The Measurement and Influence of Body Composition Phenotype in Ageing: An Analysis of Health -Related Changes in Irish Adults

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Abstract

Title: The measurement and influence of body composition phenotype in ageing: an analysis of health-related changes in Irish adults

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Ageing is a process associated with adverse effects on nearly every facet of human body composition and is generally accompanied by progressive loss in lean mass (sarcopenia) and bone mineral density (BMD) (osteoporosis) and concomitant increase in total and visceral adiposity (fat obesity). However, body mass index (BMI), the metric commonly used to quantify changes in body composition, does not adequately represent these health-related changes.

The technological advance in bone and soft-tissue imaging offered by dual energy X-ray absorptiometry (DXA) provides an accepted criterion measure of body composition into three components (adiposity, lean mass, BMD). Using DXA as the reference method of measurement, this thesis reports a cross-sectional characterisation of body composition in a convenience sample of 1,606 Irish adult men and women age 18-81 years. Interrogation of these data afforded the opportunity to challenge the validity and sensitivity of metrics such as BMI, redefine the criterion reference of adiposity and monitor age-related change and interrelationships between components of body composition.

The first study recommends measurement of a body fat mass index (BFMI) for accurate classification of adiposity that is independent of change in fat-free mass. An age-, BMI- and gender-specific reference equation is generated that allows accurate estimation ($R^2=0.9$, $SEE=1.1\%$) of BFMI for use clinically. Additionally, reference ranges of BFMI based on a young adult Z-score and centiles are presented, offering classification of the individual based on adiposity. The second study tracks the age-related change in body composition from the median young adult (18-29y), to middle age (30-49y) and older age ($\geq50y$), observing an increase in BFMI, coinciding with a re-distribution of adiposity from subcutaneous to visceral compartments. A decline in lean mass and BMD was observed to begin at age 30 years in men and women in this cohort. Examining the interrelationships between these parameters, the third study investigates the association between adiposity (BFMI and visceral) on BMD. Adiposity was shown to negatively influence BMD ($p<0.05$) to a greater extent at the whole body vs. site-specific regions and in younger vs. older adults.

Taking a combination of approaches, this thesis defines the criteria that describe ageing in an Irish cohort, and tracks the body compositional changes that may be detrimental to health. These results should form a basis to devise methods that offset the decline in composition that leads to frailty, disability, disease and loss of independence in the elderly, and promote health and functional status for longer.
**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. Susan Coote and Karen McCreeesh of the Department of Clinical Therapies 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 (Bibliography).

_______________________________
Clodagh Toomey, April 2014
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>25OHD</td>
<td>25-Hydroxyvitamin D</td>
</tr>
<tr>
<td>ALTM</td>
<td>Appendicular lean tissue mass</td>
</tr>
<tr>
<td>ALTMI</td>
<td>Appendicular lean tissue mass index</td>
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<tr>
<td>BF%</td>
<td>Body fat percentage</td>
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<tr>
<td>BFM</td>
<td>Body fat mass</td>
</tr>
<tr>
<td>BFMI</td>
<td>Body fat mass index</td>
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<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
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<tr>
<td>BM</td>
<td>Body mass</td>
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<td>BMC</td>
<td>Bone mineral content</td>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CV</td>
<td>Coefficient of variance</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>Db</td>
<td>Body density</td>
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<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<tr>
<td>ECW</td>
<td>Extracellular water</td>
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<tr>
<td>EHSREC</td>
<td>Faculty of Education and Health Sciences Research Ethics Committee</td>
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<tr>
<td>FFM</td>
<td>Fat-free mass</td>
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<td>IGF-1</td>
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<td>Interleukin</td>
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<td>ISCD</td>
<td>International Society of Clinical Densitometry</td>
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<td>IQR</td>
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<tr>
<td>IUNA</td>
<td>Irish Universities Nutrition Alliance</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>LTM</td>
<td>Lean tissue mass</td>
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<td>LTMI</td>
<td>Lean tissue mass index</td>
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<td>LSC</td>
<td>Least significant change</td>
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<td>NANS</td>
<td>National Adult Nutrition Survey</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral quantitative computed tomography</td>
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<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SEE</td>
<td>Standard error of estimation</td>
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<tr>
<td>SOP</td>
<td>Standard operating procedure</td>
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<td>Type I diabetes mellitus</td>
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<td>Total bone mineral density</td>
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<td>Total body water</td>
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<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
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<td>ULBC</td>
<td>University of Limerick Body Composition Study</td>
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<td>UWW</td>
<td>Under-water weighing</td>
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<td>VAT</td>
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<td>Volumetric bone mineral density</td>
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</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
</tr>
<tr>
<td>YA</td>
<td>Young adult</td>
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Chapter 1

Introduction
1.1 Background

The exponential rise in research relating to the measurement of body composition of late is largely attributable to a greater awareness of the health consequences associated with excessive deviations from normal. The assessment of body composition changes in response to nutritional, exercise or lifestyle interventions, progression of disease, ageing and rehabilitation, is of great importance to decision making in clinical care and in response to the worldwide increase in preventable lifestyle conditions and non-communicable disease.

Obesity is defined as abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired, and is a condition that is now considered to be a worldwide epidemic (WHO 2000). In a systematic analysis of epidemiological studies from 199 countries, 1.46 billion adults worldwide were estimated to be overweight in 2008 and of these, 502 million were obese (Finucane et al 2011). In Ireland specifically, 37% of adults are overweight and 24% are obese (IUNA 2011). The burden of obesity includes an increased number of fatal and non-fatal diseases—including diabetes, coronary heart disease, stroke, cancer, and osteoarthritis—which imposes substantial costs from medical treatment and productivity losses (Wang et al 2011). In the Republic of Ireland, the direct and indirect costs of overweight and obesity in 2009 were estimated at €1.17 billion (Dee et al 2013).

Obesity is commonly diagnosed by having a body mass index (BMI; body mass divided by height squared) greater than 30kg/m\(^2\). While use of BMI to define obesity has been an invaluable tool in epidemiologic studies and health promotion endeavours, it is the excess of adiposity, and not body mass, which is detrimental to health. Since BMI does not discriminate between fat mass and fat-free mass, or reflect the fat mass distribution in the body, it tends to misclassify at the individual level, and has a low sensitivity for determination of excess adiposity (Okorodudu et al 2010). Technological advances in body composition imaging have allowed a move towards more direct measures of adiposity that will offer a superior definition of health-related risk. Increasing availability of criterion techniques such as dual-energy x-ray absorptiometry (DXA) allows accurate estimation of three components of body composition i.e. fat, lean and bone tissue, while also providing a reference method for validation of surrogate measures of body fat i.e. skinfolds, ultrasound (Leahy et al 2012). Provision of an accurate estimation of adiposity also requires a suitable expression of adiposity. Use of a body fat mass index (BFMI) by normalising for height\(^2\)
may be appropriate to detect changes in body composition due to ageing, illness or intervention and to define a new classification system for ‘fat obesity’.

Adipose tissue has recently been defined as a dynamic organ that stores excess fat and a secretory organ that produces adipokines and cytokines, both of which play critical roles in human biology (Kershaw & Flier 2004). It is also suggested that the function of adipose tissues may depend on the location of fat deposition, i.e. visceral or subcutaneous. This is supported by the increased risk of cardiovascular disease (CVD), metabolic syndrome, diabetes, and mortality with elevated visceral adipose tissue (VAT) as opposed to subcutaneous adipose tissue (SAT) (Goodpastor et al 2003, Kang et al 2011). Therefore, the distribution of adiposity may be as relevant as the total amount of adiposity when defining the health risk of an individual.

Aging is associated with progressive changes in total and regional fat distribution that have negative health consequences (Kuk et al 2009). By 2041, there will be 1.4 million people in Ireland aged 65 and over. This older group will make up 22% of the total population, compared to 11.6% of the population in 2011 (Central Statistics Office 2012), which will undoubtedly increase the financial burden on the existing healthcare system. The body composition-related health risks in ageing cannot be evaluated by adiposity volume or distribution alone. Elderly individuals have less muscle and bone mass, expanded extracellular fluid volumes, and reduced body cell mass compared to younger adults (Baumgartner et al 2000). This loss of lean tissue mass with age (sarcopenia) and loss of bone mineral density (osteoporosis) are disease states associated with reduced independence, functionality and quality of life. Incidence of falls and fractures related to osteoporosis are important public health issues that have been well investigated to date. In Ireland between 2000 and 2004, rates of fracture for the total population aged >50 years were 407 and 140 per 100,000 per year for females and males, respectively. Assuming a stable incidence rate, the absolute number of hip fractures occurring on an annual basis is expected to increase by 100% by the year 2026 (Dodds et al 2009). Prevention of frailty and bone loss may be achieved through early detection of sarcopenia and osteoporosis. However, the rate of loss of lean and bone mass with age or prevalence rates in Ireland have not yet been identified.

Determination of the amount of adiposity, lean and bone mass in youth and through ageing may help define the Irish anthropometric phenotype in comparison to other ethnicities and
nationalities. While reference values for an American reference population are often presented (National Health and Nutrition Examination Survey (NHANES) – Kelly et al (2009)), recent recommendations from the International Society for Clinical Densitometry (ISCD) suggest a need for more country-specific reference datasets to be developed (Petak et al 2013). Mechanisms by which adiposity increases with age are not only subcutaneous- or visceral-specific, but can include fatty infiltration in skeletal muscle or bone marrow, thus further contributing to incidence of sarcopenia and osteoporosis respectively (Kuk et al 2009). Higher body fat has traditionally been considered favourable for skeletal health since mechanical loading produces a positive osteogenic response and adipose tissue enhances oestrogen metabolism (Reid et al 1992). However, the potential beneficial effects of increased body fat on bone conflict with the adverse effects of obesity on various health outcomes, necessitating more specific evaluations of the association between each body component and bone mineral density.

Accurate estimates of total or regional body composition are essential outcome measures in health research, particularly as the global epidemic of obesity, and the ageing population continues to rise. The clinical consequences of altered body composition should be taken into account in a nutritional or health assessment of the individual in order to prevent disease development or progression. As the accuracy of methods to assess body composition improves, the transfer of this evidence-based knowledge to clinicians and the public is a vital step in the promotion of healthy lifestyle choices, encouragement of self-management and improvement of health outcomes.
1.2 Thesis Aims

This thesis profiles the DXA-measured body composition of an Irish convenience sample of 1,606 adults aged between 18 and 81 years of age. Primary thesis aims were as follows:

1. To establish the most appropriate metrics to represent total and regional adiposity in body composition analysis;
2. To develop a young adult reference of classification criteria for obesity and sarcopenia based on valid metrics;
3. To track the cross-sectional and longitudinal health-related body composition changes associated with ageing;
4. To determine the influence of adiposity on bone mineral density.

1.3 Thesis Structure

This thesis examines the methods used to assess body composition, while also considering the potential associations between body composition and healthy ageing. Chapter 2 presents a review of the literature on four main research areas including measurement methods of body composition, the expression of body composition metrics, the age-related changes associated with body composition and the relationship between adiposity and bone health. Chapter 3 describes the standard operating procedure and methodology used for all prospective chapters and gives recommendations based on minimisation of technical and biological error in DXA scanning. It also includes a technical analysis examining the short-term precision of DXA to measure total and regional lean, fat and bone mass and bone mineral density. Chapter 4 is the first main data chapter. It explores the relationship between BMI and DXA-based measures to determine the most appropriate metrics of body composition (i.e adiposity and fat-free mass). Alternative criterion indices are suggested based on fat and lean mass and a generalised prediction equation based on BMI is developed for use clinically. The relationship of visceral fat with total and abdominal fat is also reported. Chapter 5 advances on the analysis in Chapter 4, introducing a reference range of young Irish adults with which to define criteria for classification of fat obesity and sarcopenia based on body fat mass index (BFMI) and appendicular lean tissue mass index (ALTMI) respectively. Chapter 6 describes the changes in body composition that occur with
ageing and is divided into two parts: a cross-sectional analysis describing changes observed from youth (18-29 years), through maturation (30-49 years) and towards old age (>50 years); and a longitudinal analysis describing the annual change observed in a smaller cohort of 50-70 year olds. Chapter 7, the final data chapter, explores the complex relationship between adiposity and bone, and determines whether total and regional adiposity has a positive or negative influence on bone mineral density in the total body and at site-specific regions. Finally, Chapter 8 critically analyses and discusses the findings of this thesis. The implications of these results are discussed, while potential areas of future research are highlighted.
Chapter 2

*Literature Review*
2.1 Introduction

This literature review is divided into four sections. The first will describe the origination of body composition measurement in human nutrition and health, with detail on the accuracy and precision associated with DXA and different methods of measurement. The second section will consider the form in which body composition parameters are expressed, since this has an impact on the classification or diagnosis of body composition-related health outcomes. The third section will explore the health-related changes in body composition that are observed with advancing age in different international populations. These trends will be assessed in terms of three main outcomes i.e. adiposity, lean mass and bone health, with the anticipation of comparison to an Irish population later in this thesis. In the final section, this chapter will critically review the current literature describing the association between adiposity and bone health in adults. Both total and visceral adiposity will be examined, including interpretation of the mechanisms by which this interaction may occur.

