An analysis of body composition
and its measurement in a sample of Irish adults
aged 18-81 years

Siobhan Leahy

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Supervised by: Dr. Cian O’Neill and Professor Phil Jakeman

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Abstract

Title: An analysis of body composition and its measurement in a sample of Irish adults aged 18-81 years

Author: Siobhan Leahy

Obesity is a global epidemic and is defined as excess fat accumulation to the extent that health may be impaired (WHO 2000). However, body mass index (BMI), the metric used to quantify obesity, does not adequately represent fat tissue mass (FTM). A metric that quantifies obesity according to FTM is required. Methods that accurately measure total and segmental FTM and are suitable for use in large scale studies are necessary.

Using dual energy x-ray absorptiometry (DXA) as the reference method of measurement, this thesis documents the body composition, specifically FTM and its distribution, of 1136 Irish adults aged 18-81 years. Fat tissue mass index (FTMI, kg/m$^2$) was chosen as the most appropriate metric to define ‘fat obesity’ and to compare the difference in total body fat according to age and sex. Reference FTMI values were derived from young adult (YA) z-scores. FTMI was found to increase with age in men and women, as did the proportion of FTM deposited abdominally. 40% of men and 45% of women defined as ‘fat obese’ by FTMI were not ‘obese’ by BMI.

Compared to DXA, the accuracy of the prediction methods i.e. bioelectrical impedance analysis (BIA), anthropometry and ultrasonography, for the measurement of total and/or segmental body fat has been established. Prediction equations derived from site-specific skinfold and girth measures accurately estimated % body fat in men ($r=0.91$, standard error of the estimate (SEE) =2.5%) and women ($r=0.92$, SEE=3.0%). Prediction equations derived from site-specific subcutaneous adipose tissue thickness using ultrasonography accurately estimated % body fat in YA men ($r=0.95$, SEE=1.9%) and women ($r=0.91$, SEE=3.0%). BIA was found to underestimate % body fat and did not provide an accurate measure of body composition in YA women or YA men with >25% body fat ($p<0.001$).

This thesis finds fat tissue mass, and its distribution, to be age and sex specific. BMI was found to be an inappropriate measure of ‘fat obesity’ and not indicative of the difference in total and segmental FTM between the sexes or across the lifespan. The thesis concludes that body composition can be accurately predicted from anthropometric and ultrasonography measures in healthy adults and these methods are suitable for use in large scale studies.
Authors Declaration

I hereby declare that the work contained within this thesis is entirely my own work other than the counsel of my supervisors Dr. Cian O’Neill and Professor Phil Jakeman of the Physical Education and Sport Sciences Department, University of Limerick. This work has not been submitted for any academic award, or part thereof, at this or any other educational establishment. Where the use has been made of the work of other people it has been fully acknowledged and referenced (Appendix F).

_______________________________
Siobhan Leahy, November 2011
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>%BF</td>
<td>% body fat</td>
</tr>
<tr>
<td>Σ3SF</td>
<td>Sum of 3 skinfolds</td>
</tr>
<tr>
<td>Σ4SF</td>
<td>Sum of 4 skinfolds</td>
</tr>
<tr>
<td>Σ7SF</td>
<td>Sum of 7 skinfolds</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Analysis</td>
</tr>
<tr>
<td>BM</td>
<td>Body mass</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variance</td>
</tr>
<tr>
<td>Dₜ</td>
<td>Body density</td>
</tr>
<tr>
<td>DoHC</td>
<td>Department of Health and Children</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>ECF</td>
<td>Extra-cellular fluid</td>
</tr>
<tr>
<td>ECS</td>
<td>Extra-cellular solids</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat Free Mass</td>
</tr>
<tr>
<td>FFMI</td>
<td>Fat Free Mass Index</td>
</tr>
<tr>
<td>FTM</td>
<td>Fat Tissue Mass</td>
</tr>
<tr>
<td>FTMI</td>
<td>Fat Tissue Mass Index</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>Health ABC</td>
<td>Health, Aging and Body Composition</td>
</tr>
<tr>
<td>IAAT</td>
<td>Intra-abdominal adipose tissue</td>
</tr>
<tr>
<td>IAF</td>
<td>Intra-abdominal fat</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra-class correlation coefficient</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>ISAK</td>
<td>International Society for Advancement of Kinanthropometry</td>
</tr>
<tr>
<td>IUNA</td>
<td>Irish Universities Nutrition Alliance</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
</tbody>
</table>
kV  kilovolt
LoA  Limits of Agreement
LTM  Lean tissue mass
m    meter
ml   millilitre
MRI  Magnetic resonance imaging
mSv  millisievert
N    Nitrogen
NHANES  National Health and Nutrition Examination Survey
NIH  National Institute of Health
OECD Organisation for Economic Co-ordination and Development
r    Correlation coefficient
ROI  Region of interest
SAT  Subcutaneous Adipose Tissue
SD   Standard deviation
SEE  Standard error of the estimate
SEM  Standard error of measurement
SF   Skinfold
SRD  Smallest real difference
S:V  Subcutaneous to visceral fat ratio
TBW  Total body water
TEM  Technical error of measurement
ULBC University of Limerick Body Composition Study
ULREC University of Limerick Research Ethics Committee
US   Ultrasound
VAT  Visceral adipose tissue
WC   Waist circumference
WHO  World Health Organisation
WHtR Waist-to-height ratio
y    years
YA   Young adult
Δ    Difference (Delta)
Chapter 1

Introduction
1.1 Background

Obesity refers to excess fat accumulation in adipose tissue to the extent that health may be impaired. This condition is now considered to be a worldwide epidemic with prevalence of adult obesity more than doubling to 500 million since 1980 (World Health Organisation 2011). In Ireland, approximately 25% of the adult population is obese (Irish Universities Nutrition Alliance 2011). Obesity is defined as having a body mass index (BMI, weight divided by height squared) of greater than 30kg/m$^2$. While BMI is a useful indicator to compare trends at a population level, it is a crude measure of fatness and cannot distinguish between fat and lean tissue, leading to misclassification in some individuals such as athletes (Nevill et al., 2010) and the elderly (Jackson et al., 2011). To this end, the Strategic Plan for NIH (National Institute of Health) Obesity Research (NIH 2011) has called for the development of ‘accurate and reliable tools to measure total and regional body composition across the lifespan that are applicable to clinical and population study settings’.

Multi-component models, described in Chapter 2, are the criterion method of body composition measurement. However these models require expensive equipment and are time consuming, making them unsuitable for use at a population level. Traditionally, hydrodenstiomtry has been used to measure body density and thus fat mass. This method is based on the assumption that fat tissue mass (FTM) and fat free mass (FFM) have a constant density of 0.9g/cm$^3$ and 1.1g/cm$^3$ respectively. The density of FFM in particular has been shown to vary with age and sex (Wang et al., 2003) and hydrodenstiomtry is not now recommended for use as a body composition reference measure in heterogeneous populations (Ellis 2000). Dual energy x-ray absorptiometry (DXA) is used
extensively as a reference method of total and segmental body composition. Though based on the assumption that lean tissue mass has a constant hydration of 0.73 (Wang et al., 1998), the method has been shown to accurately measure % body fat in young and older healthy adults when compared to multi-component models (r=0.88, Δ=0.7%, Clasey et al., 1999). DXA has been used in population studies such as the National Health and Nutrition Examination Survey (NHANES, Kelly et al., 2009); however its use of ionizing radiation and its non-portable nature make it impractical for use in some populations.

Prediction methods of body composition are more commonly used in large scale studies. In Ireland, the only available body composition data at a population level (IUNA 2001; 2011) are derived from bioelectrical impedance analysis (BIA). This method involves passing an electric current through the body and measuring the resistance offered. Body water and thus FFM and FTM are estimated using the manufacturer’s proprietary algorithms (Kyle et al., 2004). However the method is reported to be inaccurate in subjects with high levels of fat mass, underestimating % body fat by as much as 4.3% in obese men and 2.7% in obese women (Sun et al., 2005). BIA can also provide segmental body composition analysis, though the accuracy of this has not been established.

Anthropometry, specifically the measurement of skinfold thickness and girth measures, is also a widely used prediction method of body composition measurement. Algorithms exist to predict body density and thus % body fat from anthropometric measures obtained from selected body sites. The most commonly used prediction equations in a European context are those of Durnin and Womersley (1974), which are age and sex specific and based on the sum of four ($\Sigma 4$) skinfold thickness measures at the biceps, triceps, subscapula and suprailiac
sites. However these equations have never been validated on an Irish population, and given the change in phenotype due to the rise in body weight over the past three decades (WHO 2011), may not be accurate in the 21st century. In addition, it is assumed that skinfold thickness measures represent a double layer of skin and underlying adipose tissue (Marfell-Jones et al., 2006), thereby providing a direct measure of subcutaneous adiposity. Several authors have investigated the relationship between skinfold thickness measures obtained using manual calipers and subcutaneous adipose tissue (SAT) thickness measured using ultrasonography at various body sites. Correlations ranging from ‘very weak’ (r=0.299, Kuczmarski et al., 1987) to ‘very strong’ (r=0.927, Selkow et al., 2011) have been reported; however, the relationship between the two measures is not proportional, i.e. a single layer SAT measure obtained using ultrasonography does not correspond to half of a double layer skinfold thickness (Booth et al., 1966; Selkow et al., 2011). These findings suggest that anthropometry, particularly skinfold thickness measures, may not represent body density/% body fat and that the relationship between the two variables is incidental.

Ultrasonography is reported to be an accurate method of measurement of both subcutaneous (Fanelli and Kuczmarski 1984) and visceral (Mook-Kanamori et al., 2009; Emmons et al., 2011) adipose tissue thickness. It is likely to provide a more accurate estimate of total and segmental body composition than anthropometry, as it is a direct measure of SAT thickness. It also overcomes the limitations associated with skinfold thickness measures such as inter-operator error and difficulty obtaining measures in very obese persons due to the limited opening of the caliper jaws (Kuczmarski et al., 1987). The development of portable scanning instruments has led to the investigation of ultrasonography as a
field method of body composition measurement (Pineau et al., 2007; Duz et al., 2009). In order for this technique to be effectively applied, accurate, generalised prediction equations to estimate total or segmental composition from ultrasound measurement of SAT thickness, using a standardised methodology, must be established on a reference population.

1.2 Thesis aims

This thesis presents the body composition data of 1136 healthy adult men and women in Ireland measured as part of the University of Limerick Body Composition (ULBC) Study from October 2008 to July 2011. Dual energy x-ray absorptiometry, bioelectrical impedance analysis, anthropometry and ultrasonography have been employed to provide a detailed analysis of body composition. For the purposes of this research, DXA is used as the reference method to describe total and segmental body composition and to investigate the accuracy of alternative measures of body composition. The thesis has two primary aims;

1. to describe total and segmental body composition, and specifically body fat mass and its distribution, in a large sample of healthy Irish adults;

2. to investigate the accuracy of field methods, namely bioelectrical impedance analysis, anthropometry and ultrasonography for the quantification of total and segmental fat mass in this population.
1.3 Thesis structure

This is an article-based thesis, comprising an introduction (Chapter 1), brief literature review (Chapter 2), four data chapters (Chapters 3-6) and a summary/conclusion (Chapter 7). Chapter 3 provides an analysis of total and segmental fat distribution in the total group and across different age cohorts of the adult population, as measured by dual energy x-ray absorptiometry. The young adult subjects are used to provide reference values for fat tissue mass index (FTMI) and thus define obesity and its prevalence in terms of fat mass. The relationship between fat tissue mass in the abdominal region and total body fat mass is also reported. Chapter 4 investigates the accuracy of BIA in the measurement of total body composition and regional fat and fat free mass in healthy young adults aged 18-29 years. This chapter has previously been published in the European Journal of Applied Physiology (Appendix F, Leahy et al., 2011). Chapter 5 describes the validation and cross validation of novel prediction equations for % body fat using selected skinfold thickness and girth measures in the total adult sample. This article is currently under review in the British Journal of Nutrition. Chapter 6 contains three related articles on the use of ultrasonography to measure SAT thickness and thus % body fat. The first article is a technical paper providing recommendations for the accurate measurement of SAT using real time, B-mode ultrasound and has been published previously (Appendix F, Toomey et al., 2011). The second article investigates the relationship between skinfold thickness measures, obtained using manual calipers, and ultrasound measured SAT thickness in a sample of healthy young adults aged 18-29 years. The final article describes the relationship of SAT thickness measured at specific anatomical sites to total and segmental body fat mass in the
same sample of young adults. Prediction equations for % body fat derived from ultrasound measured SAT thickness at specific sites are also validated for the subject group. This article has been accepted for publication in Ultrasound in Medicine and Biology (Appendix F, Leahy et al., 2011).

In keeping with the article based format, each data chapter has a self-contained abstract, introduction, methodology, results, conclusion and reference section with an additional bibliography included at the end of the thesis. There are minor deviations from the styles of articles in their published form, for example all abstracts are in monograph form and the Harvard UL referencing system is used throughout. Tables and figures are numbered to reflect the chapter number and so do not appear as in their published form.
1.4 References


Leahy, S., Toomey, C., McCreesh, K., O’Neill, C. and Jakeman, P. (2011b) 'Ultrasound measurement of subcutaneous adipose tissue thickness accurately predicts total and segmental body fat of young adults', *Ultrasound in Medicine and Biology*, IN PRESS.


fat in a large, healthy population', *The American Journal of Clinical Nutrition*, 81, 74-78.


Chapter 2

Literature Review
2.1 Obesity, Body Mass Index and waist circumference

The purpose of section 2.1 is to discuss the use of body mass index (BMI) and waist circumference (WC) as measures of fat mass and its distribution. These metrics are currently recommended by the World Health Organisation (WHO 2000) as a means to classify individuals into health risk categories.

Obesity refers to excess fat accumulation in adipose tissue to the extent that health may be impaired (WHO 2000). It is estimated that the prevalence of obesity has more than doubled worldwide since 1980, with approximately 500 million adults worldwide now obese and a further one billion overweight (WHO 2011). Ireland has the second highest rate of obesity in Europe, with 23% of the adult population affected, compared to the European average of 15.5% (OECD 2010). A recent National Adult Nutrition Survey (IUNA 2011) found that 25.8% of Irish men and 21.3% of Irish women aged 18-64 are obese, figures that have grown considerably since 1991 when the corresponding prevalence was 7.8% in men and 12.9% in women. BMI, the metric used to classify individuals into risk categories (Table 2.1.1), is calculated as weight divided by height squared (kg/m²).

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI</th>
<th>Risk of comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
<td>Low (but risk of other clinical problems increased)</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50-24.99</td>
<td>Average</td>
</tr>
<tr>
<td>Overweight:</td>
<td>≥25.00</td>
<td></td>
</tr>
<tr>
<td>Preobese</td>
<td>25.00-29.99</td>
<td>Increased</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00-34.99</td>
<td>Moderate</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00-39.99</td>
<td>Severe</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥40.00</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

(Reproduced from WHO (2000))

Overweight and obesity are the fifth leading risk of global deaths, with a BMI of greater than 25kg/m² recognised as a risk factor for various cardiovascular, musculoskeletal and psychological disorders, type II diabetes mellitus and certain
cancers (NIH 2011; WHO 2011). In Ireland, approximately 2,000 deaths annually are attributed directly to obesity, at significant cost to the economy (DoHC 2005).

BMI, however, is a crude measure of ‘fatness’. While it is a useful tool for between-populations comparisons, it may correspond to different levels of fatness between individuals within a population as it does not distinguish between fat and fat free mass (FFM; WHO 2000). It can be particularly misleading in groups such as athletes (Nevill et al., 2010) who have a larger proportion of lean mass than the general population, and elderly populations where BMI has been shown to increase up to the age of 80 despite a decline in fat free mass (Jackson et al., 2011). Furthermore BMI does not account for the wide individual variation in body fat distribution and may not correspond to the same level of risk in all individuals (WHO 2000).

Numerous authors have shown BMI to be a poor predictor of % body fat (%BF) when compared to methods that directly measure %BF. Frankenfield et al. (2001) found that 30% of men (n=53) and 46% of women (n=88) with a BMI<30kg/m² were classified as obese according to their %BF. Similarly in a study of 182 men and 234 women ranging in age from 18-70y and BMI of 17.0-41.9kg/m², Deurenberg et al. (2001) found that 41% of men and 32% of women classified as ‘fat obese’ according to their %BF were not obese according to their BMI. In both studies, cut-off points of >25%BF in men were used to define ‘fat obesity’; the cut-offs of >30% and >35% were used respectively by Frankenfield et al. (2001) and Deurenberg et al. (2001) in women. Curtin et al. (1997) demonstrated similar results in 15-86 year olds when using the lower cut-off points of BMI >27.8kg/m² and %BF >20% in men and BMI >27.3 kg/m² and %BF >25% in women to define obesity.
Deurenberg *et al.* (1991) and Gallagher *et al.* (2000) have attempted to address the limitations of BMI as a measure of %BF. Using dual energy x-ray absorptiometry (DXA) as the reference method of %BF measurement in a sample of 749 adults aged 16-83, Deurenberg *et al.* (1991) found that an algorithm including age and sex in addition to BMI shared a greater proportion of variance with %BF ($R^2=0.79\%$) than did BMI alone ($R^2=0.38$). Gallagher *et al.* (2000) demonstrated a curvilinear relationship between BMI and %BF in 1626 adults and replaced BMI with $1/BMI$ to improve linearity. Similar to the findings of Deurenberg *et al.* (1991) it was shown that an equation including age, sex and $1/BMI$ had a strong correlation to %BF ($r=0.90$) in the total subject group, whereas the correlation between %BF and $1/BMI$ alone ranged from 0.68 to 0.89. Further, Gallagher *et al.* (2000) demonstrated that these predictions differed according to ethnicity, where Asians had a significantly higher %BF for any given BMI than either White or African American subjects. More recently Heymsfield *et al.* (2007) established in 1757 adults that FFM shares a greater amount of variance with height ($R^2=0.42$) than does FTM ($R^2=0.02$) and concluded that subjects of the same BMI but who differ in stature have similar, but not identical, body composition.

Mean values for % body fat have been reported in various populations (NHANES, Kelly *et al.*, 2009; NANS, IUNA 2011). However, ideal ranges of % body fat do not exist (Gallagher *et al.*, 2000). Jackson *et al.* (2011) found that neither BMI nor %BF were suitable measures of body composition in healthy older men as they mask the decline in fat free mass which occurs with ageing. Therefore body composition should be reported in terms of FTM and FFM when comparing age groups or tracking body composition across the lifespan. Heymsfield *et al.* (2007) also recommend normalising body composition to height squared ($kg/m^2$) to
eliminate difference in composition due to stature. This approach has been adopted by Kelly et al. (2009), who established ‘normal’ values for fat tissue mass index (FTMI) from NHANES data. ‘Normal’ FTMI values of 3-6 kg/m² for men and 5-9 kg/m² for women are recommended, with FTMI >9 kg/m² in men and >13 kg/m² in women defining ‘obese’. However, these values were established by matching the prevalence of each FTMI category to BMI prevalence in young adults. As discussed earlier, BMI is a flawed measure of obesity and this may not be an appropriate approach to defining obesity according to fat tissue mass.

In addition to BMI, the World Health Organisation (2000) recommends the measurement of waist circumference as a convenient and simple measurement which ‘may provide a more practical correlate of abdominal fat distribution and associated ill-health’, with separate cut-offs recommended for men and women (Table 2.1.2).

<table>
<thead>
<tr>
<th>Risk of metabolic complications</th>
<th>Waist circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Increased</td>
<td>≥94</td>
</tr>
<tr>
<td>Substantially increased</td>
<td>≥102</td>
</tr>
</tbody>
</table>

(Reproduced from WHO (2000))

Waist circumference is a useful tool for identifying individuals with a ‘normal’ BMI (i.e. 18.5-25 kg/m²) who may be at risk of abdominal obesity, however it is thought that waist circumference measurement is unlikely to affect clinical management in those who are also classified as obese according to their BMI (Klein et al., 2007). While waist circumference has been shown to correlate well with intra-abdominal adipose tissue (IAAT) as measured by magnetic resonance imaging (MRI) and computed tomography (CT, Han et al., 1997; Klein et al., 2007; Ashwell and Gibson 2009), it cannot be considered an independent predictor of cardiovascular risk and is only of benefit where additional information on blood pressure, diabetes history and
Cholesterol measures are not available (The Emerging Risk Factors Collaboration 2011). Additionally, the stated cut-offs for waist circumference were derived from regression against BMI and concerns exist that the same cut-offs may not be relevant in all populations (Klein et al., 2007; Ashwell and Gibson 2009). To overcome this problem, the use of a waist to height ratio (WHtR) has been proposed (Ashwell et al., 1996), with a ratio of 0.5 or above indicating increased abdominal adiposity. A recent meta-analysis of ten studies found WHtR to be a better discriminator of cardiovascular disease risk factors than WC or BMI in adult men and women (Lee et al., 2008).

The anatomical location at which WC is measured is a subject of much debate, with up to four different sites widely used (Klein et al., 2007). The midpoint between the lower border of the rib cage and the iliac crest is recommended by the WHO (2000). However, the level of the umbilicus is also commonly used and can give a measure up to 10% higher than that taken at the recommended site (Han et al., 1997). Although the strong association with WC and IAAT is well documented, WC cannot distinguish between subcutaneous and visceral fat depots in the region (Klein et al., 2007; Wajchenberg 2000). The importance of measuring these depots independently is discussed in section 2.2.3.

It is clear that BMI and waist circumference are useful measures to compare health risk between different populations; however they do not provide a direct measure of body fat mass and may be misleading when used to classify individuals into risk categories.
2.2 Measurement of human body composition

The aim of section 2.2 is to describe the theoretical framework surrounding the organisation of human body composition and to discuss the commonly used methods of measuring total and segmental body composition. These methods are divided into two categories- reference methods (section 2.2.2.1) and prediction methods (section 2.2.2.2). Measurement of segmental body composition is discussed in section 2.2.3.

2.2.1 Organisation of human body composition

The anthropometric measures of BMI and WC discussed above do not directly measure body composition and are merely estimates of body fat mass and its distribution. There are five levels of organisation of human body composition; atomic, molecular, cellular, tissue system and whole body. The components of each level are shown in Figure 2.2.1.

![Figure 2.2.1 The five levels of body composition. *N=Nitrogen, CHO=Carbohydrate](image)

(Reproduced from Wang et al. (1992) in Heymsfield et al. (2005))

The atomic level describes the elemental composition of the human body; approximately 98% of body weight is made up of six elements (oxygen, carbon, hydrogen, nitrogen, calcium, phosphorous), and body composition may be
reconstructed using neutron activation analysis (Wang et al., 1992). Body composition measurements at the atomic level are the most reliable, as there are few underlying assumptions (Ellis 2000). The molecular level describes composition in terms of five components; water, protein, glycogen, mineral and lipid, with fat tissue mass (FTM) being a sub category of total lipid. Measurement of total body water (TBW) by isotope dilution and bone mineral content (BMC) by DXA are examples of body composition measurement at the molecular level. The cellular level has three components; cells, extracellular fluids (ECF) and extra cellular solids (ECS). ECF is the only cellular component that can be directly measured through isotope-dilution techniques. The fourth tissue-system or tissue-organ level comprises adipose tissue, bone and non-adipose soft tissue, or lean tissue mass (LTM). MRI and CT are the gold standard techniques for body composition measurement at the tissue-system level. The final, whole-body level refers to factors such as body weight, body volume and stature and can be measured using techniques such as anthropometry and hydrodensitometry (Wang et al., 1992).

2.2.2 Methods of human body composition measurement

Methods of measurement of body composition can be broadly described as either reference methods or prediction methods. Reference methods directly measure one or more aspect of composition and from this can evaluate a number of other components, while prediction methods (also known as field methods) provide an indirect estimate of an aspect of composition, usually based on regression equations derived by comparison to a reference method.
2.2.2.1 Reference methods of body composition measurement

There is no ‘gold-standard’, method of body composition measurement. The methods discussed below are each subject to measurement error and rely on basic assumptions which do not always hold true. However, ‘reference’ methods exist against which new methods of body composition measurement are validated (Heyward and Wagner 2004). Two main criteria define a reference method; the underlying principles of the method must not be based on major assumptions, and the method must have maximal precision (Wang et al., 1998). Reference measurements of body composition can be described by the number of components they measure, i.e. 2-component, 3-component, 4-component.

**Multi-component models**

Multi-component models refer to those methods measuring four or more components at the atomic or molecular level and are accepted as the criterion method of body composition measurement as they account for the largest number of components (Ellis 2000). Though models measuring as many as six components have been reported (e.g. $BM = FTM + water + protein + bone mineral + soft tissue mineral + glycogen$; Wang et al., 1998), four component models are the most widely used criterion method. These models measure fat mass by hydrodensitometry or air plethysmography, bone mineral content (BMC) by DXA, and TBW by hydrometry or dilution techniques; the remaining mass is the protein component of FFM. By combining these methods, inter individual variability in the characteristics of fat free mass are accounted for, allowing for a more accurate estimation of body composition (Heyward 2001; Williams et al., 2006). Due to the number of measures that must be taken, multi-component models are too time-consuming to be used in a clinical
setting or in studies requiring large numbers of subjects and thus are not widely used. Methods such as hydrodensitometry and DXA are more time efficient and have been used extensively (Durnin and Womersley 1974; Jackson and Pollock 1978; Gallagher et al., 2000).

**Hydrodensitometry**

Hydrodensitometry, or hydrostatic weighing, established in 1942 by Behnke et al. (Heyward and Wagner 2004), was the earliest method of body composition measurement. It is a 2-component model and measures fat mass and fat free mass. Hydrodensitometry involves weighing a subject in air and underwater, with a correction made for residual air in the lungs and digestive tract. Body density ($D_b$) is then calculated and converted to % body fat using the equations of Siri (1956);

$$% \text{ Body Fat} = \left[\frac{(4.95/D_b) - 4.500}{100}\right]$$

or Brozek et al. (1963);

$$% \text{ Body Fat} = \left[\frac{(4.57/D_b) - 4.142}{100}\right]$$

(Maud and Foster 1995)

This method is time consuming as several repeat trials need to be carried out, with some researchers conducting up to 10 trials per subject (Durnin and Rahaman 1967; Jackson and Pollock 1978). Hydrodensitometry is unsuitable for some groups such as children and the elderly who may not tolerate being submerged in water. A key assumption underlying this 2-component model is that the density of fat free mass is uniform at 1.1 g/cm$^3$ across all ages, sexes, body types and activity levels. However, the density of fat-free mass has been shown to vary by 0.002-0.004 g/cm$^3$ between men and women and by 0.003-0.005 g/cm$^3$ between those age <60 years and those ≥60 years (Wang et al., 2003). For this reason, hydrodensitometry is no longer
Dual energy X-ray Absorptiometry

Dual energy X-ray Absorptiometry (DXA) was developed in the early 1990’s (Wong et al., 2002). It is an advance on hydrodensitometry as it is a 3-component model measuring FTM and the LTM and BMC components of FFM (Heyward 2001). Using a low energy (40 kilovolt (kV)) and a high energy (70-100kV) x-ray beam, all three components are measured simultaneously (Ellis 2000). Compared to hydrodensitometry or other multi-component models, DXA scanning is time efficient, practical, requires no active subject involvement and imposes minimal risk (Pietrobelli et al., 1998). A standard scan using the Lunar iDXA™ instrument (GE Healthcare, Chalfont St Giles, Bucks., UK) takes less than seven minutes to perform and exposes the subject to a radiation dose of 0.03mSv (millisievert).

