6 regression equations were applied and compared. Descriptive statistics (mean, standard deviation [SD]) for each of the dependent variables were determined and independent 't' tests were used to indicate sex differences. No reference method was used for comparison of the results. One way ANOVA and paired 't' tests post hoc procedures were used to examine differences for each regression equation among the mean estimated %BF values and Pearson Product Moment correlation analysis was used to examine the relationship among SFT and BIA measurements of body composition. In their discussion of the study the authors indicated that this investigation demonstrated that great variation existed among %BF values calculated using different BIA regression equations. This paper was designed as a review of various equations used with BIA with a view to making recommendations as to which of the equations are most suitable. However, with the lack of any valid reference method, conclusions about the validity of any of the equations reached by Jurimae *et al* (1998) are not appropriate.

A recent study to evaluate the use of whole BIA in children was by Lohman *et al* (2000). They reported on the estimation of body fat from anthropometry and BIA in Native American children. Their main objective was to derive equations for use with this population due to the increase in obesity levels of this group and the lack of prediction equations for estimating body fatness in this population. They used ^2H_2O dilution as their reference method in 98 Native American children between the ages of 8 and 11 and compared various published equations for children of that age group. The equations used however were for non-native American children. The previously published equations consistently under-estimated %BF in this population in comparison with both the reference method and the equations derived from this population. The authors speculated that this could be as a result of not only physiological differences between the population groups, but also the fact that the previously published equations had been derived from multi - component reference methods as opposed to TBW alone. This conclusion merely underpins the need for the informed choice of equation and reference method when using BIA analysis for the estimation of body composition in any group irrespective of race or age.

In light of the limited research that has been conducted using whole body BIA with children a number of very important points are evident. The validity of this method for use with children is still unclear. The need for validation is extremely important since this method is highly dependent on the equation chosen and on the population being tested. Whole body BIA must be tested against an appropriate multi-component reference method in order to determine its validity for use with children and adolescents.

1.7 Leg to Leg Impedance: Review of use in Children and Adolescents

Abbott and Davies (2001) validated the use of the leg to leg BIA method for use in children (n=32) between the age of 6 and 10. TBW was predicted in the children by use of the leg to leg impedance measurement and then compared to TBW as measured by $^{2}H_2O$ dilution. The Bland and Altman (1986) approach was applied to compare the measured TBW result with the estimated one from the BIA method. In comparison with TBW measures Abbott and Davies (2001) found that the leg to leg impedance method overestimated TBW on average by 2.8 litres, or approximately 17% of the measured value. However, they noted that this bias was not constant over the age range and actually increased as the measured volume increased, resulting in an overestimation of as much as 6.0 litres at the extremes of their data. In body composition terms they concluded that this would equate to an error of up to 8kg in

FFM in a child weighing only 37kg; clearly unacceptable in terms of accuracy (Abbott and Davies, 2001). Moreover, they concluded that the majority of these errors could safely be assigned to problems with the BIA method since in various previous research studies conducted within their laboratory using the ^2H_2O dilution technique, this method has provided precision within 1%. Furthermore, they expressed concerns over the significant correlation between the mean of the measurements and their difference. According to the researchers this clearly shows that as the volume of TBW increases, which one could presume equates with the child getting physically larger, the error increases dramatically. This could cause considerable concern for use with children over longitudinal studies where the child is growing or for any estimation of body composition in children declared as clinically obese or for studies where older and younger children are compared.

Sung *et al* (2001) recently evaluated the measurement of body fat using leg to leg BIA in Hong Kong Chinese children. They compared the leg to leg method against DXA in 49 children between the ages of 7 and 18. From their study they concluded that the leg to leg system could readily be used in place of DXA. They found that although the BIA method consistently over estimated fat mass in comparison to DXA, their 95% limits of agreement were within an acceptable margin. This is in conflict with some of the previous research mentioned. However, it should be noted that this study used the predictions of body composition from the manufacturer's equations (unknown) and did not compare any other previously validated equation for impedance measurement in children. Moreover, in this particular leg to leg system, age is not entered in to the software but a 'child measurement' is chosen. This presumes that all ages of children should be entered into an identical equation which does not take their age into account, but only their Ht and Wt and sex.

Furthermore, it is not clear at what age the manufacturer feels a child becomes an adult and therefore qualifies for the adult equation. In addition, since Sung *et al* (2001) did not use a reference method to measure body composition it cannot be considered as a validation study.

Rowlands and Eston (2001) investigated the comparisons between whole body BIA and leg to leg BIA for estimating body composition in children aged 8 to 10 years old. The measures of %BF and FFM as provided by the leg to leg system and the whole body system were compared with predictions from SFT measurements using equations for SFT that had been validated in children using a multi-component reference method (equations of Slaughter *et al*, 1988). Bland and Altman (1986) 95% limits of agreement were applied to the results and this study found that the prediction of FFM and %BF from the leg to leg system and the whole body system were not significantly different. However, they did find that in girls both impedance measurements predicted results, which consistently overestimated %BF, compared to SFT measurements. Furthermore, they expressed concern over the regression equation within the leg to leg impedance system. The manufacturer's equation was the only one available for use with this method, and with this system being marketed for home and health club use where there is no access, or no desire to access alternative equations, it is important that the equation is suitable. For this reason, Rowlands and Eston (2001) recommended that further research is required in validating the use of this method with children. Unfortunately, the advice they gave was to compare the leg to leg impedance method with DXA which does not acknowledge the need for a multi-component reference method.

Tyrrell *et al* (2001) reported results predicted by the leg to leg BIA system compared to DXA – derived body composition in a multi-ethnic group of European, New Zealand Maori and Pacific Island children aged 4 to 11 (n=82). Like Sung *et al* (2001), this study found that the results from the leg to leg BIA system correlated highly with DXA in the estimation of FFM, FM and %BF (r = 0.98, 0.98 and 0.94 respectively). However, unlike Sung *et al* (2001), they concluded that the 95% limits of agreement between the methods were too wide to render the leg to leg system an acceptable replacement for DXA in the measurement of body composition in children. Although they did concede that the mean differences between the two methods were close enough for the BIA system to be acceptably used for large group studies. Tyrrell *et al* acknowledged that there has been criticism for the use of DXA as a reference method (Ogle *et al,* 1995, Reilly *et al* 1996),

1.8 Review of Individual Methods - Body Density: Air Displacement Plethysmography

The BODPOD® (Life Measurement Instruments, Concord, CA) ADP body composition system measures BV and hence, Db by application of gas laws within a two-chambered plethysmograph (McCrory *et al,* 1995). Small volume changes are produced within the chambers and the corresponding pressure change is measured (Dempster and Aitken, 1995, Nunez *et al,* 1999). BV is equal to the reduction in chamber volume caused by introduction of the subject (McCrory *et al,* 1995). The measured BV is then converted to Db with the equation of Goldman and Buskirk (1961) and fat mass then calculated with the Siri equation (Siri, 1961).

This non-invasive method for determining Db, and subsequent calculation of body composition provides many advantages over the conventional hydrodensitometry technique. The test procedure is quick, convenient and requires minimal compliance with very little or no discomfort to the subject. It appears that this method is more suitable for a wide variety of subjects where hydrodensitometry is not practical, including the obese, elderly, children and those who are uncomfortable with water submersion (Elia and Ward, 1999, Fields *et al*, 2000, Wells and Fuller, 2001).

1.9 Air Displacement Plethysmography: Review of use in Adults

Dempster and Aitkens (1995) reported on a new ADP method for the determination of human body composition (BODPOD® Body Composition System). The system's ability to measure inanimate objects was evaluated for accuracy, reliability and linearity. Using a wide range of volumes approximating human size (25 – 150 l), they produced an equation to assist in the measurement of BV with this system. They found the reliability over repeated trials was excellent (SD <14ml) and mean percent error was less than 0.1%. From this high level of precision they suggested that this method should be very useful for the determination of body composition. However, this study on inanimate objects does not have the complications offered by the study of human body composition due to the confounding effects of lung volume (LV) and surface area artefact (SAA). The human body is not consistent in its volume, not just from one human body to the next but also within the same body the volume may vary dependent upon time of day, fluid volumes, whether the subject has recently eaten or is suffering from a respiratory problem. It is therefore beneficial to be able to measure or predict LV and SAA in each subject.

McCrory *et al* (1995) also evaluated the BODPOD® for measuring body composition, however, their subjects were not inanimate objects, but 68 adults between the age of 20 and 56 (26 female, 42 male). Predictions of %BF from the BODPOD® were compared with those of hydrodensitometry. Using the Bland and Altman method, (1986) 95% limits of agreement between the 2 methods were assessed. McCrory *et al* (1995) found that the 95% limits of agreement between these methods were very narrow and concluded that in comparison to the conventional method of hydrodensitometry, this new ADP method was valid. Consequently, they suggested that the BODPOD® could provide an easier and more practical method for predicting %BF in special populations such as the elderly and the disabled. However, it is important to note that they recommended further research in a variety of populations to assess the validity of this method.