2.2 Measurement of Human Body Composition in Relation to Health

In order to establish the nutrition and health status of an individual, knowledge of the complete representation of the anthropometric phenotype is necessary i.e. measurement of adiposity, lean mass and bone. In vivo body composition research uses various methods that rely on particular assumptions which may not always hold true. Therefore, the need for accurate and valid methodologies for estimation of body composition has led to the emergence of new technologies, with superior ability to differentiate the multiple components of the body. The various models of body composition are briefly described, preceding a more detailed review of each component.

2.2.1 Models of Body Composition

At its most simple form, a two-component model of body composition divides the body into a body fat mass (BFM) component and a fat-free mass (FFM) component. Two-component methods such as hydrodensitometry or air displacement plethysmography assume a constant chemical composition and hence density of BFM and FFM (Siri et al 1956, Brozek et al 1963). While these models have served the field of body composition assessment for over
four decades, measurement technique can be demanding for the participant and can lead to inaccuracies if ‘constants’ are not valid (Pietrobelli et al 2001). Three-component models of body composition offer the advantage of differentiation of a third component, bone mineral content. Methods such as dual-energy x-ray absorptiometry (DXA) are simple and non-invasive, but accuracy is also subject to certain assumptions. Increasingly referred to as ‘gold standard’, DXA measurement will be the focus of this review. A four-component model of body composition is obtained by combining several measurement techniques to divide body mass into water, mineral, protein and fat (Figure 2.2.1). It is therefore acknowledged as the true criterion method of reference (Baumgartner et al 1991, Pietrobelli et al 2001). However, the time and expense of utilising multiple measurement tools limits its use in clinical settings or in large population studies.

Figure 2.2.1 Models of body composition with two (2-C), three (3-C), or four (4-C) components
(Reproduced with permission from Toombs et al 2011)

2.2.1.1 Dual-Energy X-Ray Absorptiometry

DXA is a method that provides simultaneous body component (BFM, FFM and bone mineral content) measurement through transmission of X-rays through the body at high and low energies. Current models (Lunar iDXA™, GE Healthcare, Chalfont St Giles, Bucks., UK)
have a narrow-angle fan-beam that use a high-definition cadmium zinc telluride staggered array detector and a more powerful X-ray tube than previous densitometers, thereby enhancing image resolution and bone edge detection and, subsequently, the development of superior algorithms for body composition assessment (Toombs et al 2012). A whole body iDXA scan is estimated to have an effective dose of 0.96 µSv in thin and standard mode, and 1.92 µSv in thick mode, which is considerably less than the worldwide average background radiation dose of ~4 µSv per day (based on 2400 µSv for a human being per year, Rothney et al 2012). Compared to hydrodensitometry or multicomponent methods, DXA scanning time is efficient, requires minimal active subject involvement and imposes minimal or ‘trivial’ risk (Pietrobelli et al 1998). DXA also has the ability to offer multiple regional analysis of body composition, which may be important in the assessment of health-related markers.

2.2.2 Measurement of Adiposity

2.2.2.1 Total Adiposity

In the assessment of body composition-related health, measurement of adiposity appears to be an essential factor in the determination of disease states such as cardiovascular disease, metabolic syndrome and diabetes (Zhu et al 2003). Accuracy (or trueness) and precision of a method is therefore important for correct diagnosis or classification. The accuracy of a measurement is how close a result comes to the true value or ‘gold standard’ reference. The precision of a measurement system is the degree to which repeated measurements under unchanged conditions show the same results.

Excellent precision has been reported for iDXA measurement of BFM in adults, with a coefficient of variance (CV%) between 0.7 and 1% (Hind et al 2011, Rothney et al 2012). While the iDXA instrument has shortened scanning time and improved quality and image resolution compared to previous systems, the ability of the Lunar iDXA to accurately assess body composition has not yet been tested against the gold standard four-component model. Previous densitometers have shown differences in body fat percentage (BF%) (~5.3 to 2.9%), with a tendency to underestimate BF% in leaner individuals and overestimate it among individuals with a higher BF% (Toombs et al 2012). Initial results from studies comparing the iDXA to older models have indicated that BFM and BF% are slightly higher on the iDXA
than on the Lunar Prodigy for BF% <30%, and less on the iDXA then on the Lunar Prodigy for BF% > 30% (Toombs et al 2012). This may indicate a more accurate representation of the four-component model, but currently warrants further investigation. Although DXA measurement of body composition cannot yet be claimed to be as accurate as a four-component model, it has become a widely accepted criterion method for the measurement of body composition due to its excellent precision, relative ease of use and extensive availability. This is highlighted from its current use as an assessment tool in large multicentre research groups such as the National Health and Nutrition Examination Survey (NHANES, Kelly et al 2009) and the Health, Ageing and Body Composition Study (Health ABC, Newman et al 2005) in the United States. However, two limitations of DXA measurement of adiposity are its weight limit (typically 200kg) and height and width restrictions (197cm X 66cm), which compromises scanning accuracy for individuals who exceed these specifications.

Since DXA may not be widely available for clinical use, surrogate or prediction methods of body composition also require review. Such methods provide an indirect estimate of body composition parameters, and are usually based on regression equations derived by comparison to a reference method. Their availability and use in research and clinically has grown in recent years since they generally do not require expensive equipment. Bioelectrical impedance analysis (BIA) involves the passing of a small electrical current through the body and measuring the resistance offered. This current is resisted or impeded to different levels depending on the type of tissue it passes through, thereby differentiating between BFM and FFM. Validity of BIA using DXA as the reference method has previously been examined by this research group (Leahy et al 2012a). BIA was found to underestimate median BFM by 0.3kg in men and 1.7kg in women aged 18-29 years. Similarly, in older adults, Sun et al (2005) reported a tendency of BIA to underestimate percentage body fat in men and women compared to DXA. Most discrepancies tended to occur outside the ‘normal’ body fat ranges (15-25% in men, 25-33% in women). Therefore, its use may be limited in obese subjects or athletes.

Anthropometry, specifically skinfold and girth measurement is an indirect, prediction-based assessment of BF%. The commonly used prediction equations of Durnin & Womersley (1974) have recently been updated by this research group using a cohort of 1,136 men and women aged 18-81 years with a body fat range of 5-56.8% to incorporate the changing
human phenotype (Leahy et al 2012). These equations accurately predict BF% using three skinfold sites (tricep, mid-axilla and supraspinale) in men and three skinfold sites (mid-axilla, biceps and medial calf) and one girth site (abdominal) in women with a standard error of estimate (SEE) of 2.5-3%. While the Durnin & Womersley (1974) equations tended to underestimate BF% by between 0.8 and 1% in adults, these equations offer a highly accurate ($R=0.90$ to 0.95) representation in this population. Alternatively, the use of B-mode ultrasound for prediction of BF% is a method that offers certain advantages over anthropometry. While use of a skinfold caliper causes deformation of the subcutaneous layer to produce a measurable fold, an ultrasound probe measures a single-layer construction and can distinguish adipose from dermal tissue with minimal deformation. This method has been previously described by this research group (Toomey et al 2011), and validated using DXA in 135 young adults aged 18-29 years (Leahy et al 2012c). Prediction equations generated using quantile regression found subcutaneous adipose tissue thickness at the abdominal and thigh sites to accurately predict BF% in men (SEE=1.9%) and at the abdominal and medial calf sites in women (SEE=3%).

**2.2.2.2 Regional Adiposity**

Regional adipose distribution, in particular the estimation of intra-abdominal or visceral adipose tissue (VAT) has been considered to be more important in understanding the link to the many facets of the metabolic syndrome, including impaired glucose tolerance, hypertension, dyslipidemia, and insulin resistance (Wajchenberg et al 2000). However, measurement of VAT is limited due to availability of imaging methods that accurately quantify this compartment, and distinguish VAT from subcutaneous adipose tissue (SAT).

Computed tomography (CT) is considered the most accurate and reproducible technique of VAT assessment (Rossner et al 1990), although technical difficulties, high cost, radiation exposure, and variations in the definition of adipose tissue compartments limit the use of CT in routine clinical practice. Magnetic resonance imaging (MRI) yields excellent concordance with CT (Gomi et al 2005), is without radiation exposure but is more expensive. The L4-L5 spinal segment is the site most commonly used to define VAT by CT and MRI. While VAT measures in the mid-abdomen (i.e. L2-L3, L3-L4) are stronger correlates of total VAT volume, this does not translate into an improved ability to predict metabolic risk, or changes through intervention than the L4-L5 site (Kuk et al 2010).
Recent technological advances in densitometry have allowed VAT estimation within the android (abdominal) compartment of DXA acquisition (GE CoreScan™ software). Detection of the layer thickness of SAT at sides of the android region allows the software to estimate the total SAT compartment and indirectly derive VAT by subtracting SAT from the total android fat mass. This method has shown an excellent association ($R^2=0.96$) with CT, examined in 124 men and women of varying age (18-90 years) and representing a wide range of BMI (18-40kg/m$^2$) (Kaul et al 2012). Precision has been characterised on both an anthropometric imaging phantom across ten iDXA systems and in a clinical population of obese women ($n=32$) (Rothney et al 2013). Phantom scanning (VAT values 0-1800g) yielded a precision estimate of 4.8% CV for a 1kg VAT mass. Repeat measurement in obese women ($n=32$) has shown a precision of 5.1% CV for a 1kg VAT mass and a non-significant bias of +15.4g. However, precision error is yet to be evaluated in a human population of non-obese subjects. Those with very low quantities of VAT (e.g. athletes) or very high quantities of VAT (e.g. obese men) may yield different results.

Abdominal ultrasound is an alternative method to assess the amount of VAT since it can distinguish between layers of adipose tissue. It is highly correlated ($R=0.81$) to CT and MRI (Stolk et al 2001), even in obesity ($R=0.71$) (Ribeiro-Filho et al 2003) and the elderly ($R=0.80-0.82$) (De Lucia-Rolfe et al 2010). Since ultrasound combines safety, cost-effectiveness, and accuracy, its use in both clinical practice and epidemiological studies is increasing. As the most common surrogate measure of abdominal adiposity, waist circumference (WC) is widely used in clinical and public health settings. Cut-offs for increased risk of metabolic complication are defined as >94cm in men and >80cm in women (WHO 2000). Adiposity distribution can also be estimated by measurements of the ratio of waist to the hip circumference (WHR). Individuals with low WHR, (subcutaneous or pear-shaped obesity) are at low risk for metabolic complications, whereas individuals with a high WHR (>0.5; visceral or apple-shaped obesity) are at high risk for these complications (Kissebah and Krakower 1994). Both WC and WHR are reported to have high intra-rater reliability ($R=0.99$ and 0.97 respectively) (Chen et al 2001), while correlations with CT-measured VAT are moderate (WC; $R=0.64-0.80$ and WHR; $R=0.59-0.73$) (Hill et al 1999).

The main limitation of WC and WHR measurements is that they cannot distinguish between SAT and VAT in the abdominal compartment. This distinction is essential in the interpretation of health-related risk since VAT is known to be metabolically distinct from
SAT (Wajchenberg 2000). In a collaborative analysis of 58 prospective studies, The Emerging Risk Factors Collaboration (2011) stated that neither WC nor WHR can be considered independent predictors of CVD and are only of benefit when additional information on blood pressure, history of diabetes and lipids are not available.

In summary, a number of factors are important in the determination of an appropriate measurement tool for the measurement of adiposity. These dynamics include the accuracy and reproducibility (precision) of the tool, the availability and cost, and, most importantly, the representation of health-related outcome. DXA appears to be an appropriate measure in this regard in terms of both total and regional adiposity measurement.

### 2.2.3 Measurement of Lean Mass

Skeletal muscle or lean tissue mass (LTM) is essential in the performance of exercise, maintenance of optimal function and activities of daily living. Altered muscle metabolism plays a key role in many common pathologic conditions and chronic diseases including cachexia, starvation, HIV, sarcopenia (reviewed in more detail in section 2.4.2), osteoporosis, insulin resistance and diabetes (Wolfe 2006, Wilson et al 2013). The role of LTM in some of these conditions is often underappreciated but is evidently important in the assessment of body composition, nutrition and health.

Measurement of fat-free mass (FFM), composed of LTM and bone mineral content, is based on an assumption that the water content of FFM is constant at 0.73±0.03 (Wang et al 1999). This hydration constant is thought to maintain relatively stable throughout the adult lifespan (Schoeller 1989, Lohman 2000), despite likely increases in the variance of the extracellular to intracellular water (ECW:ICW) ratio with age (Baumgartner et al 1991). However, hydration of FFM has been shown to vary from 0.69 to 0.81 in cadavers, with predicted variation range of FFM hydration for healthy young adult humans approximating 0.69 to 0.77 (Wang et al 1999). Therefore, stringent efforts should be made to ensure subject measurement occurs in a normal or euhydrated state.