However, DXA is not without limitations. It is assumed that the composition of the soft tissue layer overlaying bone has the same fat-to-lean ratio as that for non-bone pixels in the same region. As bone is contained in approximately 40-45% of pixels in whole body scans, lean and fat composition is therefore based only on 55-60% of the body image obtained from DXA (Ellis 2000). It is also believed that DXA measurements are not affected by the anterior-posterior thickness of the body. The use of phantom calibration blocks to routinely check the accuracy of the DXA instrument and the availability of ‘thick scan’ mode for larger subjects attempt to correct for this limitation (Heyward and Wagner 2004). Finally, DXA is based on the assumption that the hydration of lean tissue mass is constant at 0.73 (Wang et al., 1998). Hydration of FFM has been shown to vary from 0.686 to 0.808 in human
cadavers (Wang et al., 1999). Going et al. (1993) scanned 17 adults at three intervals during a dehydration-rehydration protocol designed to induce changes of ~2% body mass (BM). A 2% decrease in BM was achieved following dehydration; no differences were found between baseline, dehydration and rehydration estimates of FTM and BMC. DXA detected increases in BM (+1.21kg), total tissue mass (+1.13kg) and LTM (+1.3kg) following rehydration. Subsequently Thomsen et al. (1998) demonstrated a mean increase in total tissue (+898g) and LTM (+812g) following ingestion of 1000g of water in 10 subjects, with no change observed in FTM or BMC. It is suggested that under normal conditions or in clinical conditions not associated with large changes in fluid balance, variation in soft tissue hydration does not limit the accuracy of DXA body composition measures (Pietrobelli et al., 1998).

Conflicting evidence exists regarding the accuracy of DXA measurement of total body composition when compared to multi-component models in adults. Williams et al. (2006) demonstrated that DXA overestimates %BF in non-obese young adult (mean age = 20.4 years) men (+1.7%; n=26) and women (1.2%; n=44) when compared to a 4-component model. This bias was greater in obese women (+2.2%; n=14). Van der Ploeg et al. (2003) found DXA to underestimate %BF by 1.6% in men aged 18.8 to 58.7 years and indicated that this bias increased with subjects’ age. Visser et al. (1999) showed that DXA shared 98% of the variance with a 4-component model for the measurement of total body FFM in 58 older adults (70-79 years); however there was a mean underestimate of 1.8kg FFM by DXA. Clasey et al. (1999) indicated that there was no difference in %BF measurement between DXA and a 4-component model in a group of 78 young and old men (mean age 23.4 and 66.5 years respectively) and women (mean age 24.2 and 65.8 years.
respectively). Wang et al. (1998) showed very high agreement ($R^2$=0.97) in 23 adults between DXA and the 6-component model described earlier for total body FTM, with no significant difference between the two methods.

Two possible explanations for the conflicting findings outlined above are the differences between DXA hardware and software used in each study, and the difference in measured body mass (BM) and that estimated by DXA. Three different manufactures produce DXA instruments – Hologic Inc. (Waltham, MA, USA), Lunar Corp. (Madison, WI, USA), and Norland Medical Systems (Fort Atkinson, WI, USA) and body composition results have been found to vary with manufacturer, software version, generation of DXA instrument and beam mode (Kistorp and Svendsen 1998). Differences of 1.5kg in FTM and 1.1kg in LTM have been reported between different software versions used to analyse the same scans in 15 overweight women (van Loan et al., 1995). In the study by Williams et al. (2006) discussed above, DXA-measured BM was significantly underestimated by 0.5kg in obese women and overestimated by 1.0kg in non-obese men. Lohman et al. (2000) suggests that agreement within 1kg is acceptable between the two methods.

The Lunar iDXA (GE Healthcare, Chalfont St Giles, Bucks., UK) used in the research presented here utilises a staggered array of 64 detectors (compared to 16 in the previous Lunar Prodigy™ instrument) which enhances the precision of the instrument, creating a high resolution image for analysis (Hull et al., 2009). Excellent precision has been demonstrated for iDXA measurement of total body composition in adults. Hind et al. (2011) reported a coefficient of variation (CV) ranging from 0.5-0.86% for total body BMC, LTM, FTM and %BF in 52 adults with BMI’s ranging from 16.7-42.7kg/m$^2$. Similarly Huizenga et al. (2007) reported CV
values of 0.78-1.22% for total body composition measurements in 29 very obese subjects (mean BMI=43.6kg/m²).

It is clear from the above information that DXA measures of body composition cannot yet be considered accurate to the level of multi-component models. However due to its relative ease of use and wide availability, the method is now widely accepted as a reference method of body composition measurement and is currently used as an assessment tool in the National Health and Nutrition Examination Survey (NHANES, Kelly et al., 2009) and the Health, Aging and Body Composition Study (Health ABC, Newman et al., 2005) in the United States.

MRI and CT are also examples of reference methods of body composition measurement. However they are not discussed here as their high cost and limited availability make them unsuitable for use in large scale studies.

2.2.2.2 Prediction methods of body composition measurement

As stated earlier, prediction methods of body composition indirectly estimate individual body components. Prediction methods are suitable for widespread use as they rarely require expensive equipment and are portable. For the purposes of this research, bioelectrical impedance analysis, anthropometry and ultrasound scanning are discussed in detail below. Other examples of prediction methods include infrared interactance (Conway et al., 1984) and 3D laser scanning (Pepper et al., 2010).

Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) involves the passing of an electric current through the body and measuring the impedance, or resistance offered. It is based on the principle that electric current flows at different rates through the body
depending upon composition (Dehghan and Merchant 2008). Aqueous tissue (i.e. lean tissue mass) is a major conductor of electric current, whereas fat tissue mass and bone are poor conductors (Ellis 2000). BIA is a popular method of body composition measurement as it is widely available commercially, is relatively cheap and non-invasive and skilled operators are not required. Single frequency BIA involves the passing of a single current at 50Hz through the body and offers an estimate of total body water (TBW) and fat free mass (FFM) whereas multi-frequency BIA uses several frequencies and can estimate the intra-cellular and extracellular components of TBW (Kyle et al., 2004).

Several authors have demonstrated a tendency of BIA to underestimate %BF by as much as 6.8% in men and 8.8% in women (Webber et al., 1994; Wattanapenpaiboon et al., 1998; Jebb et al., 2000). This underestimate is more apparent in those with higher %BF (Deurenberg et al., 2001; Sun et al., 2005). Sun et al. (2005) compared BIA to DXA on a sample of 591 adults and found an underestimation of % body fat in the group as a whole. When the sample was categorised into ‘lean’ ‘normal’ and ‘obese’, almost perfect agreement was found for those in the ‘normal’ category, while those in the ‘lean’ and ‘obese’ groups had their % fat significantly overestimated (+3.0% in men, 4.4% in women) and underestimated (-4.3% in men, 2.7% in women) respectively by BIA (Figure 2.2.2).
Multi-frequency, 8-polar BIA can also be used for segmental body composition analysis of the arm, leg and trunk regions. Malavolti et al. (2003) reported that at a resistance of 500Hz BIA shared 98% of the variance of leg LTM and 86% of arm LTM in 110 adults aged 21-82 years. Similarly Pietrobelli et al. (1998a) showed that equations including age, gender and impedance at a resistance of 300Hz accounted for 93% of arm and 88% of leg skeletal muscle mass in 49 adults. Though these studies indicate a relationship between impedance and LTM, BIA is based on an estimate of FFM and cannot distinguish between LTM and BMC. Therefore reports on lean tissue mass obtained from BIA such as those outlined above must be interpreted with caution. Furthermore, it is not clear what anatomical boundaries are used to define each body segment by BIA.

Various factors have been shown to influence the accuracy of bioelectrical impedance analysis, and differences in protocol between investigators may explain the wide variation in results between BIA studies. Numerous equations exist to convert raw impedance scores to % body fat and the equations chosen can influence
the accuracy of BIA. Wattanapenpaiboon et al. (1998) found differences of 7.7% in
the prediction of %BF between equations in a sample of 196 adults aged 26-86y.
Ingestion of a meal and strenuous exercise up to five hours prior to taking BIA
measurements have both been found to decrease impedance (Deurenberg et al.,
1988; Gallagher et al., 1998), while the time of day that measurements are taken may
also affect results (Oshima and Shiga 2006). These factors must be accounted for and
their influence minimised, particularly when BIA is being used to determine
reference body composition data.

Anthropometry

Anthropometry and specifically skinfold thickness measures have been used
for almost a century to generate prediction equations for body density and % body
fat (Durnin and Rahaman 1967). It is assumed that a skinfold thickness measure
represents a double fold of skin plus the underlying adipose tissue (Marfell-Jones et
al., 2006). To ensure maximal accuracy and precision of skinfold thickness
measures, a carefully standardised protocol must be followed by a highly trained
investigator. Sites being measured must be carefully landmarked and measures taken
according to the protocol of the International Society for the Advancement of
Kinanthropometry (ISAK) with a high-quality, spring-loaded calipers calibrated to a
force of 10g/mm$^2$ (Marfell-Jones et al., 2006). It has been shown that skinfold
thickness measures taken as little as one centimetre away from a defined ISAK site
produce significantly different measurement results at the majority of skinfold sites
assessed (Hume and Marfell-Jones 2008). Reliability of skinfold thickness measures
are assessed using technical error of measurement (TEM). An inter-tester TEM of
$<$10% and intra-tester TEM of $<$5% is considered an acceptable level of reliability
for experienced testers (Perini et al., 2005; Marfell-Jones et al., 2006). Circumference measures are subject to the same standardised protocol according to ISAK and require an inter-rater TEM of <2% and an intra-rater TEM of <1% (Marfell-Jones et al., 2006; Daniell et al., 2010).

As with BIA, several equations exist to predict body composition from anthropometric measures in various populations (Durnin and Rahaman 1967; Brook 1971; Eston et al., 2005), however the equations of Durnin and Womersley (1974), Jackson and Pollock (1978) and Jackson et al., (1980) remain widely used today (Davidson et al., 2011). Durnin and Womersley (1974) investigated the relationship between the sum of four skinfolds (Σ4SF; biceps, triceps, subscapular and suprailiac) and body density as measured by hydrodensitometry in a sample of 272 men and 209 women aged 17-65 years. The relationship between skinfold thickness and body density was found to be curvi-linear, thus logarithmic transformations were carried out. Age and sex specific equations were generated with correlations ranging from 0.7 to 0.9 and standard error of the estimate (SEE) ranging from 3.5% in women to 5% in men.

Jackson and Pollock (1978) and Jackson et al., (1980) attempted to improve on the findings of Durnin and Womersley (1974) by using validation and cross validation samples as a means to derive generalised regression equations that would provide unbiased body density estimates for men and women varying in age and body composition. The body density of 403 men and 335 women aged 18-61 years were measured using hydrodensitometry. Prediction equations were generated from the sum of seven log transformed skinfold thickness measures (Σ7SF; chest, axilla, triceps, subscapular, abdominal, suprailiac and thigh). In women a hip circumference was also included in the prediction equation. The equations had an R² value of 0.81
in men and 0.76 in women, with a standard error of 3.6% body fat for each equation. Similar results were obtained when using the sum of three skinfolds in men ($\sum 3SF$: chest, abdominal and thigh) and women (triceps, suprailiac and thigh).

While these equations have been very useful, the use of hydrodensitometry as the reference method by both investigators, as well as the undoubted changes in phenotype worldwide over the past 30 years (WHO 2000; OECD 2010) indicates that these equations may not be applicable in the 21st century. Several researchers have investigated the validity of the equations against newer reference methods and found significant differences in % body fat estimated from these predictions when compared to DXA and a 4-component model. Clasey et al. (1999) applied the equations of Jackson and Pollock (1978) and Jackson et al. (1980) to a sample of 76 adult men and women and found that %BF was underestimated by 5.9% compared to a 4-component model, with a correlation of 0.77. Similarly Friedl et al. (2001) showed that the equations of Jackson et al. (1980) underestimated %BF by 5.2% in 150 adult women, whereas the equations of Durnin and Womersley (1974) closely predicted % body fat for the total group. Conversely, Wattanapenpaiboon et al. (1998) reported a mean bias of 3% between Durnin and Womersley predictions and DXA measured %BF in women aged 26-72; this underestimate increased to 7.4% in a subgroup of obese women (n=13). Scherf et al. (1986) also observed an overestimation of 7.1%BF using the Durnin and Womersley (1974) predictions compared to hydrodensitometry in 23 formerly obese adults. The equations of Jackson and Pollock (1978) and Jackson et al. (1980) closely predicted %BF in the same group with a non-significant difference of 0.7%.

It is evident that the accuracy of skinfold prediction equations varies according to the population studied. Given the changes in human phenotype over the
past three decades, there is a clear gap in the literature for a comprehensive and updated review of anthropometric predictions of %BF, particularly in an Irish population where no such work has been undertaken to date.

**Ultrasonography**

Real time, B-mode ultrasonography has been shown to be an accurate method of measurement of both subcutaneous (Booth *et al.*, 1966) and visceral (Mook-Kanamori *et al.*, 2009; Emmons *et al.*, 2011) adipose tissue thickness. Ultrasound works by emitting an ultrasonic wave via a transducer probe placed on the skin which is in part reflected by the fat-muscle interface (Pineau *et al.*, 2007). Adipose tissue thickness can then be measured on screen using electronic calipers. The relationship between skinfold thickness obtained using calipers and subcutaneous adipose tissue (SAT) thickness measured using ultrasound has been investigated at various sites. Booth *et al.* (1966) found a correlation of 0.81 between skinfold thickness and SAT measures at the abdominal and infrascapular areas combined in adult men and women. Correlations ranging from 0.299 at the waist (Kuczmarski *et al.*, 1987) to 0.927 at the thigh (Selkow *et al.*, 2011) have been reported in obese and healthy adults respectively. However, these relationships are not proportional, i.e. a single layer SAT measure obtained using ultrasound does not correspond to half of a double layer skinfold thickness (Booth *et al.*, 1966; Selkow *et al.*, 2011), even when corrected for compression (Fanelli and Kuczmarski 1984; Kuczmarski *et al.*, 1987).

These findings suggest that skinfold thickness measures obtained using manual calipers may not represent a double layer of dermal and subcutaneous adipose tissue and that the ability of skinfold prediction equations to estimate body density and thus %BF may be fortuitous. Improvements in ultrasound technology
and the advent of portable scanners have led to renewed interest in ultrasonography as a field method of body composition measurement. As with anthropometry, prediction equations have been derived to estimate body density or % body fat from ultrasound measured SAT, but with conflicting results. Recently, Pineau et al. (2007) demonstrated good agreement between DXA and a prediction equation including SAT and circumference measures at the abdominal and thigh in 83 adults aged 18-60 years. A correlation of 0.98 and standard error of the estimate of 2.03% was obtained. Duz et al. (2009) validated a three site %BF prediction equation from SAT thickness measures at the chest, abdominal and thigh in young men (n=104, aged 18-26) and at the triceps, suprailiac and thigh in young women (n=104, aged 18-26). While a strong correlation was obtained (r=0.94) there was poor agreement between the ultrasound prediction and DXA measured %BF, with a mean underestimate by the ultrasound equation of 6.6% in men and 3.4% in women.

The studies discussed above have failed to document subject positioning, transducer orientation, scanning protocol and the method of SAT measurement from ultrasound images making the methodology difficult to reproduce.

2.2.3 Measurement of segmental fat mass

Measurement of fat mass in the abdomen is of particular interest as excess fat mass accumulation in this region has been linked to increased risk of type II diabetes, hyperlipidemia, hypertension and atherosclerosis (Wajchenberg 2000). As discussed in section 2.1, waist circumference is recommended as a surrogate measure of abdominal adiposity by the WHO (2000) where more appropriate measures of health risk are not available. Similar to BMI, WC is a crude measure and may not correspond to the same level of risk in all individuals. MRI and CT
scanning are the only available reference methods which directly measure abdominal fat mass and can independently quantify subcutaneous and visceral fat depots. However, as with multi-component models for measurement of total body composition, these scanning techniques are expensive and not widely available, thus are unsuitable for use in large scale studies.

Researchers have recently begun to examine the efficacy of estimating visceral fat content from total abdominal fat mass measured by DXA. Although DXA cannot distinguish between subcutaneous and visceral fat, it is more readily available than either CT or MRI and emits a much lower radiation dose per scan when compared to CT. Segmental analysis is performed by defining the region of interest (ROI) according to body landmarks such as spinal vertebrae or the iliac crests. An optimum region of interest to estimate visceral fat mass has not been defined universally, with most researchers using various sub-regions defined from the lumbar vertebrae. Park et al. (2002) compared abdominal fat mass measured by DXA in four lumbar ROIs to visceral fat mass measured by MRI in 90 healthy non-obese adult men. All regions were found to correlate well with MRI values, with the DXA measured FTM at the L2-L4 ROI explaining 73% of the variance in total visceral adipose tissue (VAT). Kamel et al. (1999) also studied non-obese subjects and found that WC and FTM at the L2–L3 region defined by DXA correlated equally to MRI measured intra-abdominal fat in men (r=0.89). However, in women, L2-L3 FTM had a stronger correlation (r=0.87) than WC (r=0.77) to MRI measured abdominal fat. A group of men and women with % body fat ranging from 8-58% was subsequently studied by Glickman et al. (2004). Abdominal fat mass measured in the L1-L4 region combined with WC explained 84% of the variance of intra-abdominal fat as measured by CT at the L4-L5 spinal level. The authors also found
that DXA was a valid and reliable method of measuring abdominal adiposity compared to CT, with an intraclass correlation coefficient of 0.97 for inter-rater reliability of fat mass measurement in the L1-L4 ROI. Each of the authors listed above suggest that whilst waist circumference was a good indicator of IAAT in non-obese subjects, DXA measured abdominal fat mass was a more sensitive measure than anthropometry in obese subjects.

It has thus far been established that an increase in intra abdominal or visceral fat is more detrimental to health than subcutaneous fat deposition, and this can now be estimated using DXA. However, normative values for fat distribution do not exist. It is likely that such values will differ by age and sex and will be related to stature. Normative values for existing health markers such as bone mineral density are defined by z-scores obtained from healthy young adult samples, where standard deviations are used to categorise patients into risk categories. Similar values for intra abdominal fat mass would greatly add to the fight against obesity and related diseases.

2.3 Conclusions

Various methods of body composition measurement have been discussed in this chapter. While questions still surround the accuracy of DXA measurement of total and segmental body composition in some groups, the method has excellent precision and has the added advantage above multi-component models of providing segmental body composition analysis. It is now widely used as a reference method in both cross-sectional (NHANES, Kelly et al., 2009) and longitudinal (Health ABC Study, Newman et al., 2005) population studies. The prediction method of bioelectrical impedance analysis is widely available and has been used to establish reference body composition data in children (McCarthy et al., 2006). However it has
been shown to be inaccurate in subjects with high fat mass (Sun et al., 2005) and its accuracy in the measurement of segmental composition has not yet been established. Similarly anthropometry is a popular prediction method of body composition measurement due to the low cost and portable nature of the equipment. The accuracy of existing equations for the prediction of %BF from anthropometric measures varies according to the population studied. The human phenotype has changed dramatically over the past 30 years as the prevalence of adult obesity has more than doubled (WHO 2000). Thus prediction equations validated in the 1970’s (Durnin and Womersley 1974; Jackson and Pollock 1978) may not be accurate in today’s population. Advances in technology have led to the emergence of ultrasonography as a prediction method of body composition measurement, which is desirable due to its portable and time efficient nature. However, lack of a standardised measurement protocol means that results from previous studies (Pineau et al., 2007; Duz et al., 2009) are not easily reproducible.
2.4 References


index and from impedance in samples of five European populations', *European Journal of Clinical Nutrition*, 55(11), 973-979.


body composition in obese subjects before and after weight loss', *Clinical Nutrition*, 13, 177-182.


Chapter 3

An analysis of total body fat mass and its distribution in healthy Irish adults aged 18-81 years
3.1 Abstract

An analysis of total body fat mass and its distribution in healthy Irish adults aged 18-81 years

The aim of this investigation was to undertake an analysis of total and regional body fat mass in an adult population. Fat tissue mass (FTM) of the total body, arm, leg and trunk segments and the L1-L4 region of interest (ROI) was measured by dual-energy x-ray absorptiometry (DXA) in 1136 Irish men and women aged 18 to 81 years. Fat tissue mass index (FTMI) was used to describe the distribution of FTM among the young adult population. The resultant z-scores provided a reference range of fatness that could be applied to the total subject population. ‘Overfat’ and ‘fat obese’ were defined as >1 standard deviation and >2 standard deviations above the young adult mean z-score, respectively. FTMI increased with age in men and women and more accurately described the increase in FTM in older adults than % body fat or body mass index (BMI). Defined by FTMI, the prevalence of ‘fat obesity’ was 13% in men (66/518) and 15% in women (94/618). BMI alternatively classified 10% of men and 9% of women as ‘obese’. Compared to the young adult population, the deposition of FTM in the trunk segment (L1-L4) was 14.8% greater in men and 10.6% greater in women aged 55+ years. Waist-to-height ratio (WHtR), an established index of excess abdominal fat, was found to be strongly related to total body FTMI (r=0.88 in men and women) and abdominal fat mass (r=0.91 in men, 0.92 in women). In conclusion, total and abdominal fat mass increase with age in men and women aged 18 to 81 years. BMI is not sensitive to changes in FTM and its distribution across the age range investigated. WHtR may be a useful measure of total and abdominal fat mass when direct measures of body composition are not available.
3.2 Introduction

Obesity is defined as excess accumulation of fat mass to the extent that health may be impaired. The condition is now considered a worldwide epidemic, affecting 500 million adults globally (WHO 2011). However, body mass index (BMI), the metric used to classify persons as obese, does not directly measure fat mass and can misclassify some individuals such as the elderly (Jackson et al., 2011). A metric which defines obesity according to fat mass is required.

Measurement tools that accurately quantify total and regional body composition across the lifespan are sought (NIH 2011). Percent body fat values derived from anthropometry (ACSM 2006), bioelectrical impedance analysis (IUNA 2011) and more recently dual energy x-ray absorptiometry (Kelly et al., 2009) are widely reported for specific populations; however, accepted healthy ranges of % body fat do not exist (Gallagher et al., 2000). Jackson et al. (2011) suggest that % body fat fails to identify the loss of fat free mass in elderly populations and instead recommend the use of fat tissue mass (FTM) to compare composition across age groups. Further, Heymsfield et al. (2007) recommend the use of a fat tissue mass index (FTMI) to eliminate differences in body composition due to stature. Similar to % body fat, ‘normal’ ranges of FTMI have not been established. In this study, FTMI is used to define ‘normal’, ‘overfat’ and ‘fat obesity’.

The distribution of fat mass around the body is of particular interest as individuals with a high proportion of fat deposited abdominally (i.e. ‘android obesity’) are at increased risk of metabolic and cardiovascular diseases such as type II diabetes, hyperlipidemia, hypertension and atherosclerosis (Wajchenberg 2000). Waist-to-height ratio (WHtR) has been suggested as a simple metric to diagnose excess abdominal adiposity, with a ratio of >0.5 indicating central fat distribution
(Ashwell and Gibson 2009). This metric has been shown to have a stronger relationship with cardiovascular risk factors than BMI (Lee et al., 2008). However, like BMI, WHtR does not directly measure abdominal fat mass. Abdominal fat mass measured at the L1-L4 lumbar spine region using dual energy x-ray absorptiometry (DXA) is a valid and reliable method of measuring abdominal adiposity compared to computed tomography (CT), and fat mass in this region correlates strongly (r=0.967) to intra-abdominal adipose tissue (IAAT) or ‘visceral fat’ (Glickman et al., 2004).

In the current study, DXA is used to measure total and segmental body composition in 1136 Irish adults ranging in age from 18 to 81 years. The relationships between % body fat, BMI and FTMI are investigated. Using young adult subjects as the reference population, FTMI values corresponding to ‘overfat’ and ‘fat obese’ are derived. The distribution of FTM by age and sex is described and the relationship between fat mass in the abdominal L1-L4 region, FTMI and WHtR is explored.

3.3 Methods

The study was approved by the University of Limerick Research Ethics Committee (ULREC 08/07).

3.3.1 Participants

Following written, informed consent (Appendix A) 1136 healthy adults (518 men and 618 women) aged 18 and over were recruited from the University of Limerick (UL) campus and surrounding community as part of the UL Body Composition Study (www.ul.ie/bodycompositionstudy). Subjects were instructed to refrain from exercise for 12 hours, to refrain from eating for 3 hours and to consume
500ml of water one hour before testing. Subjects were also required to empty their bladder immediately prior to measurement. Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, United Kingdom) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.).

3.3.2 Circumference measures

Waist circumference (WC) was measured using the procedures of the International Society for the Advancement of Kinanthropometry (ISAK) and was identified as the circumference of the abdomen at the narrowest point between the lower border of the 10th rib and the upper border of the iliac crest (Marfell-Jones et al., 2006).

3.3.3 Dual Energy X-ray Absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ 2007 v.11 software was used to capture total body scans. Daily calibration of the scanner employed a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to the manufacturer’s recommendations and instructed to remain as still as possible for the duration of the scan. Where subjects were too wide to fit within the boundary of the scan, the right hand side of the body was scanned and results doubled. This procedure has been validated specifically for the iDXA by Rothney et al. (2009). The coefficient of variance (CV) of the iDXA for repeated measures of whole body composition analysis was 0.6%. The enCORE software provided segmental analysis of the total body into arm, leg and trunk segments defined by the following anatomical landmarks (Figure 3.3.1);
Arm; all tissue extending from a line drawn through the centre of the arm socket to the phalange tips

Leg; all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips

Trunk; all tissue distal to the lowest point of the skull, excluding that contained in the arm and leg segments.

Figure 3.3.1 Dual energy x-ray absorptiometry scan with segmental partitioning of the arm, leg and trunk regions

The trunk was further segmented to investigate the lumbar (L1-L4) region. The method of Glickman et al. (2004) was used as follows; a quadrilateral box was manually drawn around the L1–L4 region of interest, bounded inferiorly by the horizontal line identifying the L4/L5 vertebral space and superiorly by the horizontal line identifying the T12/L1 vertebral space. Brightness and contrast of each scan was adjusted to ensure that all soft tissue was encompassed by the quadrilateral box (Figure 3.3.2).
Reliability for FTM measurement at this site is excellent, with an inter-rater intra-class correlation coefficient (ICC) of 0.999 and CV of 0.33% and intra-rater ICC=0.998 and CV=1.5%. All DXA-derived composition data were derived from the reconstructed mass, calculated by the enCORE™ software. The mean difference between the measured and reconstructed body mass in this group was 0.4% of weighed body mass for men and 0.1% for women.

3.3.4 Statistical analyses

Statistical analyses were performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.) and lmsChartMaker Light Version 2.43 (Pan and Cole 2011). Male and female data were analysed separately in all instances. A Kolgomorov Smirnov test was conducted to assess whether variables were normally or non-normally distributed. Mean values and standard deviation (SD), median and interquartile range (IQR) and ranges are reported for descriptive statistics. A Mann Whitney U Test or Kruskal-Wallis Test was used, as appropriate, to undertake between-sex and between-age comparisons of the dependent variables of interest. Scatter plots and Spearman’s rho correlation was used to investigate relationships between variables of interest.

A curve fitting procedure called LMS was used to generate age related reference curves for BMI, % body fat, FTMI and fat free mass index (FFMI) because...
it is capable of handling the relatively common situation where the underlying reference data are skew, i.e. the data are not normally distributed. It does so by normalising the underlying reference data by dividing the independent measure (age) into groups and then applying a power transformation which extends one tail of the distribution and contracts the other, eliminating skewness in the variable under analysis. A smooth curve is fitted to the normalizing power transformation for each age group, generating an optimum ‘‘L’’ (power) curve that normalizes the dependent measure, e.g. % body fat, over the entire age range (Kelly et al., 2009). Young adult z-scores were calculated as follows for fat tissue mass index (FTMI) in men and women;

\[
\frac{\text{Observed FTMI} - \text{YA median FTMI}}{\text{YA FTMI standard deviation}}
\]

Statistical significance (two-tailed) was set at \(p<.05\) for all analyses.