1.10 Air Displacement Plethysmography: Review of use in Children and Adolescents

Nunez *et al* (1999) compared the use of ADP against hydrodensitometry and DXA for measuring Db. In their study they tested adults for comparison to previous research, but the main purpose of their study was to assess the use of ADP for measuring body composition in children in comparison to other traditional validated methods. Firstly measurements for Db from hydrodensitometry and ADP were compared in adults to evaluate the system. Nunez *et al* (1999) argued that it was not necessary to compare body fatness as predicted by these two methods since they both use the Siri equation (Siri, 1961) and would therefore gain no more insight than that already offered by the Db result. For this reason they used %BF as predicted by DXA with which to compare %BF as predicted by ADP. Subjects were grouped by age (adults ≥ 20, n = 72) (children ≤ 19, n = 48) and measurements by all 3 methods were taken. Results were compared using various statistical analyses including Bland and Altman (1986) 95% limits of agreement. The researchers found that the results from the children differed from the adults. Their findings strongly supported body composition estimation by ADP for use with adults and confirmed earlier research observations (McCrory *et al*, 1995). Although their results for the children between ADP and hydrodensitometry were also highly correlated a small but statistically significant bias was detected. Similarly, a small bias was observed between ADP %BF estimates compared to corresponding %BF estimates by DXA. Nunez *et al* (1999) concluded that this was due to a minor system calibration problem with the BODPOD® ADP system. Furthermore, they hypothesised that this problem could have been linked to the 12 children within their subject group who weighed less than the recommended weight for use with this system (40kg). Moreover, they encouraged further research in this area to identify and correct these problems for use with children since this method is more practical and acceptable than hydredensitometry for subject groups such as children. However, it should be noted that Nunez *et al* (1999) did not use an acceptable reference method.

Lockner *et al* (2000) compared ADP with hydrodensitometry DXA for assessing body composition in 54 10 – 18 year olds. Subjects in New Mexico were measured for Db using all three methods and results were compared using paired t-tests and linear regression analysis. The researchers discussed the problems associated with hydrodensitometry as a reference method for use with this population due to the practical issues with water submersion and expressed that DXA may be a more appropriate reference method for use with children. However, they acknowledged that previous research had produced acceptable results and comparisons between hydrodensitometry and ADP in adults so the hydrodensitometry was included in this study and all three methods compared. Db results obtained from the ADP method and hydrodensitometry were converted to %BF using the 2-component model conversion formula of Siri (Siri, 1961). Questions regarding the validity of these results arise from this as it has been frequently published, and readily accepted amongst researchers, that a multi-component reference method is essential when measuring body composition in children and the Siri formula is inappropriate for paediatric use. As previously stated, this is due to often inaccurate assumptions made regarding the composition of the FFM when an adult 2-component model is applied (Reilly, 1998). However, Lockner *et al* (2000) found a bias in Db measurement by the ADP method, which was related to body size and suggested that further research to identify a small correction factor may improve the accuracy of this method for use with children.

Dewit *et al* (2000) considered the acceptability and feasibility ADP in children to determine its precision and agreement with hydrodensitometry. Measurements of BV were obtained using the ADP system, and the raw BV noted before these values were transformed into Db by the system. The measurements are adversely influenced by conditions created by the subject's presence in the chamber (isothermal air is more compressible than adiabatic air), therefore the manufacturers apply certain correction factors to the software for predicting thoracic gas volume (TGV) and volume of air next to the skin (SAA) to adjust isothermal conditions for obtaining each subject's actual BV (ABV) (Dempster and Aitkens, 1995). Equations involving TGV and SAA are then applied to obtain ABV. However, whole body ADP estimates TGV from functional residual capacity (FRC) prediction equations derived in adults aged 15 – 91 years (Crapo *et al*, 1982). Dewitt *et al* (2000) therefore substituted these with the children derived prediction equations of Rosenthal *et al* (1993) and Zapletal *et al* (1976) to calculate BV. These results of BV were compared with BV results obtained by hydrodensitometry where BV was calculated as the difference of body mass in air and in water, correcting for density of water and measured LV (Fuller *et al*, 1992). In this study by Dewit *et al* (2000), agreement between the hydrodensitometry and the ADP method was assessed using the method of Bland and Altman (1986). Comparisons between BV as calculated by the BODPOD® and by using the child specific prediction equations for TGV were also assessed using the method of Bland and Altman (1986). The researchers found that the precision of volume measurements and of body composition assessments by plethysmography was approximately twice as good as obtained by hydrodensitometry. Moreover, they reported an improvement in the results when the child specific equations for the prediction of TGV were applied. Dewit and colleagues (2000) acknowledged that the 2-component model of body composition used in this study makes the assumptions of a constant FFM density, though this assumption is not valid for children. Therefore, they recommend more complex models of body composition analysis be used as reference models when validating any particular method for use with children. However, these 3- and 4-component models all require measurements of BV. These researchers argued that ADP is a valid and more suitable method for this role in analysing body composition in children as part of a multi-component model.

Wells and Fuller (2001) recently reported on the precision of measurement by whole body ADP. They investigated methodological and biological precision for ADP across a wide range of body sizes in 28 adults and 30 children aged between 5 and 48 years. Repeated measures of BV and Wt were made using the BODPOD® from which they derived results for Db, FFM and fat mass. The raw BV results (BV before corrections were applied for the effects of isothermal conditions as described earlier and cited in Dempster and Aitkens, 1995) were recorded and hence BV calculated using the adult and child specific correction factors as appropriate (Dewit *et al*, 2000). Fat and FFM in the children were then derived from Db using the child specific equations of Lohman (Lohman, 1989). As a result of their repeated measures, Wells and Fuller (2001) concluded that the ADP system showed good precision for BV and Db across a wide range of body sizes, subject to appropriate correction factors for TGV and SSA, and the appropriate use of equations for predicting fat mass and FFM.

1.11 Review of Individual Methods - Skinfold Thickness

Skinfold body composition analysis is based on the assumption that approximately half of an adult's body fat is in the subcutaneous tissues, that is, the tissues immediately beneath the skin (Tritschler, 2000). Overall body composition is estimated from SFT measurements taken at selected sites on the body using a set of specially designed calipers. There are more than 100 equations to estimate %BF from SFT (Heyward, 1997). The equations differ by number and location of skinfold sites. The use of calipers for measuring SFT as an estimation of body fatness has for many years been recognised and generally accepted as an extremely useful tool. This method is easily portable, cheap, fairly non-invasive, is quick and does not require extensive training (Durnin *et al*, 1997). However, problems arise with the use of SFT measurements with the large number of published equations for use with this method. Often inappropriate equations which are age/sex or population specific are used incorrectly (Slaughter *et al*, 1988, Reilly, 1998). Furthermore, many of the SFT prediction equations are based upon the 2-component model of body composition where the body is divided into two compartments for measurement, fat mass and FFM, and the assumption is made that FFM has a constant composition. This however should not be assumed in the case of children where their FFM is continually changing through puberty and should not therefore be presumed to be constant (Slaughter *et al*, 1988, Janz *et al*, 1993, Reilly, 1998, Jurimae *et al*, 1998). For this reason it is important that when using SFT measurements for the estimation of body composition in children to choose the appropriate regression equations for the subject population (Slaughter *et al*, 1988, Janz *et al*, 1993, Reilly, 1998).

1.12 Skinfold Thickness: Review of use in Adults

There have been numerous studies which have looked at the use of SFT as an indication of %BF in adults and as a result many varying regression equations have been derived to assist in the interpretation of the measurements (Brozek and Keys, 1951, Pascale *et al*,1956, Durnin and Rahaman,1967, Durnin and Womersley, 1974, Jackson and Pollock ,1978). In the UK the equations of Durnin and Womersley (1974) are probably the most widely used (Jebb and Elia, 1993). These equations are based on measurements made at four sites – biceps, triceps, subscapular and suprailiac and a logarithmic transformation of the sum of the four skinfold thicknesses produces a linear relationship with Db which is age- and sex-specific. Fat mass is then calculated using the equation of Siri (Siri, 1961). The regression equations of Durnin and Womersley (1974) were derived from a study of 481 men and women aged between 16 and 72 comparing results with those obtained from Db as measured by hydrodensitometry. These equations have been shown to accurately predict body fatness in Caucasian adults but different equations are required for use with children or specific ethnic groups. It is generally accepted amongst practitioners that the problems arising from the SFT method do not rest solely with the chosen equation, but also with the location of the measurements and the accuracy of the observer taking the measurements (Jebb and Elia, 1993). In longitudinal studies it is advised to maintain the measurements using the same observer to reduce error at the stage of measurement. However, a recent study by Durnin *et al* (1997) has cast doubt on this problem. In their study of 53 women and 45 men they made SFT measurements at the traditional sites as set out by Tanner *et al* (1953) and Edwards *et al* (1955) and then again at deliberately chosen sites approximately 20mm from the 'correct' sites. Durnin *et al* (1997) found that the effect of this procedure resulted in differences, in most cases, of less than 1% in terms of body fatness. However, it could be argued that they were indeed still following standard procedures. Even at these incorrect sites as it was the same trained observer taking each measurement and the repeated measurements were taken at the same point (20mm away from the 'correct' site) each time, therefore not making allowances for random measurement or indeed variation of observer. If in further study however, it was found that random measurements and variation of observers, trained and untrained, came to the same conclusions, this would spark a renewed faith in the use of this method for predicting body composition both in the field and at the bedside.