DXA, by measuring three components – BFM, LTM and bone - is not free of the assumption of uniform hydration since it cannot distinguish total body water from LTM. Intra-subject variability may occur in DXA FFM or LTM estimation due to the variation in hydration that
occurs with food and fluid intake, prior exercise and other physiological processes throughout the day. Going et al (1993) demonstrated an apparent increase in DXA-measured LTM (+1.3kg) after rehydrating following a -2% body mass (BM) dehydration protocol in 17 adults. No differences were found between baseline, dehydration and rehydration estimates of BFM or bone mineral content (BMC). Within-day biological variation was examined in a group of active young men and women (n=31) by Nana et al (2012). Introduction of a breakfast meal consisting of cereal, reduced-fat milk and water increased total (0.9-1.5%) and trunk (2-3.2%) LTM ~40 minutes post-meal. Change in BFM did not exceed the measurement error. A study by the same authors demonstrated changes in total (-0.4%) and trunk (-1.5%) LTM in 14 male cyclists following a 110±42 minute cycling bout (Nana et al 2013). Although no quantifiable measurement of hydration status was used, these studies highlight the importance of a pre-scan protocol or standard operating procedure (SOP) in order to ensure the participant is both fasted and rested to maintain an euhydrated state, and thus, not confound the LTM measurement.

It is suggested that under normal conditions not associated with large changes in fluid balance, variation in FFM hydration does not limit the accuracy of DXA body composition measurement (Pietrobelli et al 1998). Although no validation study comparing iDXA to a 4-component model exists, Williams et al (2006) evaluated an older model (Lunar Prodigy, GE Medical Systems, Madison, WI) in comparison to the criterion 4-component model in non-obese adults. A non-significant bias in FFM was observed in men (-0.37kg; p>0.05), with a significant underestimation observed in women (-1.19kg; p<0.01). These results should be interpreted with caution since no hydration or fasting protocol was declared. Also, an improved image resolution in the newer iDXA model may result in improved accuracy of FFM and LTM measurement but remains to be investigated. Excellent precision has been reported for iDXA measurement of total LTM in adults, with CV% reported as between 0.4 and 0.5% (Hind et al 2011, Rothney et al 2012).

Use of imaging modalities such as CT and MRI offer certain advantages over DXA since they are capable of measurement of fatty infiltration in LTM, and thus provide an estimation of muscle quality. Altered fat deposition in LTM is linked to reduced insulin-stimulated glucose uptake and increased muscle wasting diseases, particularly in the elderly (Ross 2003) and may offer a more complete representation of condition than LTM quantity alone. These
methods have been validated against cadavers (\( R=0.99, \ SEE=3.8-3.9\text{cm}^2, \ p<0.001 \)) by Mitsiopoilos et al (1998).

Alternative methods of LTM measurement include total body potassium (TBK), which is an index of the body’s cell mass. It is estimated that 60% of the body’s potassium is found in skeletal muscle, and the remainder is found in other organs and tissues and has therefore been used as an index of skeletal lean mass in previous studies (He et al 2003). Total body protein has also been used as a surrogate for functional LTM since most protein is in LTM, and is not dependent on hydration. Despite its cost, radiation exposure and clinical inaccessibility, neutron activation analysis (NAA) is considered the gold standard measure for \textit{in vivo} total body protein (Lukaski et al 1981). Recently, a simplified method to measure total body protein using DXA and BIA was validated by Wilson et al (2013), showing good correlation (\( R^2=0.87 \)) against the NAA method. This may provide a more clinically accessible technique to measure total body protein in future but requires comparison against markers of physical function.

### 2.2.4 Measurement of Bone Mineral Density

DXA is the gold standard measurement tool for both bone mineral density (BMD) and bone mineral content (BMC) and can be measured by whole body or regional/site-specific scans. BMD explains approximately 70-75% of the variance in bone strength (Njeh et al 1997) and consequently remains the most significant factor in the assessment of bone health, osteoporosis and fracture risk. It corresponds to the ratio between BMC and the bone area scanned and is therefore not a volumetric density but an areal density given in units of g/cm\(^2\).

#### 2.2.4.1 Site-Specific BMD

BMD varies depending on the assessment point on the individual. The hips and particularly the spine are the first regions to lose a significant amount of BMD since they have a relatively high trabecular to cortical bone ratio, making them more susceptible to bone turnover and hormonal change (Jergas & Genant 1997). The negative contributors that lead to reduction in BMD within the cortical bone at any period of life are neutralised slower and to a lesser extent than within the trabecular bone. Substantial cortical bone loss begins in middle life in women but begins mainly after age 75 in men. In contrast, substantial trabecular bone
loss begins in young adult women and men and continues throughout life with acceleration during peri-menopause in women (Riggs et al 2008). Low BMD in the hip and vertebrae are more detrimental as they are associated with a higher mortality rate post fracture. Therefore, the spine (vertebrae L1-L4) and both hips (femoral neck or total proximal femur) are the recommended scanning sites for the diagnosis of osteoporosis and osteopenia by the International Society for Clinical Densitometry (ISCD; Schousboe et al 2013). The WHO international reference standard for osteoporosis diagnosis in postmenopausal women and men age 50+ is a T-score of -2.5 or less at the lumbar spine, total hip or femoral neck (WHO 1994). This corresponds to 2.5 standard deviations below the mean BMD for a young healthy adult. The reference standard to obtain this T-score uses the data from the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) database (female, white, 20-29 years) (Looker et al 1998). The minimum acceptable precision for an individual technician as outlined by the ISCD is 1.9% (lumbar spine), 1.8% (total hip) and 2.5% (femoral neck) (Schousboe et al 2013).

2.2.4.2 Total and body region BMD

To determine total BMD (TBMD), a whole body DXA scan is carried out which can be analysed to quantify ROIs similar to a whole body composition scan. This method is currently being used by the NHANES following a switch from site-specific BMD measurement at the proximal femur in 1999. However, the ability of TBMD to predict fracture risk or response to treatment is less well known. Despite this, it is often used in place of recommended diagnostic measures in large population based studies when site-specific data is unavailable or unknown (Katzmarzyk et al 2011). Melton et al (2005) compared site-specific BMD with sub-regions of TBMD scans in 348 males and 350 females, age 21-90 years. They found an excellent correlation between site-specific lumbar spine and whole body lumbar spine BMD ($R^2=0.92$), but weaker correlations for total hip compared to pelvis region ($R^2=0.72$) or between total wrist and left arm sub-region from the whole body scan ($R^2=0.83$). While overall estimates of osteoporosis prevalence were comparable, there was some measurement disagreement in individuals. Measurements at whole body regions underestimated osteoporosis as assessed at the femoral neck or total wrist. Among postmenopausal women, only 14% would have been classified as osteoporotic on the basis of pelvic region BMD from the whole body scan compared to 27% by measurement of femoral neck BMD, whereas the spinal region was less inaccurate (6.9 versus 7.4%). Therefore,
regional analysis of a whole body scan to measure bone health may only be appropriate at the spine.

Regional BMD can alternatively be measured using peripheral quantitative computed tomography (pQCT), which has the advantage of the ability to separate measurement of the cortical and trabecular compartments of bone. This may be relevant in monitoring bone loss, where a loss in one compartment may be disguised by a gain in the other, thus resulting in no overall bone mineral change. *In vivo* precision of pQCT at numerous long bone sites has been reported as between 0.9 and 2.7% for trabecular bone and between 0.5 and 6.8% for cortical bone (Sievanen *et al* 1998).

Response or changes in site-specific BMD have been employed as an endpoint in most randomized controlled clinical trials of currently approved osteoporosis therapies. In addition, current understanding of the epidemiology and pathophysiology of osteoporosis is derived from hip and spine BMD, not TBMD. While total and regional BMD and BMC cannot be substituted for site-specific BMD in the recommended clinical diagnosis of osteoporosis, they may have a role to play in the determinants of bone health at non-specific fracture sites and in monitoring the change in bone health following intervention.

### 2.2.5 Summary

Body composition can be quantified at several levels, depending on clinical concerns. The use of CT and MRI appear to be excellent representations of body composition but are not always practical due to availability, radiation dose and expense. A 4-component model offers high accuracy but does not provide regional body composition assessment. The clinical use of DXA for body composition analysis is increasing as it offers a complete health-related assessment of both total and regional body compartments in the classification of the anthropometric phenotype and has the potential to offer new diagnostic insights for disease in a variety of areas e.g. HIV therapy, sarcopenia, bariatric surgery and obesity (Kendler *et al* 2013). However, differences in the use of specific DXA software packages and scanning algorithms should be considered along with the use of a standardisation protocol to limit technical error and biological variation in hydration status.
2.3 **Expression of Body Composition Measurement**

While the accuracy and precision of the body composition measurement tool is important, so, too, is the manner in which the measured variable is expressed. Conclusions derived from studies using inappropriate metrics of body composition can incorrectly influence policy and clinical decision-making. Reviewed in this section are three metrics that classify body composition: BMI, BF% and the body fat mass index/fat-free mass index.

### 2.3.1 Body Mass Index

BMI derives from “Quetelet’s index” (body mass/height$^2$) which was developed in the 1800’s to chart the range of heights and weights of army conscripts after observation of the relationship between proportions of body mass to height. BMI was subsequently suggested as an indicator of body fat content (Garrow & Webster 1985), and thereafter became one of the most common parameters used in nutritional, metabolic and cardiovascular studies.

Obesity is viewed simply as an excess of body fat, which has accumulated to the extent that health is adversely affected (WHO 2000). However, BMI is an imperfect measure of adiposity as it cannot distinguish BFM from LTM and therefore may not offer the best representation of this definition of ‘obesity’. Nonetheless, it is commonly relied on as an indicator of adiposity in epidemiologic studies and has been incorporated into clinical practice due to its ease of measurement and recommendation by the World Health Organisation (WHO). Classification criteria for the definition of obesity that were independent of age and sex were defined as $<18.5$kg/m$^2$ (underweight), $18.5-24.9$kg/m$^2$ (normal), $25-29.9$kg/m$^2$ (overweight) and $\geq30$kg/m$^2$ (obese) (WHO 1995). The incidence of BMI-defined obesity has grown rapidly over the last three decades, with worldwide estimates now over 500 million, having increased at a rate of 0.4-0.5kg/m$^2$ per decade since 1980 (Finucane et al 2011).

The relationship of BMI to BF% as measured by DXA in the NHANES database (12,901 adults) reveal a significant correlation that decreases with age ($R=0.72-0.79$ in men, $R=0.72-0.84$ in women). Using arbitrary increments of five percentage points, categories of BMI were prevalence-matched to categories of BF%. This analysis revealed only 46% of men and 49% of women were in the same category by both BF% and BMI (Flegal et al 2009). Gallagher et al (1996) investigated the relationship between BMI and BF% in 706 subjects,
finding that BMI alone accounted for 25% of between-individual differences in BF%. Adding age and sex as independent variables to the regression model increased the prediction to 67%. These results suggest that BMI is age and sex dependent when used as an indicator of body fatness, although currently not classified as such by the WHO.

Indices of diagnostic performance used in various studies include sensitivity, defined as the probability that a person who actually has the condition of interest will have a positive test result; and specificity, defined as the probability that a person who does not have the condition of interest will have a negative test result. Okorodudu et al (2010) performed a systematic review and meta-analysis that calculated a pooled sensitivity of 0.50 and specificity of 0.90 of BMI to identify excessive body adiposity (BF% >25% in men, >35% in women), suggesting BMI is an excellent tool for predicting when obesity (BMI >30kg/m$^2$) is not present. However, approximately half of individuals with excess BF% are labelled as non-obese. This suggests obesity is presently being under-diagnosed due to continued reliance on BMI, if the criterion for BF% is correct.

The negative impact of obesity on health has been thoroughly investigated and a ‘J’ or ‘U’-shaped association between BMI and mortality has been identified (Figure 2.3.1, Calle et al 1999), where higher risk of death is found in those who are above or below the apparent optimum of 22.5-25kg/m$^2$ (Prospective Studies Collaboration 2009). Adults identified as ‘obese’ have been consistently linked with adverse health conditions such as diabetes, osteoarthritis and hypertension (Patterson et al 2004). However, recent reports have identified individuals classified as ‘overweight’ as having better outcomes than those classified as ‘normal’ (Flegal et al 2013). This may in fact reflect intrinsic limitations of BMI in differentiating adiposity from lean mass in intermediate BMI ranges from 18.5-25kgm$^2$. For an individual who is 1.75 m tall the variation in body mass within the ‘normal’ range of BMI constitutes 20 kg, while the between-group difference between ‘normal’ and ‘overweight’ can be as little as 0.1 kg. It is, therefore, naïve to treat all those within a category as a homogeneous group when there may be substantial differences within a group, and minimal differences between groups at the boundaries (Nicholls et al 2013).
The widespread and longstanding application of BMI contributes to its utility at the population level. Its value cannot be underestimated in the endeavour to increase availability of published population data that allows public health professionals to make comparisons across time, regions, and population subgroups. However, at the individual level, BMI does not measure adiposity directly and should not be used as a diagnostic tool (Centers for Disease Control and Prevention 2009). While valuable for its convenience and simplicity in public health surveillance and screening it lacks the sensitivity to be used as a diagnostic tool.