3.4 Results

3.4.1 Total body fat mass

Descriptive statistics for total body composition in men and women are given in Table 3.4.1. Men were taller (+0.15m) and heavier (+17.2kg) than women, with a higher body mass index (BMI; +1.5kg/m\(^2\)), fat free mass (FFM; +21.7kg) and fat free mass index (FFMI: +4.1 kg/m\(^2\), all \(p<0.05\)); women were older than men (+9.7y), with a higher fat tissue mass (FTM: +4.3kg), fat tissue mass index (FTMI; +2.6kg/m\(^2\)) and % body fat (+12.7%, all \(p<0.05\)).
### Table 3.4.1 Descriptive statistics for body composition variables in total men (n=518) and women (n=618) and age subgroups

<table>
<thead>
<tr>
<th></th>
<th>Men (n=518)</th>
<th>18-29y (n=329)</th>
<th>30-54y (n=123)</th>
<th>55+y (n=66)</th>
<th>Women (n=618)</th>
<th>18-34y (n=311)</th>
<th>35-54y (n=140)</th>
<th>55+y (n=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>31.7 (14.6)</td>
<td>21.9 (4.0)</td>
<td>39.0 (15.7)</td>
<td>61.3 (6.6)</td>
<td>39.5 (17.0)</td>
<td>34.2 (32.9)</td>
<td>18.0 – 81.4</td>
<td>24.1 (6.6)</td>
</tr>
<tr>
<td><strong>Ht (m)</strong></td>
<td>1.79 (0.07)</td>
<td>1.80 (0.09)</td>
<td>1.79 (0.08)</td>
<td>1.75 (0.08)</td>
<td>1.64 (0.06)</td>
<td>1.64 (0.09)</td>
<td>1.48 – 1.85</td>
<td>1.65 (0.08)</td>
</tr>
<tr>
<td><strong>Mass (kg)</strong></td>
<td>82.6 (11.2)</td>
<td>79.4 (13.5)</td>
<td>85.6 (17.3)</td>
<td>84.4 (16.2)</td>
<td>66.1 (11.0)</td>
<td>64.0 (12.5)</td>
<td>43.9 – 117.5</td>
<td>62.3 (9.8)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.7 (3.2)</td>
<td>24.3 (3.0)</td>
<td>26.5 (4.4)</td>
<td>27.3 (3.7)</td>
<td>24.6 (4.2)</td>
<td>23.7 (4.9)</td>
<td>17.2 – 45.8</td>
<td>22.5 (3.2)</td>
</tr>
<tr>
<td><strong>FTM (kg)</strong></td>
<td>17.9 (8.0)</td>
<td>13.3 (7.1)</td>
<td>21.2 (10.5)</td>
<td>23.8 (7.6)</td>
<td>22.6 (8.6)</td>
<td>20.8 (10.1)</td>
<td>7.8 – 66.3</td>
<td>18.3 (6.4)</td>
</tr>
<tr>
<td><strong>FTMI (kg/m²)</strong></td>
<td>5.6 (2.6)</td>
<td>4.1 (2.2)</td>
<td>6.6 (3.5)</td>
<td>7.7 (2.3)</td>
<td>8.5 (3.4)</td>
<td>7.7 (4.2)</td>
<td>2.8 – 25.9</td>
<td>6.4 (2.3)</td>
</tr>
<tr>
<td><strong>FFM (kg)</strong></td>
<td>65.1 (7.2)</td>
<td>65.8 (9.6)</td>
<td>64.5 (9.6)</td>
<td>61.0 (8.5)</td>
<td>43.4 (5.0)</td>
<td>43.1 (6.8)</td>
<td>31.4 – 61.7</td>
<td>43.8 (6.5)</td>
</tr>
<tr>
<td><strong>FMI (kg/m²)</strong></td>
<td>20.2 (1.7)</td>
<td>20.2 (2.2)</td>
<td>19.9 (2.7)</td>
<td>19.6 (2.3)</td>
<td>16.1 (1.5)</td>
<td>16.0 (1.9)</td>
<td>12.6 – 24.0</td>
<td>15.9 (1.9)</td>
</tr>
<tr>
<td><strong>% body fat</strong></td>
<td>21.0 (7.4)</td>
<td>16.8 (8.0)</td>
<td>24.7 (9.5)</td>
<td>28.0 (6.7)</td>
<td>33.4 (7.7)</td>
<td>32.9 (11.2)</td>
<td>12.2 – 56.8</td>
<td>29.5 (7.9)</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>86.8 (9.3)</td>
<td>81.8 (6.8)</td>
<td>90.3 (12.6)</td>
<td>95.6 (9.7)</td>
<td>78.1 (10.0)</td>
<td>75.6 (12.6)</td>
<td>59.1 – 127.2</td>
<td>72.6 (7.5)</td>
</tr>
<tr>
<td><strong>WHtR</strong></td>
<td>0.48 (0.06)</td>
<td>0.46 (0.04)</td>
<td>0.50 (0.04)</td>
<td>0.55 (0.04)</td>
<td>0.48 (0.07)</td>
<td>0.47 (0.09)</td>
<td>0.36 – 0.77</td>
<td>0.43 (0.05)</td>
</tr>
</tbody>
</table>

1 indicates total women significantly different from total men (p<0.05), 2 indicates significantly different from men aged 18-29y (p<0.025)
3 indicates significantly different from men aged 30-49y (p<0.025), 4 indicates significantly different from women aged 18-34y (p<0.025)
5 indicates significantly different from women aged 35-54y (p<0.025)
Correlations between % body fat, BMI, FTMI and WHtR in men and women are given in Table 3.4.2. The relationships between % body fat and BMI, % body fat and FTMI and % body fat and WHtR are illustrated in Figures 3.4.1-3.4.3. FTMI shared the greatest amount of variance with % body fat in men (94%) and women (88%). WHtR shared a greater amount of variance with % body fat than did BMI in men (71% vs. 53%) and women (67% vs. 63%).

Table 3.4.2 Correlation between % body fat, body mass index (BMI), fat tissue mass index (FTMI) and waist:height ratio (WHtR) in 1136 adult men and women

<table>
<thead>
<tr>
<th></th>
<th>Men (n=518)</th>
<th>Women (n=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% body fat</td>
<td>0.713(^1)</td>
<td>0.797(^1)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FTMI (kg/m(^2))</td>
<td>0.985(^1)</td>
<td>0.973(^1)</td>
</tr>
<tr>
<td>% body fat</td>
<td>0.818(^1)</td>
<td>0.907(^1)</td>
</tr>
<tr>
<td>WHtR (kg/m(^2))</td>
<td>0.830(^1)</td>
<td>0.815(^1)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>0.854(^1)</td>
<td>0.887(^1)</td>
</tr>
<tr>
<td>FTMI (kg/m(^2))</td>
<td>0.880(^1)</td>
<td>0.877(^1)</td>
</tr>
</tbody>
</table>

\(^1\) indicates significant correlation (p<0.01)

Figure 3.4.1 Scatter plot of BMI vs. % body fat in men (n=518) and women (n=618)
The changes in BMI, % body fat, FTMI and FFMI across the age span for men and women are illustrated in Figures 3.4.4-3.4.7. A summary of the data used to construct these graphs is given in Appendix B. In men, median (50th centile) BMI appears to increase steadily through adulthood, peaking at approximately the age of 55 years before decreasing in subjects aged 60+. Median % body fat and FTMI both increase in men from the age of 18 through to 55 years; thereafter %
body fat appears to increase slightly while FTMI decreases. FFMI remains relatively stable until the age of 55 years before decreasing. In women, BMI, FTMI and % body fat begin to increase from approximately 35 years and plateau at approximately 55 years. FFMI remains stable across the age span, decreasing slightly after 55 years.

**Figure 3.4.4** 3rd, 50th and 97th centiles for body mass index (BMI) across adulthood in men (n=518) and women (n=618)

**Figure 3.4.5** 3rd, 50th and 97th centiles for % body fat across adulthood in men (n=518) and women (n=618)
3.4.2 Defining ‘fat obese’

Based on the trends observed in the centile graphs, subjects aged 18-29 years in men and aged 18-34 years in women were used as the young adult reference group to obtain z-scores for FTMI. Descriptive statistics of each age subgroup are given in Table 3.4.1. ‘Overfat’ was defined as FTMI greater than
one standard deviation above the young adult z-score (i.e. >6.6 kg/m$^2$ in men, >9.2 kg/m$^2$ in women); ‘fat obese’ was defined as FTMI greater than two standard deviations above the young adult z-score (i.e. >8.7 kg/m$^2$ in men, >11.5 kg/m$^2$ in women; Table 3.4.3).

<table>
<thead>
<tr>
<th>Table 3.4.3 ‘Overfat’ and ‘fat obese’ cut-offs derived from young adult FTMI z-scores in men and women (n=640)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young adult men</strong> (n=329)</td>
</tr>
<tr>
<td>FTMI (Median (SD)) (kg/m$^2$)</td>
</tr>
<tr>
<td>z-score (Mean (SD)) (kg/m$^2$)</td>
</tr>
<tr>
<td>‘Overfat’ (kg/m$^2$)</td>
</tr>
<tr>
<td>‘Fat obese’ (kg/m$^2$)</td>
</tr>
</tbody>
</table>

Figures 3.4.8 (men) and 3.4.9 (women) illustrate the prevalence of ‘abdominal obesity’ defined as WHtR>0.5, ‘obesity’ defined as BMI >30 kg/m$^2$ and ‘fat obesity’ defined as FTMI >2SD above the young adult z-score in the total group and in subgroups by age.

![Figure 3.4.8](image_url)  
**Figure 3.4.8** Prevalence of ‘abdominal obesity’ defined as WHtR >0.5, ‘obesity’ defined as BMI >30 kg/m$^2$ and ‘fat obesity’ defined as FTMI >2SD above the young adult z-score in men
Categorised by WHtR >0.5, the prevalence of abdominal obesity approximated to 30% in the total sample (men=32%, women=31%). The percentage of subjects defined as ‘abdominally obese’ using this criteria increased approximately seven-fold between the 18-29 year olds and 55+ year olds in men (12% to 89%) and between 18-34 year olds and 55+ year olds in women (9% to 65%). The FTMI criteria (Table 3.4.3) indicated a prevalence of ‘fat obesity’ of 13% and 15% in total men and women respectively. Similar to the WHtR criteria, prevalence increased five-fold between 18-29 and 55+ year old men (6% to 32%) and 18-34 and 55+ year old women (5% to 33%). BMI predicted a prevalence of ‘obesity’ of 10% in total men and 9% in total women. In men, prevalence of BMI obesity was greater in 30-54 year olds and 55+ year olds (both 18%) than in 18-29 year olds (5%). In women, prevalence of obesity defined by BMI increased across all age groups from 3% in 18-34 year olds to 18% in 55+ year olds.

All men defined as ‘obese’ by BMI had a WHtR >0.5, however 21% of these subjects were not defined as ‘fat obese’ by FTMI. 40% of men defined as
‘fat obese’ by FTMI were not ‘obese’ by BMI. In women, all subjects who were defined as obese by BMI were also obese by WHtR and FTMI. However, only 55% of women defined as ‘fat obese’ by FTMI were ‘obese’ by BMI; the remaining 45% were ‘overweight’.

3.4.3 Segmental body fat mass

As illustrated in Figure 3.4.10 and Tables 3.4.4 and 3.4.5, FTM distribution in the arm, leg and trunk regions differed between age groups in men and women.

![Figure 3.4.10 Distribution of absolute FTM in the arm leg and trunk segment across three adult age groups in men (n=518) and women (n=618)](image)

In men, absolute FTM (kg) in the arm segment was 0.7kg greater in 30-54 year olds than 18-29 year olds and a further 0.3kg greater in 55+ year olds than 30-54 year olds. FTM in the trunk segment was 5.3kg greater in 30-54 year olds than in 18-29 year olds and a further 2.7kg greater in 55+ year olds than 30-54 year olds. FTM in the leg segment was 1.5kg greater in 30-54 year olds than 18-29 year olds but did not differ between 30-54 and 55+ year olds. Proportionally, the percentage of total body FTM contained in the arm segment did not differ between age groups; however %FTM in the leg segment was 7.5% lower in 30-54 year olds than 18-29 year olds.

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year olds than 18-29 year olds and a further 3.3% lower in 55+ year olds. In the trunk segment, % FTM was 11.6% greater in 30-54 year olds than 18-29 year olds and a further 3.2% greater in 55+ year olds (all p<0.05, Table 3.4.4).

Table 3.4.4 Absolute and relative fat tissue mass distribution in the arm, leg and trunk segments and L1-L4 region of interest in men of different age groups, reported as median (interquartile range)

<table>
<thead>
<tr>
<th></th>
<th>Total group (n=518)</th>
<th>18-29y (n=329)</th>
<th>30-54y (n=123)</th>
<th>55+y (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTM (kg)</td>
<td>FTM (%)</td>
<td>FTM (kg)</td>
<td>FTM (%)</td>
</tr>
<tr>
<td>Arm</td>
<td>1.6 (1.1) 9.6 (1.4)</td>
<td>1.3 (0.7) 9.6 (1.3)</td>
<td>2.01 (0.9) 9.4 (1.3)</td>
<td>2.32 (0.9) 9.8 (1.6)</td>
</tr>
<tr>
<td>Leg</td>
<td>5.6 (2.8) 35.3 (9.6)</td>
<td>5.1 (2.8) 38.0 (6.6)</td>
<td>6.61 (3.0) 30.51 (6.1)</td>
<td>6.4 (2.3) 27.22 (4.1)</td>
</tr>
<tr>
<td>Trunk</td>
<td>8.0 (7.4) 49.1 (12.8)</td>
<td>6.0 (4.0) 44.5 (8.2)</td>
<td>11.31 (7.3) 56.11 (7.4)</td>
<td>14.02 (4.3) 59.32 (4.5)</td>
</tr>
<tr>
<td>L1-L4 ROI</td>
<td>1.8 (2.3) 11.4 (5.5)</td>
<td>1.3 (1.0) 9.6 (3.4)</td>
<td>2.91 (2.3) 14.21 (3.7)</td>
<td>3.92 (1.7) 16.22 (1.8)</td>
</tr>
</tbody>
</table>

1 indicates significant difference from 18-29y (p<0.025)
2 indicates significant difference from 30-54y (p<0.025)

Similar patterns were observed in women, where absolute FTM (kg) in the arm and trunk segment was 0.6kg and 3.3kg greater respectively in 35-54 year olds than 18-29 year olds and a further 0.5kg and 3.1kg greater respectively in 55+ year olds. FTM in the leg segment was 1.1kg greater in 35-54 year olds than 18-34 year olds but did not differ between 35-54 and 55+ year olds. Relative FTM was 0.2% greater in the arm segment in 35-54 year olds compared to 18-34 year olds; %FTM in the leg segment was 5% lower in 35-54 year olds than in 18-29 year olds and a further 4.5% lower in 55+ year olds. In the trunk segment, %FTM was 5.7% greater in 35-54 year olds than 18-29 year olds and a further 4.9% greater in 55+ year olds (all p<0.05, Table 3.4.5).
Table 3.4.5 Absolute and relative fat tissue mass distribution in the arm, leg and trunk segments in women of different age groups, reported as median (interquartile range)

<table>
<thead>
<tr>
<th></th>
<th>Total group (n=618)</th>
<th>18-34y (n=311)</th>
<th>35-54y (n=140)</th>
<th>55+y (n=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTM (kg)</td>
<td>FTM (%)</td>
<td>FTM (kg)</td>
<td>FTM (%)</td>
</tr>
<tr>
<td>Arm</td>
<td>2.3(^1)</td>
<td>10.8(^1)</td>
<td>1.9</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>(1.1)</td>
<td>(1.6)</td>
<td>(0.8)</td>
<td>(1.3)</td>
</tr>
<tr>
<td>Leg</td>
<td>8.2(^1)</td>
<td>40.5(^1)</td>
<td>7.8</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>(2.9)</td>
<td>(9.9)</td>
<td>(2.7)</td>
<td>(6.3)</td>
</tr>
<tr>
<td>Trunk</td>
<td>9.3(^1)</td>
<td>44.6(^1)</td>
<td>7.5</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>(6.8)</td>
<td>(11.2)</td>
<td>(3.6)</td>
<td>(7.1)</td>
</tr>
<tr>
<td>L1-L4</td>
<td>2.1(^1)</td>
<td>10.3(^1)</td>
<td>1.6</td>
<td>8.8</td>
</tr>
<tr>
<td>ROI</td>
<td>(2.0)</td>
<td>(4.5)</td>
<td>(0.9)</td>
<td>(2.5)</td>
</tr>
</tbody>
</table>

\(^1\) indicates total women significantly different from total men (p<0.05)
\(^2\) indicates significant difference from 18-34y (p<0.25)
\(^3\) indicates significant difference from 35-54y (p<0.25)

3.4.4 Trunk FTM (L1-L4 Region of Interest)

Similar to the total trunk region, FTM in the L1-L4 region was greater in 55+ year old men than 18-29 year old men (Table 3.4.4, +2.6kg/6.6%, p<0.05) and 55+ year old women than 18-34 year old women (Table 3.4.5, +2.1kg/4.3%, p<0.05). The relationship between FTM in the L1-L4 region and total body FTMI is shown in Figure 3.4.11. 94% of variance was shared between the two variables in men and 91% in women, suggesting that fat tissue mass index can predict abdominal fat deposition.

![Regression equations](image)

**Regression equations;**

Men: \( y=1.5507x + 1.9861 \)

Women: \( y=2.0459x + 3.2416 \)

Figure 3.4.11 Relationship between FTMI and L1-L4 ROI FTM (kg) in men (n=518) and women (n=618)
The relationship between FTM in the L1-L4 region and WHtR was stronger in women where 85% of the variance was shared compared to 83% in men (Figure 3.4.12).

![Figure 3.4.12](image)

**Figure 3.4.12** Relationship between WHtR and L1-L4 ROI FTM (kg) in men (n=518) and women (n=618)

Regression equations:
- Men: y=0.0316x + 0.4112
- Women: y=0.0386x -0.3784

Using equations generated from these regression lines, values for ‘overfat’ and ‘fat obese’ corresponding to those calculated for FTMI were derived for WHtR and L1-L4 FTM. These figures are given in Table 3.4.6.

**Table 3.4.6** FTMI, L1-L4FTM and WHtR values indicating ‘overfat’ and ‘fat obese’ in men (n=518) and women (n=618)

<table>
<thead>
<tr>
<th></th>
<th>Men (n=518)</th>
<th>Women (n=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTMI (kg/m²)</td>
<td>6.6</td>
<td>9.2</td>
</tr>
<tr>
<td>L1-L4 FTM (kg)</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.51</td>
<td>0.49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>‘Overfat’</th>
<th>‘Fat obese’</th>
<th>‘Overfat’</th>
<th>‘Fat obese’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.6</td>
<td>8.7</td>
<td>9.2</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>4.3</td>
<td>2.9</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>0.51</td>
<td>0.55</td>
<td>0.49</td>
<td>0.53</td>
</tr>
</tbody>
</table>
3.5 Discussion

This chapter describes total and segmental body fat mass in 1136 healthy Irish adults ranging in age from 18 to 81 years. In the total subject group, all body composition variables studied differed between men and women. With the exception of fat tissue mass (FTM) in 55+ year olds, these differences were also evident in each age subgroup. Men were taller and heavier with higher BMI, WC, WHtR, FFM and FFMI values but lower FTM, FTMI and % body fat values than women (Table 3.4.1, all p<0.05).

In Ireland, body composition data at a population level is provided by the National Adult Nutrition Survey (NANS, IUNA 2011). Table 3.4.7 illustrates mean values for body composition variables reported in NANS and in the current study.

Table 3.4.7 Comparison of body composition variable and obesity prevalence in Irish adults reported from National Adult Nutrition Survey (n=1274) and University of Limerick Body Composition Study (n=1136). Data are mean values.

<table>
<thead>
<tr>
<th></th>
<th>NANS (18-64y)</th>
<th>ULBC (18-72y)</th>
<th>NANS (18-64y)</th>
<th>ULBC (18-81y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kg)</td>
<td>86.2</td>
<td>82.6</td>
<td>70.0</td>
<td>66.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77</td>
<td>1.79</td>
<td>1.63</td>
<td>1.64</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5</td>
<td>25.7</td>
<td>26.4</td>
<td>24.6</td>
</tr>
<tr>
<td>% body fat</td>
<td>23.3</td>
<td>21.0</td>
<td>33.9</td>
<td>33.4</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>95.5</td>
<td>86.8</td>
<td>86.3</td>
<td>78.1</td>
</tr>
<tr>
<td>Prevalence of obesity by BMI (%)</td>
<td>25.8</td>
<td>9.8</td>
<td>21.3</td>
<td>8.6</td>
</tr>
</tbody>
</table>

NANS= National Adult Nutrition Survey
ULBC= University of Limerick Body Composition Study

Mean values reported in the NANS for BMI, % body fat and WC are higher than those reported in the present study in men and women. 25.8% of men and 21.3% of women in the NANS study were obese according to their BMI; these values are 10% and 9% in the current study. Subjects in the NANS study were selected to be
representative of Irish adults with respect to age, gender, social class, and urban/rural location. In the current study a convenience sample of subjects were recruited from the University of Limerick campus and the local community, with age and gender the only demographic characteristics accounted for. Subjects recruited in this manner are unlikely to be as fully representative of the total population. Despite the difference in BMI-defined obesity prevalence between studies, the mean % body fat values were similar in men (23.3% vs. 21.0%) and women (33.9% vs. 33.4%). This suggests that % body fat relates differently to BMI in the two studies. % body fat values reported from NANS were obtained using bioelectrical impedance analysis (BIA) and those reported in the current study were obtained using DXA. BIA has previously been shown to underestimate % body fat in adults (Sun et al., 2005) and may explain the differences noted above. The accuracy of BIA in measuring total and segmental body fat when compared to DXA has been reported by the author (Leahy et al., 2011) and is discussed in Chapter 4.

Percent body fat increased continuously across adult age groups from a median of 16.8% in 18-29 year old men to 28% in 55+ year old men. These values were 29.5% and 39.5% for 18-34 year old and 55+ year old women respectively. BMI and FTMI also increased with age group; however, in men, centile graphs (Figures 3.4.3 and 3.4.4) indicate that these variables tend to decrease from approximately 55 years of age. FFMI did not differ between age groups. However, centile graphs indicate that FFMI also decreased from the age of 55 in men, accounting for the lack of change observed in % body fat. Similar findings have been reported in a longitudinal study by Jackson et al. (2011) who found that BMI and % body fat were not sensitive in detecting change in FFM in older adults and
recommend that FTM and FFM should both be reported when measuring body composition and ageing. These trends were not evident in women in the current study where % body fat, BMI, FTMI and FFMI all appeared to plateau from approximately 55 years of age.

As discussed earlier, FTMI is reported in the current study to eliminate differences in FTM due to stature. There is a very strong correlation between FTMI and % body fat in men (r=0.985) and women (r=0.973). The weaker relationship between the two variables in women appears to be due to the ‘fat obese’ subjects (FTMI >11.5kg/m\(^2\)). Increases in FTMI in this range are associated with smaller increases in % body fat than at lower values of FTMI (Figure 3.4.1). This suggests a concurrent increase in FFMI in this subject group.

To overcome the limitations of BMI and % body fat discussed above, FTMI was used to define subjects as ‘overfat’ and ‘fat obese’ in the current study. The young adult (YA) population was used as a reference group; in men this was defined as 18-29 year olds as there was a steady increase in BMI, % body fat and FTMI from the age of 18 years onwards. In women, these variables remained relatively stable until approximately 35 years; therefore the YA population was defined as 18-34 year olds. FTMI between one and two standard deviations above the young adult mean z-score defined ‘overfat’ and FTMI greater than two standard deviations above the young adult mean defined ‘fat obesity’. This approach is based on similar studies investigating appendicular lean tissue mass to define sarcopenia (Baumgartner et al., 2004). Using these criteria, 13% of men and 15% of women in the current sample were defined as ‘fat obese’; this was higher than the percentage of adults classed as ‘obese’ by BMI (10% of men and 9% of women). A further 18% of men and women were classified as ‘overfat’ by
FTMI; prevalence of overweight defined by BMI of 25-29.9kg/m² was higher at 42% in men and 29% in women (data not shown). BMI misclassified 40% of ‘fat obese’ men (28/70) and 45% of ‘fat obese’ women (44/97) as ‘overweight’ and misclassified 21% (11/52) of men with ‘overfat’ FTMI z-scores as ‘obese’. These findings indicate that BMI misclassifies as ‘normal’ some individuals who have a high fat mass classified by FTMI. Conversely, a small number of men (n=11) defined as ‘obese’ by BMI had a ‘normal’ FTMI (i.e. <1 SD above the young adult mean).

As discussed in Chapter 2, Frankenfield et al. (2001) found that 30% of men (n=53) and 46% of women (n=88) with a BMI<30kg/m² were classified as obese according to their % body fat and Deurenberg et al. (2001) found that 41% of men and 32% of women classified as ‘fat-obese’ according to their % body fat were not obese according to their BMI. In both studies, cut-off points of >25% body fat defined ‘fat obesity’ in men; the cut-offs of >30% and >35% were used respectively by Frankenfield et al. (2001) and Deurenberg et al. (2001) in women. In the present study, the ‘median man’ has a height of 1.79m and body mass of 81.2kg. The ‘overfat’ FTMI threshold of 6.6kg/m² in this man corresponds to a % body fat of 26%, while the ‘fat obesity’ threshold (FTMI=8.7kg/m²) corresponds to 34.3% body fat. The ‘median woman’ is 1.64m tall with a mass of 64.0kg. The ‘overfat’ FTMI threshold of 9.2kg/m² corresponds to 38.7% body fat in this woman, while ‘fat obesity’ of 11.5kg/m² equates to 48.3% body fat. These data suggest that the arbitrary values of % body fat used to define ‘fat obese’ in earlier studies are not appropriate in the current subject sample as the thresholds for ‘fat obesity’ are too low. For example, at a % body fat of 25%, the ‘median man’ in the current study would have a ‘normal’ FTMI of 6.3kg/m². At a body fat of 30%,
the ‘median woman’ would have a ‘normal’ FTMI of 9.1kg/m$^2$; at a body fat of 35%, median woman would have an ‘overfat’ FTMI of 10.6kg/m$^2$.

Regarding distribution of FTM, in the total subject sample the largest deposit of FTM is in the trunk region in men (49.1%) and women (44.6%). When analysed by age group %FTM in the trunk increased from 44.5% in 18-29 year olds to 56.1% in 30-54 year olds; a further increase of 3.2% was observed in 55+ year olds. In women, %FTM in the trunk increased from 41.5% to 47.2% between 18-34 and 35-54 year olds; a further increase of 4.9% occurred in 55+ year olds. Independent of age and sex %FTM contained in the arm segment remained relatively stable at ~10%. An increase in trunk %FTM was mirrored by a decrease in leg % FTM. The difference in FTM distribution between age and sex is well established (Enzi $et$ $al.$, 1986); however an increase in trunk, and thus abdominal, fat mass is not desirable due to the associated increased risk of cardiovascular disease (Wajchenberg 2000).

FTM in the L1-L4 ROI was investigated in an attempt to directly quantify abdominal fat mass, as this region is shown to correlate strongly with visceral fat measured by CT (Glickman $et$ $al.$, 2004). L1-L4 FTM was strongly related to FTMI, sharing 94% of the variance in men and 91% of the variance in women. This suggests that total body FTMI may act as a surrogate measure of visceral fat deposition. WHtR has been suggested as a measure of abdominal adiposity (Ashwell and Gibson 2009) and correlated strongly to L1-L4 FTM in men ($r=0.91$) and women ($r=0.92$) in the present study. The relationship between WHtR and FTMI was also strong in men ($r=0.880$) and women ($r=0.877$). However, using the cut-off of WHtR >0.5 appears to over-predict the prevalence of increased abdominal adiposity, particularly in older adults where 89% of men
and 65% of women aged 55+ years have a WHtR of >0.5. This indicates that WHtR is sensitive to the increase in abdominal fat deposition that occurs with age. However the cut-off of 0.5 to indicate ‘abdominal obesity’ is not appropriate as the proportion of fat tissue mass deposited abdominally differs between men and women, as does the relationship between abdominal fat and FTMI. When WHtR was regressed against FTMI in the current study, cut-offs of 0.51 and 0.49 corresponded to ‘overfat’ in men and women respectively and cut-offs of 0.55 in men and 0.53 in women corresponded to ‘fat obese’.