1.13 Skinfold Thickness: Review of use in Children and Adolescents

As previously mentioned, many of the prediction equations available for use with SFT measurement in predicting body composition are based on the 2-component model assuming that the FFM is constant. It is now commonly known that this is not true for children and therefore alternative age specific equations are essential when predicting body composition in children and adolescents. The equations of Slaughter *et al* (1988) were derived using a multi-component model which divided the FFM of 310 children into further compartments of bone mineral and TBW. The age range of the children was not given in this study, however, the children were put into groups according to their maturation stages using the Tanner Scale (Tanner, 1962) of pubertal stage development. In the reference method used by Slaughter *et al* (1988) bone mineral was estimated from radius and ulna measurements using photon absorptiometry and TBW was estimated using ^2H_2O dilution. As a result, a number of age and sex specific equations were derived for use with SFT measurements on children and adolescents using measurements from 2 sites as opposed to the 4 sites required for adult measurements.

Lohman (1989) reviewed the various methods for measuring body composition in children, including the use of SFT as an indicator of %BF. In this review Lohman concluded that the equations developed by Slaughter *et al* (1988) which take into account the effects of age on Db, eliminate the previous problems related to suppositions about relationships between Db and SFT which resulted in an overestimation of %BF upon conversion. Also, in this review Lohman underlines the need for comparisons with a multi-component reference method when undertaking any validation of methods to be used with children and adolescents.

In 1993, Janz *et al* produced a cross-validation of the Slaughter SFT equations for children and adolescents. In this cross-validation they compared a selection of the Slaughter equations against a criterion measurement (Lohman's Siri-age adjusted Db equation) in 122 subjects ranging in sexual maturation from pre- to post pubescent and ranging in age from 8 – 17. In this study Janz and colleagues found that their data indicated that the Slaughter equations "hold promise" for estimating body composition in children and adolescents, as there was a high degree of correlation between the Slaughter equations and the Lohman adjusted equation. However, since it has been established by many researchers, including Lohman, that any comparisons of methods for measuring body composition in children should include a multi-component reference method, this cross- validation should be treated with caution. On the other hand, Lohman's adjusted equation is well received amongst researchers and this study by Janz *et al* (1993) should therefore not be dismissed entirely.

Jurimae *et al* (1998) compared the different regression equations used with SFT measurements and BIA. The skinfold measurements and impedance measurements of 107 boys and 105 girls aged between 9 and 11 were taken and body fatness results compared when used with 6 different regression equations found in the literature. From their study Jurimae and colleagues found a significant difference between the results given from the various equations and concluded that this provided evidence that any regression equation should be used with caution. However, they ventured further to suggest the use of 2 specific equations from the 6 for use with children in this age group based on their findings. This advice should be treated with caution since Jurimae *et al*, have no foundations upon which to make this suggestion since no reference method, multi-component or otherwise, was included in their study.

Reilly (1998) reviewed various methods for measuring body composition in children and adolescents, and in particular, criteria for determining choice of method. In his review he also underpinned the need for cautious choice of equation when using the SFT method. He reported not only that it is essential that the equation should have been validated against a recognised multi-component method, but also that comparisons were analysed using Bland and Altman (1986) 95% limits of agreement rather than correlations coefficients only. This is due to the fact that body composition methods tend to be highly correlated, though this does not necessarily mean that they agree. Therefore, although it appears from much of the literature available that the Slaughter equations are the most appropriate for use with children and adolescents, it also appears that these recommendations could be flawed. Both of the following two fundamental conditions that should be applied to any validation concerned with body composition methods for children – they should be compared to a multi-component reference method and analysed for 95% limits of agreement as depicted by Bland and Altman (1986) (Reilly, 1998, Wells *et al*, 1999).

1.14 Review of Individual Methods -Total Body Water

It is apparent that the total amount of body water can be determined by drying various tissues to constant weights (desiccation method). However, due to the nature of this method, the requirement for tissue removal, this is not appropriate in living humans (Chien *et al*, 1962). The earliest, and still the most direct, method for measuring TBW in living humans is that based on the isotope dilution principle. In this technique a tracer dose of labelled or 'heavy' water is used and 2 samples of body fluid (blood, urine or saliva) are collected, one pre-dose and one post-dose after an accepted equilibration time of 2-3 hours (Ellis, 2000). The method of analysis is dependent upon the tracer used, but the most commonly used tracer is ^2H_2O, which is analysed by mass spectroscopy. In general, TBW values obtained by dilution methods are considered as the reference methods for comparison against other techniques. Problems can arise however with the sample collection, especially with children, and the conversion of TBW results to calculate %BF for the purposes of body composition analysis (Westerterp, 1999).

1.15 Review of Individual Methods - Body Mass Index

In 1953 the Metropolitan Life Insurance Company developed the first tables of desirable BWt for men and women according to their Ht. These tables and subsequent revisions of them were based on actuarial data from insured persons; the data did not represent all ages, races, and socio-economic groups. Despite their ease of use and interpretation and appropriateness for some persons, Ht-BWt tables are insufficient for body composition assessment (Tritschler, 2000). Their use may lead to invalid conclusions, particularly for those who have a large muscle mass, who may be perceived as overweight according to the tables. Similarly, a person who has very little muscle mass but excessive body fat may be perceived as having a desirable BWt or even under weight according to the tables. BMI is an improvement over the Ht-BWt tables, although Ht and BWt are still the only factors used in the calculation of the index. BMI is defined as the ratio of BWt in kilograms to the square of Ht expressed in meters as follows

$$BMI = BWt/Ht^2$$

Estimation of body composition from BMI is widely used in large health studies and can be very informative but its standard error is quite large. It is especially prone to errors in children and the elderly, whose muscle and bone weight in relationship to their Ht are changing rapidly (Tritschler, 2000). In 1995, Cole *et al* published centile curves for BMI in British children from birth to age 23years based on the same large representative sample that was used to update the Ht and BWt references (appendix 1). These centile curves are commonly used in paediatrics to assess for the degree of fatness over or under the national average for a child of a specific BWt and Ht.

1.16 Study Aims and Objectives

The ability of BIA to accurately predict body composition is largely dependent on the equation chosen. (Reilly *et al*, 1996, Jurimae *et al*, 1998). The relatively new BIA technique, based on lower body (leg to leg) impedance measurement has become widely available for paediatric use, but its validity is questionable for use with children. Software for body composition estimation contained in commercially available instruments is a commercial secret, and is often very inaccurate in children and adolescents. Since leg to leg BIA is even quicker and cheaper than the conventional whole body BIA, while being equally acceptable to the child, there is an urgent need to validate it in comparison with a multi-component reference model (Jebb *et al*, 2000, Rowlands and Eston, 2001).

To my knowledge, the leg to leg system has not currently been validated against an accepted reference method for use with children. This is a critical age for body composition assessment due to the many changes in the child's body as it goes through the various stages of puberty. Many of the equations available for BIA do not take these factors into account and can often result in inaccurate predictions of body composition. Many BIA equations have been derived but none for use specifically with leg to leg BIA until the study by Tyrrell *et al* (2001), which developed such an equation based on comparisons between leg to leg BIA and DXA. However, this equation was developed for children between the ages of 4 and 11 years and has not been validated in any other population other than the test group. Moreover, since estimates of body composition derived from BIA are based on theories relating to TBW, a number of researchers believe that any reference method used to validate impedance measurements should incorporate TBW measurements (Abbot and Davies 2001, Wells *et al* , 1999, Lohman, 1989, Jackson & Pollock, 1988). In addition, the use of a multi-component reference method or even a gold standard is desirable as described above, especially when making measurements of body composition in children. A 2-component method rests on the assumption of constant composition of fat mass and FFM (Lohman, 1986, Reilly,1998, Rowlands and Eston, 2001). This can be readily assumed in adults, but in children this can not be assumed due to the changing chemical composition through childhood into adulthood. Since there are very few studies which compare the simpler methods with an accepted reference method, or indeed compare as many of the simpler methods with an accepted reference method, it is the main purpose of this study to compare and validate the use of these widely available methods for measuring body composition in children and adolescents against an accepted multi-component reference method.

2.0 Methods

2.1 Study Rationale and Reference Method

The rationale for the study was that a 3 - component model of body composition using TBW and Db provides measurements of fat mass and FFM which can be used as a reference method against which other simpler methods can be compared and validated (Fuller *et al,* 1992, Wells, 1999).