2.3.2 Body Fat Percentage

Body fat mass is a direct measure of adiposity, that can be expressed as a percentage (BF%) for a more appropriate representation of adiposity relative to the body mass of an individual. Numerous reference and surrogate measures of BF% are available as described in Section 2.2.2.1 that vary in cost, ease of use and accuracy. Often judged as the criterion metric of adiposity, BF% has been shown to be an improved representation of health risk compared to BMI. In 6,171 ‘normal range BMI’ subjects from the Third National Health and Nutrition Examination Survey (NHANES III), BF% in the highest tertile (>23% in men, >33% in women) has been shown to be associated with the presence of metabolic syndrome, higher prevalence of dyslipidaemia, hypertension in men, and cardiovascular (CV) disease in women. (Romero-Corral et al 2010). These individuals were termed ‘normal weight obese’.
However, these BF% values may be underestimated since body composition measurement was undertaken using BIA.

Mean values or centiles of BF% in various populations using DXA have been described (USA NHANES, Kelly et al 2009; Italian, Bazzochi et al 2012). However, ideal ranges of BF% do not exist. Currently, a cut-off of 25% for men and 35% for women have been suggested by the American Association of Clinical Endocrinology/American College of Endocrinology (AACE/ACE 1998) and World Health Organisation (WHO 1995), but these have not been validated in large epidemiological studies for health outcomes and mortality. Gallagher et al (2000) developed an approach for establishing healthy BF% ranges that correspond to published BMI ranges. However, this method may be inadequate due to the limitation of BMI and classification of obesity, as described. Gallagher et al (2000) also developed age-, sex- and ethnicity- specific formulas for estimating BF% from BMI using 1,626 participants from three ethnic groups (Caucasian, African American, and Asian) from the USA, UK and Japan. Both four-component models and DXA were used as the criterion measure of BF% measurement. 1/BMI term was used as an independent variable to linearise the data and to avoid the need for logarithmic conversion, resulting in a stronger correlation with BF% ($R=0.68$ to $0.89$) compared to previous studies (Gallagher et al 1996). Inclusion of age, sex and ethnicity showed a stronger correlation to BF% ($R=0.74$ to $0.92$) than using BMI alone. This analysis was repeated in a more recent study by Heo et al (2012) using DXA data from the U.S. 1999-2004 NHANES population (n=12,906) on three ethnic groups (Caucasian, African American, Mexican American), also resulting in a stronger correlation ($R=0.74$ to $0.89$). These algorithms provide a better estimate of adiposity that is specific to the populations studied.

However, like BMI, the expression of BF% has limitations in measurement of adiposity in certain groups. Without prior knowledge of total body mass, the fraction (BF%), just like the surrogate measure (BMI), contains information about two components of body composition (BFM and FFM) and cannot distinguish each other effectively. BF% could result from high BFM, and/or low FFM and may therefore not be a suitable metric for tracking the changes in adiposity that occurs for example in relation to age or resulting from intervention. In a longitudinal study on 7,265 men, Jackson et al (2012) found that the modelled age-related change in body composition was not detected by BMI or BF%. As BF% continues to rise with age, it is not known whether body fat is increasing or lean mass is decreasing.
2.3.3 Body Fat Mass and Fat-Free Mass Indices

Both lean mass and fat mass scale with height to approximately the power of two (Heymsfield \textit{et al} 2011), establishing an analytic framework for height-scaled indices. To overcome some of the limitations associated with expressing BFM or FFM in absolute terms or as % of body mass, VanItallie \textit{et al} (1990) proposed the use of indices of body fat and fat-free mass to be a more relevant assessment of nutrition status. FFM index (FFMI) and BFM index (BFMI) are calculated by dividing the numerator (BFM or FFM) by height$^2$.

VanItallie \textit{et al} (1990) calculated these indices in 32 non-obese young men (from the Minnesota Study) before, during, and after 12 weeks of experimental semi-starvation using a two-component model of body composition (total body electrical conductivity (TOBEC)). As protein-calorie malnutrition became more severe, an increase in FFM% was observed due to a faster decrease in BFM than FFM. Also, the extent of the decrease in BF% was underestimated due to the concurrent decline in FFM. Use of values for FFMI and BFMI below the reference cohort’s 5$^{th}$ percentile, however, were able to accurately diagnose ranges for malnutrition.

This method merits a reappraisal and appears to be relevant in the classification of underweight/‘underfat’ patients and overweight/‘overfat’ or ‘fat obese’ individuals. Using BFMI, abnormalities in fat mass can be assessed without interference from other unrelated components such as an excess or deficit of FFM or water. Reference values or centiles of indices have been reported in past research by BIA method in Europeans (Kyle \textit{et al} 2001, Schutz \textit{et al} 2002) and by DXA in Americans (NHANES – Kelly \textit{et al} 2009). Conforming to the classical BMI cut-off points set out by the WHO, Kyle \textit{et al} (2001) and Schutz \textit{et al} (2002) took corresponding values for FFMI and BFMI using regression analysis. Similarly, Kelly \textit{et al} (2009) established ranges by matching the prevalence of each BFMI category to BMI prevalence in young adults. Again, a dependency on BMI is observed, which may negate the positive steps towards re-defining obesity through use of an adiposity-based index.

Measured by BIA, Liu \textit{et al} (2013) investigated the relationship between BFMI and presence of metabolic syndrome in 1,698 Chinese adults (aged 20-79y). After adjusting for age, BMI, BF%, total cholesterol, low-density-lipoprotein cholesterol (LDL), C-reactive protein (CRP), smoking status and physical activity, BFMI was significantly related to higher odds of metabolic syndrome in men and women (p<0.01)This indicates that BFMI appears to be a
better screening tool for health risk markers than BMI and BF%. In a Danish prospective study, 27,178 men and 29,875 women were assessed using BIA to determine BFMI and FFMI, which was subsequently studied in relation to all-cause mortality after a median of 5.8 years (Bigaard et al 2004). J-shaped associations were found between BFMI and mortality adjusted for FFM and smoking, while reverse or mirror J-shaped associations were found between FFMI and mortality (Figure 2.3.2). These results suggest that the U-shaped association between BMI and all-cause mortality reflects the combination its subcomponents i.e. a J-shaped association for BFMI and a reverse J-shaped association for FFMI, indicating opposite, but joint effects, and again, highlighting the inaccurate conclusions derived in studies measuring BMI and mortality. Similar studies to establish ranges based on epidemiological evidence of health risk and mortality based on a more accurate measurement tool (i.e. DXA) are warranted.

![Figure 2.3.2 The ‘reverse J-shaped’ association between FFMI and mortality (left) and the ‘J-shaped’ association between BFMI and mortality (right) the in the Danish ‘Diet, Cancer and Health’ Study, 1993-2001. F, Female; M, Male.](Reproduced with permission from Bigaard et al 2004)

2.3.4 Summary

Accurate measurement and expression of adiposity is important both at the individual and population level for the correct classification of ‘fat obesity’ and subsequent obesity-related disorders. While values of BMI and BF% are widely cited in the literature and in clinical practice, variation in muscularity represents a confounding factor in their use as a phenotypic expression of adiposity. BFMI is advantageous, in that adiposity can be classified regardless of the amount of FFM, while still adjusting for body size. A recent ISCD official position for body composition analysis reporting has indicated that use of body composition adiposity...
measures (BF% or BFMI) may be useful in risk-stratifying patients for cardiometabolic outcomes (Petak *et al* 2013). However, based on the literature reviewed, it would appear that BFMI is the preferable metric for use. Specific thresholds to define ‘fat obesity’ based on BFMI have not yet been established.

**2.4 Health-related Changes in Body Composition Associated with Ageing**

Ageing is a process that has either direct or indirect consequences on multiple measures of body composition. Characterisation of these changes is important since progression of some or all of these features can often culminate in disease. Increases in adiposity, the loss of lean mass and bone health are associated with obesity, sarcopenia and osteoporosis respectively; disease states that can result in severe consequences for independence, functionality and quality of life. While the magnitude of change is likely influenced by gender, genetics and lifestyle, ageing itself is thought to have a prominent role. This section describes the current literature that observes the cross-sectional and longitudinal changes that occur in adiposity, lean mass and BMD with advancing age.

**2.4.1 Changes to Adiposity with Age**

Ageing is generally associated with an increase in BFM. This increase has been observed to occur up until the age of ~60 to 70 years in men and women, when it begins to decrease again. This trend has been reported in cross-sectional DXA studies on Americans (NHANES; Kelly *et al* 2009), Italians (Coin *et al* 2008; Bazzocchi *et al* 2012), Spanish (Henche *et al* 2008) and Australians (Shaw *et al* 2007) to varying extents and described in more detail below. Increases in adiposity during maturation are suggested to be due to a chronic positive energy balance throughout the lifetime, resulting from subtle decreases in physical activity and basal metabolic rate that are not matched with decreases in energy intake (Enzi *et al* 1986, Kuk *et al* 2009). However in older age, a failure to adequately regulate food intake due to altered hormonal and neurotransmitter regulation and side-effects of medication can cause a physiologic ‘anorexia of aging’ and decrease in adiposity (Morley 1997).
The NHANES study population, with 9,304 DXA records on 8-85 year olds, observed a peak BFMI (8.8kg/m$^2$ in men, 12.1 kg/m$^2$ in women) at ~65 years of age and a subsequent decline in BFMI in American men and women thereafter (Kelly et al 2009). Coin et al (2008) has also used BFMI to describe the change in Italian body composition by decade, and detected a peak value at age 70 years in 1,866 participants. However, peak BFMI was much lower than measured in Americans (7.1 kg/m$^2$ in men, 9.7 kg/m$^2$ in women). Using an absolute measure of fat, Henche et al (2008) observed a peak BFM of 27.7kg in men at the age of 65, and a peak BFM of 35.9kg in women at the age of 55 in 1,113 Spanish participants aged 0-80 years. However, results of this study are interpreted with caution since the use of 16 age categories reduced the number of participants in each band. Shaw et al (2007) examined 731 older Australian adults aged 50-79 years to determine the change in fat with older age. Peak BFM was found to occur at the 50-59 year age band in men (26.0kg), and slightly older at the 60-69 year age band in women (32.6kg). Despite this plateau, the authors concluded that adiposity continues to increase with age since they observed that BF% continued to rise. However, this is likely due to a decrease in LTM. Therefore, careful interpretation of ageing studies reporting BF% is advised.

Prospective longitudinal studies that track body composition changes over time offer an improved understanding of fluctuations in adiposity as a result of age. This is due to the secular trend, or birth cohort effect that may bias results when analysing cross-sectional change caused by age. Generations born in later years are likely to have different physical activity, eating and lifestyle habits than those born in earlier years. This is highlighted in a study by Ding et al (2007), who analysed the effect of birth cohort on changes in 1,786 70-79 year old adults in the Health, Ageing and Body Composition (Health ABC) Study from 1997 to 2003. Later cohorts (i.e. participants born in later years) were found to have a greater BFM (0.45kg in men, 0.34kg in women per birth year, p<0.001) than did earlier cohorts. This observation may suggest that the increase in adiposity with ageing concluded in many cross-sectional studies is over-estimated.

The Fels Longitudinal Study (Guo et al 1999) recorded serial measures BFM and FFM in 102 men and 108 women from the USA aged 40-66 years using underwater weighing. Although length of follow-up was extensive (1-20 years), there were very few numbers in the higher age categories (e.g. at age 66 years, n=6). In men, peak BFM was reached at age 64 years (31.4kg), while in women peak BFM was 27.7kg at age 66. Since no data was available
in participants >66 years, this may not be a peak value. Jackson *et al* (2012) undertook a large analysis on 7,265 American men with multiple body composition measurements using hydrostatic weighing and skinfolds from the age of 20 to 80 years. A linear mixed regression model showed BFM to increase from 20 years and level off at 80 years. Analysing mean trends by age decade, BFM appeared to plateau from age 50-70 years (18.3kg) and decline slightly thereafter. Regardless of observation type, BFM results from these studies appear to vary from peak values in cross-sectional studies. Apart from influence of a secular trend, this is likely due to the method of measurement. Long term prospective studies using DXA are lacking and only encompass 1-5 years of measurement. Gallagher *et al* (2000) analysed DXA-measured BFM in 24 men and 54 women aged 60-90 years over a 5-yr period. On average, men gained 0.3kg/yr and women lost 0.1kg/yr. Visser *et al* (2003) analysed 2,040 70-79 year olds from the Health ABC study at baseline and after a 1 and 2-yr follow-up. On average, men gained 0.1kg/yr and women lost 0.2kg/yr of BFM. Whilst useful for predicting trends in BFM, longer term DXA studies are required to measure rate of loss at different decades of age.