### 3.6 Conclusions

This investigation details total and segmental body composition of 1136 Irish adults ranging in age from 18-81 years. Reference values of fat tissue mass index (FTMI) established from young adult subjects indicate a prevalence of ‘fat obesity’ of 13% in total men and 15% in total women. Total and segmental body fat mass deposition differs according to age and sex. FTMI increases with age in men and women. The proportion of total fat tissue mass deposited in the trunk region is higher in men than in women, accounting for 59% and 52% of total FTM in men and women respectively aged 55+ years. Body mass index does not adequately measure fat tissue mass and its distribution. Waist-to-height ratio is strongly correlated to total and segmental fat tissue mass, where a ratio of 0.55 in men and 0.53 in women corresponds to ‘fat obesity’ as defined by FTMI. However, FTMI, L1-L4 FTM and WHtR must be related to disease indicators before these metrics are used to categorise individuals according to health risk.
3.7 References


Chapter 4

A comparison of dual energy x-ray absorptiometry and bioelectrical impedance analysis to measure total and segmental body composition in healthy young adults
4.1 Abstract

A comparison of dual energy x-ray absorptiometry and bioelectrical impedance analysis to measure total and segmental body composition in healthy young adults

S. Leahy, C. O’Neill, R. Sohun and P. Jakeman

The aim of this study was to investigate the accuracy of BIA in the measurement of total body composition and regional fat and fat free mass in healthy young adults. Four hundred and three healthy young adults (167 women and 236 men) aged 18 to 29 years were recruited from the Mid-West region of Ireland. Multi frequency, 8-polar bioelectrical impedance analysis (BIA) and dual energy x-ray absorptiometry (DXA) were used to measure total body and segmental (arm, leg and trunk) fat mass and fat free mass. BIA was found to underestimate percentage total body fat in men and women (p<0.001). This underestimate increased in men with >24.6% body fat and women with >32% body fat (p<0.001). Fat tissue mass in the trunk segment was overestimated by 2.1kg (p<0.001) in men and underestimated by 0.4kg (p<0.001) in women. BIA was also found to underestimate fat free mass in the appendages by 1.0kg (p<0.001) in men and 0.9kg (p<0.001) in women. Compared to dual energy x-ray absorptiometry, bioelectrical impedance analysis underestimates fat mass and overestimates lean tissue mass in healthy young adults. BIA should, therefore, be used with caution in the measurement of total body composition in women and men with >25% total body fat. Though statistically significant, the small difference (~4%) between methods indicates that BIA may be used interchangeably with DXA in the measurement of appendicular fat free mass in healthy young adults.
4.2 Introduction

Accurate measurement of body composition is of benefit in the assessment of nutritional status within and between populations and is a valuable diagnostic/evaluative tool in obesity, metabolic syndrome, type II diabetes and sarcopenic obesity. Reference values for body composition are established from healthy young adults representative of the general population. In this study of 403 young adult men and women from the Mid-West region of Ireland a comparison was made of total and segmental body composition measured by dual energy x-ray absorptiometry and bioelectrical impedance analysis.

Dual energy x-ray absorptiometry (DXA) is an accepted method of measurement of body composition (Ellis 2000; Rubiano et al., 2000). DXA is a 3 compartment model of fat mass (FTM) and two components of fat free mass (FFM) i.e., bone mineral content (BMC) and lean tissue mass (LTM). DXA, however, is not widely available outside of the clinical or research setting.

Bioelectrical Impedance Analysis (BIA) is a valid method of body composition analysis that is widely available, inexpensive and without a requirement for high level operator training. BIA measures the resistance to an electric current, with least resistance offered by fat-free mass due to its high water content (Baumgartner 1996). Proprietary algorithms then convert raw impedance scores to % body fat. The algorithm (Wattanapenpaiboon et al., 1998), the time of measurement (Oshima and Shiga 2006), eating or exercise prior to measurement (Deurenberg et al., 1988; Gallagher et al., 1998) have been shown to affect the accuracy of BIA measurement and must be accounted for to minimise error (Kyle et al., 2004).
Both DXA and multi-frequency, 8-polar BIA allow measurement of segmental composition partitioned into three regions- arm, leg and trunk. This is particularly useful, for example, in the measurement of regional changes in adiposity (Glickman et al., 2004) and appendicular fat-free mass (FFM).

Using DXA as the reference method, this study investigated the accuracy of BIA as a measure of total body composition and regional fat mass and fat free mass in healthy young adults.

4.3 Methods

4.3.1 Participants

Following written, informed consent (Appendix A), 403 healthy young adults (167 women and 236 men) between the age of 18 and 29 years were recruited from the University of Limerick campus and surrounding community. The study was approved by the University of Limerick Research Ethics Committee (ULREC 08/07).

4.3.2 Protocol

Subjects were instructed to refrain from exercise for 12 hours, to refrain from eating for 3 hours and to consume 500ml of water one hour before testing (Kyle et al., 2004). Subjects were also required to empty their bladder immediately prior to measurement.

4.3.3 Anthropometric measurements

Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, United Kingdom) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.).
4.3.4 Bioelectrical Impedance Analysis

BIA measurement was carried out prior to DXA scanning in all subjects. A multi frequency bioelectrical impedance analyzer (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.), set to use the ‘normal’ (non-athletic) proprietary algorithm, was used for the impedance measurement. Subjects stood with the ball and heel of each foot in contact with metallic electrodes on the floor scale. Once weight had been recorded, subjects were instructed to grasp the hand grips and hold down by their sides with metallic electrodes in contact with the palm and thumb. Arms were extended and abducted away from the body according to manufacturer’s instructions. Coefficient of variance (CV) of the impedance measure was 0.4%. In addition to total body values the Tanita GMON software (v1.7.0) generated values for FTM and FFM for the arm, leg and trunk segments, but it was not possible to ascertain the anatomical boundaries of these regions of interest.

4.3.5 Dual Energy X-ray Absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ 2007 v.11 software was used to perform total body scans. Daily calibration of the scanner was performed using a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to the manufacturer’s recommendations and instructed to remain as still as possible for the duration of the scan. Where subjects were too wide to fit within the boundary of the scan, the right hand side of the body was scanned and results doubled. This procedure has been validated for general DXA use by Tataranni and Ravussin (1995) and specifically for the iDXA by Oates et al. (2007). The coefficient of variance of the iDXA for measuring body
composition is <1% (Huizenga et al., 2007). The enCORE software provided segmental analysis of the total body into arm, leg and trunk segments defined by the following anatomical landmarks:

Arm; all tissue extending from a line drawn through the centre of the arm socket to the phalange tips

Leg; all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips

Trunk; all tissue distal to the lowest point of the skull, excluding that contained in arm and leg segments

All DXA-derived composition data were derived from the reconstructed mass, calculated by the enCORE™ software. The mean difference between the measured and reconstructed body mass in this group was 0.4% of weighed body mass for men and 0.1% for women.

4.3.6 Statistical Analyses

Statistical analyses were performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.). A Kolgomorov Smirnov test was conducted to assess whether variables were normally or non-normally distributed. Paired t-tests and Wilcoxon Signed ranks tests were used as appropriate to compare BIA and DXA measures of total body and segmental body composition. Pearson’s and Spearman’s correlation and Bland Altman (Bland and Altman 1986) plots were used to investigate agreement and bias between the two methods. Limits of agreement were calculated as the mean of difference between methods ± 1.96-standard deviation of the difference. One factor analysis of variance and Mann Whitney U tests were conducted as appropriate to explore difference between sexes for all variables and to investigate differences between methods
according to sex and % body fat category. Statistical significance (two-tailed) was set at p<.05 for all analyses.

4.4 Results

Subject characteristics are given in Table 4.4.1. As not all data were normally distributed, the median and interquartile ranges (IQR) are reported as well as the mean and standard deviation and range. Overall, men were taller (+0.14m; p<0.001) and heavier (+17.5kg; p<0.001) than women and had a higher body mass index (BMI) (+1.8kg/m$^2$; p<0.001).

4.4.1 BIA vs. DXA total body analysis

BIA underestimated % body fat when compared to DXA by 2.1% (p<0.001) in the total subject group. When subjects were partitioned according to sex, the underestimate of % body fat measured by BIA was found to be greater in women (mean difference of -3%) than men (median difference of -0.1%) (Table 4.4.2).

Bland Altman plots showed that underestimation by BIA became more evident as % body fat increased (Figure 4.4.1). In men BIA secured similar, though statistically different, median % body fat (-0.1%; p<0.001) in subjects with <24.6% body fat and -4.2% (p<0.001) lower median scores in subjects with ≥24.6% body fat (i.e. 8.5% of men), limits of agreement (LoA); -5.1% to +7.1%. In women BIA underestimated % body fat by -2.5% in subjects with <32% body fat and -4.8% (p<0.001) in subjects with ≥32% body fat (i.e. 21.6% of women), LoA; -3.5% to +9.5%.
### Table 4.4.1  Subject Characteristics (Mean and standard deviation (SD), median and interquartile range (IQR) and range; n=403)

<table>
<thead>
<tr>
<th></th>
<th>All (n=403)</th>
<th>Men (n=236)</th>
<th>Women (n=167)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
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<td>22.1</td>
<td>22.4</td>
</tr>
<tr>
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<td>3.2</td>
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<tr>
<td>Median</td>
<td>22.0</td>
<td>21.5</td>
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</tr>
<tr>
<td>IQR</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Range</td>
<td>18-29</td>
<td>18.9-40.0</td>
<td>18-29</td>
</tr>
<tr>
<td>Height (m)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
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<td>1.80</td>
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<tr>
<td>SD</td>
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<td>154-1.83</td>
</tr>
<tr>
<td>Mass (kg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>80.7</td>
<td>63.4</td>
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<tr>
<td>SD</td>
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<td>10.5</td>
<td>8.0</td>
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<tr>
<td>Median</td>
<td>73.1</td>
<td>79.8</td>
<td>79.8</td>
</tr>
<tr>
<td>IQR</td>
<td>17.8</td>
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<tr>
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<td>23.0</td>
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<tr>
<td>SD</td>
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<td>2.7</td>
<td>2.6</td>
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<tr>
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<td>24.4</td>
<td>22.6</td>
</tr>
<tr>
<td>IQR</td>
<td>3.2</td>
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<tr>
<td>Range</td>
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<td>18.9-40.0</td>
<td>17.2-33.5</td>
</tr>
</tbody>
</table>

1 indicates normal distribution  
2 indicates significantly different from men (p<.05)

### Table 4.4.2  DXA and BIA measured fat tissue mass (FTM), fat free mass (FFM) and % body fat in men and women aged 18-29 years (n=403)

<table>
<thead>
<tr>
<th></th>
<th>All (n=403)</th>
<th>Men (n= 236)</th>
<th>Women (n=167)</th>
</tr>
</thead>
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<tr>
<td>FTM (kg)</td>
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<td></td>
<td></td>
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<tr>
<td>DXA</td>
<td>16.7</td>
<td>14.9</td>
<td>19.2</td>
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<tr>
<td>BIA</td>
<td>15.4</td>
<td>14.0</td>
<td>17.4</td>
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<td>15.9</td>
<td>13.4</td>
<td>18.4</td>
</tr>
<tr>
<td>SD</td>
<td>6.7</td>
<td>6.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Median</td>
<td>15.9</td>
<td>13.4</td>
<td>18.4</td>
</tr>
<tr>
<td>IQR</td>
<td>3.2</td>
<td>7.1</td>
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<tr>
<td>Range</td>
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<td>4.6-48.9</td>
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</tr>
<tr>
<td>FFM (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA</td>
<td>57.0</td>
<td>66.1</td>
<td>44.2</td>
</tr>
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<td>BIA</td>
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<td>66.7</td>
<td>46.2</td>
</tr>
<tr>
<td>Mean</td>
<td>58.6</td>
<td>65.7</td>
<td>46.1</td>
</tr>
<tr>
<td>SD</td>
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<td>Median</td>
<td>58.6</td>
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<tr>
<td>IQR</td>
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<td>53.9-91.1</td>
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<tr>
<td>% Body Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA</td>
<td>22.9</td>
<td>18.0</td>
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</tr>
<tr>
<td>BIA</td>
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<td>26.9</td>
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<tr>
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<td>8.4</td>
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<td>5.0</td>
</tr>
<tr>
<td>Median</td>
<td>22.3</td>
<td>16.7</td>
<td>27.2</td>
</tr>
<tr>
<td>IQR</td>
<td>13.7</td>
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<tr>
<td>Range</td>
<td>8.9-41.0</td>
<td>8.9-41.0</td>
<td>14.9-41.3</td>
</tr>
</tbody>
</table>

1 indicates normal distribution  
2 indicates significant correlation between BIA and DXA measurement (p<0.01)  
3 indicates significant difference of BIA compared to DXA measurement (p<0.05)
Compared to DXA, BIA underestimated median FTM by 0.3kg (-2.2%) in men (LoA; -4.2 to +6.0) and 1.7kg (-9.2%) in women (LoA; -2.3 to +5.9) indicating that BIA is more likely to underestimate FTM in subjects with higher FTM (Figure 4.4.2). In these subjects FTM was underestimated by BIA in men with >27kg FTM (i.e. 4.7% of men, p<0.001) and women with >23.3kg FTM (i.e. 14.4% of women, p<0.001).
BIA was found to overestimate mean FFM by 0.6kg (0.9%) in men (LoA; -6.0 to +4.9) and 2.0kg (4.5%) in women (LoA; -6.2 to +2.1). This overestimate was consistent across the range of FFM values (Figure 4.4.3).

![Figure 4.4.3](image)

**Figure 4.4.3** Bland Altman plots of fat free mass with mean difference (---) and 95% limits of agreement (…) in men and women

### 4.4.2 BIA vs. DXA Segmental Body Analysis

Figure 4.4.4 illustrates the distribution of fat tissue mass (FTM) between arm, leg and trunk as measured by BIA and DXA. Estimates of FTM differed between the two methods in each segment (p ≤ 0.006). These differences were larger in men where BIA underestimated the median value for leg FTM by 1.6kg (31.4%) (p<0.001) and overestimated the mean value for trunk FTM by 2.1kg (35.6%) (p<0.001).
Similarly, a major difference in FFM was observed in the trunk segment where BIA overestimated FFM by a median of 5.8kg (19.7%) in men (p<0.001) and a mean of 6.2kg (30.2%) in women (p<0.001). In contrast, only a minor difference was seen in FFM in the appendages where BIA underestimated arm and leg FFM by 0.2kg (2.4%) and 0.8kg (3.4%) respectively in men and 0.2kg (4.5%) and 0.7kg (4.4%) respectively in women (p<0.001) (Figure 4.4.5).

BIA tended to overestimate % fat in the arm segment (+2%, p<0.001) and underestimate % fat in the leg segment (-4.3%, p<0.001) in men, with no difference in the trunk segment (1.9%, p=0.131). In women, % fat in the arm (-1%, p=0.001)
and trunk (-5.6%, p<0.001) was underestimated by BIA, with no difference between the methods for leg % fat (-0.6%, p=0.05) (Figure 4.4.6).

![Figure 4.4.6 Segmental % fat measurement in men and women by BIA and DXA (Mean ± 95% confidence intervals)](image)

4.5 Discussion

The data from this study detail total body and segmental body composition analysis of a representative population of healthy, young men and women recruited from the Mid-West region of Ireland.

A principal finding was that total body percentage fat and fat tissue mass were underestimated using bioelectrical impedance analysis when compared to dual energy x-ray absorptiometry. This bias was more apparent in women and greater in subjects with high total percentage body fat where BIA underestimated % body fat in young adult men with >25% body fat (approximately 10% of sample) and young adult women with >32% body fat (approximately 20% of the sample).

Comparative data between BIA and DXA for the young adult population is limited. In a sample of 108 American sedentary college aged students, Bowden et al. (2005) found BIA to overestimate total percentage body fat by 6% in men and 7% in women. These differences were not found to be statistically significant. Duz et al. (2009) studied college students aged 18-26 (104 men, 104 women) and found BIA to
underestimate % body fat by 4.8% in men and 9.2% in women with an increase in bias as the mean percentage body fat increased, but this bias was not investigated further. In older adults BIA is reported to underestimate percentage body fat in men and women (Deurenberg et al., 2001; Sun et al., 2005; Wattanapenpaiboon et al., 1998). As in the present study, Deurenberg et al. (2001) and Sun et al. (2005) noted that bias in percentage body fat increased in those with high percentage body fat, the interpretation between these studies differing only in the categorisation of high fat mass. In the current study, men (≥24.6%) and women (≥32%) of high fat mass were selected based on interpretation of the bias identified in the Bland Altman plots, Sun et al. (2005) used a percentage body fat >30% in men and >33% in women and Deurenberg et al. (2001) a percentage body fat >25% in men and >35% in women. At present there is no consensus on the range of total body percentage fat to be employed to categorise an individual’s level of health risk (Gallagher et al., 2000). Methodological bias in the measurement of total body percentage fat should be avoided in the identification and monitoring of those ‘at risk’ (i.e. of high % fat). The results of the present study indicate that BIA is a valid measure of % body fat in men with <25% body fat, but is to be used with caution in women and in men with ≥25% body fat.

Compared to total body, region-specific body composition analysis offers a discriminate level of health risk for the individual. An evaluation of difference between methods in the region-specific body composition found BIA to significantly overestimate trunk FTM in men by 35.6% (2.1kg) compared to DXA with a smaller underestimate in women (0.4kg). In terms of fat free mass, a substantial overestimation of FFM in the trunk segment was found in all subjects, with a mean difference of 5.8kg in men and 5.2kg in women. As it was not defined by the
manufacturer, we have assumed that the head was included in trunk segment analysis by BIA. Subtracting the DXA estimate of the head segment of 3.9kg of FFM and 0.9kg of FTM in men and 3.4kg of FFM and 0.8kg of FTM in women, the residual overestimation of FFM by BIA was found to be 1.9kg in men and 1.8kg in women. Similarly the pelvic area is included in trunk segment analysis by the DXA software. Technical notes for the Tanita MC-180 used in the current study state that ‘current flows from one leg to the other via the lower abdomen’. This suggests that the pelvic area is likely to be included in the leg segment by the BIA software. If this is the case, one would expect to find lower values for both FTM and FFM in the trunk segment and higher values in the leg segment when measured by BIA, which was not evident in the current study. Concerns have been raised about the ability of previous DXA models to accurately measure composition in areas of high bone mass such as the thorax (Genton et al., 2002; Roubenoff et al., 1993). However the Lunar iDXA used in the current study employs 64 detectors compared to 16 in the previous model (Lunar Prodigy). This allows for enhanced precision and increased image resolution than earlier systems (Hull et al., 2009).

Despite the differences in FTM and FFM outlined above, we found no difference between BIA and DXA in the measurement of % trunk fat in men but an underestimate of 5.6% in women. These results indicate that, at least for the trunk segment, fat should be reported as the absolute mass (kg) of tissue rather than a relative percentage of this segmental mass.

To the authors’ knowledge there is no comparable study of 8-polar BIA measurement of segmental composition in a young adult population, previous data being confined to small subject numbers dispersed over a wide age range. In 333 healthy children aged 6-13 years, Kriemler et al. (2009) found 8-polar BIA to be an
accurate predictor of whole body FFM (mean difference = 0kg, LoA -1.8 to +1.8) and segmental LTM ($r^2$=0.93-0.96, root mean squared error $\leq$0.1kg for arms, $\leq$0.24kg for legs). Furstenberg and Davenport (2011) reported significant correlation between 8-polar BIA and DXA measured whole body FTM and FFM in 53 haemodialysis patients. Mean bias was lower than that reported in the current study (FTM; +0.6kg, FFM; -0.53kg); however 95% limits of agreement were wider (FTM; -7.9 to +7.6kg, FFM; -8.1 to +9.1kg). Regarding segmental composition, highest bias (+0.86kg) was noted in the trunk segment for lean tissue mass with an underestimate by BIA similar to the current study reported for the arm (<0.2kg) and leg segments (<0.9kg). Both studies discussed above reported values for whole body and segmental lean tissue mass obtained from BIA, as have previous authors (Pietrobelli et al., 2004; Malavolti et al., 2003). However, it is not possible to distinguish lean tissue and bone mineral components of fat free mass from impedance measures.

4.6 Conclusions

In conclusion, we find the small difference in measurement of total body percentage fat in men whose body fat is <25% allows DXA and BIA to be used interchangeably in this group. Close agreement between methods was also found in the measurement of trunk fat tissue mass in women, but BIA significantly overestimated fat tissue mass in the trunk region in men. Furthermore, when measured by BIA, adiposity in the abdominal region should be reported as the absolute mass rather than as a percentage of the segmental mass. Agreement between methods of measurement of FFM in the arm and leg segments confirms BIA to be an accurate measure of appendicular composition. However, the large overestimate of FFM in the trunk region implies that BIA may not be used accurately for this body segment.
4.7 References


Sun, G., French, C. R., Martin, G. R., Younghusband, B., Green, R. C., Xie, Y.,
Mathews, M., Barron, J. R., Fitzpatrick, D. G., Gulliver, W. and Zhang, H.
(2005) 'Comparison of multifrequency bioelectrical impedance analysis
with dual-energy X-ray absorptiometry for assessment of percentage body fat
in a large, healthy population', *The American Journal of Clinical Nutrition*,
81, 74-78.

absorptiometry in obese individuals', *The American Journal of Clinical
Nutrition*, 62(4), 730-734.

Wattanapenpaiboon, N., Lukito, W., Strauss, B. J. G., Hsu-Hage, B. H. H.,
measurement and bioelectrical impedance analysis (BIA) methods with
dual energy X-ray absorptiometry (DEXA) in estimating total body fat in
Chapter 5

Generalised equations for the prediction of % body fat by anthropometry in adult men and women aged 18-81 years
5.1 Abstract

Generalised equations for the prediction of % body fat by anthropometry in adult men and women aged 18-81 years

S.Leahy, C. O’Neill, R. Sohun, C. Toomey and P. Jakeman

Anthropometric data indicate that the human phenotype is changing. Today’s adult is greater in stature, body mass and fat mass. Accurate measurement of body composition is necessary to maintain surveillance of obesity within the population and to evaluate associated interventions. The aim of this study was to construct and validate generalised equations for % body fat prediction from anthropometry in 1136 adult men and women. Reference values for % body fat were obtained using dual energy x-ray absorptiometry (DXA). Skinfold thickness (SF) at 10 sites and girth (G) at 7 sites were measured on 736 men and women aged 18-81 (% body fat 5.1-56.8%). Quantile regression was employed to construct prediction equations from age and log-transformed skinfold thickness and girth measures. These equations were then cross-validated on a cohort of 400 subjects of similar age and fatness. The following generalised equations were found to most accurately predict % body fat;

Men: \(\text{Age} \cdot 0.1 + \log_{10}\text{tricepsSF} \cdot 7.6 + \log_{10}\text{midaxillaSF} \cdot 8.8 + \log_{10}\text{suprspinaleSF} \cdot 11.9 - 11.3\)  
(Standard error of the estimate: 2.5%, 95% limits of agreement: -4.8, +4.9)

Women: \(\text{Age} \cdot 0.1 + \log_{10}\text{abdominalG} \cdot 39.4 + \log_{10}\text{midaxillaSF} \cdot 4.9 + \log_{10}\text{bicepsSF} \cdot 11.0 + \log_{10}\text{medialcalfSF} \cdot 9.1 - 73.5\).  
(Standard error of the estimate: 3.0%, 95% limits of agreement: -5.7, +5.9)

These generalised anthropometric equations accurately predict % body fat and are suitable for the measurement of % body fat in adult men and women of varying levels of fatness across the lifespan.
5.2 Introduction

Valid and reliable methods of measurement of whole body and regional fat mass are required to inform public health policy and for the measurement and treatment of obesity (NIH 2011), an epidemic affecting 500 million adults worldwide (WHO 2011). The Lancet (2011) recently highlighted the need to accurately monitor and evaluate obesity interventions as one of the key factors required for the control of this epidemic worldwide.

Dual energy x-ray absorptiometry (DXA) is an accepted reference method of body composition measurement and has been utilised in both cross sectional (Kelly et al., 2009) and longitudinal (Newman et al., 2005) body composition studies. Close agreement has been shown between DXA and the criterion multi-component models in young and older healthy adults (Wang et al., 1998; Clasey et al., 1999). However DXA is not appropriate in the conduct of large scale, field-based studies as it is non-portable and is deemed unsuitable for some populations sensitised to the use of ionizing radiation (NIH 2011).

Anthropometry and, specifically, the measurement of skinfold thickness and body girths is an indirect, prediction-based method of assessment of body fat that is applicable to large scale studies (Wang et al., 2000). In Europe, the age and sex-specific equations of Durnin and Rahaman (1967) and Durnin and Womersley (1974) relating skinfold thickness at four sites (biceps, triceps, subscapular and suprailiac) to % body fat are widely used. Referencing the body density and thus body fat to that obtained by hydrodensitometry, the Durnin and Womersley (1974) equations were constructed from a sample of 481 men and women aged 16-72 years.

Forty years on from Durnin and Rahaman’s (1967) original report, the current study re-examines the concept of formulating an equation for calculating body fat
from the measurement of skinfold thicknesses. Using DXA as the reference method, the aim of the current study was to construct and validate generalised prediction equations for % body fat from anthropometric measures using a robust regression technique in a large sample of adult Irish men and women.

5.3 Methods

The study was approved by the University of Limerick Research Ethics Committee (ULREC 08/07).

5.3.1 Participants

Following written, informed consent (Appendix A), 1136 healthy adults (518 men and 618 women) aged 18 or over were recruited from the University of Limerick campus and surrounding community as part of the UL Body Composition Study (www.ul.ie/bodycompositionstudy). Subjects were instructed to refrain from exercise for 12 hours, to refrain from eating for 3 hours and to consume 500ml of water one hour before testing. Subjects were also required to empty their bladder immediately prior to measurement. Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, United Kingdom) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.).

5.3.2 Dual Energy X-ray Absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ 2007 v.11 software was used to capture total body scans. Daily calibration of the scanner employed a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to the manufacturer’s recommendations and instructed to remain as still as possible for the duration of the scan. Where subjects were too wide to fit within the boundary of the
scan, the right hand side of the body was scanned and results doubled. This procedure has been validated specifically for the iDXA by Rothney et al. (2009). The coefficient of variance (CV) of the iDXA for repeated measures of whole body composition analysis was 0.6%.

5.3.3 Anthropometry

Using Harpenden calipers (Assist creative resources Ltd., Wrexham, United Kingdom), skinfold thickness measures were obtained on all subjects at ten sites (forearm, biceps, triceps, subscapular, midaxilla, iliac crest, supraspinale, abdominal, front thigh and medial calf) with an additional chest site measured on men only (Appendix C). Corresponding girth measures were taken at seven body sites (forearm, upper arm, waist, abdominal, hip, mid thigh and calf) on all subjects using a Physiomed tape measure (Physio-med, Nottinghamshire, United Kingdom) with an additional chest girth taken on men only (Appendix C). These sites were chosen to represent all body segments and were identified in accordance with the International Society for the Advancement of Kinanthropometry (ISAK) standards (Marfell-Jones et al., 2006). Three trained investigators carried out the anthropometric measures. Inter-tester technical error of measurement (TEM) was <10% for skinfold thickness measures and <2% for girth measures. Intra-tester TEM was set at <5% for skinfold thickness measures and <1% TEM for girth measures; if TEMs were greater than these values a third measure was taken and the median value was used for analysis.

5.3.4 Statistical Analyses

Statistical analyses were performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.) and TIBCO Spotfire S+ 8.1 (TIBCO Software Inc. Palo Alto, CA.). Male and female data were analysed separately in all instances. A
Kolgomorov Smirnov test was conducted to assess whether variables were normally or non-normally distributed. Mean values and standard deviation, median and interquartile range and ranges are reported for descriptive statistics. A Mann Whitney U Test was used, as appropriate, to undertake between-sex comparisons of the dependent variables of interest. Two hundred men and two hundred women were randomly selected from the total sample for use as a cross validation sample. Prediction equations were generated on the remaining subjects (n=318 men, 418 women).

As most variables were found to be non-normal in their distribution, quantile regression was used to generate prediction equations for % body fat from anthropometric measures in men and women. The relationship between anthropometric measures and % body fat was curvilinear in several instances, therefore all anthropometric variable were log transformed prior to regression analysis. Spearman’s rho correlations were used to investigate the relationship between anthropometric measures at each site and also between each site and % body fat. The anthropometric measure that had the strongest correlation to % body fat was retained in all regression investigations. To avoid violating the assumptions of regression analysis, pairs of anthropometric measures that had a higher correlation than either individual site had with % body fat were not included in regression analyses together.