In the present study the 3 – component model developed by Fuller *et al* (1992) and applied by Dewit *et al* (2000) was used which separates the body into 3 components: water, fat and fat free dry mass (fat free dry mass is essentially protein and mineral) as the reference method against which all other methods would be compared. For this method, fat mass was calculated using BWt and BV obtained by ADP having been adjusted for LV and SAA to give ABV, and TBW measured by deuterium dilution, using the equation of Fuller *et al* (1992). Methods for measurement of TBW and BV are given below

$$\text{Fat Mass (kg)} = [(2.20 \times \text{ABV}) - (0.764 \times \text{TBW})] - (1.465 \times \text{BWt})$$

2.2 Subjects

56 healthy boys and 8 healthy girls aged between 10 and 14 were included in the study. The University of Glasgow Ethics Committee and the Ethics Committee for the Glasgow City Council Education Department approved the study (App. 2 & 3).

Informed written consent was obtained from the parents or guardian of the child (App. 4). One parent or guardian was present during all stages of the testing and every effort was made to ensure the comfort of the child during the test procedures. The subjects were recruited voluntarily by letter through local schools and football teams (App. 5).

Prior to all tests a home visit was arranged by telephone and carried out by the researcher in order to brief the parents and the child fully with regards to the exact nature of the tests. This also provided opportunity for clarification of any point of confusion or allowed an additional option of withdrawal from the study and could withdraw consent at any time. At this meeting the subject's BWt was also taken, using the same calibrated scales to be used for the testing, for the purpose of accurate dose preparation for the TBW analysis (part of the 3-component reference method). All tests were carried out at the University laboratory in the Department of Human Nutrition within the Glasgow Royal Infirmary. Only apparently healthy children were included in the study, and the results of the children who for whatever reason did not complete all of the tests (n = 14) have been excluded. Poor recruitment of females, due mainly to unwillingness to have body composition measured, resulted in insufficient numbers which led to the study being based on the male subjects only.

2.3 Laboratory Protocol – Anthropometry: height, weight and body mass index

In all subjects standing Ht was measured to the nearest 0.1cm using a floor standing stadiometer (Leicester Height Measure, Child Growth Foundation, London).

BWt was measured to the nearest 0.1kg using calibrated electronic scales (SECA ALPHA model 770) with the child measured while wearing swimming trunks/swimming costume. BWt was also measured as an integral part of the ADP procedure however, for the calculations presented throughout this study the values obtained by the SECA ALPHA scales were used.

BMI was also calculated for the subjects using Quetelet's index (Rolland – Cachera *et al*, 1982, Dietz and Robinson, 1998). BMI values were then expressed as centiles relative to UK 1990 reference data (Cole *et al*, 1995) for descriptive purposes so that the sample could be assessed in terms of their degree of underweight / overweight. (results section 3.2, Table 5).

2.4 Laboratory Protocol – Estimates of Fatness from Skinfold Thickness

SFT was measured in triplicate with Harpenden Calipers in all subjects at the triceps and subscapular sites (fig 1 & 2). Previous studies relative to a 2-component reference method (hydrodensitometry) have suggested that measurement at these two sites in children and adolescents are both more practical, and as informative as multiple site measurement for predicting adiposity in children (Reilly *et al*, 1995).

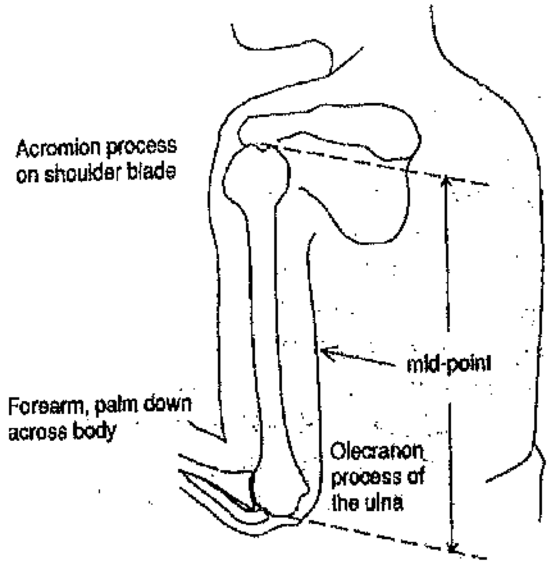

fig 1: Location of tricep skinfold site. Reproduced from Robbins GE, Trowbridge FL. In: Nutrition Assessment: A Comprehensive Guide for Planning Intervention by M.D. Simko, C.Cowell, and J.A. Gilbride, p. 87.

fig. 2: Location of subscapular skinfold site. Reproduced from Robbins GE, Trowbridge FL. In: Nutrition Assessment: A Comprehensive Guide for Planning Intervention by M.D. Simko, C.Cowell, and J.A. Gilbride, p. 87.

All SFT measurements were made by the same observer (the author) after a period of training and standardisation. The age and sex specific equations of Slaughter *et al* (1988) were used to calculate an estimate of %BF from the average measurement at each site.

For a sum of triceps and subscapular less than 35mm, the following equation was applied (Slaughter *et al*, 1988).

$$\text{Boys \% BF} = 1.21\,(\text{triceps} + \text{subscapular}) - 0.008\,(\text{triceps} + \text{subscapular})^2 - 1.7$$

For a sum of triceps and subscapular greater than 35mm, the following equation was applied (Slaughter *et al*, 1988).

$$\text{Boys \%BF} = 0.783\,(\text{triceps} + \text{subscapular}) + 1.6$$

These equations were derived by Slaughter *et al* (1988) using a multi-component method utilising measurements of Db, TBW and bone mineral content of the radius and ulna of 66 children (mean age 9.8 years) from the USA. These were used in the present study because they were more accurate when compared to a 2-component model than alternative published skinfold thickness equations in children and adolescents (Reilly *et al*, 1995).

2.5 Laboratory Protocol – Estimates of Fatness from Total Body Water

TBW was calculated from the distribution of a dose of ^2H_2O in body water. Each subject was given a sterilised dose of 99.9% ^2H_2O manufactured by SIGMA-Aldrich Chemical Company, Poole, UK, in the form of a single drink (diluted 1:10 with tap water). The dose given was 0.05g of ^2H_2O per kilogram of BWt as described by Wells *et al,* 1999, and Prosser and Scrimgeour (1995). The precise amount of ^2H_2O consumed was determined by adding the weights of the container, water and ^2H_2O and then subtracting an aliquot for dilution analysis and subtracting the weight of the bottle. Prior to consuming the drink the subjects provided a urine sample in a sterile universal container to establish the natural abundance levels of deuterium within the subject's body water. After the dose consumption, fruit juice or drinking water was used to rinse the container that contained the dose and this was also consumed. Subjects were required to measure the volume, note the time and collect a sample (approximately 10ml) of all urine produced for up to 5 hours post dose, or for 3 samples post dose, whichever was longer. These urine samples were stored in a freezer at –20^{0}C until taken for analysis at the University of Glasgow Human Nutrition Laboratory within Yorkhill Hospital. All urine samples, diluted doses and tap water used to make up the diluted doses were analysed for deuterium content by continuous-flow isotope ratio mass spectrometry (Hydra, PDZ Europa). Samples were analysed in triplicate after equilibration with a reference gas (5% hydrogen in helium) over a platinum catalyst. The mass spectrometer was calibrated using gravimetric standards of known deuterium content, which were prepared and analysed with each batch of samples. (Prosser and Scrimgeour, 1995, Scrimgeour *et al,* 1993). TBW was calculated by dilution of the deuterium dose after correction for non-aqueous hydrogen exchange taken as deuterium dilution space/1.04 and for deuterium passed in the urine (Reilly, 1998).

Hydration of FFM changes during childhood and adolescence (Reilly *et al*, 1998). In order to calculate FFM from TBW in children and adolescents, it is therefore necessary to assume an age and sex specific constant for hydration of FFM. In the present study FFM was calculated incorporating Lohmans hydration factor for children of each specific age group the validity of which has been confirmed previously (Wells *et al*, 1999) Body fat was calculated by the difference between BWt and FFM.

2.6 Laboratory Protocol – Estimates of Fatness from Whole Body Bioelectrical Impedance Analysis

Whole body bioelectrical impedance was measured using the Bodystat 1500MDD (Bodystat UK, Isle of Man) hand to foot method. In this method a current of 800μA at a single frequency of 50kHz was passed between electrodes on the right hand and right foot of the subject. The surface area of the skin was cleaned with alcohol wipes and the current electrodes were placed on the dorsal surfaces of the right hand and the right foot at the distal metacarpals and metatarsals, respectively. The detector electrodes were applied to the right pisiform prominence of the wrist and between the medial and lateral malleoli at the ankle, according to the manufacturer's instructions (see fig. 3 & 4).

Fig 3 Electrode placement at right hand. Fig 4 Electrode placement at right foot.

The drop in voltage between the two points was calculated to give an estimate of impedance or resistance.