Changes with age do not only occur in the amount of adiposity, but also the distribution. The increase in abdominal or visceral deposition has important clinical implications since VAT is related to negative health outcomes in men and women independent of ages and race/ethnicity (Kuk *et al* 2009). A cross-sectional using CT by Kotani *et al* (1994) reported that visceral fat increases at rates of 0.39% per annum in middle-aged men. Pre-menopausal women increase at a much slower rate (0.15% per annum) until menopause, after which their visceral fat accumulation mirrors that of men. Also observed was a decrease in subcutaneous fat in the abdomen, thighs and calves. Similarly in obese women, Zamboni *et al* (1997) reported an age-dependent increase in visceral abdominal fat, and decrease in subcutaneous abdominal fat despite no significant BMI changes. Longitudinal assessments using CT on elderly participants have all observed that VAT continues to increase with age in men aged 30-72 years (Matsushita *et al* 2012) and women pre- and post-menopause (Franklin *et al* 2009). In this study and others (Sumino *et al* 2012), the menopause-induced oestrogen deficiency has been shown to be associated with an acceleration of visceral fat accumulation.
2.4.2 Changes to Lean Mass with Age

Fat-free or lean mass is seen to reach peak mass at the age of ~30 years, with declines thereafter reflective of declines in total body potassium, an index of body cell mass (He et al 2003). Sarcopenia has been defined as the loss of skeletal muscle mass and function that occurs with advancing age. Prevalence in 60–70-year-olds is reported as 5–13%, while the prevalence ranges from 11 to 50% in those >80 years (Morley et al 2008). The loss of LTM with age is often judged alongside the loss of appendicular LTM (ALTM) due to the decline in functionality of the limbs and ability to carry out activities of daily living (ADL).

Cross-sectional studies that have described the loss of LTM with ageing have shown less variation between populations than was shown with adiposity. Age of peak FFM or LTM in men and women was found to be between ages 30-40 years in Americans (Kelly et al 2009), Italians (Coin et al 2008) and Australians (Shaw et al 2007). Loss of ALTM or ALTM Index (ALTMI) was additionally measured by Kelly et al (2009) who found a peak ALTMI at age 40 in men (9.1 kg/m$^2$) and women (7.0 kg/m$^2$).

Longitudinal studies using two-component models have reported a rate of FFM decline of between 0.06 and 0.11kg/yr in men and women respectively (Guo et al 1999), and 0.07kg/yr in men from maturation to old age (Jackson et al 2012) An accelerated loss of 0.14kg/yr from age 61-71 years is observed in men (Jackson et al 2012). Prospective studies on older adults using DXA have observed a greater annual decline in men (0.25kg/yr) compared to women (0.05kg/yr) aged 70-79y (Visser et al 2003). In this study, men lost 0.10kg/yr of ALTM compared to a gain of 0.01kg/yr in women, while a previous study by Gallagher et al (2000) reported an annual loss of ~0.20kg/yr in men compared to ~0.10kg/yr in women over age 60 years by DXA. These results suggest that women preserve total and appendicular LTM better than men, likely due to larger initial muscle mass in men.

Classification of sarcopenia in terms of lean mass has been described by Baumgartner et al (1998), using a young adult reference base from the Rosetta study (229 Caucasian men and women aged 18-40 years). Sarcopenia was defined as ALTMI being less than two standard deviations below the mean of the young reference group (7.3kg/m$^2$ in men, 5.5kg/m$^2$ in women). Defined in this way, sarcopenia was significantly associated with physical disability in a population-based survey of 883 elderly Hispanic and non-Hispanic white men and women living in New Mexico. This association and was independent of ethnicity, age,
comorbidity, health behaviours and fat mass. Newman et al (2003) developed ranges based on the lowest 20th centile ALTMI of American 70-79 year olds in the Health ABC Study (1,435 men, 1,549 women). These ranges were similar (7.2kg/m² in men, 5.7kg/m² in women) to those developed by Baumgartner et al (1998), and were associated with smoking, poorer health, lower activity and impaired lower extremity function. These ranges, in combination with a gait speed of less than 1 m/s, were undertaken by the International Working Group on Sarcopenia as part of consensus definition (Fielding et al 2011).

Many authors suggest that adiposity should be considered in the estimation of prevalence of sarcopenia, since an increase in adiposity can co-occur with a decline in LTM. Often termed as ‘sarcopenic obesity’, the cumulative risk derived from each of the two individual body composition phenotypes increases the predictive value for disability (Prado et al 2012). Changes in muscle composition are also important, e.g. fat infiltration into muscle has been shown to lower muscle quality and work performance (Visser et al 2003). In the definition of sarcopenia, ALTMI additionally adjusted for fat mass was found to be strongly associated with impaired lower extremity function in women in the Health ABC Study (Newman et al 2003). Since no prospective studies on defining thresholds for sarcopenia are yet available, consensus guidelines await confirmation. However, the ISCD has recommended that ‘low LTM’ could be defined using ALTMI with Z-scores derived from a young adult, race and gender-matched population (Petak et al 2013).

The loss in lean mass is due to a decline in both muscle fibre size and type, with a preferential loss of type II fibres with advancing age. Factors involved in pathogenesis are numerous, and may include genetic heritability, nutritional status (protein intake, energy intake, and vitamin D status), physical activity, hormonal changes (declines in serum testosterone and growth hormone), insulin resistance, atherosclerosis and changes in circulating pro-inflammatory cytokines (Fielding et al 2011). The extent to which each of these factors may contribute to the onset of sarcopenia is beyond the scope of this review. Regardless of definition, the decline of lean mass with age is an important area of research since sarcopenia represents an impaired state of health that is associated with mobility disorders, risk of falls and fractures, impaired ability to perform ADL, disabilities, loss of independence and increased risk of death (Cruz-Jentoft et al 2010).
2.4.3 Changes to Bone Mineral Density with Age

Given current demographic changes in the world, osteoporosis is becoming an important socio-economic problem. Annual incidence of hip fractures in Europe has been estimated as 179,000 for men and 611,000 for women, while costs of all low-energy fractures are almost 25 million per year (Melton et al 2003). Within each bone metabolic unit bone formation by osteoblasts and bone resorption by osteoclasts is coupled tightly in a delicate balance to maintain bone mass and strength to resist deformity. With aging this balance shifts in a negative direction, favouring greater bone resorption and less bone formation. This combination of bone mass deficiency and reduction in strength ultimately results in osteoporosis and fractures.

Looker et al (2013) reported total body BMD (TBMD) data for 28,454 persons aged 8 years and over who participated in the 1999–2006 NHANES survey. They found TBMD remained relatively stable in young adulthood until approximately age 50, after which it declined with age in both sexes. An analysis of site-specific BMD of NHANES 2005-2008 was carried out by Looker et al (2012). Lumbar spine BMD increased until early adulthood. After age 50 years, lumbar spine BMD remained stable or increased slightly in men but declined in women (Figure 2.4.1). Both femoral neck and total proximal femur BMD declined after adulthood for both sexes.
Changes in site-specific BMD have also been described in longitudinal studies such as the Framingham Osteoporosis Study (Hannon et al 2000). This US population-based study took repeat BMD examinations over 4 years on 765 men and women aged 67-95 years and reported a -0.9% and -0.4% loss of BMD per annum in women and men respectively at the femoral neck, while loss in the lumbar spine site was -1.1% in women and -0.8% in men. An accelerated period of bone loss has been shown to occur in women post-menopause. This was shown by Zhai et al (2008) in a 15-yr prospective study on 955 women aged 45-68 years at baseline. A linear decline in femoral neck BMD (-1.7% per annum) was observed throughout the study, while a quadratic decline was observed in the spine (-3.1% per annum). This accelerated period slowed again by 0.02% per squared age increase.

The clinical significance of osteoporosis rests with the fractures that arise as a consequence of the condition, and their consequent morbidity and mortality. Many prospective studies using DXA, particularly in elderly women, indicate that the risk of fracture about doubles for
2.4.4 Summary

This section described the health-related changes in body composition that occur through age - from maturation to decline. A review of the available literature suggests BFM increases linearly with age until ~60-70 years where a plateau and subsequent decline is observed. Despite a decrease in total adiposity, distribution of fat is seen to change from a gynoid to android or abdominal distribution. This has clinical implications due to the shift from subcutaneous to visceral adiposity, a change which has been associated with increased risk of cardiometabolic disease. The decline in lean mass with age (sarcopenia) has been observed to begin after age 30 years in men and women, with a more substantial decline occurring in men, particularly after the age of 60 years. The decline in bone health (osteoporosis) appears to affect women more than men, due to the effect of oestrogen loss during and after menopause. Knowledge of these trends is worthwhile for timely planning of interventions to offset the development of age-related disease. Early implementation of a physical activity, nutrition and lifestyle change may be successful in reducing prevalence of largely preventable non-communicable disease.

2.5 Adiposity and Bone Health

Obesity and osteoporosis, two disorders of body composition, are becoming increasingly deleterious public health concerns in Ireland and throughout the world. Since both disorders can become more prevalent with advancing age, it is important that we recognise the complex relationship that exists between the two. The negative effect of adiposity on health outcomes such as CV disease, metabolic syndrome, diabetes and muscle quality has been long established. However, the relationship between fat and bone health has only recently become a topic for debate. Previously, excess adiposity was recognised to exert a positive influence on bone health due to physical loading, from studies which described a correlation between higher body mass/BMI and BMD (De Laet et al 2005, Reid et al 1992). However, overweight status and obesity represent consequences of a gain in BFM as well as an increase
in LTM. Therefore, identification of the specific roles adiposity plays in bone regulation is important. With the recognition of fat as a metabolically active endocrine organ, the effect of total and visceral fat mass on bone may extend beyond its mechanical load on the skeleton (Bhupathiraju et al 2011). The relationship between both total and visceral adiposity and BMD will be reviewed, along with potential pathways for non-mechanical interaction.

2.5.1 The Relationship between Total Adiposity and Bone Mineral Density

Ample evidence supports the view that fat mass, a component of total body mass and one of the most important indices of obesity, has a beneficial effect on bone health, increasing BMD and reducing the risk of osteoporosis. Skeletal tissue is highly responsive to its mechanical environment, and any increase in loading stimulates bone formation by decreasing apoptosis and increasing the proliferation and differentiation of osteoblasts and osteocytes (Ehrlich et al 2002). However, the association between BFM and bone in non-weight bearing as well as weight bearing sites (Reid et al 1992), along with the recognition of adipose tissue as an endocrine organ, suggests the adipose-bone link operates through multiple pathways.

Extensive data over the last three decades has cross-sectionally investigated the influence of BFM on bone, with results depending largely on the type of analysis undertaken and the population under investigation. Since bivariate or multiple linear regression is the most powerful analysis for determining the strength of association between body composition variables, studies undertaking this analysis appropriately in the relation between adiposity and bone will be the focus for this review.

This type of analysis was first carried out by Reid et al (1992a,b), who found a positive association ($R=0.5$) between BFM and total body and site-specific (lumbar spine, proximal femur) BMD ($p<0.001$) in 140 healthy post-menopausal women. When analysis was repeated, BFM had a significant positive association with bone in 68 pre-menopausal women, and had no association with bone in 51 men. Several more recent groups have repeated this enquiry in various populations with larger cohorts. With and without adjustment for LTM, the positive association with total and site-specific BMD has been found to be both significant (Chen et al 1997, Mizuma et al 2006, Ho-Pham et al 2010, Fu et al 2011, Park et al 2012) and non-significant (Kawamura et al 2011) in normal post-menopausal women in
Asian and American cohorts (n=50-267), and significant in osteoporotic post-menopausal women at the femoral neck only in a Polish cohort (n=92) (Dytfeld et al 2011). A consistent significant positive association (p<0.05) between BFM and total and site-specific BMD has been found in healthy pre-menopausal woman in multi-ethnic cohorts (African-American, Caucasian, Latino and Asian) (n=260-921) (Wang et al 2005, Mizuma et al 2006, Fu et al 2011, Park et al 2012, and in one study specifically on men (n=762) (Park et al 2012).

The potential beneficial effects of increased BFM on BMD conflict with the adverse effects of excess adiposity on various health outcomes, necessitating more specific evaluations of the association between each body component and BMD. Conclusions from the above studies may be confounded by the mechanical loading effects of total body mass on the skeletal system. In a cross-sectional study, Zhao et al (2007) measured body composition in a large sample (n=6477) of different ethnicity (Chinese and American Caucasians), gender and age (19-90y). A positive association between BFM and BMD at the lumbar spine and femoral neck was initially found. However, when the mechanical loading effect of total body mass was statistically adjusted for by its inclusion in the regression model, BFM became negatively associated (β=-0.12, p<0.01) with bone mass, suggesting that high fat mass may actually have a detrimental effect on bone. Similar results were found by Choi et al (2010) and Bhupathiraju et al (2011) in Korean (n=461) and Puerto-Rican (n=629) cohorts respectively after dividing participants into men (β=-0.008 to -0.146, p<0.05) and women (β=-0.006 to -0.215, p<0.05) and adjusting for factors such as age, smoking, regular alcohol consumption, regular exercise, calcium intake, plasma 25-hydroxyvitamin D status, menopausal status, height and body mass. In a recent Korean study by Yoo et al (2012), healthy participants (n=502) were sub-divided by gender and menopausal status. After adjustment for age and body mass, the influence of BF% on BMD at the lumbar spine (L1-L4) was found to be significantly negative only in pre-menopausal women (β=-0.38, p<0.01) and not post-menopausal women or men.