Percent body fat measures within sex were compared using Wilcoxon Signed ranks tests. Standard error of the estimate (SEE), scatter plots, Spearman’s rho correlations and Bland Altman (Bland and Altman 1986) plots were used to investigate agreement and bias between % body fat derived from DXA and that derived from anthropometric prediction equations. 95% limits of agreement were
calculated as the mean of difference between methods ± 1.96-standard deviation of the difference.

Statistical significance (two-tailed) was set at p<.05 for all analyses.

5.4 Results

5.4.1 Descriptive statistics

Five hundred and eighteen men and six hundred and eighteen women participated in the study. Descriptive statistics are provided in Table 5.4.1. All variables differed between sexes with women being older (+9.7y) with a higher % body fat (+12.7%) and fat tissue mass (+4.3kg) but lower height (-0.15m), mass (-17.3kg) and body mass index (BMI; -4.5kg/m^2) than men (all p<0.05).

<table>
<thead>
<tr>
<th>Table 5.4.1 Descriptive statistics of men (n=518) and women (n=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (n=518)</strong></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Ht (m)</td>
</tr>
<tr>
<td>Mass (kg)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
</tr>
<tr>
<td>% body fat</td>
</tr>
<tr>
<td>FTM (kg)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation. IQR, interquartile range. BMI, Body Mass Index. FTM, Fat Tissue Mass
^1indicates normal distribution
^2indicates significantly different from men (p<0.001)
5.4.2 Anthropometry

The relationship between percentage body fat and individual anthropometric variables was curvilinear, thus all skinfold thickness and girth measures were log transformed prior to regression analysis. Figure 5.4.1 illustrates the midaxilla skinfold in women before and after log transformation. Table 5.4.2 illustrates values for log transformed anthropometric variable obtained in men and women.

Figure 5.4.1 Relationship of % body fat and midaxilla skinfold thickness in women (n=618) before and after log transformation
Table 5.4.2 Log transformed skinfold thickness (mm) and girth (cm) measures in men (n=518) and women (n=618)

<table>
<thead>
<tr>
<th>Site</th>
<th>Men (n=518)</th>
<th>Women (n=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Log Skinfold thickness (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forearm</td>
<td>0.77 (0.15)</td>
<td>0.75 (0.20)</td>
</tr>
<tr>
<td>Biceps</td>
<td>0.68 (0.17)</td>
<td>0.65 (0.22)</td>
</tr>
<tr>
<td>Triceps</td>
<td>1.01 (0.19)</td>
<td>1.01 (0.26)</td>
</tr>
<tr>
<td>Subscapular</td>
<td>1.11 (0.21)</td>
<td>1.06 (0.31)</td>
</tr>
<tr>
<td>Chest</td>
<td>1.05 (0.28)</td>
<td>1.05 (0.48)</td>
</tr>
<tr>
<td>Midaxilla</td>
<td>1.03 (0.26)</td>
<td>1.00 (0.43)</td>
</tr>
<tr>
<td>Iliac crest</td>
<td>1.22 (0.23)</td>
<td>1.23 (0.34)</td>
</tr>
<tr>
<td>Supraspinale</td>
<td>1.01 (0.23)</td>
<td>0.99 (0.36)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>1.31 (0.26)</td>
<td>1.35 (0.40)</td>
</tr>
<tr>
<td>Front thigh</td>
<td>1.16 (0.22)</td>
<td>1.15 (0.31)</td>
</tr>
<tr>
<td>Medial calf</td>
<td>0.91 (0.20)</td>
<td>0.90 (0.29)</td>
</tr>
<tr>
<td>Log Girth (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forearm</td>
<td>1.45 (0.03)</td>
<td>1.45 (0.03)</td>
</tr>
<tr>
<td>Upper arm</td>
<td>1.51 (0.04)</td>
<td>1.51 (0.05)</td>
</tr>
<tr>
<td>Chest</td>
<td>2.01 (0.03)</td>
<td>2.00 (0.04)</td>
</tr>
<tr>
<td>Waist</td>
<td>1.94 (0.05)</td>
<td>1.93 (0.07)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>1.95 (0.05)</td>
<td>1.95 (0.07)</td>
</tr>
<tr>
<td>Hip</td>
<td>2.01 (0.03)</td>
<td>2.01 (0.03)</td>
</tr>
<tr>
<td>Mid thigh</td>
<td>1.74 (0.03)</td>
<td>1.74 (0.04)</td>
</tr>
<tr>
<td>Calf</td>
<td>1.59 (0.03)</td>
<td>1.59 (0.04)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation. IQR, interquartile range

\(^{1}\) indicates normal distribution
5.4.3 Generalised prediction equations

Generalised equations to predict % body fat were generated for men and women with age included as an independent variable in addition to anthropometric measures. Skinfold thickness at the midaxilla had the strongest correlation to % body fat in both men (r=0.918, p<0.01) and women (r=0.854, p<0.01) (Appendix D).

In men, an equation with age and three skinfold thickness measures (midaxilla, triceps and supraspinale) predicted % body fat in the validation group with a correlation coefficient (r) of 0.95, SEE of 2.6% and limits of agreement of -4.9% to +5.1% (Table 5.4.3). When the prediction equation was applied to the cross-validation sample, there was no difference (p=0.672) between % body fat measured by DXA and that predicted from the skinfold equation, with r=0.95, a SEE of 2.4% and limits of agreement of -4.8 to +4.6% (Table 5.4.3, Appendix E). Similar results were observed when the validation and cross-validation groups were combined; Figure 5.4.2 illustrates the Bland Altman analysis of agreement between methods in the total group (n=518).

Table 5.4.3 Generalised regression equation in men aged 18-72 (n=518)

<table>
<thead>
<tr>
<th>Equation</th>
<th>%BF (DXA)</th>
<th>%BF (predicted)</th>
<th>Δ</th>
<th>r</th>
<th>SEE</th>
<th>95% LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age·0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ log triceps·7.6</td>
<td>Validation</td>
<td>21.5 (7.3)</td>
<td>21.3 (6.8)</td>
<td>0.2</td>
<td>0.95</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Cross-validation</td>
<td>20.3 (7.4)</td>
<td>20.4 (7.1)</td>
<td>-0.1</td>
<td>0.95</td>
<td>2.4</td>
</tr>
<tr>
<td>+ log midaxilla·8.8</td>
<td>Total men</td>
<td>21.0 (7.4)</td>
<td>21.0 (6.9)</td>
<td>0.0</td>
<td>0.95</td>
<td>2.5</td>
</tr>
<tr>
<td>- log supraspinale·11.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 11.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BF, body fat. DXA, dual energy x-ray absorptiometry. Δ, difference. r, correlation coefficient. SEE, standard error of the estimate. LoA, limits of agreement.
Figure 5.4.2 Bland Altman analysis of agreement between DXA measured and skinfold predicted %
body fat in men (n=518) with mean difference (---) and 95% limits of agreement (…).

In women, an equation with age, abdominal girth and three skinfold thickness
measures (bicep, midaxilla and medial calf) predicted % body fat in the validation
group with r=0.93, a SEE of 2.7% and limits of agreement of -5.3 to +5.3% (Table
5.4.4, Appendix E). When the equation was applied to the cross-validation sample
(n=618), there was no difference (p=0.176) between % body fat measured by DXA
and that obtained using the prediction equation. The SEE was 3.5% with r=0.93 and
limits of agreement were -6.5 to + 7.1. When the validation and cross-validation
samples were combined, r=0.90, SEE=3.0% and limits of agreement were -5.7 to +
5.9%. Figure 5.4.3 illustrates the Bland Altman analysis of agreement between
methods in the total group (n=618).
**Table 5.4.4** Generalised regression equation in women aged 18-81 (n=618)

<table>
<thead>
<tr>
<th>Equation</th>
<th>Validation (n=418)</th>
<th>Cross-validation (n=200)</th>
<th>Total women (n=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-0.1</td>
<td>% BF (DXA)</td>
<td>% BF (predicted)</td>
<td>Δ</td>
</tr>
<tr>
<td>+ logabdominalgirth·39.4</td>
<td>33.5 (7.5)</td>
<td>33.4 (7.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>+ logmidaxilla·4.9</td>
<td>33.2 (8.1)</td>
<td>32.9 (7.6)</td>
<td>-0.7</td>
</tr>
<tr>
<td>+ logbiceps·11.0</td>
<td>33.4 (7.7)</td>
<td>33.3 (7.3)</td>
<td>0.1</td>
</tr>
<tr>
<td>- 73.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BF, body fat. DXA, dual energy x-ray absorptiometry. Δ, difference. r, correlation coefficient. SEE, standard error of the estimate. LoA, limits of agreement.

**Figure 5.4.3** Bland Altman analysis of agreement between DXA measured and skinfold predicted % body fat in women (n=618) with mean difference (—) and 95% limits of agreement (…)

The prediction equations of Durnin and Womersley (1974) were applied to the total sample. Table 5.4.5 illustrates the comparison of these equations to DXA derived % body fat in men and women respectively.
Table 5.4.5 Comparison of Durnin and Womersley (1974) prediction equations and DXA derived % body fat in men (n=518) and women (n=618)

<table>
<thead>
<tr>
<th>Equation</th>
<th>%BF (DXA)</th>
<th>%BF (predicted)</th>
<th>Δ</th>
<th>r</th>
<th>SEE</th>
<th>95% LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td>1.1765-0.0744·log(Σ4SF)</td>
<td>21.0 (7.4)</td>
<td>20.0&lt;sup&gt;1&lt;/sup&gt; (6.3)</td>
<td>1.0</td>
<td>0.92</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>1.1567-0.017·log(Σ4SF)</td>
<td>33.4 (7.7)</td>
<td>32.6&lt;sup&gt;1&lt;/sup&gt; (5.7)</td>
<td>0.8</td>
<td>0.86</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Abbreviations: BF, body fat. DXA, dual energy x-ray absorptiometry. Δ, difference. r, correlation coefficient. SEE, standard error of the estimate. LoA, limits of agreement. Σ4SF=sum of four skinfold thickness measures biceps, triceps, subscapula and iliac crest<sup>1</sup>indicates significantly different from DXA derived % body fat

Figure 5.4.4 illustrates the Bland Altman analysis of agreement between DXA derived % body fat and that predicted from the Durnin and Womersley (1974) equations in men and women.

![Figure 5.4.4](image)

**Figure 5.4.4** Bland Altman analysis of agreement between % body fat derived from DXA and that predicted from Durnin and Womersley skinfold equations in men (n=518) and women (n=618) with mean difference (- - -) and 95% limits of agreement (…)

5.5 Discussion

The purpose of this study was to construct and validate generalised prediction equations to estimate % body fat from anthropometric measures in a large sample of adult men and women (n=1136) ranging widely in age (18-81 years) and % body fat
Cross-validated on an independent subject sample, data indicate that % body fat is accurately predicted from skinfold thickness and girth measures in this group when compared to DXA derived % body fat. Heyward and Wagner (2004) recommend that the correlation coefficient (r) for body composition prediction equations should exceed 0.80 and limits of agreement should be within 5% when compared to the reference method. SEE ≤3.0% between methods is considered ‘very good’ and SEE ≤2.5% is considered ‘excellent’. In men, skinfold thickness measures from three body sites (triceps, midaxilla and supraspinale), combined with age, predicted % body fat with a correlation coefficient (r) of 0.95, SEE of 2.5% and 95% limits of agreement of <5% (n=518). In women, an equation containing age, abdominal girth and skinfold thickness measures at the biceps, midaxilla and medial calf sites predicted % body fat with r=0.92, SEE of 3.0% and 95% limits of agreement of -5.7 to +5.9% for the total subject group (n=618).

The strong correlation of midaxilla skinfold thickness to % body fat and the presence of a second predictor variable from the trunk region in both men (supraspinale skinfold thickness) and women (abdominal girth) reflects the deposition of approximately 50% and 45% of the total fat mass in the trunk segment in men and women respectively (Leahy et al., 2011). The presence of an abdominal girth in place of a skinfold thickness measure in women may reflect an increased ratio of subcutaneous to visceral trunk fat deposition compared to men (Enzi et al., 1986). The importance of including lower limb skinfold thickness measures in body fat prediction equations has been highlighted previously (Eston et al., 2005). However, while medial calf skinfold thickness was found to be a significant predictor of % body fat in women in the current study, no lower limb measure was
found to be statistically important for inclusion in the final prediction equation in men.

The application of the Durnin and Womersley (1974) prediction equations to the current sample resulted in a small but statistically significant underestimate of mean % body fat values by 1% in men and 0.8% in women compared to those obtained using DXA. The SEE values of 3.4% in men and 4.1% in women, while higher than those obtained from the equations presented in this paper, are considered ‘good’ and ‘fair’ respectively (Heyward and Wagner 2004). In the original Durnin and Womersley study, SEE values of 3.5% for women and 5% for men were reported, with correlation coefficients ranging from 0.7 to 0.9. Bland Altman analysis of agreement between methods (Figure 5.4.4) suggests that as subject’s % body fat increases, the Durnin and Womersley equations are more likely to underestimate % body fat when compared to DXA. This bias is more apparent in women than in men and explains the higher SEE value and wider limits of agreement observed in women compared to men. These findings indicate that while Durnin and Womersley’s equations are still valid at a population level, the equations presented in this paper provide a better estimate of % body fat in individuals, particularly those with very high or very low % body fat.

Previous studies have used linear regression to derive body composition prediction equations from anthropometric variables (Durnin and Womersley 1974; Jackson and Pollock 1978). In the current study, neither anthropometric variables nor % body fat were normally distributed, therefore quantile regression was used to construct prediction equations. Quantile regression is a robust regression which has been recommended for use in developing prediction equations for body composition (Heymsfield et al., 2005). It reduces the influence of outliers on the regression
outcome and may explain why there is uniform agreement across the wide range of % body fat between DXA and the equations presented here. Additionally, skinfold thickness and girth measures from multiple sites were not summed in the current study as each site did not contribute an equal amount to the prediction equations.

The anthropometric prediction equations presented here accurately estimate % body fat in a large sample of adult men and women ranging widely in age and % body fat. The equations of Durnin and Womersley closely predict mean % body fat of the total group but appear to become less accurate as % body fat increases. The new equations are therefore more appropriate for use in subjects with higher % body fat and may be more beneficial in the measurement and treatment of obesity.

5.6 Conclusions

The anthropometric prediction equations presented here accurately estimate % body fat in a large sample of adult men and women ranging widely in age and % body fat. The equations of Durnin and Womersley (1974) closely predict mean % body fat of the total group but appear to become less accurate as % body fat increases. The new equations are therefore more appropriate for use in subjects with higher % body fat and may be more beneficial in the monitoring and evaluation of obesity interventions.
5.7 References


Chapter 6

Use of ultrasound measured subcutaneous adipose tissue thickness to predict % body fat in healthy young adults
6.1 Technical considerations for accurate measurement of subcutaneous adipose tissue thickness using B-mode ultrasound

6.1.1 Abstract

C. Toomey, K. McCreech, S. Leahy and P. Jakeman

The search for valid, reliable and inexpensive methods of body composition is an on-going issue for many researchers. In particular, the measurement of subcutaneous adipose tissue is carried out by numerous methods, each with its own drawbacks. Skinfold thickness measurement is the most common in-field method, but it is limited by its tendency to deform the adipose layer, the limited caliper opening which prevents measurement of larger skinfolds and the lack of correction for elastic properties of tissue between individuals. Therefore non-invasive field measures which overcome these limitations would be desirable. Ultrasound scanning provides such a device due to its portability, cost and availability, allowing reduced tissue compression and on-screen views of the adipose tissue. Despite a number of papers referring to the use of US for measuring adipose tissue, the method of measurement has never been fully described. This paper describes our work in determining an accurate method for the measurement of subcutaneous adipose tissue at different body sites, including a comparison of scanning directions and sites. We also describe our investigations into the degree to which compression force through the transducer affects adipose tissue measurement, and the reliability and sensitivity of our methods. We conclude with a recommended reliable scanning protocol for the measurement of subcutaneous adipose tissue thickness.
6.1.2 Introduction

There is widespread and increasing interest in the study of human body composition, in particular body fat mass due to its association with disease risk. Accurate measurement of total and regional body fat requires expensive equipment of limited availability, i.e. dual energy x-ray absorptiometry (DXA), therefore surrogate methods of measurement such as the use of the skinfold thickness have, customarily, been used by healthcare professionals. Using a standardised protocol (Marfell-Jones et al., 2006), skinfold thickness is deemed to provide a measure of subcutaneous adipose tissue (SAT) thickness which, when incorporated into a prediction algorithm, provides an indirect estimate of percentage body fat (Durnin and Womersley 1974). However, the accuracy of body fat estimation using skinfold thickness measurements has been questioned (Lukaski 1987) because of the error introduced from inter-individual variation in tissue compressibility and degrees of tissue deformation by the caliper jaws. There is also a practical limitation in the skinfold width that can be measured using calipers, i.e. as occurs in a person of high body fat mass.

Ultrasound imaging is proposed as a viable alternative measurement of subcutaneous adiposity that overcomes the specific limitation of skinfold calipers i.e. tissue distortion and skinfold width. Ultrasound (US) has the capability to measure full subcutaneous fat thickness in persons of high fat mass with minimal compression. However there has been no published assessment of the degree of tissue compression incurred by the operator in placement of the transducer, and/or the reliability of estimate when measuring SAT thickness by ultrasound. Previous studies (Fanelli and Kuczmarski 1984; Pineau et al., 2007; Duz et al., 2009) have failed to document subject positioning, transducer orientation, scanning protocol and
the method of SAT measurement from US images. In our experience, accurate measurement of SAT by ultrasound is influenced by the orientation and compression force applied through the transducer. The present paper presents a technical analysis of the use of ultrasound for the measurement of SAT thickness.

The aim of this technical study was to develop a method for the accurate and reliable ultrasound measurement of SAT thickness. For the purpose of this study, seven anatomical sites commonly used in skinfold measurement to predict percentage body fat were selected for analysis. Objectives were as follows:

- To determine the effect of operator force through the transducer on SAT thickness;
- To determine if there is a difference in SAT thickness when measured by transverse or longitudinal orientation of the transducer centred around a specific anatomical site;
- To investigate the within-session and between-day intra-rater reliability of the ultrasound technique in the measurement of SAT thickness

6.1.3 Methods

Ethical approval for this study was granted by the University of Limerick Research Ethics Committee (ULREC 08/07). All participants provided written, informed consent (Appendix A).

A total of 37 participants (n=23 men, n=14 women; aged 18-35 years), recruited from the UL Body Composition Study (http://www.ul.ie/bodycompositionstudy), took part in the study. Participant characteristics are shown in Table 6.1.1.
### Table 6.1.1 Subject characteristics (N=37; Males n=23, Females n=14)

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.2 (4.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.0 (8.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.9 (13.9)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.9 (3.2)</td>
</tr>
</tbody>
</table>

#### 6.1.3.1 Instrumentation

SAT thickness was assessed using a GE Logiq e B-mode ultrasound scanner with a 12MHz 12L-RS linear array transducer (GE Medical, China). All scanning was carried out by an operator with two years experience and in adherence with the safety guidelines issued by the British Medical Ultrasound Society (2010). A Mecmesin Basic Force Gauge (Mecmesin, UK) with a limit of 50N and accuracy of ±0.25% of the full scale was used to measure transducer compression.

#### 6.1.3.2 Scanning Protocol

Ultrasound scans were taken at six anatomical sites commonly used in the measurement of percentage body fat by skinfold (Leahy et al., 2009). In males, these sites were identified as; triceps, midaxilla, iliac rest, abdominal, front thigh and medial calf; those for females were triceps, iliac crest, supraspinale, abdominal, front thigh and medial calf. Each site was identified in accordance with the International Society for Kinanthropometric Assessment (ISAK) guidelines (Marfell-Jones et al., 2006) and marked using a surgical pen. The anatomic location of each site is presented in Table 6.1.2.
**Table 6.1.2** Location markings for each skinfold site. All ultrasound scans taken 1cm distally to the point at which the right thumb and forefinger are placed when raising a skinfold

<table>
<thead>
<tr>
<th>Skinfold Site</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triceps</td>
<td>Mid-acromiale-radiale line on the posterior surface of the right arm</td>
</tr>
<tr>
<td>Midaxilla*</td>
<td>Mid-axillary line at the level of the xiphoid process of the sternum</td>
</tr>
<tr>
<td>Iliac crest</td>
<td>Immediately superior to the iliac crest at the mid-axillary line (i.e. above the crest on the mid-line of the body). The fold runs anteriorly downwards</td>
</tr>
<tr>
<td>Supraspinale</td>
<td>The intersection of the border of the ilium (project a horizontal line from the iliac crest mark) and a line from the spinale to the anterior axillary border (armpit)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>A vertical fold on the right side 5cm lateral to, and at the level of, the omphalion (midpoint of the navel)</td>
</tr>
<tr>
<td>Front thigh</td>
<td>Midpoint of a line drawn at the half-distance between the inguinal crease and anterior patella with the subject seated and foot resting on a box</td>
</tr>
<tr>
<td>Medial calf</td>
<td>Vertical fold on the relaxed medial right calf at the estimated level of the greatest circumference flexed to an angle of 90° at the knee by placing the foot on a box</td>
</tr>
</tbody>
</table>

*Midaxilla is not an ISAK skinfold site

The site for ultrasound measurement was identified with an ‘X’ located approximately 1cm below the thumb when the skinfold was pinched between the thumb and forefinger. In order to ensure that a standard area of the on-screen image was measured, a wound closure strip (Steroplast Wound Closures 6mm x 75mm) was placed from left to right (horizontally) across the ‘X’ of each skinfold site prior to scanning (Figure 6.1.1). This caused a shadow to appear on the ultrasound screen. Measurement of SAT thickness was taken at the point of this shadow, to ensure standardisation of the area of on-screen measurement of tissue thickness.
Subcutaneous adipose thickness was measured on-screen using electronic calipers, defined by the perpendicular distance between the upper border of the dermal/adipose interface and the upper border of the adipose/muscle interface at the level of the wound closure strip (Figure 6.1.2).

6.1.3.3 Compression

A subset of five participants (n=3 men, n=2 women) was used to investigate the degree of adipose tissue compression that occurs with varying operator-dependent ultrasound transducer force (N) measured in series. Three representative sites were chosen for this part of the study: an upper limb (triceps), trunk (abdominal) and lower limb (front thigh) site. Sites were identified and marked as described above. In order to stabilise the testing site the participant was positioned prone for measurement of the triceps, and supine for the abdominal and front thigh.

Three levels of compression force were applied at each site. Minimal compression force, defined as the minimum force applied through the transducer to achieve an on-screen image of the subcutaneous adipose tissue (Figure 6.1.3A); maximal compression force, defined as the force applied through the transducer at
which no further change in SAT thickness occurred on-screen (Figure 6.1.3B) and 50% of the measured maximal compression force (Figure 6.1.3C). Using the electronic caliper, SAT thickness was measured for each of the three levels of force applied through the ultrasound transducer at each site.

![Figure 6.1.3A Minimal force (0.5N) applied to the abdomen site. Adipose thickness 24.6mm](image)

![Figure 6.1.3B Maximal force (11.2N) applied to the abdomen site. Adipose thickness 14.6mm](image)

![Figure 6.1.3C Half maximal force (5.5N) applied to the abdomen site. Adipose thickness 15mm](image)

6.1.3.4 Scanning Orientation and Estimate of Reliability

Thirty-four participants (n=21 men, n=13 women) were used to determine difference in SAT thickness measurement obtained from images taken in longitudinal and transverse scanning direction, and to determine measurement reliability. Ultrasound scanning protocol was as described above using six sites for men and women. Participants were positioned as per the recommended guidelines for skinfold measurement (Marfell-Jones et al., 2006). Triceps scans were taken with the participant in sitting with the upper limb in mid-prone position. Midaxilla, iliac crest, supraspinale and abdominal scans were taken in standing. Front thigh scans were taken in sitting with the knee extended and the foot resting on a low stool and medial calf scans were taken in standing with the knee flexed to 90° and with the foot on a low stool. For each site, the transducer was placed in a longitudinal orientation at the skinfold site, perpendicular to the wound closure strip to form a longitudinal image. For the transverse scans, a second wound closure strip was
placed longitudinally across the skinfold site and the transducer was rotated 90° clockwise to form a transverse image (Figure 6.1.4).

![Image](image_url)

**Figure 6.1.4** Wound closure strip shadow and midline measurement of a transverse abdominal image. 1= Adipose tissue + dermal layer, 2= Adipose layer

Generous amounts of contact gel and constant observation of the real-time image kept the pressure of the transducer to a minimum and prevented distortion of the layer of gel standoff and the underlying tissues. An average measure of SAT thickness obtained from a minimum of two scans was recorded in each direction at each site. When a discrepancy of more than 1mm in recorded SAT thickness occurred, a third scan was obtained and the two closest values averaged and recorded. Using this measurement protocol the coefficient of variation (CV) for the measurement of SAT thickness averaged 3% across all sites.

Between-day reliability of longitudinal and transverse imaging was determined on five female participants. The operator, protocol, skinfold markings and wound closure strips remained the same between. As women only were brought back for the second day of testing, there was no between-day measurement of the midaxilla skinfold site.
6.1.3.5 Statistical analysis

Data were tested for normality using a Shapiro-Wilk test and were found to be normally distributed. Within-session intra-rater reliability was calculated for each imaging method (i.e. longitudinal and transverse) using intraclass correlation coefficients $^{3,1} (ICC)$ and the standard error of measurement (SEM) (pooled Standard Deviation $\times \sqrt{(1 - ICC)}$ (Bruton et al., 2000). $ICC_{3,2}$ and SEM was used to assess between-day intra-rater reliability. In addition, the smallest real difference (SRD) ($SRD = 1.96 \times \sqrt{2} \times SEM$: Lexell and Downham 2005)) was employed to determine the minimum change in measurement between days that could be interpreted as a true difference. Paired $t$-tests were used to evaluate difference between scanning methods. Statistical analysis was performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.).

6.1.4 Results

6.1.4.1 Compression

Figure 6.1.5 shows the non-linear relationship between relative transducer force and SAT thickness. Compared to minimal transducer force, the use of maximal transducer force reduced SAT thickness by a mean of 36% at the triceps site, 37% at the abdominal site and 25% at the front thigh site. The application of half maximal force reduced SAT thickness by a mean of 32%, 36% and 24% at triceps, abdominal and front thigh sites respectively (Table 6.1.3).
Figure 6.1.5 Subcutaneous adipose thickness (SAT) (Mean with standard error) in relation to transducer force

Table 6.1.3 Mean Minimal, Maximal and Half Maximal applied transducer forces (N) with standard error (SE) and corresponding subcutaneous adipose thickness (SAT) (mm) using longitudinal scanning

<table>
<thead>
<tr>
<th>Site</th>
<th>Min Force (N)</th>
<th>SAT (mm)</th>
<th>Max Force (N)</th>
<th>SAT (mm)</th>
<th>Relative Change¹ (%)</th>
<th>Half Max Force (N)</th>
<th>SAT (mm)</th>
<th>Relative Change¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triceps</td>
<td>1.1 (0.3)</td>
<td>10.7</td>
<td>9.0 (2.0)</td>
<td>6.9 (1.1)</td>
<td>36%</td>
<td>4.6 (1.0)</td>
<td>7.3 (1.1)</td>
<td>32%</td>
</tr>
<tr>
<td>Abdominal</td>
<td>0.8 (0.1)</td>
<td>20.6</td>
<td>13.0 (1.3)</td>
<td>13.0 (2.2)</td>
<td>37%</td>
<td>6.4 (0.7)</td>
<td>13.1 (2.2)</td>
<td>36%</td>
</tr>
<tr>
<td>Front</td>
<td>0.7 (0.2)</td>
<td>10.8</td>
<td>14.1 (3.6)</td>
<td>8.0 (1.8)</td>
<td>25%</td>
<td>7.2 (2.0)</td>
<td>8.2 (1.7)</td>
<td>24%</td>
</tr>
<tr>
<td>Thigh</td>
<td>0.7 (0.2)</td>
<td>10.8</td>
<td>14.1 (3.6)</td>
<td>8.0 (1.8)</td>
<td>25%</td>
<td>7.2 (2.0)</td>
<td>8.2 (1.7)</td>
<td>24%</td>
</tr>
</tbody>
</table>

¹Relative change is % reduction in SAT compared to the minimum force condition

6.1.4.2 Scanning Direction and Reliability

With the exception of the iliac crest, there was no significant difference in SAT thickness measured using transverse or longitudinal scans. Excellent intra-rater reliability was found for within-session measurement, with ICCs above 0.99 for all sites and accompanying narrow 95% confidence intervals (Table 6.1.4). The mean
standard error of measurement (SEM) was 0.2mm for longitudinal and 0.3mm for transverse measurement.