It was important to ensure consistency among subjects, therefore, subjects were asked to fast for 4 hours prior to the measurements and to refrain from any physical exercise for the same period. Subjects were rested in the supine position for 5 minutes before the measurements were taken, to allow body fluid compartments to equilibrate (Kushner and Schoeller, 1986, Reilly, *et al* 1996). FFM was calculated using the impedance value corrected for Ht and BWt using the Houtkooper equation (Houtkooper *et al,* 1996). The Houtkooper equation has previously been shown to be most accurate of those available when compared to 2-component reference methods in children and adolescents (Reilly *et al* 1996)

$$\text{FFM (kg)} = 0.61\,(\text{Ht}^2/\text{Z}) + 0.25\ \text{BWt} + 1.31$$

Where Ht = height (cm), Z = impedance (Ω) and BWt = weight (kg).

The results from these calculations were compared to the 3-component model, as were the results predicted by the Bodystat 1500MDD software using the manufacturers equations, which are unfortunately unavailable to the user.

2.7 Laboratory Protocol – Estimates of Fatness from Leg to Leg Bioelectrical Impedance Analysis.

Working on the same principles of electricity conduction as for the hand to foot method, again a small current of 800μA at a single frequency of 50kHz was passed, this time between two electrodes in the form of metal foot plates on a set of specially designed scales. For the purpose of this study the TANITA model TBF-521 was used (fig. 5). The current was passed from one foot to the other via the legs only. The subject stood on the metal plates for this procedure. As with all bioelectrical impedance measurements, the position of the electrodes was important to the measurement (Jebb and Elia, 1993), therefore, the positioning of the subjects' feet were kept the same as far as possible and prepared by cleaning and drying prior to stepping on the electrodes. The subjects were also asked to stand completely still for the duration of the measurement (approximately 20 seconds).

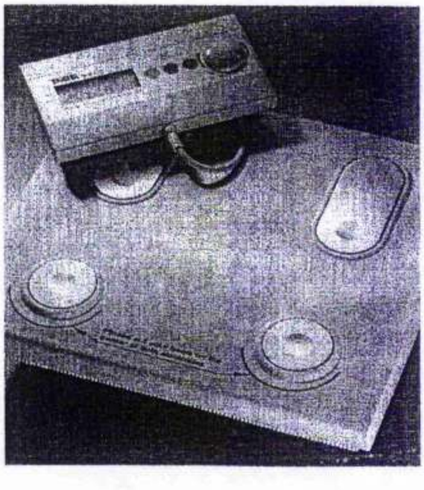

fig 5 TANITA leg – leg bioelectrical impedance model TBF-521.

The ability of the leg to leg method for predicting body composition using the manufacturer's software was compared to the 3-component model (again, these equations are not available to the user) and the actual impedance measurement cannot be obtained from this model.

2.8 Laboratory Protocol – Estimates of Fatness from Whole Body Air Displacement Plethysmography

Db and BV were measured using ADP by means of the BODPOD® body composition system (Life Measurement Instruments, California, USA) (McRory *et al*, 1995, Dempster and Aitkens, 1995, Dewit *et al*, 2000, Wells and Fuller, 2001). Measurements were taken according to the manufacturer's instructions with subjects wearing a tight fitting swimming costume and swimming cap and all jewellery removed in order to minimise the amount of trapped air between the subject and their clothing, which can result in an inaccurate measurement (Dempster & Aitkens 1995, McCrory *et al*, 1995, Dewit *et al* 2000).

Before each subject was measured, a volume calibration was conducted using a known volume metal cylinder. Subjects entered the BODPOD® and were required to sit completely still and breathe normally for the duration of the measurements (less than 2 minutes). Subjects sat inside the anterior chamber of the BODPOD® which was connected to a measuring chamber at the rear of the system via oscillating diaphragms used to induce pressure changes in the anterior chamber (fig. 6).

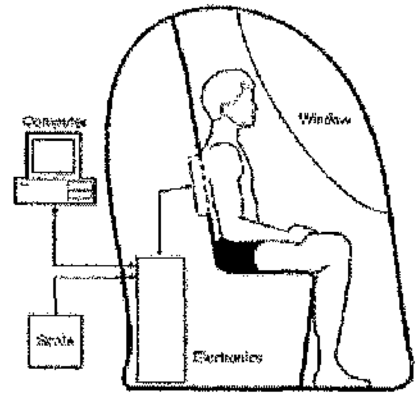

Fig 6 Diagrammatic representation of major system components of BODPOD®. Dempster and Aitkens, Med. Sci. Sports Exerc.Vol. 27, No. 12, pp. 1692-1697

Two measurements of BV lasting approximately 50 seconds each were performed. A third measurement was occasionally required when the first two measurements differed by more than 150ml (Dempster and Aitkens, 1995, Dewit *et al*, 2000, Wells and Fuller, 2001). The mean of the closest two measurements was then recorded and the whole procedure repeated to improve method precision (Dewit *et al*, 2000, Wells and Fuller, 2001).

The ability of the whole body ADP system to predict body composition was tested using 2 approaches. Firstly the manufacturer's software, the adult-specific equation of Siri (1961) was used to give a predicted value for %BF. Secondly, by using the children's age-specific densities of FFM of Lohman (1989) incorporated into the fundamental 2-component equation presented by Brozek *et al* (1963) (table 2).

Age (years)	Male Fat-free density kg/l	Female Fat-free density kg/l
1	1.068	1.069
1-2	1.071	1.071
3-4	1.075	1.073
5-6	1.079	1.075
7-8	1.081	1.079
9-10	1.084	1.082
11-12	1.087	1.086
13-14	1.094	1.092
15-16	1.096	1.094

Table 2. Density of FFM corrected for age. T. G. Lohman, Pediatric Exercise Science, 1989, Vol. 1, pp. 19 - 30

$$\%F = 1 / Db\ [(d_1 d_2) / d_1 - d_2] - [d_2 / (d_1 - d_2)] \times 100$$

(Brozek *et al*, 1963)

Where Db = body density as provided by the BODPOD®,

d_1 = Density of FFM according to the tables of Lohman (1989)

d_2 = Density of fat (0.90kg/l for all ages)

The results predicted from these equations were compared with the 3-component model which used the ABV calculated by the ADP system. The equations of Rosenthal *et al* (1993) and Zapletal *et al* (1976) to correct for TGV and the equations of Dubois and Dubois (1916) to correct for SAA were then applied to the raw body volume measurement (Wells and Fuller, 2001), to give ABV.

$$ABV = RBV + 0.4TGV - SAA$$

(Dempster and Aitkens, 1995)

Firstly surface area (SA) was calculated using the Dubois and Dubois (1916) equation:

$$SA = 71.84 \times BWt^{0.425} \times Ht^{0.725}$$

Where BWt was in kg and Ht was in cm, this is then multiplied by a constant k to give SAA:

$$SAA = k \times SA$$ (Dewit *et al*, 2000)

where $k = -4.67 \times 10^{-5}$ (Wells, 2001 personal communication)

TGV was then calculated by

$$TGV = FRC + 0.5TV$$

Where FRC is functional residual capacity and TV is tidal volume, all values in litres. The equations for FRC (Rosenthal *et al*, 1993) are different for males and females and are Ht dependent.

For males below the height of 162.6cm the following equation was used (Ht is in cm)

$$FRC = (0.02394 \times Ht) - 1.716$$

(Rosenthal *et al*, 1993)

For males above the height of 162.5cm the following equation was used (Ht is in cm)

$$FRC = (0.05918 \times Ht) - 7.036$$

(Rosenthal *et al*, 1993)

The equations for TV (Zapletal *et al*, 1976, published in Czech, JCK Wells personal communication) for both sexes, using log-ten values with Ht in cm, are as follows

$$LogTV = (1.8643 \times LogHt) - 1.3956$$

(Zapletal *et al*, 1976)

Using BV obtained from the above equations, TBW from the ^2H_2O dosing and BWt, fat mass was calculated from the following equation (Dewit *et al*, 2000).

$$FM\ (kg) = (2.20\ BV) - (0.764\ TBW) - (1.465\ BWt)$$

And %BF calculated from the equation

$$\%BF = [\text{Fat mass(kg)} / \text{BWt (kg)}] \times 100$$

2.9 Statistical Analysis - Tests of Validity: comparisons with the reference method

The limits of agreement between the various methods were investigated by plotting the individual between-method differences against their respective means (Bland-Altman plot). Heterocedasticity was examined by plotting the absolute (positive) differences against individual means and calculating the Spearman's correlation coefficient. If the heterocedasticity correlation was close to zero and the differences were normally distributed (Shapiro-Wilk's test), the mean bias and the 95% limits of agreement were calculated as mean $\pm$ 1.96 SD of the between-method differences (results section 3.3, fig 7 and table 4).

3.0 Results

3.1 Recruitment of subjects and drop out

56 apparently healthy 10 –14 year old boys were recruited from the Glasgow area through schools and local amateur football teams. 42 of the subjects tested complied with all the necessary procedures and the present study is based on these. The other 14 dropped out at crucial stages in the testing or failed to comply with the study protocol either by not completing the tests, failing to supply urine samples, or taking the urine samples too soon after the ^2H_2O had been administered.