Although DXA-measured areal BMD is the most common clinical measurement in the diagnosis of osteoporosis, it does not distinguish between cortical and trabecular bone compartments. Risk factors for fracture are also influenced by bone quality, as a composite of bone structure, composition, microarchitecture, and micro-damage which contribute to bone strength independently of BMD (Seeman et al 2003). Sukumar et al (2011) found differences in trabecular and cortical bone in the distal tibia that were detected using pQCT that were not
observed with areal BMD analysis in 211 women aged 25-71y. BFM had a positive influence on trabecular vBMD ($\beta=0.41$, $p<0.001$) and a negative influence on cortical vBMD ($\beta=-0.2$, $p=0.007$) after adjustment for age, parathyroid hormone (PTH) and 25-hydroxy-vitamin D (25OHD). Additionally, when pre- and postmenopausal women were analysed separately without age in the model, BFM explained greater variance for all bone variables in pre-menopausal women ($R^2\leq62\%$) compared post-menopausal women ($R^2<35\%$). Similar results were found by a second American study (Ng et al 2013) in 218 women and 291 men. After adjustment for body mass, BFM was found to have a negative association with cortical vBMD in the femoral neck and cortical thickness in the lumbar spine in post-menopausal women ($p<0.05$) and a negative association with cortical vBMD in the femoral neck, cortical area in the ultradistal radius and trabecular vBMD in the lumbar spine in young men (age 20-49y) ($p<0.05$). In contrast to Sumukar et al (2011), no significant associations were found for pre-menopausal women or older men. These findings may be important in explaining the apparent discrepancy in studies examining the effect of BFM on areal BMD. Low cortical vBMD over a longer duration may compromise bone quality and explain findings of increased fracture risk in obese individuals despite normal areal BMD (Holmberg et al 2006, Hsu et al 2006, Premoar et al 2009).

The qualitatively different relationship between fat mass and bone mass appears to be dependent on whether bone mass is unadjusted or adjusted for total body mass, with a predominant positive association seen in unadjusted analysis and negative association found in adjusted analysis. While recent large studies that have contended that mass-adjusted fat mass has a deleterious effect on bone, a review by Reid (2010) has argued that some of these analyses are confounded by the co-linearity between the variables studied, and therefore have produced misleading results. Because of strong co-linearity between fat mass and body mass, most epidemiologic studies that had small sample sizes could not reliably explore the effects of fat mass on bone independent of body mass, unlike the studies mentioned above. Therefore, readers should use caution when interpreting results of studies using multiple regression analysis, ensuring that formal statistical tests for the influence of multi-co-linearity are carried out to ensure analysis is sound. Also, no longitudinal or intervention studies to date have correctly adjusted for body mass in a fat-bone analysis. Therefore, a cause-effect relationship cannot be established.
2.5.2 The Relationship between Visceral Adiposity and Bone Mineral Density

It is suggested that the function of adipose tissues may depend on the location of fat deposition. Ageing is not only associated with increased adiposity, but also a redistribution in the pattern of adiposity (Kuk et al 2009b, Leahy 2011). A preferential increase in abdominal fat, in particular VAT, combined with a decrease in lower body SAT are commonly cited in the literature. It is likely that the varied results describing a relationship between total adiposity and bone are due to the inability of these surrogate measures to distinguish VAT from other tissues. This is particularly important since visceral fat is known to be metabolically distinct from subcutaneous fat, being more strongly associated with metabolic disease (Wajchenberg 2000) and associated with increased risk for mortality above and beyond that associated with total adiposity (Bigaard et al 2005).

Several studies have attempted to evaluate the relationship of VAT with bone using surrogate measures of VAT - that include waist circumference, WHR and android fat measured by DXA. Evidence regarding the association between central obesity and BMD has been reported as both positive (Nguyen et al 2005, von Muhlen et al 2007) and negative (Choi et al 2010, Kim et al 2009, Zillikens et al 2010, Fu et al 2011, Bhupathiraju et al 2011), likely due to the inability of these measures to distinguish visceral from subcutaneous fat. Therefore, this review focuses on studies reporting measurement of VAT through gold standard measurement tools (e.g. CT and MRI) in adults.

Gilsanz et al (2009) was the first to distinguish optimally measured VAT from SAT using CT in 100 healthy American women aged between 15 and 25 years. They found that VAT at the umbilicus was negatively associated with the structure and strength of the femur (femoral cross-sectional area, cortical bone area, principal moment maximum, principal moment minimum, and polar moment (all p values <0.03)). Interestingly, while VAT was found to be a negative predictor of bone health, SAT was observed to have a positive protective effect on bone (p<0.01), using leg length and thigh musculature as covariates. Post-estimation procedures were used to exclude any possibility of multi-collinearity in the regression model. Thus, these results may explain the large body of conflicting data describing the relationship between total fat or total abdominal fat and bone to date.

Studies describing the effect of VAT on DXA-measured areal BMD found similar results. Choi et al (2010) used CT to describe both VAT and SAT at the L4-L5 region in both males
and females aged between 18 and 83 years and subsequently compared results to BMD at the lumbar spine, femoral neck and total hip. Regression analysis adjusting for body mass showed that VAT had a significantly negative relation with BMD at all sites in both sexes. Contrasting to Gilsanz et al (2009), SAT showed no statistically significant relationships with BMD at any site except one negative relation measured at the lumbar spine in men. In Caucasian and African-American adults (n=1,081), Katzmarzyk et al (2012) found VAT and SAT to be negatively related to TBMD in the total sample as well as in all sex-by-race groups, after adjustment for lean tissue mass and menopausal status in women.

Finally, three recent studies have investigated the effect of VAT on QCT-measures of bone microarchitecture and mechanical properties in different age groups and gender. VAT was a negative predictor of trabecular volumetric density, trabecular thickness and failure load in obese young men at the distal radius (p=0.002-0.04) (Bredella et al 2012) and a negative predictor of total, trabecular and cortical volumetric density at the femoral neck and lumbar spine in healthy young men (p<0.01) (Ng et al 2013). Similar significant (p<0.01) results were found for post-menopausal women but not healthy older men or pre-menopausal women (Ng et al 2013). However, obesity may play a role in this relationship as trabecular BMD (lumbar spine) in obese pre-menopausal women was significantly negatively influenced by VAT, independent of age (p=0.003) (Bredella et al 2011). Thus, VAT would appear to negatively influence bone quality in young men and post-menopausal women, while the association in pre-menopausal women may be dependent on BMI.

2.5.3 Adiposity and Bone: Potential Mechanisms of Interaction

Studies of adipocyte function have revealed that adipose tissue is not just an inert organ for energy storage, but is considered an active endocrine organ which modulates energy homeostasis. Several potential mechanisms have been proposed to explain the relationship between adiposity and bone through modulating activity of bone cells. However, this relationship appears to be complex and dynamic in nature, occurring through a multitude of potential pathways that may be multi-directional in effect.
2.5.3.1 Bone-active hormones

The prevailing paradigm in skeletal biology is that differentiation and functions of the two bone-specific cell types, osteoblasts and osteoclasts, are determined by secreted molecules that can either be cytokines acting locally or hormones acting systemically (Lee et al 2007). Bone remodelling, the process whereby bones renew themselves, is regulated by multiple hormones.

2.5.3.1.1 Beta Cell Hormones

The secretion of bone-active hormones from the pancreas (including insulin, amylin, and preptin) is increased in obesity, and may partly explain the relationship between adiposity and BMD. Insulin is a potential regulator of bone growth, since osteoblasts have insulin receptors (IR) as well as insulin-like growth factor 1 (IGF-1) receptors, which can also mediate the effects of insulin.

Clinical, *in vivo*, and *in vitro* studies all suggest that insulin acts as an anabolic agent and improves bone formation via pro-osteoblastic mechanisms (Thrailkill et al 2005). Mice lacking IR in osteoblasts have low circulating undercarboxylated osteocalcin and reduced bone acquisition due to decreased bone formation and deficient numbers of osteoblasts. With age, these mice develop marked peripheral adiposity and hyperglycemia accompanied by severe glucose intolerance and insulin resistance (Fulzele et al 2010). These results indicate the existence of a bone-pancreas endocrine loop through which insulin signaling in the osteoblast ensures osteoblast differentiation and stimulates osteocalcin production, which in turn regulates insulin sensitivity and pancreatic insulin secretion. *In-vivo* laboratory studies have shown amylin and preptin, two hormones that are co-secreted with insulin, to have similar influence on bone in mice (Cornish et al 2009), and therefore may reinforce the direct effect of insulin on bone. Thus, the β-pancreatic cell exerts a cluster of anabolic effects on bone, all of which are accentuated with high fat mass.

In humans, circulating insulin levels are positively related to femoral neck BMD in normal postmenopausal women, independent from that of adiposity, with no association at the total body or femoral trochanter (Reid et al 1993). However, a recent study by Sayers et al (2012) found that insulin levels are inversely related to tibial cortical BMD in adolescents after accounting for associated differences in body composition. Investigating specifically markers of bone turnover, Basu et al (2011) found that acute changes in insulin levels, as would occur
during meals and over the course of a day, do not regulate bone turnover, undercarboxylated osteocalcin, or osteoprotegerin levels in humans. This data does not exclude the possibility that physiological variations in insulin may regulate bone metabolism over a longer time frame.

Insulinopenia as occurs in type 1 diabetes (T1DM) or resistance to the metabolic actions of insulin as occurs in type 2 diabetes (T2DM), are both associated with several deleterious consequences for skeletal health. Low BMD and diabetic osteoporosis is increasingly recognised as a significant comorbidity of T1DM (Kemink et al 2000). In contrast, T2DM, a state of hyperinsulinemia, is typically associated with increased bone density, yet seemingly decreased bone strength contributing again to an increased risk of fracture (Strotmeyer et al 2004, Schwartz et al 2001). The persistence of fracture risk in these states may suggest a threshold for insulin in promoting healthy bone. This clearly demonstrates the complex nature of the insulin-bone interaction.

2.5.3.1.2 Oestrogen

Oestrogen, a well-known hormone with a bone-protecting effect, is produced by adipocytes, with subcutaneous preadipocytes having higher aromatase activity in comparison to visceral ones (McTernan et al 2002). Reid et al (1992) confirmed a relationship between circulating oestrone levels and bone density in pre-menopausal women, but has shown this to be both independent of the effects of BFM and substantially weaker. This implies that oestrogen is not the only pathway by which adiposity influences bone density, particularly in premenopausal women, in whom the adipocyte is a relatively minor source of oestrogens. However, adipose tissue is the principle source of oestrogen in postmenopausal women, so it is not surprising that BFM is significantly correlated with BMD and hip fracture in women over age 50–55 years.

2.5.3.1.3 Adipokines

Obesity may affect bone metabolism directly or indirectly through adipocyte-derived cytokines (adipokines) such as leptin and adiponectin. Obesity is associated with significant increase in serum leptin (Considine et al 1996) and decrease in adiponectin (Arita et al 1999). Leptin has been found to have both a positive and negative effect on bone dependent on the mode of action (central or peripheral effects). Systemic administration of leptin in intact or leptin-deficient animals results in increases in bone formation and skeletal mass and reduced
bone fragility (Cornish et al 2002). Leptin directly increases proliferation and differentiation of osteoblasts and inhibits osteoclastogenesis through reducing the expression of nuclear factor κ ligand (RANKL) and increasing osteoprotegerin (OPG) levels. However, indirect effects of leptin on bone were discovered by infusion of leptin into the third ventricle of the brain (Ducy et al 2000). With central administration, leptin caused bone loss in leptin-deficient and wild-type mice through inhibition of bone formation and stimulation of bone resorption.

In human studies, the relationship between leptin concentration and BMD is unclear. No significant association of leptin concentration with bone density, independent of hormonal influence was found in men, premenopausal or postmenopausal women using various definitions of bone mass and risk of fracture (Jurimae et al 2005, 2008, Ruhl & Everhart 2002, Barbour et al 2011).

Adiponectin is another cytokine secreted by adipocytes and has an anti-inflammatory effect. In animal studies, adiponectin has been reported to inhibit osteoclastogenesis, reduce bone resorption, and increase bone mass in trabecular, not cortical bone (Oshima et al 2005). Adiponectin has been shown to have different effects in humans and also between men and women, although evidence pertaining to BMD is conflicting. In the Rancho Bernardo study (Araneta et al 2009), it has been shown that adiponectin was inversely associated with DXA-measured BMD in both men and women, but it was associated with vertebral fractures in men only. However, Napoli et al (2010) found a significant inverse association between serum adiponectin and total, trabecular and cortical bone in women only. While in-vitro studies on the effect of adiponectin on bone cells yield contradictory results, the majority of available data suggest that adiponectin has an anabolic effect on osteoblasts and inhibits osteoclastogenesis, likely independently of RANKL and OPG which are two critical factors in osteoclast differentiation (Williams et al 2009).