Table 6.1.4 Within-session reliability and difference between longitudinal and transverse scans at each site

<table>
<thead>
<tr>
<th>Site/US Method</th>
<th>n</th>
<th>Mean SAT (SD) (mm)</th>
<th>Within-session ICC (95% CI)</th>
<th>SEM (mm)</th>
<th>Mean diff (mm)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triceps:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>34</td>
<td>10.0 (5.2)</td>
<td>0.999 (0.998→1)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.534</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>9.9 (5.4)</td>
<td>0.999 (0.999→1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Midaxilla:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>21</td>
<td>8.5 (5.5)</td>
<td>0.997 (0.993→0.999)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>8.3 (5.6)</td>
<td>0.999 (0.998→1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Iliac crest:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>34</td>
<td>14.8 (8.3)</td>
<td>0.999 (0.997→0.999)</td>
<td>0.3</td>
<td>-0.6</td>
<td>0.007†</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>15.4 (8.5)</td>
<td>0.999 (0.999→1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Supraspinale:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>13</td>
<td>15.1 (10.2)</td>
<td>0.999 (0.997→1)</td>
<td>0.3</td>
<td>-0.6</td>
<td>0.212</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>15.7 (9.8)</td>
<td>0.999 (0.997→1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Abdominal:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>34</td>
<td>23.5 (11.1)</td>
<td>0.999 (0.999→1)</td>
<td>0.4</td>
<td>0</td>
<td>0.859</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>23.5 (10.9)</td>
<td>0.993 (0.987→0.997)</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Front thigh:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>34</td>
<td>9.9 (4.7)</td>
<td>0.999 (0.998→0.999)</td>
<td>0.1</td>
<td>-0.1</td>
<td>0.511</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>10 (4.7)</td>
<td>0.999 (0.998→0.999)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medial calf:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>34</td>
<td>6.3 (3.2)</td>
<td>0.998 (0.996→0.999)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.159</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>6.1 (3.1)</td>
<td>0.997 (0.994→0.999)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: US, ultrasound; SD, standard deviation; ICC, intraclass correlation coefficient (model 3,1); CI, confidence interval; SEM, standard error of measurement. †P<0.05 using paired t-test

Reliability between days was also excellent with ICCs for each method >0.99 at all sites and SEM was less than 0.4mm (Table 6.1.5) As indicated by the SRD, the method was highly sensitive to detect change that, dependent on the anatomical location of measurement, required between 0.3-1mm of difference to be considered a true change in SAT thickness.
### Table 6.1.5 Between-day reliability and sensitivity for longitudinal and transverse methods at each site

<table>
<thead>
<tr>
<th>Site/US Method</th>
<th>n</th>
<th>Mean SAT (SD)(mm)</th>
<th>Between-day ICC (95% CI)</th>
<th>SEM (mm)</th>
<th>SRD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triceps:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>12.9 (2.8)</td>
<td>0.992(0.958→0.999)</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>12.5 (2.2)</td>
<td>0.997(0.985→1)</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Iliac crest:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>16.0 (9.1)</td>
<td>0.999(0.997→1)</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>15.5 (9.2)</td>
<td>0.999(0.997→1)</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Supraspinale:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>10.9 (5.2)</td>
<td>0.997(0.984→1)</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>11.8 (5.8)</td>
<td>0.999(0.994→1)</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Abdominal:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>21.4 (9.5)</td>
<td>0.999(0.996→1)</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>20.8 (8.7)</td>
<td>0.998(0.991→1)</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Front thigh:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>10.8 (2.5)</td>
<td>0.997(0.985→1)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>10.6 (2.6)</td>
<td>0.992(0.959→0.999)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Medial calf:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>5</td>
<td>6.6 (3.2)</td>
<td>0.998(0.990→1)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Transverse</td>
<td></td>
<td>6.1 (3.2)</td>
<td>0.995(0.977→0.999)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Abbreviations:** US, ultrasound; SD, standard deviation; ICC, intraclass correlation coefficient (model 3,2); CI, confidence interval; SEM, standard error of measurement; SRD, smallest real difference

### 6.1.5 Discussion

This study aimed to examine the technical aspects of using ultrasound to measure SAT thickness. We found that the amount of transducer force applied by the operator influenced the size of SAT thickness. The relationship between SAT thickness and operator-applied transducer force was non-linear. Maximal force reduced SAT thickness by between 25% and 37% depending on the site. This percentage of compression did not change when force was reduced to half of the maximal amount, i.e. SAT thickness was still reduced by 24-36%. Reduction in SAT thickness under compression may be due, in part, to the “squeezing out” of water contained in adipose tissue and in the compression of small blood vessels. The percentage water content in adipose cells has been variously reported as 7.4% (Querleux *et al.*, 2000), 14% (Wang and Pierson 1976) and 20% (Baker 1969) which may lead to difference in compressibility and, thereby, the variability in the
measurement of SAT thickness between subject and anatomical location. There is no
standard value of transducer force to undertake an ultrasound scan – the skilled
operator generally determines the appropriate degree of force to best suit the
structures under examination. These results highlight the care that must be taken by
the operator in applying the least force possible through the transducer in order to
prevent tissue compression – best achieved by consistent observation of the on-
screen image as feedback.

In comparing methods, no statistically significant difference was found
between longitudinal and transverse scanning, with the exception of the iliac crest
site. However this equated to a mean difference of only 0.6mm, and may not be of
clinical significance. Using the protocol described we find ultrasound to be a reliable
and sensitive method of measurement of SAT thickness across a wide range of
anatomical sites. The reported ICCs of 0.99 for the within-session and between-day
measurements compare favourably to previous studies (Ishida et al., 1992; Bellisari
et al., 1993). An analysis of the SEM between measurements indicated low
variability across all sites for both longitudinal and transverse scans. An average
sensitivity to change of 0.6mm for both longitudinal and transverse scans constituted
a real difference measureable within subjects and indicates that ultrasound is
sensitive to detect changes in subcutaneous adipose thickness. Therefore, the
statistically significant difference of 0.6mm between longitudinal and transverse
scans at the iliac crest site may be interpreted as measurement error. By conducting
this study it is possible to recommend a standardised protocol for the use of
ultrasound in the measurement of SAT thickness. Both longitudinal and transverse
imaging methods were found to be similar in measure, equally reliable and sensitive
with low variability. However, for accuracy of measurement, the authors would
recommend the use of longitudinal transducer orientation for future studies. This is
due to the convexity of certain sites such as the triceps, front thigh and medial calf,
which were more difficult to capture using minimal compression in transverse
orientation. This is due to reduced transducer-skin contact, which resulted in rounded
edges disappearing off-screen. The use of longitudinal imaging eliminated this
convexity and resulted in faster scanning time and higher quality images. The use of
a wound closure strip over the ISAK landmark in our protocol is also recommended
as it allows accurate transducer placement and midline thickness measurement, thus
improving rater reliability.

6.1.6 Conclusions

This technical report concerning the ultrasound imaging of subcutaneous adipose tissue has shown it to be a non-invasive, reliable and accurate tool. Results from the current study have led to recommendations for future measurement of SAT thickness by ultrasound (Table 6.1.6).

Table 6.1.6 Protocol Recommendations for the measurement of subcutaneous adipose tissue using B-mode ultrasound

- Site identification and subject positioning using ISAK guidelines
- Longitudinal scanning of skinfold sites using a high frequency (12MHz) linear transducer
- Use of an on-skin wound closure strip to guide consistent on-screen measurement of images
- Close observation of real-time imaging along with the use of generous amounts of gel to ensure minimal compression of the underlying tissues

This protocol overcomes some of the limitations of skinfold calipers in the measurement of SAT and may therefore better represent the true thickness of subcutaneous adipose tissue at each site. However, US cannot be proposed as a replacement for skinfold thickness measures unless this method is shown to be a superior predictor of body fat percentage, due to the extra expense involved for
equipment and training. Since this study has not looked at changes in adiposity over time, further assessment of this scanning protocol is needed in the form of a longitudinal study. However, our results have established that a highly accurate and reproducible method may now be applied in order to further validate ultrasound as a body composition measurement tool.
6.2 Comparison of skinfold thickness obtained using manual skinfold calipers and ultrasound measured subcutaneous adipose tissue thickness

6.2.1 Abstract

The aim of this study was to investigate the relationship between skinfold thickness measures obtained using manual skinfold calipers and subcutaneous adipose tissue (SAT) thickness measured by ultrasound. Skinfold thickness and SAT thickness measures were obtained at five body sites (triceps, iliac crest, abdominal, front thigh and medial calf) in men (n=83) and women (n=52) aged 18-29 years. Site-specific and general correlations between skinfold thickness and SAT thickness were investigated separately in men and women. Skinfold thickness and SAT thickness measure were strongly correlated at each site in men (r=0.839 to 0.955) and women (r=0.731 to 0.920). The proportional difference ranged from -22.3% to -119.4% of SAT in men and -7.1% to 139.2% of SAT in women. Skinfold thickness measures at selected body sites are strongly correlated to, but not representative of, ultrasound measured subcutaneous adipose tissue thickness.
6.2.2 Introduction

The use of skinfold thickness measures to predict body fat as discussed in Chapter 5 is based on the assumption that skinfold thickness provides a direct measure of subcutaneous adiposity at a given site. Therefore, a double layer skinfold should correlate strongly and proportionally to the single subcutaneous adipose tissue (SAT) layer as measured by ultrasound (Fanelli and Kuczmarski 1984; Selkow et al., 2011). However, several factors may affect the accuracy of skinfold thickness measures using manual calipers. For example, variations in skinfold compressibility between individuals, inability to palpate the fat-muscle interface and difficulty obtaining measures on subjects with excessive amounts of fat tissue may lead to measurement inaccuracies (Fanelli and Kuczmarski 1984). Inter and intra-rater reliability for skinfold thickness measures can also be poor particularly when a standardised protocol is not used (Scherf et al., 1986).

The relationship of skinfold thickness measures to ultrasound measured SAT thickness has been studied previously, with correlations between methods ranging from 0.299 (Kuczmarski et al., 1987) to 0.927 (Selkow et al., 2011) However, these relationships are not proportional, i.e. a single layer SAT measure obtained using ultrasound does not correspond to half of a double layer skinfold thickness (Booth et al., 1966; Selkow et al., 2011), even when corrected for compression (Fanelli and Kuczmarski 1984; Kuczmarski et al., 1987). As discussed in section 6.1, the methodology used to measure SAT using ultrasound has been poorly reported in previous studies.

The aim of the current study is to investigate the relationship of skinfold thickness measures obtained using manual calipers to ultrasound measured subcutaneous adipose tissue thickness at selected body sites in men and women.
6.2.3 Methods

6.2.3.1 Participants

Following written, informed consent (Appendix A) 135 healthy young adults (52 women and 83 men) between the age of 18 and 29 years were recruited from the University of Limerick campus and surrounding community as part of the UL Body Composition Study (www.ul.ie/bodycompositionstudy). Subjects were instructed to refrain from exercise for 12 hours, to refrain from eating for 3 hours and to consume 500ml of water one hour before testing. Subjects were also required to empty their bladder immediately prior to measurement.

6.2.3.2 Anthropometric measures

Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, United Kingdom) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.).

6.2.3.3 Skinfold thickness and ultrasound SAT thickness measures

Using methodology outlined in section 5.3.3 and 6.1.3, skinfold thickness and SAT thickness measures were obtained at five body sites (triceps, iliac crest, abdominal, front thigh and medial calf) in all subjects.

6.2.3.4 Statistical analysis

Statistical analysis was performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.). Male and female data were analysed separately. A Kolgomorov Smirnov test was conducted to assess whether variables were normally or non-normally distributed. Mean values and standard deviation, median and
interquartile range and ranges are reported for descriptive statistics. A Mann Whitney U Test was used, as appropriate, to undertake between sex comparisons. As all SAT and skinfold thickness measures were non-normally distributed, Spearman’s rho was used to investigate the general and site-specific relationship between these variables. Statistical significance (two-tailed) was set at $p<0.05$ for all analyses.

### 6.2.4 Results

Subject characteristics are shown in Table 6.2.1. Men were taller (+0.14m) and heavier (+16.6kg) with higher BMI’s (+2.0kg/m$^2$) than women (all $p<0.05$).

<p>| Table 6.2.1 Subject characteristics (mean and standard deviation (SD), median and interquartile range (IQR) and the range (min – max); n=135). |
|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=83)</td>
<td>21.9 (3.0)</td>
<td>21.0 (5.0)</td>
<td>18-29</td>
<td>22.1 (3.0)</td>
<td>21.0 (4.8)</td>
<td>18-28</td>
</tr>
<tr>
<td>Women (n=52)</td>
<td>21.0 (5.0)</td>
<td>21.0 (4.8)</td>
<td>18-28</td>
<td>22.1 (3.0)</td>
<td>21.0 (4.8)</td>
<td>18-28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Height (m)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=83)</td>
<td>1.80 (0.06)</td>
<td>1.80 (0.07)</td>
<td>1.66-1.97</td>
<td>1.66 (0.06)</td>
<td>1.66 (0.08)</td>
<td>1.55-1.77</td>
</tr>
<tr>
<td>Women (n=52)</td>
<td>1.66 (0.06)</td>
<td>1.66 (0.08)</td>
<td>1.55-1.77</td>
<td>1.66 (0.06)</td>
<td>1.66 (0.08)</td>
<td>1.55-1.77</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mass (kg)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=83)</td>
<td>81.7 (12.3)</td>
<td>79.1 (15.6)</td>
<td>56.9-120.8</td>
<td>64.3 (10.1)</td>
<td>62.5 (10.3)</td>
<td>47.7-98.8</td>
</tr>
<tr>
<td>Women (n=52)</td>
<td>79.1 (15.6)</td>
<td>79.1 (15.6)</td>
<td>56.9-120.8</td>
<td>62.5 (10.3)</td>
<td>62.5 (10.3)</td>
<td>47.7-98.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI (kg/m$^2$)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=83)</td>
<td>25.0 (3.3)</td>
<td>24.3 (3.8)</td>
<td>17.5-35.4</td>
<td>23.5 (3.8)</td>
<td>22.3 (4.3)</td>
<td>17.2-40.2</td>
</tr>
<tr>
<td>Women (n=52)</td>
<td>24.3 (3.8)</td>
<td>22.3 (4.3)</td>
<td>17.2-40.2</td>
<td>23.5 (3.8)</td>
<td>22.3 (4.3)</td>
<td>17.2-40.2</td>
</tr>
</tbody>
</table>

1 Indicates normal distribution
2 Indicates significantly different from men ($p<0.05$)

All SAT and skinfold thickness measures differed significantly between men and women (all $p<0.001$). Table 6.2.2 shows the SAT and skinfold thickness results, the correlations and differences between the two methods in men. SAT and skinfold thickness measures were strongly correlated ($p<0.01$) but significantly different ($p<0.001$) at all sites, with the SAT measure being lower than the skinfold thickness measure. The magnitude of this difference varied widely from -22.3% of SAT thickness at the abdominal to -119.4% at the front thigh.
Table 6.2.2 Ultrasound and skinfold thickness measures taken at five sites in men (median and interquartile range, Spearman’s correlation and differences between methods; n=83)

<table>
<thead>
<tr>
<th>Site</th>
<th>SAT (mm)</th>
<th>Skinfold thickness (mm)</th>
<th>Spearman’s rho</th>
<th>Δ (US-SF) (mm)</th>
<th>% Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>6.0 (3.3)</td>
<td>9.5 (5.5) 1</td>
<td>0.839 2</td>
<td>-3.5</td>
<td>-58.3%</td>
</tr>
<tr>
<td>Iliac crest</td>
<td>11.6 (7.3)</td>
<td>14.6 (10.6) 1</td>
<td>0.888 2</td>
<td>-3.0</td>
<td>-25.9%</td>
</tr>
<tr>
<td>Abdominal</td>
<td>13.9 (14.2)</td>
<td>17.0 (15.9) 1</td>
<td>0.955 2</td>
<td>-3.1</td>
<td>-22.3%</td>
</tr>
<tr>
<td>Front thigh</td>
<td>6.2 (4.1)</td>
<td>13.6 (10.7) 1</td>
<td>0.952 2</td>
<td>-7.4</td>
<td>-119.4%</td>
</tr>
<tr>
<td>Medial calf</td>
<td>4.2 (3.0)</td>
<td>8.0 (5.4) 1</td>
<td>0.908 2</td>
<td>-3.8</td>
<td>-90.5%</td>
</tr>
</tbody>
</table>

1 indicates significant difference from US measure (p<0.05)
2 indicates significant correlation (p<0.01), Δ = difference (SAT – skinfold thickness)

Similarly in women, SAT and skinfold thickness measures were strongly correlated (p<0.01) but significantly different (p≤0.05) at all sites except the abdominal. Table 6.1.3 shows the median SAT and skinfold thickness measures, the correlations and differences between the two methods in women. SAT measures at the triceps (-3.1mm, p<0.001), iliac crest (-3.1mm, p=0.025), front thigh (-19.9mm, p<0.001) and medial calf (-10.3mm, p<0.001) sites were lower than skinfold thickness measures; the relative magnitude of the difference varied from -14.6% at the iliac crest to -139.2% at the front thigh.

Table 6.2.3 Ultrasound and skinfold thickness measures taken at five sites in women (median and interquartile range, Spearman’s correlation and differences between methods; n=52).

<table>
<thead>
<tr>
<th>Site</th>
<th>SAT (mm)</th>
<th>Skinfold thickness (mm)</th>
<th>Spearman’s rho</th>
<th>Δ (US-SF) (mm)</th>
<th>% Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>14.7 (5.8)</td>
<td>17.8 (10.0) 1</td>
<td>0.731 2</td>
<td>-3.1</td>
<td>-21.1%</td>
</tr>
<tr>
<td>Iliac crest</td>
<td>21.2 (10.8)</td>
<td>24.3 (14.9) 1</td>
<td>0.762 2</td>
<td>-3.1</td>
<td>-14.6%</td>
</tr>
<tr>
<td>Abdominal</td>
<td>28.2 (14.1)</td>
<td>30.2 (19.7)</td>
<td>0.884 2</td>
<td>-2.0</td>
<td>-7.1%</td>
</tr>
<tr>
<td>Front thigh</td>
<td>14.3 (6.9)</td>
<td>34.2 (22.5) 1</td>
<td>0.891 2</td>
<td>-19.9</td>
<td>-139.2%</td>
</tr>
<tr>
<td>Medial calf</td>
<td>9.8 (4.6)</td>
<td>20.1 (9.3) 1</td>
<td>0.920 2</td>
<td>-10.3</td>
<td>-105.1%</td>
</tr>
</tbody>
</table>

1 indicates significant difference from US measure (p<0.001)
2 indicates significant correlation (p<0.01)
To investigate the general relationship between SAT and skinfold thickness measures, data obtained from each site was pooled. Figure 6.2.1 demonstrates the relationship between SAT and skinfold thickness measures in men and women, independent of site. In women the relationship between skinfold thickness and SAT thickness is poor, with only 38% of the variance shared between the two measures. This relationship is much stronger in men, where 75% of the variance is shared.

![Figure 6.2.1](image)

**Figure 6.2.1** Relationship of skinfold thickness and subcutaneous adipose tissue (SAT) thickness in men (n=83) and women (n=52)

### 6.2.5 Discussion

The results presented in this investigation suggest that although skinfold thickness measures obtained using manual calipers are strongly correlated to ultrasound measured subcutaneous adipose tissue thickness, this relationship is not proportional and varies depending upon the site measured. In men, the relationship between the two variables was strongest at the abdominal site (r=0.955), with ultrasound measured SAT being 22.3% lower than the skinfold thickness measure. If the skinfold thickness was a true measure of a double layer of SAT, the % difference should be closer to 100%. In women, the relationship between variables was
strongest at the medial calf site ($r=0.920$). SAT thickness was 105% lower than the skinfold thickness measure, suggesting that skinfold thickness is representative of SAT thickness at this site. Similarly in men, SAT thickness was 90.5% lower ($r=0.908$) than the skinfold thickness measure at the medial calf site.

Of the five sites measured in this study, the medial calf skinfold thickness is the only measure not commonly used in skinfold prediction equations (Durnin and Womersley 1974; Jackson and Pollock 1978; Jackson et al., 1980). These findings suggest that skinfold thickness measures do not represent site specific subcutaneous adipose tissue thickness and may not be an appropriate method for predicting % body fat.

When the overall relationship between SAT thickness and skinfold thickness was analysed independent of body site, the correlation between variables was much stronger in men ($r=0.86$) than in women ($r=0.57$). As demonstrated in chapter three, women have a greater amount of total fat tissue mass than men and it has been suggested that a greater proportion of this fat mass is deposited subcutaneously in women (Enzi et al., 1996). Therefore, skinfold thickness measures are larger in women than men (Table 6.2.2 and 6.2.3) and consequently are more difficult to obtain accurately (Kuczmarski et al., 1987).

### 6.2.6 Conclusions

Skinfold thickness measures obtained using manual calipers at specific body sites are strongly correlated to but not representative of ultrasound measured subcutaneous adipose tissue thickness. Ultrasound measured SAT thickness may be a more useful predictor of total and segmental body fat than commonly used skinfold prediction equations.
6.3 Ultrasound measurement of subcutaneous adipose tissue thickness accurately predicts total and segmental body fat of young adults

S. Leahy, C. Toomey, K. McCreesh, C. O’Neill and P. Jakeman

6.3.1 Abstract

This study evaluated the ability of ultrasound measurement of subcutaneous adiposity to accurately determine whole body and segmental body fat in young adults aged 18-29 years. Subcutaneous adipose tissue (SAT) thickness was measured by ultrasound at five body sites in 135 subjects (83 men, 52 women) and compared to the corresponding segmental fat mass measured by dual energy x-ray absorptiometry (DXA). Ultrasound measures of SAT thickness were strongly correlated to segmental fat mass and total percentage body fat \( (r=0.697-0.907, p<0.01) \). Prediction equations generated using quantile regression found SAT thickness at the abdominal and thigh to accurately predict \% body fat in men (SEE=1.9\%, LoA\%; -3.6\% to +3.8\%) and SAT thickness at the abdominal and medial calf to accurately predict \% body fat in women (SEE=3.0\%, LoA\%; -6.5\% to +5.4\%). These data indicate that ultrasound measurement of SAT thickness proportionally reflects segmental fat mass and accurately predicts \% body fat in young adults.
6.3.2 Introduction

The strategic plan for obesity research (NIH 2011) identifies the need for accurate and reliable estimates of total and regional body composition that are applicable to the general and clinical populations across the lifespan. In studies of small subject numbers and specific to a patient population ultrasound has been shown to be an accurate, non-invasive measurement technique with the potential to provide site (Pineau et al., 2007; Duz et al., 2009) and tissue specific (Stolk et al., 2003; Mook-Kanamori et al., 2009; Emmons et al., 2011) fat content without recourse to radiation exposure. The utility of ultrasound to provide an accurate and reliable estimate of total or site-specific body fat in a larger population has yet to be established.

Regional measures of subcutaneous adiposity, such as skinfold thickness, are widely used in population studies to predict body composition and, specifically, body fat mass. In this approach site-specific skinfold thickness is deemed to represent segmental fat content, though this has yet to be established. Algorithms, generated by comparison to a reference method of measurement of body composition, identify the body segments and the corresponding site(s) of measurement that offer the most accurate prediction of total body fat (Durnin and Womersley 1974; Jackson and Pollock 1978). However, the manual measurement of skinfold thickness using anthropometric calipers is technically demanding (Marfell-Jones et al., 2006) and limited, for example, by the limited span of the caliper jaws.

In a recent study we found ultrasound to provide an accurate and reliable measurement of the thickness of subcutaneous adipose tissue (SAT) at sites representative of the major body segments that overcame many of the limitations attributed to the measurement of skinfold thickness (Toomey et al., 2011). It was
reasoned that algorithms based on the accurate measurement of segmental SAT by ultrasound would provide a more accurate prediction of total and regional body fat that could be applied to population studies and clinical settings. Therefore, using dual energy x-ray absorptiometry (DXA) as the reference method of measurement of whole body and segmental adiposity (Ellis 2000; Rubiano et al., 2000) this study evaluated the ability of ultrasound measurement of subcutaneous adiposity to accurately predict whole body and segmental body fat in young adult men and women.

6.3.3 Methods

The study was approved by the University of Limerick Research Ethics Committee (ULREC 08/07).

6.3.3.1 Participants

Following written, informed consent (Appendix A), 135 healthy young adults (52 women and 83 men) between the age of 18 and 29 years were recruited from the University of Limerick campus and surrounding community as part of the UL Body Composition Study (www.ul.ie/bodycompositionstudy). Subjects were instructed to refrain from exercise for 12 hours, to refrain from eating for 3 hours and to consume 500ml of water one hour before testing. Subjects were also required to empty their bladder immediately prior to measurement.
6.3.3.2 Anthropometric measurements

Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, United Kingdom) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.).

6.3.3.3 Ultrasound Measurements

Subcutaneous adipose tissue (SAT) thickness was assessed using a GE Logiq e B-mode ultrasound scanner with a 12MHz linear array transducer (GE Healthcare, Chalfont St Giles, Bucks., UK). All scanning was carried out in adherence with the safety guidelines issued by the British Medical Ultrasound Society (2010). Regions of interest for ultrasound measurement of SAT thickness were identified to reflect the distribution of body fat (Leahy et al., 2011) residing in the upper limb (i.e. triceps representing ~ 10% of fat mass), the trunk region (iliac crest and abdominal representing ~ 50% of fat mass) and the lower limb (front thigh and medial calf representing ~ 40% of fat mass) in men and women. Each site was located in accordance with the International Society for Kinanthropometric Assessment (ISAK) guidelines (Marfell-Jones et al., 2006) and marked using a surgical pen. A wound closure strip (Steroplast Wound Closures 6mm x 75mm) was placed from left to right across the ‘X’ marking each site prior to scanning. These strips caused a shadow to appear on screen as the ultrasound image was captured to ensure that SAT thickness was measured at the correct site directly below the ‘X’.

Ultrasound capture and measurement of SAT was undertaken according to the method of Toomey et al. (2011). Briefly, the ultrasound transducer was placed longitudinally across the wound closure strip with minimal force applied through the transducer to avoid compression of the adipose layer. Measurements of SAT
thickness were taken at the point of this shadow generated by the wound closure strip, to ensure standardisation of the area of on-screen measurement of tissue thickness. On-screen electronic callipers were used to measure SAT thickness, defined as the perpendicular distance between the upper border of the dermal/adipose interface and the upper border of the adipose/muscle interface at the level of the wound closure strip (Figure 6.3.1). The measure was repeated twice at each site and the mean value used for analysis. The coefficient of variance (CV) for repeated measures across all sites was 3%. Within- and between-day reliability has been demonstrated for this scanning protocol, with an intraclass correlation coefficient (ICC) of >0.99 and standard error of measurement (SEM) of 0.2mm reported for within-day scans. Corresponding figures for between-day scans were ICC>0.99 and SEM=0.4mm (Toomey et al., 2011).

![Ultrasound image of subcutaneous adipose tissue thickness measured at the abdominal. White circle indicates placement of wound closure strip](image)

**Figure 6.3.1** Ultrasound image of subcutaneous adipose tissue thickness measured at the abdominal. White circle indicates placement of wound closure strip
6.3.3.4 Dual Energy X-ray Absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ 2007 v.11 software was used to capture total body scans. Daily calibration of the scanner employed a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to the manufacturer’s recommendations and instructed to remain as still as possible for the duration of the scan. Where subjects were too wide to fit within the boundary of the scan, the right hand side of the body was scanned and results doubled. This procedure has been validated for the iDXA by Rothney et al. (2009). The CV of the iDXA for repeated measurement of whole body compositional analysis was 0.6%. The enCORE software was used to undertake segmental analysis of customised regions of interest. Accordingly, arm, leg and trunk segments were defined as regions of interest using the following anatomical landmarks:

Arm: all tissue extending from a line drawn through the centre of the arm socket to the tip of the phalange;

Leg: all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the tip of the phalange;

Trunk: all tissue distal to the lowest point of the skull, excluding that contained in arm and leg segments.