3.2 Physical characteristics of subjects

Mean age of the 42 male subjects was 12.9 (SD 1.1) years, median 13.0 (range 10.1 – 14.5) years. Mean height was 1.59 (SD 0.11) m, median 1.59, (range 1.36 – 1.86) m, and mean weight 51.8kg (SD11.9; median 49.4 range 35.6 – 74.6). BMI was calculated and assessed as a centile score relative to UK 1990 population reference data, as a simple index of under/overweight: mean BMI was 20.2 (SD 3.0; median 20.1, range 16.49 to 27.74)kg/m^2. Individual characteristics of subjects are given in table 3. Summary data for each method of estimating body composition are given in table 5. A graph of the BMI scores plotted on the centile curves is given in fig 9.

3.3 Biases and Limits of Agreement Relative to the Reference

Biases were generally large, and 95% limits of agreement between each method and the reference were generally wide, though 95% limits of agreement were narrowest for the 2-component approach based on TBW, which also had the smallest bias (Figure 7); Summary statistics for the biases and 95% limits of agreement are given in table 4. In all cases except skinfolds, errors were not significantly correlated with the size of the fat mass. The assessment of skinfold errors are presented separately (figure 8) where it is clear that the agreement between the skinfolds and the reference is also poor at the level of the individual.

Table 3. Characteristics of subjects (n 42)

Age	Sex	Height (m)	Weight (kg)	BMI	Centile scores for degree of over/underweight	%BF (ref. method)
12	M	1.56	67.4	27.70	**>99.6th**	45.95
10	M	1.36	42.7	23.09	**98th**	41.81
11	M	1.55	41.3	17.19	50th	17.73
13	M	1.59	50.5	19.98	75th	25.99
10	M	1.39	36.6	18.94	75th	24.35
13	M	1.60	43.8	17.11	25th	17.79
11	M	1.49	38.3	17.25	50th	29.94
12	M	1.60	43.1	16.84	25th	22.26
12	M	1.54	61.2	25.81	**98th**	42.14
14	M	1.67	46.9	16.82	9th	13.38
13	M	1.77	64.7	20.65	75th	28.14
14	M	1.57	44.9	18.22	25th	1.92
13	M	1.60	47.6	18.59	50th	22.09
13	M	1.61	59.5	22.95	91st	26.90
14	M	1.70	63.8	22.08	75th	4.94
13	M	1.64	74.6	27.74	**98th**	35.65
14	M	1.67	67.5	24.20	91st	17.97
14	M	1.71	52.5	17.95	25th	5.41
14	M	1.51	38.5	16.89	9th	6.37
11	M	1.45	35.6	16.93	25th	5.50
14	M	1.62	54.0	20.58	75th	7.98
12	M	1.46	36.4	17.08	25th	5.84
14	M	1.62	53.7	20.46	75th	8.97
12	M	1.48	36.3	16.57	25th	4.89
14	M	1.68	68.5	24.27	91st	9.47
14	M	1.50	38.9	17.29	9th	5.49
14	M	1.58	46.2	18.51	25th	6.18
14	M	1.86	73.7	21.30	75th	4.40
14	M	1.71	69.1	23.63	91st	14.40
14	M	1.76	65.7	21.21	75th	6.18
14	M	1.71	66.5	22.74	91st	5.01
13	M	1.64	54.4	20.23	75th	9.61
13	M	1.56	45.3	18.61	50th	25.90
12	M	1.57	50.7	20.57	75th	26.56
12	M	1.61	54.1	20.87	75th	8.08
13	M	1.49	36.6	16.49	9th	15.56
13	M	1.50	38.8	17.24	25th	12.29
13	M	1.51	46.6	20.44	75th	9.09
12	M	1.47	45.2	20.92	91st	26.53
14	M	1.70	70.2	24.29	91st	10.80
13	M	1.66	53.5	19.42	50th	20.23
13	M	1.59	48.2	19.07	50th	10.79
Mean 12.84		Mean 1.59	Mean 51.8	Mean 20.2		Mean 16.4
SD 1.13		SD 0.11	SD 11.9	SD 3.0		SD 11.6

Table 4. Biases and limits of agreement* (Method minus reference) for 6 methods relative to the reference

	Body Fat Percentage			Body Fat Mass (kg)		
Method	**Bias**	**SDdiff**	**Bias ± 95%Limits**	**Bias**	**SDdiff**	**Limits (+/-)**
Hand – Foot BIA	+2.4	6.0	11.8	+1.4	3.9	7.6
Leg – Leg BIA	+4.1	7.2	14.1	+2.3	4.0	7.8
BODPOD® Software	+4.7	4.9	9.6	+2.3	2.5	4.9
Db	+2.6	4.8	9.4	+1.2	2.5	4.9
TBW	+0.9	2.6	5.0	+0.5	1.5	2.9
SFT	-1.4	6.0	11.8	-0.6	3.1	6.0

*Limits of agreement calculated as Bias ±1.96 x SD of the difference

Table 5. Body Fatness estimates by each of the seven methods (Mean, SD; Median, range)

	Body Fat Percentage				Body Fat Mass (kg)			
Method	**Mean**	**SD**	**Median**	**Range**	**Mean**	**SD**	**Median**	**Range**
Reference	16.4	11.6	12.8	2.0 – 46.0	8.7	7.0	6.4	0.9 – 30.9
Hand – foot impedance	18.8	7.9	16.2	8.2 – 40.7	10.1	5.6	8.3	3.8 – 24.3
Leg – leg impedance	20.5	7.8	16.6	7.0 – 40.5	10.9	5.8	8.9	3.4 – 27.3
BODPOD Software	21.2	11.7	18.3	1.1 – 49.7	10.9	7.1	9.9	0.5 – 33.5
Body Density	19.0	11.5	16.2	-1.3 – 46.6	10.0	7.1	8.7	-0.6 – 31.4
TBW	17.3	11.7	13.3	3.4 – 45.9	9.2	7.2	7.5	1.6 – 30.9
Skinfolds	15.0	8.2	13.1	6.0 – 40.6	8.1	5.8	6.5	2.5 – 30.3

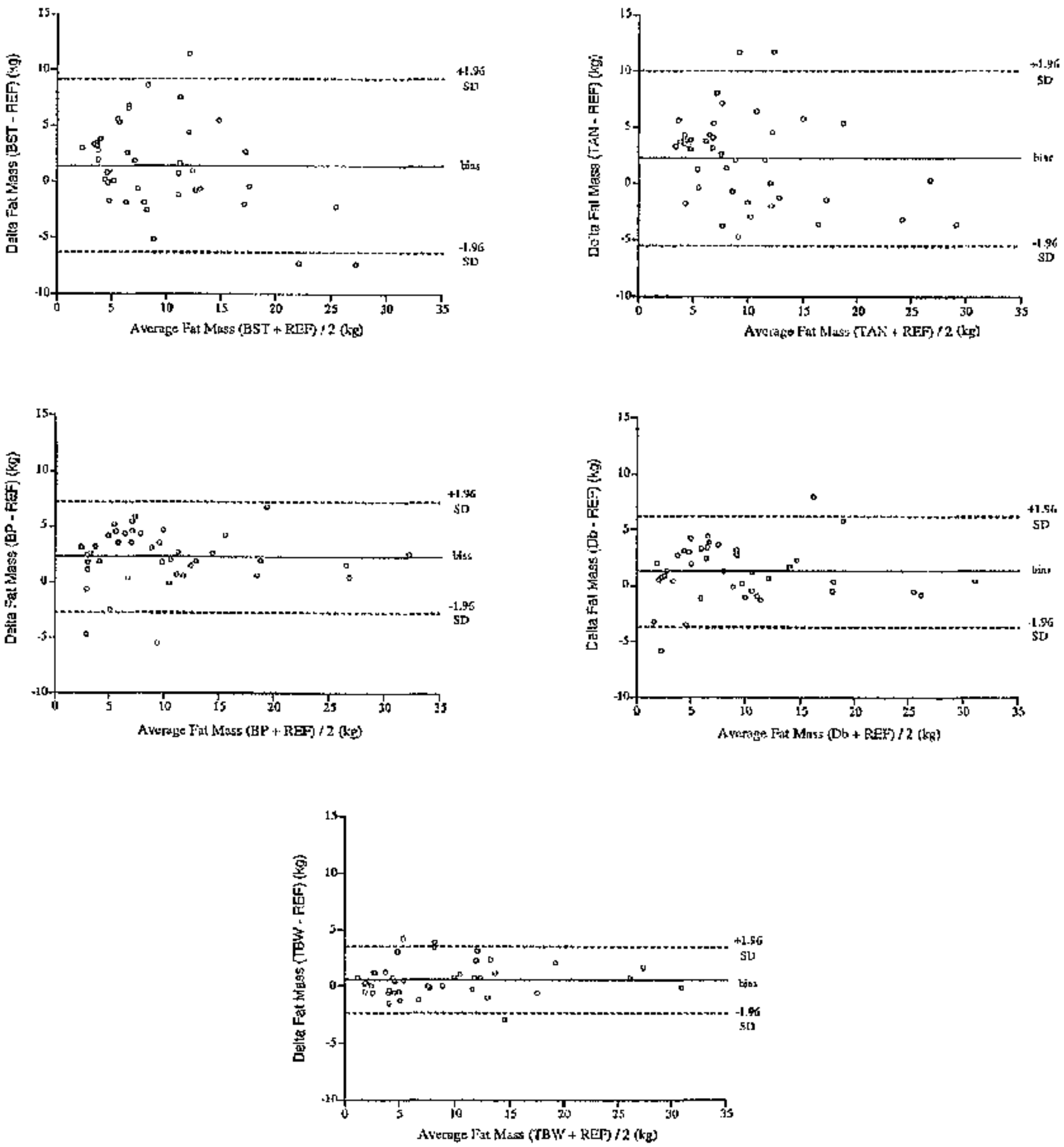

Fig. 7 Biases and limits of agreement for 5 of the 6 methods plotted against the mean of the reference plus the method (Bland/Altman 1986) TBW, total body water; BST, hand to foot impedance using bodystat; TAN, leg to leg impedance using TANITA; BP, BODPOD® software; Db, body density.