2.5.3.2 Inflammatory Markers

Cytokines operate in an intricate system that involves both pro-inflammatory and anti-inflammatory components. The balance between these two systems plays an important role in the regulation of both osteoblasts and osteoclasts. Adipose tissue is known to produce pro-inflammatory cytokines that are linked with ageing, cardiovascular and chronic disease (Tracy 2003), with VAT associated with release of greater amounts of cytokines than
subcutaneous tissue (Fain et al 2004). Both prospective and cross-sectional analyses have
indicated that higher circulating concentrations of pro-inflammatory cytokines—including C-
reactive protein (CRP), interleukin (IL)-1, IL-2, IL-6, IL-11, IL-15, IL-17 and tumour
necrosis factor-alpha (TNF-α) stimulate osteoclastic bone resorption (Mundy 2007) and are
associated with lower BMD in postmenopausal women (Lee et al 2011) and greater fracture
risk in older women and men (Cauley et al 2007). In the latter Health ABC study, median
levels of cytokines were 6–12% higher among subjects who experienced an incident fracture
in comparison with those who did not fracture, and the association was particularly strong for
subjects who had elevations in two or three markers. There is also evidence from both human
and animal studies to suggest bone loss associated with menopause may be linked to
activation of osteoclasts by pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α
(Mundy 2007). It is feasible that this is the pathway by which VAT operates to negatively
influence bone turnover and BMD.

2.5.3.3 Bone Marrow Fat

Adipocytes and osteoblasts originate from a common progenitor, the pluripotential
mesenchymal stem cell within bone marrow, which has an equal propensity for
differentiation into adipocytes or osteoblasts under the influence of several cell-derived
transcription factors. Although it remains unclear whether adipocytes and osteoblasts respond
in a unique and possibly opposite manner, this relationship can ultimately determine the
balance between adipose and bone tissue.

Several factors have been shown to influence the mesenchymal stem cell into either bone or
fat cells. The peroxisome proliferator–activated receptor-γ (PPARγ) plays a central role in
initiating adipogenesis and inhibiting osteoblastogenesis, with PPARγ-agonists such as the
thiazolidinedione class of anti-diabetes drugs (rosiglitazone and pioglitazone) causing bone
loss and increasing fracture risk by shifting stem cell differentiation into the fat lineage at the
expense of bone formation (Rzonca et al 2004).

Higher content of vertebral marrow fat has been found in subjects with morphologic evidence
of bone weakness and compression fractures, with age-related bone loss associated with
progressive infiltration of bone marrow by fat (Schellinger et al 2004). Using proton
magnetic resonance spectroscopy (1H-MRS), Bredella et al (2011) have found increased bone
marrow fat in premenopausal women with high visceral fat compared to women with low
visceral fat despite normal BMD (p=0.05), and an inverse association between vertebral bone marrow fat and trabecular BMD which remained significant after controlling for visceral adipose tissue (p = 0.03). Similarly, Shen et al (2012) demonstrated the inverse relationship between pelvic bone marrow fat and BMD to be present both in younger subjects (18-39.9 years) who have not yet experienced bone loss and also in older subjects (40-80 years).

Stem cell differentiation into the bone or fat lineage clearly influences skeletal homeostasis and skeletal fragility, suggesting a distinct role of bone marrow fat to the fat-bone relationship.

2.5.3.4 Vitamin D Interaction

Vitamin D is critical for bone development, function, and preservation through stimulation of calcium absorption and mobilisation, via parathyroid hormone (PTH) (Dawson-Hughes et al 2010). In cases of vitamin D deficiency, secondary hyperparathyroidism resulting in increased bone turnover and increased bone loss and ‘remodelling space’ may subsequently increase fracture risk (Lips 2001, Abrahamsen et al 2010). Greater adiposity is associated with lower blood levels of 25-hydroxyvitamin D (25OHD) (Parikh et al 2004). It is hypothesised that vitamin D (a fat-soluble vitamin) may get sequestered in the adipose tissue, leading to lower bioavailability in the obese state (Wortsman et al 2000). Another plausible explanation is that individuals with excess adiposity may partake in less outdoor activity leading to decreased sun exposure and therefore limiting endogenous production of vitamin D in the skin. However, adjustment for sun exposure habits has little effect on the estimates, indicating that differences in sun exposure do not explain the inverse association between 25OHD and adiposity (Harris & Dawson-Hughes 2007).

In Irish older adults (>64 years), BMI and skinfold-measured adiposity was found to have an inverse association with both baseline 25OHD and levels of 25OHD following a 22-week supplementation of 15 µg cholecalciferol per day (Forsythe et al 2012). However, no such associations were apparent in younger adults, at contrast to findings from similar studies on young men and women respectively (Frost et al 2010, Kremer et al 2009). Adiposity also appeared to attenuate the natural seasonal decline in vitamin D status over the winter months, when vitamin D synthesis is lacking. This finding may be attributed to the sequestration theory, as suggested by Wortsman et al (2000). Studies reporting these inverse relationships have also reported lower BMD, in conjunction with higher levels of PTH and bone-specific
alkaline phosphatase (BAP) in participants with inadequate vitamin D status and high adiposity (Frost et al 2010). Therefore, it is conceivable that the adipose-vitamin D interaction may have a negative effect on bone health.

2.5.4 Summary

It was previously believed that obesity and osteoporosis were two unrelated diseases, but recent studies have shown that both diseases share several common environmental and genetic factors. Adiposity is one of the most important indices of obesity, and a substantial body of evidence indicates that BFM may have beneficial effects on bone. Contrasting studies, however, suggest that excessive BFM may not protect against osteoporosis or osteoporotic fracture and may, in fact, be detrimental to bone health. Differences in experimental design, sample structure, and even the election of covariates may account for some of these inconsistent or contradictory results. Knowledge that both bone loss and VAT increase with ageing supports the notion that the deleterious effect of VAT spans throughout life. With the increase in ageing and obesity in the Irish population it is important to continue the effort in identifying the contribution of adipose tissue to BMD, bone quality and fracture.

2.6 Conclusion

Dual-energy x-ray absorptiometry offers accurate and precise health-related assessment of both total and regional body compartments. However, the precision of DXA to measure quantities of VAT less than 400g has not yet been quantified. In the expression of body composition metrics, there are limitations associated with use of BMI and BF% to define adiposity. The expression of total adiposity adjusted for height, or BFMI, may offer an improved representation. However, no consensus exists regarding the categorical criteria for the definition of adiposity. In the assessment of the health-related body composition changes associated with ageing, adiposity is observed to increase and change in distribution from subcutaneous to visceral depots. This is typically accompanied by declines in lean mass and bone mineral density. Data on age-related changes in Irish adults is currently limited to BMI, with no reference data available for DXA-measured body composition parameters. Most recent literature examining the inter-relationships between these parameters has reported
mixed evidence regarding the influence of adiposity on bone mineral density. While much advancement in body composition research have been made over the past number of years, efforts to improve the quality of evidence in this area are important in the accurate assessment of health.
Chapter 3

Methodological considerations for the measurement of body composition using dual-energy x-ray absorptiometry
3.1 Introduction

With the evolution of several technological advances in bone and soft-tissue imaging, dual energy X-ray absorptiometry (DXA) has become an accepted criterion measure of body composition since its clinical introduction in the late 1980’s. Originally developed for measurement of areal bone mineral density (BMD) to diagnose skeletal conditions such as osteoporosis, a secondary application of DXA is the assessment of soft-tissue composition i.e. fat and lean tissue mass. Both methods have reported accurate and precise measurement of composition in the most advanced DXA models (Baim et al 2008, Toombs et al 2012).

As the use of DXA for body composition measurement becomes more popular both clinically and in research institutes, there are certain methodological procedures that need to be highlighted and considered to minimise any potential measurement error. Where such procedures have not been reported or acted upon, caution should be reserved in the interpretation of results. The errors of repeated body composition assessments for a specific technique can be characterised by the difference of the true versus the mean measured value (i.e. the accuracy error), and the spread of the individual readings around the mean measurement (i.e. the precision error).

While the ability of the Lunar iDXA™ (GE Healthcare, Chalfont St Giles, Bucks., UK) to accurately assess body composition has not yet been tested against the gold standard four-component model, enhanced image resolution compared to past densitometers may suggest more accurate assessment of tissues. Accuracy is confirmed daily using a phantom spine developed for quality assurance and stability monitoring of DXA devices. Variability in DXA estimates of body composition can be divided into two types: technical error and biological variation (Lohman et al 2000). Technical or precision error is the variation generated by the instrument following calibration, or by failure to standardise positioning or regional analysis of the subject. Biological variation is the variation of the individual, which includes changes in hydration status of the tissues arising from the short-term effects of exercise and the effects of food and fluid intake in the hours before a scan. As a three-component model, a known limitation of DXA is the assumption of uniform hydration of fat-free mass. Algorithms assume electrolyte consistency and a fixed fat-free mass hydration constant of 0.73 (Wang et al 1999). Therefore a variation in hydration status can violate this assumption and alter the lean tissue mass estimation. Technical errors can also be discerned
when mass from the sum-of-parts (reconstituted mass) does not agree closely with scale mass, leading to inaccurate estimates of changes in body composition (Lohman et al 2000).

The purpose of this chapter is to review some of the methodological considerations in DXA scanning and to establish a protocol for accurate and reliable measurement of body composition. A standardised operating procedure is first described followed by a technical paper. The experimental aim of this technical analysis was to establish the precision of DXA to measure:

(i) Lean, fat and bone tissue mass at different regions;
(ii) Visceral adipose tissue at high (>400g) and low (<400g) quantities;
(iii) Bone mineral density at the lumbar spine and proximal femur.

3.2 **Standard Operating Procedure**

3.2.1 **Introduction**

The following is the standard operating procedure (SOP) followed for body composition measurement throughout this thesis. All participants were measured using DXA to assess lean tissue mass (LTM), body fat mass (BFM), visceral adipose tissue (VAT) and bone mineral content (BMC), with additional site-specific bone mineral density (BMD) scans carried out on participants aged >50 years. Total body water (TBW) was assessed by a bioelectrical impedance analyser (BIA). Differences to this methodology within specific studies are indicated in their respective chapters.

Ethical approval for this research was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18). The University of Limerick Body Composition Study (ULBC – www.ul.ie/bodycompositionstudy) is an on-going investigation of body composition measurement in adult Irish men and women aged 18 and over. Participants are recruited from the UL faculty, student body and surrounding community via email advertisement and word-of-mouth. Pregnant females are excluded from the study and all female participants are asked to confirm that the test date occurred
within 7 days of the onset of their last menstrual period to further minimise risk (Appendix A).

3.2.2 Pre-Scan Protocol

The following protocol was established in order to standardise test conditions and ensure adequate hydration levels for each participant and each scan. All procedures were in line with the official positions of the ISCD for body composition (Sheperd et al 2013) and BMD (Schousboe et al 2013) DXA scanning.

All participants included in the study were instructed to refrain from strenuous exercise in the 12 hour period before testing and to attend after an overnight fast or fasting for a minimum of 3 hours before the scan. Participants were required to consume 500ml of water one hour before testing and to empty their bladder immediately prior to measurement and defecate if required. Height was measured to the nearest 0.1cm using a stadiometer (Seca, Birmingham, UK) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyser, Tanita UK Ltd.).

3.2.2.1 Bioelectrical Impedance Analysis

Bioelectrical impedance measurement was carried out prior to DXA scanning in all participants. A multi-frequency bioelectrical impedance analyser (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.), set to use the ‘normal’ (nonathletic) proprietary algorithm, was used for the impedance measurement. Participants stood with the ball and heel of each foot in contact with the metallic electrodes on the floor scale. Once body mass was recorded, participants were instructed to grasp the hand grips held 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 the manufacturer’s instructions. The Tanita GMON software (v1.7.0) generated values for total body water (TBW). Root mean square coefficient of variance (RMS-CV% - see section 3.3) of the impedance measure was 1.9% for TBW.
3.2.2.2 Dual Energy X-ray Absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ v.14.1 software was used to capture whole body and bone density scans. Calibration by tissue phantom was undertaken as per the manufacturer’s instructions to confirm the reported accuracy of DXA instrument. The quality assurance trend graph for lean tissue is displayed in Figure 3.1.

![Figure 3.1](image)

**Figure 3.2.1 Quality assurance trend for lean tissue daily calibration of DXA**

For whole body scans, participants were asked to attend in light clothing without removable metal or jewellery and were positioned in supine on the scanner bed according to the manufacturer’s recommendations. Arms were positioned at either side with hands in a mid-prone position facing towards the body and slightly apart from the trunk. Gentle longitudinal traction was applied by the operator at the feet and head to align the spine in neutral. Where participants were too wide to fit within the boundary of the scan, the right hand side of the body was scanned and the results were doubled for the missing region. This procedure has been shown to be closely comparable \( R^2 > 0.99 \) to whole-body scanning by Rothney \textit{et al} (2009).

Where additional site-specific bone mineral density scans (lumbar spine and proximal femur) were carried out, participants were positioned in supine on the scanner bed with the
manufacturer’s hip positioning device between both ankles to internally rotate both femurs to ~15 to 25 degrees. For the lumbar scan, the laser light was positioned two inches below the iliac crests in the centre-line. Scans included the entire L1-L4 region of interest, including part of L5, T12 and the top of the iliac crests where possible. For proximal femur scans, the laser light was placed at mid-width, one hand-span below the iliac crest. The lateral aspect of the greater trochanter was centred vertically in the scan field, with an equal distance below the ischium and above the greater trochanter. Participants with hip replacements were excluded from analysis due to the confounding effect on BMD.