CV for repeated, user-defined segmental analysis was found to be 1.2%.

6.3.3.5 Statistical Analysis

Statistical analysis was performed using PASW Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL.) and TIBCO Spotfire S+ 8.1 (TIBCO Software Inc. Palo Alto, CA.). Male and female data were analysed separately. A Kolgomorov Smirnov
test was conducted to assess whether variables were normally or non-normally distributed. Mean values and standard deviation, median and interquartile range and ranges are reported for descriptive statistics. A Mann Whitney U Test was used, as appropriate, to undertake between sex comparison of the dependent variables of interest.

As most variables were found to be non-normal in their distribution, quantile regression was used to generate prediction equations of percentage body fat (% fat) from ultrasound SAT thickness (Koenker and Hallock 2001). The segmental SAT thickness that had the strongest correlation to percentage body fat was retained in all regression analyses. However, to avoid violating the assumptions of regression analysis, pairs of segmental SAT thickness’ demonstrating higher correlation than either of the individual SAT thickness had with percentage body fat were not included together in any regression analyses. Standard error of the estimate (SEE) was calculated to evaluate the strength of ultrasound prediction equations.

Scatter plots, Spearman’s rho correlations and Bland Altman (Bland and Altman 1986) plots were used to investigate level of agreement and bias between % body fat measured by DXA and that predicted from ultrasound measurement of segmental SAT. The 95% limits of agreement were calculated as the mean of difference between methods ± 1.96·standard deviation of the difference between the methods.

The difference in body fat between the methods was analysed by Wilcoxon Signed ranks tests. Spearman’s rho correlation was used to investigate the relationship between SAT and % body fat at each body segment and also the relationship between SAT across all regions of interest.

Statistical significance (two-tailed) was set at p<0.05 for all analyses.
6.3.4 Results

The characteristics of the subjects recruited to this study are reported in Table 6.3.1. Men were taller (+0.14m, p<0.001), heavier (+18.6kg, p<0.001) and had a higher BMI (+2.0kg/m\(^2\), p=0.001) but lower percentage body fat (-13.6%, p<0.001) than women. Figure 2 illustrates the distribution of fat mass in the arm, leg and trunk segments in men and women. In men, 10%, 39% and 51% of fat mass was contained in the arm, leg and trunk segments respectively. In women, this distribution was 11%, 44% and 45% respectively.

Table 6.3.1 Subject characteristics (mean and standard deviation (SD), median and interquartile range (IQR) and the range (min – max); n=135).

<table>
<thead>
<tr>
<th></th>
<th>Men (n=83)</th>
<th>Women (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.9 (3.0)</td>
<td>21.0 (5.0)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 (0.06)</td>
<td>1.80 (0.07)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>81.7 (12.3)</td>
<td>79.1 (15.6)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.0 (3.3)</td>
<td>24.3 (3.8)</td>
</tr>
<tr>
<td>% body fat</td>
<td>18.4 (6.0)</td>
<td>17.5 (7.9)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>15.6 (7.2)</td>
<td>13.8 (6.8)</td>
</tr>
</tbody>
</table>

\(^1\) indicates normal distribution  
\(^2\) indicates significantly different from men (p<0.05)
SAT thickness at each site was higher in women than men (p<0.001) as shown in Table 6.3.2.

**Table 6.3.2** Ultrasound measured SAT thickness (mm) at five sites representing body segments (median, interquartile range (IQR) and the range (min – max); n=135)

<table>
<thead>
<tr>
<th></th>
<th>Men (n=83)</th>
<th>Women (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median</strong></td>
<td><strong>IQR</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>Triceps SAT (mm)</td>
<td>6.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Iliac crest SAT (mm)</td>
<td>11.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Abdominal SAT (mm)</td>
<td>13.9</td>
<td>14.2</td>
</tr>
<tr>
<td>Front thigh SAT (mm)</td>
<td>6.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Medial calf SAT (mm)</td>
<td>4.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\(^{1}\) indicates significantly different from men (p<0.05)

Specific to the aim of this study SAT thickness at selected regions of interest was found to be highly correlated to the fat mass contained in that segment and to the percentage of total body fat measured by DXA for men (Table 6.3.3) and women (Table 6.3.4).
**Table 6.3.3** Spearman’s rho correlations of % body fat, segmental fat mass and ultrasound measured SAT thickness (mm) at five regions of interest in men (n=83)

<table>
<thead>
<tr>
<th>% body fat</th>
<th>Arm FM (kg)</th>
<th>Trunk FM (kg)</th>
<th>Leg FM (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triceps SAT (mm)</td>
<td>0.734↑</td>
<td>0.747↑</td>
<td>-</td>
</tr>
<tr>
<td>Iliac crest SAT (mm)</td>
<td>0.824↑</td>
<td>-</td>
<td>0.844↑</td>
</tr>
<tr>
<td>Abdominal SAT (mm)</td>
<td>0.907↑</td>
<td>-</td>
<td>0.911↑</td>
</tr>
<tr>
<td>Front thigh SAT (mm)</td>
<td>0.851↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medial calf SAT (mm)</td>
<td>0.714↑</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

↑indicates significant Spearman’s rho correlation (p<0.01)

**Table 6.3.4** Spearman’s rho correlations of % body fat and ultrasound measured SAT thickness (mm) at five regions of interest in women (n=52)

<table>
<thead>
<tr>
<th>% body fat</th>
<th>Arm FM (kg)</th>
<th>Trunk FM (kg)</th>
<th>Leg FM (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triceps SAT (mm)</td>
<td>0.697↑</td>
<td>0.747↑</td>
<td>-</td>
</tr>
<tr>
<td>Iliac crest SAT (mm)</td>
<td>0.760↑</td>
<td>-</td>
<td>0.842↑</td>
</tr>
<tr>
<td>Abdominal SAT (mm)</td>
<td>0.905↑</td>
<td>-</td>
<td>0.923↑</td>
</tr>
<tr>
<td>Front thigh SAT (mm)</td>
<td>0.691↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medial calf SAT (mm)</td>
<td>0.707↑</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

↑indicates significant Spearman’s rho correlation (p<0.01)

In men, SAT thickness at the abdominal had the highest correlation with % body fat (r=0.907, p<0.001) and, thus, was included in all regression analyses. The correlation between SAT thickness at the front thigh and SAT thickness at both the triceps and medial calf was found to be greater than the correlation between either of these SAT thickness measures and DXA derived % body fat. Therefore, any combination that selected front thigh and medial calf or front thigh and triceps SAT thickness as independent variables was excluded in the regression analyses. From the remaining combinations, quantile regression found that ultrasound measurement of SAT thickness at two regions of interest (abdominal and front thigh, Appendix E) gave an accurate prediction of % body fat (r=0.947, SEE=1.9%, p=0.432; Table
6.3.5). This prediction was only marginally advanced by the addition of SAT measured at the iliac crest (r=0.952, SEE=1.8%, p=0.845).

Table 6.3.5 Ultrasound prediction equations for % body fat in men and women in comparison to DXA measured % body fat. (Spearman’s correlation, SEE, median difference and 95% limits of agreement (LoA))

<table>
<thead>
<tr>
<th>Quantile Regression Equation</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>r</th>
<th>SEE</th>
<th>Δ</th>
<th>95% LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td>7.65 + (abdominal·0.36) + (front thigh·0.59)</td>
<td>18.3 (5.8)</td>
<td>16.6 (6.9)</td>
<td>0.947</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>17.95 + (abdominal·0.28) + (medial calf·0.54)</td>
<td>32.3 (6.2)</td>
<td>31.7 (5.8)</td>
<td>0.909</td>
<td>3.0</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

DXA minus ultrasound prediction (median values)

Figure 6.3.3 illustrates the relationship between DXA-derived and ultrasound predicted % body fat values with 95% confidence intervals for the two site equation and Bland Altman assessment of agreement between the two methods. Mean bias between methods was 0.1%, with narrow 95% limits of agreement (-3.6 to +3.8). No bias in measurement was evident as % body fat increased.

In women, SAT thickness at the abdominal also had the highest individual correlation with % body fat (r=0.905) and thus was included in all regression analyses. Measurements taken at the iliac crest had a stronger correlation to those taken at the abdominal than to DXA derived % body fat and were excluded from all
regression analyses. As with men, the correlation between SAT thickness at the front thigh and SAT thickness at both the triceps and medial calf was found to be greater than the correlation between either of these SAT measures and DXA derived % body fat. Therefore, any combination that selected front thigh and medial calf or front thigh and triceps SAT thickness as independent variables was excluded in the regression analyses. From the remaining possibilities, quantile regression found that ultrasound measurement of SAT thickness at two sites (abdominal and medial calf, Appendix E) combined gave the best prediction of % body fat ($r=0.909$, $p=0.591$, SEE=3.0%; Table 6.3.5).

Figures 6.3.4a and b respectively illustrate the relationship between DXA-derived and ultrasound predicted % body fat values with 95% confidence intervals and Bland Altman analysis with mean difference and 95% limits of agreement in women. Mean difference between methods was -0.4%. 95% limits of agreement (-6.2 to 5.4) are wider than those in men with no clear bias evident.

Figure 6.3.4 a; Scatter plot of DXA measured versus ultrasound predicted % body fat with 95% confidence intervals (- - -) in women. b; Bland Altman analysis of DXA measured versus ultrasound predicted % body fat with mean difference (- - -) and 95% limits of agreement (...) in women
6.3.5 Discussion

In this study ultrasound was used to measure the thickness of subcutaneous adipose tissue (SAT) to investigate whether SAT is related to segmental fat mass and total percentage body fat (% body fat) in healthy young adult men and women. To the authors’ knowledge the only comparable study found that ultrasound measured SAT predictions underestimated % body fat with a bias of 6.6% in men and 3.4% in women, with agreement between DXA measurement and the ultrasound prediction increasing as % body fat increased (Duz et al., 2009). In this study, the correlation between the individual thickness of SAT and segmental fat mass suggests that SAT proportionally reflects regional fat mass. The results also affirm a relationship between segmental SAT and % body fat. The thickness of abdominal SAT alone was found to share 82% of the variance in DXA derived % body fat in men and women. In men, SAT thickness at the abdominal and front thigh provided an accurate estimate of % body fat (r=0.947, SEE=1.9%) with a median difference of 0.9% between DXA derived and ultrasound predicted values. In women, an accurate prediction of % body fat was provided by the SAT thickness of abdominal and medial calf (r=0.909, SEE=3.0%) with a median difference between DXA measured and ultrasound predicted values of -0.6%. Body fat prediction equations with an SEE value of 3% or less are considered ‘very good’, while those with an SEE of >4.5% are considered ‘fair’ or ‘poor’ (Heyward and Wagner 2004). In both sexes the trunk and lower limb region accounted for approximately 90% of total body fat mass as measured by DXA. The inclusion of SAT measured at the trunk and lower limb reflects the deposition of the majority of body fat mass in these areas.
The results of the current study indicate that % body fat can be accurately predicted from ultrasound measurement of SAT thickness in healthy young adults aged 18-29 years. However the equations should be validated on older age groups before they can be generalised to a wider adult population.

6.3.6 Conclusions

Strong relationships between individual SAT measures and segmental fat mass indicate that SAT proportionally reflects segmental fat mass distribution. A single ultrasound measure of subcutaneous adipose tissue thickness at the abdominal was found to be highly correlated with % body fat in healthy young adult men and women. The addition of a lower limb SAT thickness provides a very good prediction of total % body fat in both sexes. These data support the use of ultrasound as an accurate and reliable tool to measure total and regional body composition that is applicable to both clinical and population study settings (NIH 2011).
6.4 References


Chapter 7

Thesis summary, conclusions and recommendations

for future work
7.1 Thesis summary

This thesis provides a detailed analysis of fat tissue mass and its distribution within the body in 1136 Irish adults aged 18 to 81 years, and evaluates the accuracy of three prediction techniques to measure body composition in this cohort.

Findings presented in Chapter 3 reinforce the view that body mass index is a crude measure of fat mass. Though BMI provides important information on body size trends within and between populations, it is insensitive to an excess accumulation of fat mass and thereby obesity as defined by the WHO (2000). In order to evaluate, treat and monitor obesity, fat tissue mass and its distribution should be measured directly.

This thesis has established that fat tissue mass and its distribution in Irish adults are age and sex specific. Defining ‘normal’, ‘overfat’ and ‘fat obese’ according to fat tissue mass index is a novel approach in which the young adult population act to generate reference criteria, similar to that established for bone health. In a previous report Kelly et al. (2009) determined FTMI cut-offs of >9kg/m$^2$ in men and >13kg/m$^2$ in women to classify ‘fat obesity’. However, these cut-offs were chosen to match the prevalence of obesity as measured by BMI in the same population. This is a questionable approach as it is assumes that BMI classifies the correct proportion of the population into each category. In this thesis, the population mean FTMI z-score and standard deviation was used to define the range for ‘normal’ ‘overfat’ and ‘fat obesity’ in men and women. This resulted in a higher prevalence of obesity than by BMI classification in each age category. Determination of fat obesity by FTMI is a promising approach as only three measures are required- % body fat, height and weight.
Abdominal adiposity is of interest as it is associated with increased risk of type II diabetes and cardiovascular disease (Wajchenberg 2000). Measurement of abdominal fat mass in the lumbar (L1-L4) region is correlated to visceral fat mass (Glickman et al., 2004). In this thesis FTMI was strongly correlated to L1-L4 FTM in men (r=0.97) and women (r=0.95). In the absence of techniques which directly measure abdominal fat mass, FTMI is a useful surrogate measure.

Bioelectrical impedance analysis and anthropometry are widely available measures of % body fat that are suitable for use in large scale studies. However bioelectrical impedance analysis does not accurately measure % body fat in subjects with high fat mass (Deurenberg et al., 2001; Sun et al., 2005). This is supported by the current study, where BIA underestimated % body fat in 18 to 29 year olds by 4.2% in men with >25% body fat and by 4.8% in women with >32% body fat. Applied to older adults, % body fat would be underestimated in 83% of men and 88% of women aged 55+ years. Regarding segmental analysis, BIA was found to accurately measure appendicular (arm + leg) composition but not abdominal composition in young adults.

The anthropometric prediction equations presented in Chapter 5 accurately estimate % body fat across a wide range of age and % body fat. Standard error of the estimate for the prediction equations was 2.5% in men and 3.0% in women. These values are considered ‘excellent’ and ‘very good’ respectively (Heyward and Wagner 2004). The use of robust quantile regression was employed due to the non-normal nature of % body fat distribution within this population. This resulted in no prediction bias in subjects with higher % body fat, which is a limitation of previous anthropometric prediction equations. Skinfold thickness measured at the midaxilla site had the highest correlation to % body fat in both men and women. The selection
of an addition trunk site in the prediction equations in men (supraspinale skinfold) and women (abdominal girth) is reflective of the large proportion of FTM deposited abdominally, particularly in older subjects.

Ultrasonography is a more appropriate method of total and segmental body fat prediction than anthropometry as it provides a direct measure of subcutaneous adipose tissue (SAT) thickness. As demonstrated in Chapter 6, skinfold thickness measures, though strongly correlated to ultrasound measured SAT thickness, are not representative of a double layer of SAT thickness. The ultrasonography equations presented in this thesis have ‘ideal’ (1.9%) and ‘very good’ (3.0%) SEE values in men and women respectively. Measurement of SAT at just two sites is required; abdominal and front thigh in men and abdominal and medial calf in women. Individual SAT measures were also strongly correlated to segmental FTM (r=0.717-0.923), indicating that ultrasonography is an accurate and reliable tool to measure total and regional body composition.
7.2 Thesis conclusions

- Fat tissue mass and its distribution differ according to age and sex in Irish adults.
- BMI is not indicative of the differences in total body fat tissue mass and its distribution evident across the age span of adults studied in this thesis.
- Fat tissue mass index and waist-to-height ratio are appropriate measures of total and segmental fat tissue mass in adult men and women.
- Bioelectrical impedance analysis is not an accurate technique for % body fat measurement in young adult men with >25% fat or in young adult women.
- % body fat can be accurately predicted from the anthropometric equations presented in Chapter 5, which are applicable across a wide range of age (18 - 81 years) and % body fat (5.1 - 56.8%) in men and women.
- Ultrasonography provides a direct measure of subcutaneous adipose tissue thickness and the prediction equations presented in Chapter 6.3 accurately estimate % body fat in young adult men and women, with just one abdominal measurement and one lower limb measurement required.
7.3 Recommendations for future work

The subjects recruited to this study were a convenience sample of staff and students of the University of Limerick and members of the surrounding community. This sample is unlikely to be representative of the wider Irish adult population. Population level data provided by the SLÁN survey (Morgan et al., 2007) and the National Adult Nutrition Survey (IUNA 2011) indicate a prevalence of obesity of ~23% in Irish adults as measured by BMI. This figure has been reported as 11% in 18-29 year olds (Morgan et al., 2007) and 12.9% and 13.4% in 18-35 year old men and women respectively (IUNA 2001). While this thesis has shown that BMI is not an adequate measure of ‘fatness’, it is likely that there is a preponderance of ‘normal’ weight individuals in the current study. Therefore the thresholds established here for ‘normal’ ‘overfat’ and ‘fat obese’ according to FTMI may be lower than if established from a young adult reference sample that is more representative of the wider population. Measurement of % body fat and subsequent definition of ‘normal’, ‘overfat’ and ‘fat obese’ FTMI should be carried out on a representative sample of young Irish adults such as those included in the IUNA (2001; 2011) surveys.

Fat tissue mass index, abdominal fat tissue mass measured at the L1-L4 region, and waist-to-height ratio are appropriate measures of total and segmental body composition. However these metrics must be related to health risk indicators before they can be used to categorise individuals as ‘at risk’ of obesity related diseases.

The accuracy of bioelectrical impedance analysis in the measurement of total and segmental body fat was studied on 18-29 year olds. This method was not accurate across the wide range of % body fat studied, particularly in women, where % body fat was underestimated by 3% in the total group and 4.8% in those with
>32% body fat. In men, % body fat was underestimated by 4.2% in subjects with
>25% body fat. There is need to validate population-specific impedance algorithms
that are accurate across a wide range of % body fat. The BIA method should be used
with caution in older adults due to their increased fat tissue mass.

Ultrasonography is a promising field method of body composition
measurement and has the capacity to accurately measure both subcutaneous and
visceral adipose tissue thickness. However, the prediction equations presented in this
thesis are constructed from a relatively small subject sample and should be further
cross-validated on both young and older adults as a measure of both regional and
total body fat.

Visceral fat can only be measured directly by magnetic resonance imaging
and computed tomography, therefore is difficult to study at a population level.
Ultrasonography is capable of measuring site-specific visceral fat thickness. This
method is currently being explored with a view to quantifying abdominal adiposity
as a ratio of subcutaneous to visceral (S:V) fat thickness.

Data presented in this study are cross-sectional. Comparing age groups, (e.g.
18-29 and 55+ year olds) does not give an indication of how a generation changes
over their lifetime. Longitudinal body composition data of Irish adults would be
invaluable in developing interventions to target health states such as obesity and
sarcopenia at a population level. Anthropometry and ultrasonography are accurate
and reliable methods of body composition measurement across the lifespan and are
suitable for use in large scale studies.
7.4 References


Bibliography


Boneva-Asiova, Z. and Boyanov, M. A. (2008) 'Body composition analysis by leg-to-leg bioelectrical impedance and dual-energy X--ray absorptiometry in non-
obese and obese individuals', *Diabetes, Obesity & Metabolism*, 10(11), 1012-1018.


Colombo, O., Villani, S., Pinelli, G., Trentani, C., Baldi, M., Tomarchio, O. and Tagliabue, A. (2008) 'To treat or not to treat: comparison of different criteria used to determine whether weight loss is to be recommended', *Nutrition Journal*, 7(5).


Frayn, K. N. (2000) 'Visceral fat and insulin resistance - causative or correlative?', *British Journal of Nutrition*, 83(S1), S71-S77.


hydration', *American Journal of Physiology Endocrinology Metabolism*, 274(37), E808-E816.


Thomson, R., Brinkworth, G. D., Buckley, J. D., Noakes, M. and Clifton, P. M. (2007) 'Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during
weight loss in overweight young women', *Clinical Nutrition*, 26, 771-777.


Appendices
Thank you for considering participating in this study. The research that you are being asked to contribute to relates to a survey of the body composition taken from a representative sample of the Irish population. Should you partake in the study you will be provided with a professional evaluation of your current body composition using the most up-to-date procedures of measurement available.

The following pages describe the research study and detail the procedures involved. Four different techniques, a DEXA scan, skinfold thickness, bioelectrical impedance and ultrasound scanning will be employed to describe your current body composition.

Please read the attached subject information sheet thoroughly. If you decide to participate in the research study you will be given a date and time of for an appointment that suits your schedule. To aid in the administration, please complete the table below and bring it with you when you attend for your appointment. Your contact details are required for the purpose of contacting you in relation to your results and to advise you of further research studies in which you may wish to participate.

*This information is kept secure and confidential.*

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Appendix A. Subject Information and Consent Form

(ULREC Approval No: 08/07)

UNIVERSITY of LIMERICK
OLLSCOIL LUIMNIGH

DEPARTMENT OF PHYSICAL EDUCATION AND SPORT SCIENCES
UL BODY COMPOSITION STUDY 08
(Volunteer Information)

INFORMED CONSENT – You have been asked to participate in a research study. You are under no obligation to participate in this study. In order to decide whether you do or do not want to participate in this study, you need to fully understand the risks and benefits to allow you make an informed decision. This process is known as INFORMED CONSENT.

THE PROCEDURE – This research focuses on factors regulating body composition. As part of this research your lean tissue and fat mass will be measured using 4 different techniques, bioelectrical impedance, skinfold thickness, ultrasound scanning and DEXA scan. Guidelines indicating what will be required of you prior to the appointment are provided on the attached sheet (page 5 of this pack). What happens to you during the appointment is explained below.

The whole process takes approximately 50 minutes to complete.

You should attend wearing a loose fitting ‘T’-shirt or Polo top and shorts.

On the day the researcher will start by measuring your stature (height) and weight then proceed as follows:

Bioelectrical Impedance: This is very simple. You will be asked to stand barefoot on what looks like a weighing scale and grip a portable handle in each hand for a period of 30-40 seconds. During this period a low level current will be applied (it is so low you will not feel any sensation resulting from this) and the resistance of your body (impedance) is measured.

Skinfold thickness: The researcher will locate key body landmarks and measure the thickness of a fold of skin pinched between two fingers using a calliper. Up to 10 skinfold sites will be measured at sites covering the arms, legs and torso whilst standing, or sitting in a relaxed position. We appreciate your co-operation in gaining
access to the skinfold site. The limb girths corresponding to these sites will be measured with a tape.

**Ultrasound scanning:** An ultrasound scanner (similar to that used to scan during pregnancy) works by sending sound waves into the body and creating a picture on-screen from the echoes that come back. It is non-invasive and does not involve radiation. There are no known biological risks with ultrasound scanning. Ultrasound scans will be taken from the same sites used for skin-fold thickness (above). The operator will place some water based gel onto your skin and then place on a probe. The probe will be positioned for an optimal picture of your tissues and the image will be stored in the machine.

**DEXA scan:** A DEXA scanner works in a similar manner to an X-ray but the radiation dose is very much less (about 1/30th) than a standard X-ray. The risk to you is described by the international authorities regulating the use of X-rays as ‘trivial’. To take a scan you will lay on the bed of the scanner, the operator will position your body moving your legs and torso. Once positioned correctly the actual scan takes 4 minutes to complete and you will be asked to remain still during this time.

**BENEFITS AND RISKS** – You would not be allowed to undertake this procedure is you are known to be, or at risk of being pregnant. Otherwise the risk to you is trivial.

You will receive a copy of your results that could be of benefit to you and inform you of your current fat and lean tissue mass and overall body composition. You will also be provided the opportunity to discuss your results with the researcher at a scheduled meeting following completion of the tests.

**CONFIDENTIALITY** of participants will be maintained at all times. No information will be provided to any other party without your written consent. All we ask is that the information may be used **anonymously** in the preparation of scientific reports for dissemination at scientific congress or in refereed publication. All information held by the University of Limerick is subject to the terms of the 1997 Freedom of Information Act, details of which are available @ www.ul.ie/foi

**Who can I contact about this study?**

Should you require any further clarification on any of the above, please do not hesitate to contact me on 061-202800 (or email Phil.Jakeman@ul.ie).

Should you wish to contact the Chairperson of the Research Ethics Committee please address your enquiry to:

EHS Research Ethics Contact Point of the Education and Health Sciences Research Ethics Committee, Room E1003, University of Limerick, Limerick.
Tel: (061) 234101 Email: ehsresearchethics@ul.ie
UL BODY COMPOSITION STUDY 08
(PRE-APPOINTMENT INFORMATION)

Please, refrain from any form of organised training or exercise session of greater than 20 minutes for a period of 12h before your appointment.

If your appointment is early morning between 8.00am and 10am, do not consume breakfast then follow the arrow at all other times

BEFORE YOUR APPOINTMENT
WHAT to do and WHEN to do it

3hours BEFORE
NO FURTHER FOOD INTAKE
↓
1hour BEFORE
DRINK 500ml WATER
↓
10minutes BEFORE
EMPTY BLADDER

Notes:
1. Please be on time for your appointment.
2. Remember to bring along the completed pre-test questionnaire and consent form.

Your cooperation is greatly appreciated

Should you be unable to attend at the appointed date and time please contact Siobhan Leahy as soon as possible.

Email; Siobhan.Leahy@ul.ie
Appendix A. Subject Information and Consent Form

UNIVERSITY of LIMERICK
OLLSCOIL LUIMNIGH
DEPARTMENT OF PHYSICAL EDUCATION AND SPORT SCIENCES

UL BODY COMPOSITION STUDY 08
(Informed Consent)

I confirm that all aspects of my participation have been fully explained to my satisfaction. I understand that there are no direct benefits to me for my participation, but realize that this research may allow a better understanding of body composition.

I confirm that I am not pregnant or at risk of being pregnant at the time of measurement and that my appointment for these measurements occurs within 7 days of my last menstrual period (LMP). If you are unsure of this risk then it is recommended that you consult with your doctor before committing to this study.

AGREEMENT TO CONSENT – If you agree to participate in this research study please sign below. You will be issued with a photocopy of this form, for your own records.

I, ………………………..(PRINT NAME) consent to participate on an anonymous basis, in the research study outlined above.

__________________________  __________________________
Signature of Participant  Date

__________________________  __________________________
Signature of Principal Researcher  Date
## Appendix B. Summary of data used to create LMS graphs

### Total men (n=518)

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<th>FTMI (kg/m²)</th>
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<th>FFMI (kg/m²)</th>
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M = Median, σ = Standard Deviation, L = Skewness

### Total women (n=618)

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M = Median, σ = Standard Deviation, L = Skewness
Appendix C. Anthropometric sites

Skinfold sites

Forearm
A fold taken parallel to the long axis of the arm at the midpoint of extensor muscle belly at the widest girth of the forearm, distal to the humeral epicondyles with wrist pronated.

Biceps
A fold taken parallel to the long axis of the arm at the point on the anterior surface of the arm in the mid-line of the mid-acromiale-radiale landmark.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Triceps
A fold taken parallel to the long axis of the arm at the point on the posterior surface of the arm in the mid-line of the mid-acromiale-radiale landmark.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Subscapular
A downward oblique fold taken from the undermost tip of the inferior angle of the scapula.

(Adapted from ISAK, Marfell-Jones et al., 2006)
Appendix C. Anthropometric sites

Chest
A fold taken diagonally half way between the anterior axillary border and the midpoint of the nipple.

(Adapted from Heyward and Wagner 2004)

Midaxilla
A fold taken parallel to the long axis of the thorax in the mid-axillary line at the level of the xiphoid process of the sternum

(Adapted from Heyward and Wagner 2004)

Iliac crest
A fold taken near horizontally at the centre of the skinfold raised immediately above the iliocristale.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Supraspinale
A fold taken obliquely and medially at the point of intersection of 1) a line marked from the iliospinale to the anterior axillary border and 2) a horizontal line at the level of the iliocristale.