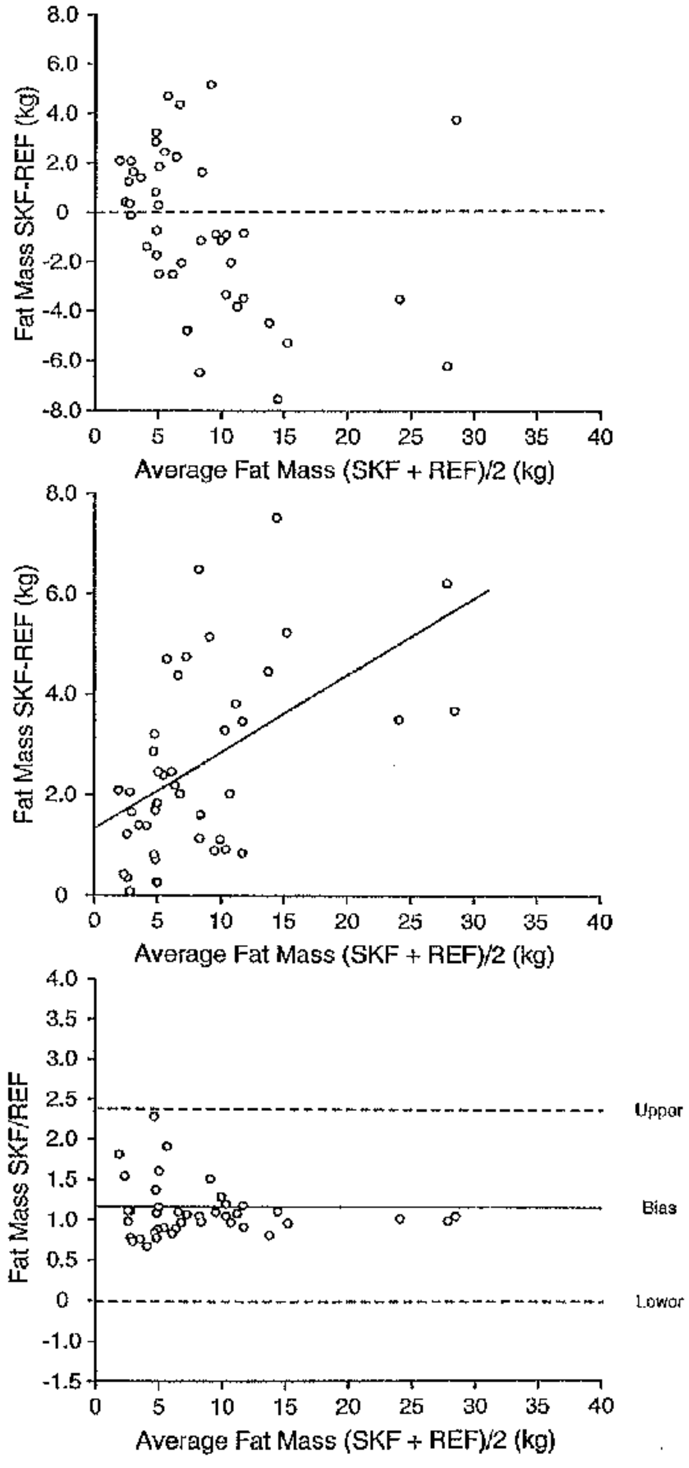

Fig 8 Errors for fat mass (kg) using skinfold method with direction of error (sign) retained (a) or removed (b); ratio of fat mass by skinfolds to reference (c); all plotted against mean fat mass by skinfolds and reference (Bland and Altman method 1986)

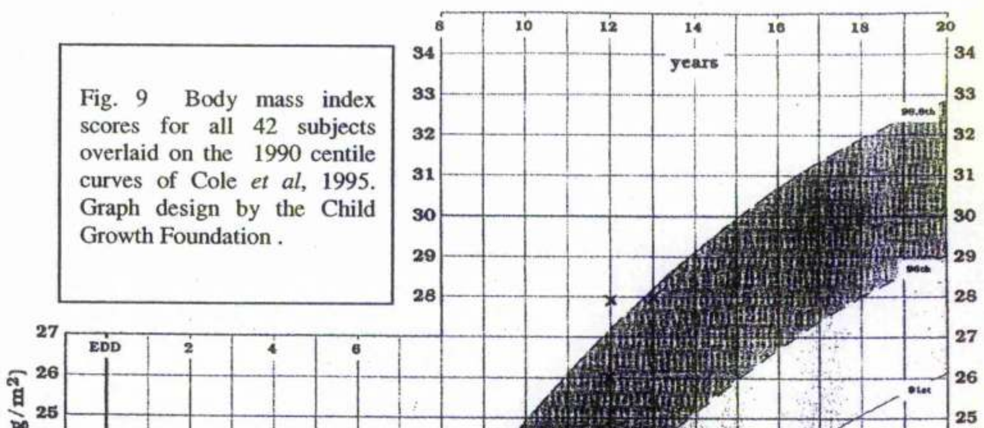

Fig. 9 Body mass index scores for all 42 subjects overlaid on the 1990 centile curves of Cole *et al*, 1995. Graph design by the Child Growth Foundation .

4.0 Discussion

4.1 Main findings and implications

As previously noted, the developed world is becoming more aware of the problems and health risks associated with increased body fatness. As well as a heightened awareness of increased BWt many more people are becoming obsessed with BWt gain/loss prompted by the present social pre-occupation with beauty and thinness advocated by current media trends. Nevertheless, a recent government health study in Scotland (Dong & Erens, 1998) has voiced concerns over the increase in levels of obesity, particularly in children, attributed to a combination of the current desire for fast food and lack of exercise. With this heightened focus on levels of body fat there is an increased awareness in the need for accurate measurement of body composition (fatness and FFM) in a range of fields from epidemiology to clinical research (Reilly, 1998).

Accurate measurement of body composition in the past has required sophisticated apparatus and techniques beyond the scope of most clinical practice and research, so workers have generally used simpler methods such as anthropometry and BIA. Many of these simpler methods are becoming more widely available not only to the researchers and practitioners, but also to the general public. With the apparent increase in awareness of body fatness, it is essential that methods publicly available for measuring body composition are safe, reliable and accurate. A recent study (Wells *et al*, 1999) has cast doubt on the accuracy of two of the most popular simpler methods, BIA and SFT. Wells *et al* (1999) and Reilly (1998) concluded that no simple methods of measuring body composition in children/adolescents had been validated and that more research was essential. Since these methods have been shown to accurately predict body composition in adults (Lukaski *et al*, 1985, Kushner and Schoeller, 1986, Segal *et al*, 1988), they are generally assumed to be reliable for use with children. However, due to the problems associated with presuming a constant FFM in children which is inherent in these methods, it can not be assumed that these simpler methods are appropriate, but should be tested and compared with a multi-component reference method. It is desirable that these methods should be validated for their use with children since the simple methods are highly suitable for use in the field or at the bedside due to their ease of use, low cost and portability. If these methods are not accurate and reliable, they are open to misinterpretation, which could result in serious repercussions, particularly in the field of medicine where a drug dosage may be dependent upon a child's body composition. The present study therefore set out to validate in children several of the simpler methods for measuring body composition against a reputable 3-component reference method.