(Adapted from ISAK, Marfell-Jones et al., 2006)
Appendix C. Anthropometric sites

Abdominal

A fold taken vertically 5cm horizontally to the right of the omphalion.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Front thigh

A fold taken parallel to the long axis of the thigh at the midpoint between the inguinal point and the patellare.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Medial calf

A fold taken vertically at the most medial aspect of the calf at the level of maximum girth, with the foot placed on a box and knee at 90°.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Girth sites

Forearm

Girth taken at the maximum girth of the forearm perpendicular to its long axis, distal to the humeral epicondyles.

(Adapted from ISAK, Marfell-Jones et al., 2006)
Appendix C. Anthropometric sites

Upper arm (relaxed)
Girth taken at the mid-acromiale-radiale site, perpendicular to the long axis of the arm.
(Adapted from ISAK, Marfell-Jones et al., 2006)

Chest
Girth of the thorax at the mesosternale site, perpendicular to the long axis of the trunk.
(Adapted from ISAK, Marfell-Jones et al., 2006)

Abdominal
Girth of the abdomen taken at the level of the omphalion, perpendicular to the long axis of the trunk at the end of normal expiration
(Adapted from Heyward and Wagner 2004)

Waist
Girth of the abdomen taken at its narrowest point between the 10th rib and the top of the iliac crest, perpendicular to the long axis of the trunk at the end of normal expiration.
(Adapted from ISAK, Marfell-Jones et al., 2006)

Hip
Girth of the buttocks at the level of the greatest posterior protuberance, perpendicular to the long axis of the trunk.
(Adapted from ISAK, Marfell-Jones et al., 2006)
Appendix C. Anthropometric sites

Mid-thigh

Girth of the thigh measured at the level of the mid-trochanterion-tibiale laterale site, perpendicular to its long axis.

(Adapted from ISAK, Marfell-Jones et al., 2006)

Calf

Maximum girth of the calf with the subject standing in an elevated position

(Adapted from ISAK, Marfell-Jones et al., 2006)
Appendix D. Anthropometric/% body fat correlations

Men (n=318)

<table>
<thead>
<tr>
<th>Girths</th>
<th>Forearm</th>
<th>Upper-arm</th>
<th>Chest</th>
<th>Abdomen</th>
<th>Waist</th>
<th>Hip</th>
<th>Mid thigh</th>
<th>Calf</th>
<th>Forcupe</th>
<th>Bicep</th>
<th>Triceps</th>
<th>Subscap</th>
<th>Chest</th>
<th>Midaxilla</th>
<th>Ilica Crest</th>
<th>Supraspin</th>
<th>Abdomen</th>
<th>Front thigh</th>
<th>Med calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BF</td>
<td>0.142</td>
<td>0.317</td>
<td>0.522</td>
<td>0.883</td>
<td>0.940</td>
<td>0.592</td>
<td>0.072</td>
<td>0.275</td>
<td>0.622</td>
<td>0.358</td>
<td>0.490</td>
<td>0.412</td>
<td>0.638</td>
<td>0.726</td>
<td>0.385</td>
<td>0.324</td>
<td>0.385</td>
<td>0.413</td>
<td>0.533</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skinfolds</th>
<th>Forearm</th>
<th>Bicep</th>
<th>Triceps</th>
<th>Subscap</th>
<th>Chest</th>
<th>Midaxilla</th>
<th>Ilica Crest</th>
<th>Supraspin</th>
<th>Abdomen</th>
<th>Front thigh</th>
<th>Med calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BF</td>
<td>0.585</td>
<td>0.776</td>
<td>0.738</td>
<td>0.869</td>
<td>0.881</td>
<td>0.918</td>
<td>0.816</td>
<td>0.879</td>
<td>0.898</td>
<td>0.683</td>
<td>0.656</td>
</tr>
</tbody>
</table>
### Appendix D. Anthropometric/% body fat correlations

#### Women (n=418)

| Girths      | Forearm | Upper arm | Abdomen | Waist | Hip | Mid thigh | Calf | Forearm | Biceps | Triceps | Subscap | Midaxilla | Iliac Crest | Supraspin | Abdomen | Front thigh | Med calf |
|-------------|---------|-----------|---------|-------|-----|-----------|------|----------|--------|---------|---------|-----------|-------------|------------|----------|-----------|-----------|---------|
| %BF         | 0.319   |           |         |       |     |           |      |          |        |         |         |           |             |           |          |           |          |
| Forearm     | 0.576   | 0.831     |         |       |     |           |      |          |        |         |         |           |             |           |          |           |          |
| Upper arm   | 0.835   | 0.489     | 0.669   |       |     |           |      |          |        |         |         |           |             |           |          |           |          |
| Abdomen     | 0.802   | 0.520     | 0.669   | 0.932 |     |           |      |          |        |         |         |           |             |           |          |           |          |
| Waist       | 0.660   | 0.619     | 0.733   | 0.756 | 0.693 |           |      |          |        |         |         |           |             |           |          |           |          |
| Hip         | 0.150   | 0.697     | 0.671   | 0.259 | 0.243 | 0.557     |      |          |        |         |         |           |             |           |          |           |          |
| Mid thigh   | 0.267   | 0.720     | 0.660   | 0.393 | 0.372 | 0.651     | 0.722|          |        |         |         |           |             |           |          |           |          |
| Calf        | 0.650   | 0.498     | 0.600   | 0.559 | 0.529 | 0.565     | 0.369 | 0.405    |        |         |         |           |             |           |          |           |          |
| Forearm     | 0.833   | 0.369     | 0.597   | 0.736 | 0.725 | 0.608     | 0.193 | 0.310    | 0.734  |         |         |           |             |           |          |           |          |
| Biceps      | 0.804   | 0.360     | 0.584   | 0.685 | 0.633 | 0.618     | 0.194 | 0.292    | 0.711  | 0.838  |        |           |             |           |          |           |          |
| Triceps     | 0.821   | 0.394     | 0.612   | 0.793 | 0.802 | 0.576     | 0.195 | 0.302    | 0.641  | 0.782  | 0.728  |           |             |           |          |           |          |
| Subscap     | 0.854   | 0.392     | 0.595   | 0.825 | 0.841 | 0.657     | 0.177 | 0.317    | 0.619  | 0.796  | 0.732  | 0.852    |             |           |          |           |          |
| Midaxilla   | 0.605   | 0.482     | 0.595   | 0.388 | 0.580 | 0.568     | 0.423 | 0.432    | 0.607  | 0.668  | 0.303  | 0.698    | 0.723      |           |          |           |          |
| Iliac Crest | 0.799   | 0.430     | 0.613   | 0.751 | 0.733 | 0.623     | 0.300 | 0.377    | 0.668  | 0.766  | 0.642  | 0.809    | 0.856      | 0.844     |          |           |          |
| Supraspin   | 0.755   | 0.411     | 0.582   | 0.737 | 0.728 | 0.619     | 0.278 | 0.368    | 0.626  | 0.707  | 0.616  | 0.777    | 0.818      | 0.782     | 0.875    |          |
| Abdomen     | 0.666   | 0.432     | 0.615   | 0.540 | 0.495 | 0.638     | 0.468 | 0.455    | 0.624  | 0.663  | 0.718  | 0.552    | 0.564      | 0.512     | 0.564    | 0.535     |
| Front thigh | 0.687   | 0.449     | 0.605   | 0.557 | 0.515 | 0.623     | 0.437 | 0.498    | 0.663  | 0.672  | 0.679  | 0.554    | 0.595      | 0.524     | 0.606    | 0.552     | 0.753    |
Appendix E. TIBCO S+ Quantile regression output

Anthropometric prediction, men

Call: rq(formula = BF ~ Age + MA + Tri + Supra, tau = 0.5, data = menvalid318)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-11.27583</td>
<td>0.31635</td>
<td>-35.64356</td>
</tr>
<tr>
<td>Age</td>
<td>0.10879</td>
<td>0.00912</td>
<td>11.93186</td>
</tr>
<tr>
<td>Midaxilla skinfold</td>
<td>8.83223</td>
<td>1.17348</td>
<td>7.52655</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>7.60596</td>
<td>0.82215</td>
<td>9.25127</td>
</tr>
<tr>
<td>Supraspinale skinfold</td>
<td>11.93060</td>
<td>1.07805</td>
<td>11.06687</td>
</tr>
</tbody>
</table>

Anthropometric prediction, women

Call: rq(formula = BF ~ Age + MA + Abdg + BicSF + M CSF, tau = 0.5, data=femvalid418)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-73.52940</td>
<td>7.71505</td>
<td>-9.53065</td>
</tr>
<tr>
<td>Age</td>
<td>0.07864</td>
<td>0.01056</td>
<td>7.44503</td>
</tr>
<tr>
<td>Midaxilla skinfold</td>
<td>4.88919</td>
<td>1.04936</td>
<td>4.65919</td>
</tr>
<tr>
<td>Abdominal girth</td>
<td>39.37585</td>
<td>4.57711</td>
<td>8.60278</td>
</tr>
<tr>
<td>Biceps skinfold</td>
<td>11.01787</td>
<td>1.18325</td>
<td>9.31153</td>
</tr>
<tr>
<td>Medial calf skinfold</td>
<td>9.06799</td>
<td>1.17458</td>
<td>7.72022</td>
</tr>
</tbody>
</table>

Ultrasound SAT thickness prediction, men

Call: rq(formula = BF ~ AbdUS + FTUS, tau = 0.5, data=USAnalysis1.M)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.648957</td>
<td>0.4673429</td>
<td>16.37</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.3588059</td>
<td>0.0237</td>
<td>15.14</td>
</tr>
<tr>
<td>Front thigh SAT</td>
<td>0.5855653</td>
<td>0.0827371</td>
<td>7.08</td>
</tr>
</tbody>
</table>

Ultrasound SAT thickness prediction, men

Call: rq(formula = BF ~ AbdUS + MCUS, tau = 0.5, data=USAnalysis1.F)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>17.94848</td>
<td>1.503811</td>
<td>11.94</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.2822266</td>
<td>0.0494143</td>
<td>5.71</td>
</tr>
<tr>
<td>Medial Calf SAT</td>
<td>0.5414576</td>
<td>0.1821012</td>
<td>2.97</td>
</tr>
</tbody>
</table>
Appendix F Journal Publications

Clodagh Toomey was the principal author of the technical paper appended below, which appears as Chapter 6.1 of this thesis. I, Siobhan Leahy, contributed to study design, aided in data collection and proof read the final manuscript for submission.

Technical considerations for accurate measurement of subcutaneous adipose tissue thickness using B-mode ultrasound

C Toomey¹, K McCreesh¹, S Leahy² and P Jakeman²

¹Department of Physiotherapy, Faculty of Education and Health Sciences, University of Limerick, Limerick, Ireland; ²Department of Physical Education and Sport Sciences, Faculty of Education and Health Sciences, University of Limerick, Limerick, Ireland

Corresponding author: C Toomey. Email: clodagh.toomey@ul.ie

Abstract

The search for valid, reliable and inexpensive methods of measuring body composition is an ongoing issue for many researchers. In particular, the measurement of subcutaneous adipose tissue (SAT) is carried out by numerous methods, each with its own drawbacks. Skinfold thickness measurement is the most common in-field method, but it is limited by its tendency to deform the subcutaneous layer, by the limited caliper opening which prevents measurement of larger skinfolds, and the lack of correlation for elastic properties of tissue between individuals. Therefore non-invasive field measures which overcome these limitations would be desirable. Ultrasound scanning provides such a device due to its portability and availability, allowing reduced tissue compression and on-screen views of the adipose tissue. Despite a number of papers referring to the use of ultrasound for measuring adipose tissue, the method of measurement has not been fully described. This paper describes our work in determining an accurate method for the measurement of SAT at different body sites, including a comparison of scanning directions and sites. We also describe our investigations into the degree to which compression force through the transducer affects subcutaneous tissue measurement, and the reliability and sensitivity of our methods. We conclude with a recommended reliable scanning protocol for the measurement of SAT.

Keywords: Ultrasound, subcutaneous adipose tissue (SAT), accuracy, methods

Ultrasound 2011; 19: 91–96. DOI: 10.1256/ultr.2011.01.0057

There is widespread and increasing interest in the study of human body composition, in particular body fat mass due to its association with disease risk. Accurate measurement of total and regional body fat requires expensive equipment of limited availability, i.e. dual energy X-ray absorptiometry; therefore surrogate methods of measurement such as the use of the skinfold thickness have, customarily, been used by healthcare professionals. Using a standardized protocol¹ skinfold thickness is deemed to provide a measure of subcutaneous adipose tissue (SAT) which, when incorporated into a prediction algorithm, provides an indirect estimate of percentage body fat.² However, the accuracy of body fat estimation using skinfold thickness measurements has been questioned³ because of the error introduced from inter-individual variation in tissue compressibility and degrees of tissue deformation by the caliper jaws.⁴ There is also a practical limitation in the skinfold width that can be measured using a caliper, i.e. in a person of high body fat mass.

Ultrasound imaging is proposed as a viable alternative method of measuring subcutaneous adiposity, and overcomes the specific limitation of skinfold calipers, i.e. tissue distortion and skinfold width. Ultrasound is capable of measuring full subcutaneous fat thickness with minimal compression in persons of high fat mass. However there has been no published assessment of the degree of tissue compression incurred by the operator in placement of the transducer, and/or the reliability of estimate when measuring SAT thickness by ultrasound. Previous studies have failed to document subject positioning, transducer orientation, scanning protocol and the method of SAT measurement from ultrasound images.⁴⁺ In our experience, accurate measurement of SAT by ultrasound is influenced by the orientation and compression force applied through the transducer. We present a technical analysis of the use of ultrasound for the measurement of SAT thickness.

The aim of this technical study was to develop a method for the accurate and reliable ultrasound measurement of SAT thickness. For the purpose of this study, seven anatomical sites commonly used in skinfold measurement to predict percentage body fat were selected for analysis. Objectives were as follows:

- to determine the effect of operator force through the transducer on SAT thickness;

Ultrasound 2011; 19: 91–96
Appendix F Journal Publications

I, Siobhan Leahy, am the principal author of the below article which appears as Chapter 4 of this thesis. I contributed to study design, data collection and analysis and drafted the final article for submission.

A comparison of dual energy X-ray absorptiometry and bioelectrical impedance analysis to measure total and segmental body composition in healthy young adults

Siobhan Leahy · Clan O'Neill · Rhoda Solan · Philip Jakeman

Received: 22 March 2011 / Accepted: 13 May 2011 © Springer-Verlag 2011

Abstract The aim of this study was to investigate the accuracy of BIA in the measurement of total body composition and regional fat and the fat free mass in the healthy young adults. Four hundred and three healthy young adults (167 women and 236 men) aged 18–29 years were recruited from the Mid-West region of Ireland. Multi frequency, eight-polar bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DXA) were used to measure the total body and segmental (arm, leg and trunk) fat mass and the fat free mass. BIA was found to underestimate the percentage total body fat in men and women (p < 0.001). This underestimate increased in men with >24.6% body fat and women with >32% body fat (p < 0.001). Fat tissue mass in the trunk segment was overestimated by 2.1 kg (p < 0.001) in men and underestimated by 0.4 kg (p < 0.001) in women. BIA was also found to underestimate the fat free mass in the appendages by 1.0 kg (p < 0.001) in men and 0.9 kg (p < 0.001) in women. Compared to dual energy X-ray absorptiometry, bioelectrical impedance analysis underestimated the total body fat mass and overestimates fat free mass in healthy young adults. BIA should, therefore, be used with caution in the measurement of total body composition in women and men with >25% total body fat. Though statistically significant, the small difference (±4%) between the methods indicates that the BIA may be used interchangeably with DXA in the measurement of appendicular fat free mass in healthy young adults.

Keywords Body fat · Fat free mass · Segmental analysis · Photon · Human

Introduction

Accurate measurement of body composition is of benefit in the assessment of nutritional status within and between populations, and is a valuable diagnostic/evaluative tool in obesity, metabolic syndrome, type II diabetes and sarcopenic obesity. Reference values for body composition are established from the healthy young adults representative of the general population. In this study of 403 young adult men and women from the Mid-West region of Ireland a comparison was made of total and segmental body composition measured by dual energy X-ray absorptiometry and bioelectrical impedance analysis.

Dual energy X-ray absorptiometry (DXA) is an accepted method of measurement of body composition (Ellis 2000; Rubiano et al. 2009). DXA is a three-compartment model of fat mass (FFM) and two components of fat free mass (FFM), i.e., bone mineral content (BMC) and lean tissue mass (LTM). DXA, however, is not widely available outside of the clinical or research setting.

Bioelectrical impedance analysis (BIA) is a valid method of body composition analysis that is widely available, inexpensive and without a requirement for high-level operator training. BIA measures the resistance to an electric current, with the least resistance offered by fat free mass due to its high-water content (Baumgartner 1996). Proprietor algorithms then convert raw impedance scores to % body fat. The algorithm (Wattanapenpaiboon et al. 1998), the time of measurement (Oshina and Shiga 2000), eating or exercise prior to measurement (Deurenberg et al. 1988, Gallagher et al. 1996) have been shown to affect the accuracy
Appendix F Journal Publications

I, Siobhan Leahy, am the principal author of the below article (conditionally accepted for publication) which appears as Chapter 6.3 of this thesis. I contributed to study design, data collection and analysis and drafted the final article for submission.

---

Sent: Wed 17/08/2011 22:56
To: Siobhan.Leahy
Cc: francois.tranquart@brg.bracco.com

Ref.: Ms. No. UMB-D-11-00217
Ultrasound measurement of subcutaneous adipose tissue thickness accurately predicts total and segmental body fat of young adults.
Ultrasound in Medicine and Biology

Dear Ms. Leahy,

Thank you for submitting your manuscript entitled, "Ultrasound measurement of subcutaneous adipose tissue thickness accurately predicts total and segmental body fat of young adults." for publication in Ultrasound in Medicine and Biology. The review process has now been completed and, guided by the referees' advice, I have decided that the paper be conditionally accepted for publication, pending revision. Copies of the referees' recommendations are appended below.

I very much hope that you and your colleagues will find the referees' comments to be both helpful and constructive. I also hope that you will be willing to revise the manuscript after giving careful consideration to the points that the referees' have made. If you are able to do this, it would be a great help to me if you would explain your response to the recommendations of the reviewers in the cover letter that accompanies your resubmission.

With kind regards,

Christy K. Holland, Ph.D.
Editor-in-Chief
Ultrasound in Medicine and Biology
U.C. Cardiovascular Center Room 3988
231 Albert Sabin Way
Cincinnati, Ohio 45267-0586
USA

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XXX
Improved prediction of body fat in healthy young Irish adults: preliminary findings from the University of Limerick (UL) Body Composition Study

S. Leahy, C. O’Neill, R. Sohun, C. MacDonncha and P. Jakeman
Faculty of Education and Health Sciences, University of Limerick, Plassey Technological Park, Limerick, Republic of Ireland

Accurate determination of body composition informs dietary management of disease. The UL Body Composition Study\(^1\) seeks to establish age- and gender-specific reference ranges for whole-body and segmental body composition based on a representative sample of the Irish population residing within the UL community. With ethical approval (ULREC 08/07) and informed written consent subjects, body composition was determined by dual-energy X-ray absorptiometry (iDXA\(^\text{TM}\); GE Healthcare, Chalfont St Giles, Bucks., UK; DXA). Skinfold thickness is commonly measured in clinical and field settings for the assessment of body fat (%BF). Using standardised procedures\(^2\), parallel measures of skinfold thickness at selected anatomical sites were analysed by stepwise regression to construct a skinfold-thickness prediction equation that most accurately represented %BF measured by DXA. For comparison, the current skinfold data were also fitted to the most-widely-used algorithm for estimation of %BF from skinfold thickness\(^3\). Bland-Altman analysis was used to test assess the limit of agreement between methods.

The present paper reports on the prediction of body fat from skinfold measurement using a two-compartment model of body composition for men and women aged between 18 and 35 years (Table).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Mass (kg)</th>
<th>BMI (kg/m(^2))</th>
<th>DXA</th>
<th>2009 Algorithm</th>
<th>1974 Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n 106) Mean</td>
<td>22.6</td>
<td>1.66</td>
<td>63.2</td>
<td>22.9</td>
<td>29.1</td>
<td>29.1</td>
<td>23.5*</td>
</tr>
<tr>
<td>Male (n 151) Mean</td>
<td>21.6</td>
<td>1.80</td>
<td>79.7</td>
<td>24.5</td>
<td>17.4</td>
<td>17.3</td>
<td>11.2*</td>
</tr>
</tbody>
</table>

Min–max, minimum–maximum. Mean values were significantly different from those for DXA and 2009 algorithm: *P < 0.001.

The sum of skinfold thicknesses (S4SF) measured at the triceps, supraspinatus, medial calf and biceps was found to be the best predictor of the percentage body fat measured by DXA for women (%BF = (0.2709*S4SF) + 14.1; R 0.916) and the S4SF measured at the abdominal, triceps, anterior thigh and iliac crest for men (%BF = (0.1965*S4SF) + 6.4; R 0.956). The limits of agreement (mean difference L2SD) approximated to L4.4%BF for women and L3.3%BF for men. Comparison of %BF determined from the equivalent age- and gender-specific algorithm\(^2\) revealed limits of agreement of + 1 to + 11.5%BF for men, and + 0.5 to + 10.8%BF for women, which would indicate that a man or woman is more likely to have their %BF underestimated from sum of four skinfolds by the Durnin and Wormersley equation\(^3\).

The greater accuracy in determining body composition from skinfold measurement presented in the present paper should aid in the dietary management of disease.

1. Faculty of Education and Health Sciences (2009) University of Limerick – Body Composition Study. www.ul.ie/bodycompositionstudy
A dual X-ray absorptiometry-based analysis of body fat distribution in healthy young adult women

P. Raftery, G. Boland, S. Leahy and P. Jakeman
Faculty of Education and Health Sciences, University of Limerick, Plassey Technological Park, Limerick, Republic of Ireland

Previous research indicates that women with a greater proportion of abdominal fat tend to be more insulin resistant, hyperinsulinemic, glucose intolerant and dyslipidemic than women with a greater proportion of gluteal/femoral fat. Accurate measurement of body fat and fat distribution is thus essential to assess the relation of regional fat mass to disease risk and change through dietary intervention; however, normative values for fat distribution do not exist. The UL Body Composition Study seeks to establish age- and gender-specific reference ranges for whole-body and segmental body composition based on a representative sample of the Irish population residing within the UL community. In this report, we analyse the relation between regional body fat and total body fat in healthy young adult women.

With ethical approval (ULREC 08/07) and informed written consent 115 young women aged 18–29 years, ranging from 15 to 47% body fat were recruited to the study. Total body fat mass and abdominal fat mass measured in the L1–L4 region was measured by dual-energy X-ray absorptiometry (iDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK; DXA) using the protocol outlined by Glickman et al.

This procedure of segmental, or region of interest, analysis has an inter-rater reliability of estimate of <5%.

The subjects’ average total fat mass 18.9 (sd, 5.6; min, 9.0; max, 39.4) kg equalled 29.5 (5.7; 14.7; 46.5) % of body mass. The average abdominal fat mass (L1–L4) of 1.71 (0.87; 0.45; 5.2) kg accounted for 8.6 (2.0; 4.3; 14.4) % of total fat mass. The % fat mass located in the abdominal was related to % body fat (r = 0.629) and body fat mass (kg; r = 0.68), accounting for 46 and 40% of the variance, respectively. Total fat mass accounted for >85% of the variance in abdominal fat mass (kg) (r = 0.932), providing a linear estimate of abdominal fat mass approximating to 150 g per kg total fat mass (Figure 1).

In this cross-sectional comparison of women whose abdominal fat ranged between 4.3 and 14% of total fat mass, we found that the DXA method provided a reliable measurement of abdominal adiposity. The results of this study indicate that abdominal fat is relatively independent of the individual’s %body fat and is best predicted by total fat mass.

Further study will confirm whether a longitudinal change in abdominal fat occurs in accordance with the linear prediction derived from these data (i.e. 150 g loss of abdominal fat per kg loss of total body fat loss) following dietary intervention.

Appendix G. Conference Proceedings

Proceedings of the Nutrition Society (2011), 70 (OCE3), E69
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Joint Irish Section and American Society for Nutrition Meeting, 15–17 June 2011, 70th anniversary: ‘Vitamins in early development and healthy ageing: impact on infectious and chronic disease’

A reference base for age-related decrease in lean tissue mass: whole body and segmental lean tissue mass in healthy young Irish men and women

S. Leahy, C. O’Neill, R. Sohun and P. Jakeman
Faculty of Education and Health Sciences, University of Limerick, Plassey Technological Park, Limerick, Republic of Ireland

Ageing is accompanied by a progressive loss of lean tissue mass. Sarcopenia is used to define age-related loss in skeletal muscle mass estimated to affect 30% of people older than 60 years and >50% of those older than 80 years. The magnitude of change in lean tissue mass with ageing is referenced to race- and gender-specific healthy young adults(1). Accurate reference body compositional data is therefore required to diagnose and investigate the prevalence of sarcopenia, its impact on health and function and to evaluate the effect of dietary and lifestyle intervention. The UL Body Composition Study(2) is a prospective study that seeks to establish age- and gender-specific reference ranges for whole-body and segmental body composition based on a representative sample of the Irish population residing within the UL community. The present paper reports on the whole body and segmental distribution of lean tissue mass for men and women aged 18–30 years.

With ethical approval (ULREC 08/07) and informed, written consent height, weight and body composition was measured by dual energy X-ray absorptiometry (DXA; iDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK) on 497 men and 392 women aged 18–30 years. Total body lean tissue mass was segmented into appendicular (legs + arms) lean tissue mass and expressed relative to stature squared generating an appendicular lean tissue mass index (ALTMI; Table 1).

Table 1. Anthropometric and body composition of young adult men and women.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Mass (kg)</th>
<th>BMI (kg/m(^2))</th>
<th>LTM (kg)</th>
<th>ALTMI (kg)</th>
<th>ALTMI (kg/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n 497)</td>
<td>Mean 22.3</td>
<td>1.80*</td>
<td>80.6*</td>
<td>24.8</td>
<td>62.4*</td>
<td>30.3*</td>
<td>9.3*</td>
</tr>
<tr>
<td></td>
<td>Std 2.8</td>
<td>0.07</td>
<td>11.7</td>
<td>3.2</td>
<td>6.9</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Min–Max 17.5–29.6</td>
<td>1.58–2.01</td>
<td>48.2–141.6</td>
<td>17.3–40.4</td>
<td>42.1–84.8</td>
<td>12.0–42.1</td>
<td>4.3–12.7</td>
</tr>
<tr>
<td>Women (n 392)</td>
<td>Mean 22.4</td>
<td>1.67</td>
<td>64.6</td>
<td>23.2</td>
<td>41.6</td>
<td>19.0</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>Std 3.1</td>
<td>0.08</td>
<td>9.5</td>
<td>3.3</td>
<td>4.5</td>
<td>2.4</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Min–Max 16.2–29.8</td>
<td>1.52–1.88</td>
<td>44.5–106.3</td>
<td>16.8–40.2</td>
<td>29.6–56.2</td>
<td>12.2–26.5</td>
<td>4.9–9.2</td>
</tr>
</tbody>
</table>

Min-Max, minimum-maximum. *P < 0.05 men v. women.

The data revealed predicted sex-specific differences in the mean values for percentage total body fat (,31.1% v. <18.6%) and lean tissue (,64.6 v. <77.4%) mass. Significant difference between the sexes was also evident in the appendicular lean tissue mass and remained when the lean tissue mass in the arms and legs was normalised to stature squared in order to account for the difference in for skeletal size.

The functional capacity of an individual, i.e. general mobility and the ability to perform activities of daily living, is determined primarily by the appendicular lean tissue mass(3). Thus, a guideline criterion of sarcopenia has been defined as an appendicular lean tissue mass index (ALTMI) value of less than -2 SD below the sex-specific mean for ALTMI in a healthy, younger adult(1). Based on the data from the present study, a criterion reference for sarcopenia in the Irish adult population would be an ALTMI value less than 7.3 kg/m\(^2\) in men and less than 5.24 kg/m\(^2\) in women.


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