The results of the present study suggest that validity, at the level of the individual child, is poor for most or all of the methods tested. Using the Bland and Altman (1986) statistical method for comparisons the results showed wide limits of agreement in all cases and large biases for most. This outlined the inaccuracy of most of the simpler methods, some of which were up to 14% over or under the result as given by the reference method. For a child whose BWt is approximately 50kg, this figure can equate to a difference of over 7kg in fat mass, which is a significant amount. Of the 2-component methods tested, TBW had highest accuracy, and this is consistent with a recent study by Wells *et al* (1999). It should be noted that even for the estimates derived from TBW, errors were also fairly large at the individual level. For example, table 4 illustrates a 0.5kg bias for fat mass (with mean fat mass 8.7kg), and 95% limits of agreement of 0.5 plus or minus 2.9kg. For the popular approaches of BIA and ADP the results of the present study are not encouraging. The greatest bias and widest 95% limits of agreement (table 4) were found using the TANITA impedance method: bias with the TANITA was 2.3kg fat mass (again with a mean fat mass for the reference of 8.7kg); 95% limits of agreement 2.3kg plus or minus 7.8kg. A correction might be applied to reduce the bias observed, but wide 95% limits of agreement indicate large errors at the individual level and these seem unavoidable. Newer methods such as the BODPOD® and TANITA systems are very attractive from a practical point of view in paediatrics, but the present study suggests that estimates of body composition obtained from them should be interpreted cautiously, and this is in broad agreement with the few comparable studies in this area. Fields and Goran (2000) for example also reported wide 95% limits of agreement for BODPOD® relative to a 4-component reference method in 14 boys and 11 girls aged 9-14 years. It is worrying that the lack of accuracy at the individual level found in these methods commonly goes unreported or unnoticed. Since many of these methods are available to the general public, the figure obtained from the chosen equipment, would be the figure used and relied upon, and it is apparent that these figures presently are inaccurate in many individual cases. This could lead to misinterpretation of the results in many ways, not only in the field of medicine as previously described, but also in the home where many of these impedance instruments are used to track levels of fat mass. This is particularly worrying in those monitoring their weight and could assist in heightening the problems, which can exacerbate many psychological disorders associated with weight management (Kerruish *et al*, 2002). The results of the present study indicate that simpler methods for measuring body composition, while they are easy, quick and relatively cheap, they are not accurate enough to be described as measuring body composition, but merely estimating it. In cases where a more accurate measurement is required, a multi-component reference method is essential. It must be noted however, that even a multi-component method only provides estimates but we know the accuracy of these estimates will be within certain limits.

The present study provided a comprehensive assessment of the validity of several body composition methods against a reference method. A recent review (Fields *et al*, 2002) concluded that there is an urgent need for more research in which several techniques are compared simultaneously against a reference method. There is also a consensus that comparing methods of unknown validity against each other does not address the issue of method validity (Lohman *et al*, 1993, Reilly, 1998, Wells *et al*, 1999, Fields *et al*, 2002) though recent paediatric body composition literature is dominated by comparisons between non reference methods (e.g. Nunez *et al*, 1999, Cole *et al*, 2000, Nicholson *et al*, 2001, Tyrell *et al*, 2001). A number of authors have recommended that future research employs larger, more homogenous samples, as here, so that conclusions as to the validity of methods for any age/sex group can be made with greater confidence. (e.g. Fields *et al*, 2002).

Conclusions can be made from this study with some confidence on the validity of most of the commonly used paediatric body composition methods, at least for 10–14 year old Caucasian boys. The relatively homogenous sample (same sex, narrow age range; n 42) tested in this study was a strength in that it permitted reasonable confidence in our conclusions at least for this sex/age group. Extrapolation of the results to girls, or children of different ages should be carried out cautiously, and the findings may best be regarded as providing testable hypotheses for other population groups. However, it should be noted that the (limited) literature comparing methods with 2- and multi-component reference methods (Reilly *et al*, 1995, Pintauro *et al*, 1996, Reilly *et al*, 1996, Reilly, 1998, Wells *et al*, 1999, Ellis, 2000, Fields and Goran, 2000, Fields *et al*, 2002) has generally produced similar conclusions as to the validity of most methods. The study design was another strength, the use of a multi-component model reference method to determine validity is essential though many of the previous studies have not included one (eg Cole *et al*, 1995, Nunez *et al*, 1997, Nicholson *et al*, 2001, Tyrell *et al*, 2001). Furthermore, multiple methods were compared with the reference giving an overall view of the simpler methods widely available for use both in the field and clinical settings.

4.2 Sources of Methodological Error

Detailed treatment of this issue is available in recent reviews (Reilly, 1998, Wells *et al*, 1999, Fields and Goran, 2000, Ellis, 2000, Fields *et al*, 2002). Our study design is now fairly standard practice in that simpler methods were compared against a multi-component reference method, and the reference inevitably included some of the individual methods tested. This is less of a problem than it might seem since when used in the multi-component model the techniques are used to provide measures of a component or property of the body, but when used as body composition estimation methods additional assumptions must be applied. For example, comparing BODPOD® estimates of fatness with the reference simultaneously tests the accuracy of the raw measurement of Db with the accuracy of the assumptions applied to the raw measure: errors observed suggest that the assumptions of constant density of FFM (presumably using Siri's equation) incorporated into the BODPOD® software are a source of error in children, as expected. Applying the Lohman constants for density of FFM did not improve accuracy greatly. This conclusion is consistent with a previous study by Wells *et al* (1999).

Similarly, for the TBW method, when used as a 2-component method, the same raw data are used in both the reference method and the 2-component estimates. The Lohman values for FFM hydration (plus the density measurements and corrections which appear to have very small error for volume measurement; Fields and Goran, 2000) are therefore effectively what is being tested. The minimal bias observed for TBW, and narrower 95% limits of agreement, imply that the Lohman hydration assumptions used are appropriate, again consistent with the earlier study by Wells *et al* (1999). For the BIA and SFT methods an analysis of sources of error is more complex since these use a variety of approaches (with or without assumptions, use of regression equations, as well as variation in the raw measurement). The main practical conclusion must be that the BIA and SFT approaches were not accurate at the individual level in this study.

4.3 Study Limitations

A few limitations of the present study should be noted. For some of the methods used the study was limited by the software incorporated in these methods. It is conceivable that the raw impedance values from the two BIA approaches tested might be incorporated into empirically derived predictions in order to improve accuracy, but such improvements may only be small (Sung *et al*, 2001) and testing this hypothesis was beyond the scope of the present study. The use of a 3-component model as reference might be seen as a disadvantage (relative to a 4-component model), but an earlier study by Wells *et al* (1999) found empirical evidence that the 3-component approach, as used here, was acceptable and it represents a significant improvement over simple comparisons between non-reference methods. A determining factor in the decision not to use a 4-component model was that the measurement of body bone mineral is likely to be hardware or software dependent to some degree (Reilly, 1998, Wells *et al*, 1999), and large errors have been reported with some combinations of hardware and software (Reilly, 1998, Wells *et al*, 1999). This study could not include all available body composition methods (DXA was not included for example). This is potentially important because DXA can provide fairly precise body composition estimates, and accuracy is potentially good (Jebb and Elia 1993), though it has recently been reported as poor relative to a 4-component model in female youth (Pintauro *et al*, 1996). Furthermore, though the homogeneity and large sample size were strengths in the present study in the sense that it permitted reasonable confidence in our conclusions, at least for this sex/age group, extrapolation of the results to girls, or subjects of different ages, or to extremes of body fatness should be carried out cautiously. Pubertal staging of subjects could not be carried out for practical and ethical reasons, potentially inclusion of information on pubertal status might improve accuracy of prediction (Slaughter *et al*, 1988). The pessimistic findings of this study may best be regarded as providing testable hypotheses for other populations. However, the (limited) literature comparing methods with reference methods (Reilly *et al*, 1995, Reilly *et al*, 1996, Reilly, 1998, Wells *et al*, 1999, Ellis, 2000, Fields and Goran, 2000, Fields *et al*, 2002, Wong *et al*, 2002) has generally produced similarly negative conclusions as to their validity.

4.4 Suggestions for Further Research

Further research aimed at improving the accuracy of simple field methods is essential. Further research on girls, subjects of different ages and extremes of body fatness is essential to gain an overall view of the accuracy of these simpler methods in comparison to a multi-component reference method. Equations for improving the accuracy of the impedance methods should be derived and tested against a reference method in large samples of relatively homogeneous subjects in order to attain valid results and conclusions. If possible, DXA should be included in any further study to provide an overall view of all available methods for estimating body composition in children and adolescents.

4.5 Main Conclusions

In conclusion, the present study found that accuracy of most of the popular field and laboratory methods for assessment of body composition was poor in 10 – 14 year old boys. This is particularly worrying since many of these methods are commonly used in paediatrics and it is not known how the results are interpreted and how much reliance is placed on their accuracy. Accuracy may be even poorer in disease states (Reilly, 1998, Wells *et al*, 1999), and this study suggests that all existing (non reference) methods are used with caution at an individual level. It is evident that if no reference method is available then TBW is more accurate than the other methods, however, if accuracy is imperative then a multi-component method is essential. Further research aimed at improving the accuracy of simple field or clinical methods of body composition estimation is essential. Alternatively, the relatively poor accuracy of the existing methods may have to be accepted. The present study again confirms that, if high accuracy of body composition estimates in individuals is essential, multi-component methods for measurement must be used without exception.

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