3.2.3 Regional Analysis

The enCORE™ software provided segmentation of the whole body into regions of interest (ROIs) as follows; with manual adjustments made where necessary (Figure 3.2): Arms; all tissue extending from a line drawn through the centre of the arm socket to the phalange tips, Legs; 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. Android; all tissue between the line joining the two superior iliac crests and extended cranially up to the 20% of the distance between this line and the chin, Gynoid; all tissue from a line joining the femoral greater trochanters, directed caudally to a distance double of the android region.

Visceral adipose tissue (VAT) within the android compartment was estimated in mass (kg) and volume (cm$^3$) using CoreScan™ (GE Healthcare, Madison, WI) software. The detection of the layer thickness of the subcutaneous adipose tissue (SAT) at sides of the android region allows the software to estimate the total SAT compartment. The amount of VAT is indirectly derived by subtracting SAT from the total android fat mass.

Site-specific L1-L4 BMD regional analysis was undertaken by ensuring all intervertebral lines were horizontal and mid-way between each vertebra. In Dual Femur analysis, the femoral neck box was centred and perpendicular to the axis line through the femoral neck, with a clear space between the ischium and the neck. Mean femoral neck, total hip and posterior-anterior lumbar spine (region L1-L4) were analysed from each scan using these
standard procedures (Baim et al 2008) (Figure 3.3). A summary of the recommended scanning protocol for standardisation of DXA scanning is presented in Table 3.2.1.

<table>
<thead>
<tr>
<th>Participant Instructions</th>
<th>Recommended Standardisation Protocol</th>
<th>Operator Instructions</th>
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</thead>
<tbody>
<tr>
<td>No moderate-strenuous exercise in previous 12 hours</td>
<td>Correct alignment and extremity positioning on scanner bed inside boundaries where possible</td>
<td>Daily calibration of machine</td>
</tr>
<tr>
<td>Fasted for minimum 3 hours</td>
<td>Correct manual adjustment of regions of interest (ROI) in post-scan analysis</td>
<td>Precision assessment after 100 scans</td>
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<tr>
<td>Light clothing – no metal/jewellery</td>
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<td>Osmolality or whole body water measurement in conjunction with scan to assess hydration</td>
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<tr>
<td>Intake of 500ml water 1 hour before scan</td>
<td></td>
<td>Check scale mass vs. reconstituted DXA mass to assess discrepancies</td>
</tr>
<tr>
<td>Urinate (defecate if required) immediately before scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lie still for scan duration</td>
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</table>
Figure 3.2.2 Dual Energy X-Ray Absorptiometry scan with segmental partitioning of the arm, leg, trunk, android (abdominal) and gynoid (hip) regions

Figure 3.2.3 Site-specific bone mineral density scan of the spine with segmental partitioning of the L1-L4 vertebrae (left); and right femur with segmental partitioning of the femoral neck, trochanter and total hip (right)
3.3 **Technical Analysis: The precision of dual-energy x-ray absorptiometry to measure body composition and bone mineral density at multiple regions**

### 3.3.1 Introduction

A standardised approach exists for DXA scanning to ensure the random (non-biological) error is kept to a minimum. Calculation of this precision error is critical in serial measurement of body composition or bone mineral density to monitor the change in body composition through longitudinal or intervention studies. Common sources of variation between scans that affect measurement precision include poor or inconsistent positioning and incomplete data acquisition. It is therefore recommended by the International Society for Clinical Densitometry (ISCD) that each technologist carries out an *in vivo* precision assessment after having performed approximately 100 scans to determine the precision error for BMD at that facility (Baim et al 2008). Recently, a similar recommendation has been made for body composition scanning (Hangartner et al 2013), without indication of prior technologist scanning experience. A second source of potential variation is inconsistent placement or sizing of regions of interest (ROI) during analysis. The importance of correct regional analysis is highlighted in a precision assessment by Rothney *et al* (2012), who found small but statistically significant differences between automatic and manual ROI placement in non-obese adults. Differences were small in whole body, leg, trunk, and android and gynoid regions (0.004-2.8%), but larger in the arm region (3.0-6.3%).

Measurement precision has important implications on study design, such as sample size determination and optimisation of monitoring intervals between measurements. Short-term precision refers to the precision obtained with a short time interval between tests (typically minutes or hours). Precision error is characterised by the root mean square standard deviation (RMS-SD) in g/cm² of a set of measurements, or the coefficient of variation (CV), the RMS-SD divided by the mean and expressed as a percentage. To accurately interpret serial measurements, knowledge of the least significant change (LSC) is necessary to govern confidence that true loss or gain in body composition parameters has occurred. Precision error for whole body fat tissue mass, lean tissue mass and bone tissue mass has been estimated as <1% (Toombs *et al* 2012) but is less well-defined for regional body
composition measurement by iDXA. The minimum acceptable precision for an individual technologist as set out by the ISCD is 3% for BFM and 2% for LTM (Hangartner et al 2012)

A new tool to quantify visceral adipose tissue (VAT) mass and volume with iDXA has recently been described (Kaul et al 2012). CoreScan™ (GE Healthcare, Madison, WI) is reported to have a strong association ($R^2=0.957$) with computed tomography (CT), examined in 124 men and women of varying age (18-90 years) and representing a wide range of BMI (18-40kg/m$^2$). CoreScan™ precision has been characterised by Rothney et al (2013) on an anthropometric imaging phantom across 10 iDXA systems and in a clinical population of obese women (n=32). Phantom scanning (VAT values 0-1800g) yielded a precision estimate of 47.6g for VAT mass, which corresponds to a 4.8% CV for a 1kg VAT mass. Repeat scanning of obese women (n=32) has shown a precision of 56.8g on an average VAT mass of 1110.4g, corresponding to a 5.1% CV and a non-significant bias of +15.4g. Precision error is yet to be evaluated in a human population of non-obese subjects. Those with very low quantities of VAT (e.g. athletes) or very high quantities of VAT (e.g. obese men) may yield different results.

Knowledge of precision error for BMD measurement is important since non-biological measurement variability can obscure the rates of true bone loss (0.5 – 2.0%/year) that occur in adults throughout their lifetime, and suggest bone loss when there has been no real biological change (Baim et al 2008). The minimum acceptable precision for an individual technologist is a coefficient of variance (CV) of 1.9% in the lumbar spine, 1.8% in the total femur and 2.5% in the femoral neck (ISCD Official Positions 2007).

The aim of this technical paper is to assess technologist precision for whole body and site-specific BMD, BFM and LTM measurement. Novel to the ISCD recommendations was an assessment of the precision of CoreScan™ to quantify VAT across a range of visceral adiposity.
3.3.2 Methods

3.3.2.1 Participants and Measurement

To determine whole body composition precision, repeat scans were carried out as described in Section 3.2 using a Lunar iDXA™ scanner. A convenience sample of 87 participants (48 women, 39 men) from the ULBC study were selected to take part in a second scan and asked to give consent in line with the ‘Precision Assessment Information for Patients’ as referenced in the White Paper of the International Society for Clinical Densitometry (Baim et al 2008) (See Appendix B). After the first scan was complete, participants were asked to stand up before being subsequently re-positioned for a second scan (~1 minute between scans). Precision was examined for the measurement of whole and regional LTM, BFM, BMC and VAT in the android compartment.

A similar protocol was established for repeat bone mineral density scans at the lumbar spine and dual femur after at least 100 scans had been completed by the operator, as recommended by the International Society for Clinical Densitometry (ISCD) (Schousboe et al 2013). This assessment was carried out on older adults (age >50 years), representative of the ageing cohort who received BMD scans in the ULBC study population. Two consecutive BMD scans were conducted for each participant, with re-positioning in between scans. All scanning and subsequent analysis was carried out by the author.

3.3.2.2 Statistical Analysis

Regional analysis was carried out as described in Section 3.2.3 with enCORE™ v.14.1 software. Dependent variables were extracted into Microsoft EXCEL (Office Professional Plus 2010) for further analysis. Precision results are reported as the root mean square standard deviation (RMS-SD), calculated as \( \sqrt{(\sum SD^2)/n} \); root mean square coefficient of variance (RMS-CV%), calculated as (RMS-SD/mean). Least significant change (LSC) at the 95% confidence interval (CI) was calculated as (RMS-SD*2.77). This is the recommended precision analysis for repeat scan analysis by the ISCD (Baim et al 2008). LSC was also calculated at the 80% CI (RMS-SD*1.81) (Gluer et al 1999).
3.3.3 Results

Participants included in the body composition precision analysis were deemed to be representative of the study population. 87 participants (48 women, 39 men) undertook repeat whole body DXA measurement (Mean (SD), range; age 25.7 (30.7), range 18-71y; BMI 24.7 (3.1), range 19-34kg/m\(^2\)). For BMD precision analysis, repeat measurement of 33 older participants (27 women, 6 men) (age 60.6 (5.7), range 50-72y; BMI 26.6 (3.9), range 20-35kg/m\(^2\)) was analysed.

Precision error is presented in Table 3.3.1 as RMS-SD and CV and least significant change (LSC) at 95% and 80% confidence interval. Precision error was <1.2% for whole body composition variables and <3.3% for regional measurements. LSC for 95% CI was established as 0.72kg for whole body LTM and 0.58kg for whole body FM. These values will determine levels of true biological change for future intervention research.

Six participants from the whole body scan precision analysis measured zero grams of VAT on at least one scan. These values were deemed to be below the lower limit of detection (LLOD) and were therefore excluded from any further VAT analysis. Remaining participants (n=81; 39 men and 42 women) had a mean age of 36.1 (17.8) years (range 18-71) BMI of 24.8 (3) kg/m\(^2\) (range 19-34) and mean VAT mass of 473 (437)g (range 0.1-1,963g). Precision error was calculated as 47g with a CV of 27.9% (Table 3.3.2). An additional analysis was carried out in participants with less than and greater than 400g VAT to confirm measurement reliability across varying levels of adiposity. The level of 400g was chosen to be comparable with the population assessed in Rothney et al (2013) (mean VAT 1110 (435); range 404-2,297g). Precision error was 31g (CV 35.5%) for VAT mass <400g and 64g (CV 8.4%) for VAT mass >400g.
Table 3.3.1 Precision of short-term repeat measurement of whole body composition of 87 adult subjects (47 women and 39 men) acquired by iDXA

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
<th>RMS-SD</th>
<th>RMS-CV%</th>
<th>LSC (95%)</th>
<th>LSC (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>Lean (kg)</td>
<td>51.3 (12)</td>
<td>31.8-75.7</td>
<td>0.26</td>
<td>0.55</td>
<td>0.718</td>
</tr>
<tr>
<td></td>
<td>Fat (kg)</td>
<td>20.0 (7.6)</td>
<td>8.6-38.5</td>
<td>0.21</td>
<td>1.20</td>
<td>0.583</td>
</tr>
<tr>
<td></td>
<td>BMC (kg)</td>
<td>3.0 (0.7)</td>
<td>1.8-4.4</td>
<td>0.02</td>
<td>0.52</td>
<td>0.043</td>
</tr>
<tr>
<td>Arms</td>
<td>Lean (kg)</td>
<td>6.1 (2.1)</td>
<td>3.1-10.9</td>
<td>0.16</td>
<td>2.07</td>
<td>0.453</td>
</tr>
<tr>
<td></td>
<td>Fat (kg)</td>
<td>2.2 (0.9)</td>
<td>0.8-5.0</td>
<td>0.07</td>
<td>3.33</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td>BMC (kg)</td>
<td>0.4 (0.1)</td>
<td>0.2-0.6</td>
<td>0.01</td>
<td>1.72</td>
<td>0.023</td>
</tr>
<tr>
<td>Legs</td>
<td>Lean (kg)</td>
<td>18.4 (4.7)</td>
<td>10.7-28.1</td>
<td>0.34</td>
<td>1.60</td>
<td>0.932</td>
</tr>
<tr>
<td></td>
<td>Fat (kg)</td>
<td>73.5 (2.8)</td>
<td>3.1-14.1</td>
<td>0.12</td>
<td>2.06</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>BMC (kg)</td>
<td>1.1 (0.3)</td>
<td>0.7-1.8</td>
<td>0.01</td>
<td>0.63</td>
<td>0.023</td>
</tr>
<tr>
<td>Trunk</td>
<td>Lean (kg)</td>
<td>23.7 (5.1)</td>
<td>15.2-34.4</td>
<td>0.37</td>
<td>1.52</td>
<td>1.029</td>
</tr>
<tr>
<td></td>
<td>Fat (kg)</td>
<td>9.6 (4.3)</td>
<td>2.9-19.9</td>
<td>0.20</td>
<td>2.22</td>
<td>0.560</td>
</tr>
<tr>
<td></td>
<td>BMC (kg)</td>
<td>0.9 (0.2)</td>
<td>0.5-1.5</td>
<td>0.01</td>
<td>1.49</td>
<td>0.038</td>
</tr>
<tr>
<td>Android</td>
<td>Fat (kg)</td>
<td>1.5 (0.9)</td>
<td>0.3-3.8</td>
<td>0.05</td>
<td>3.08</td>
<td>0.139</td>
</tr>
<tr>
<td>Gynoid</td>
<td>Fat (kg)</td>
<td>3.5 (1.4)</td>
<td>1.3-7.1</td>
<td>0.07</td>
<td>2.03</td>
<td>0.193</td>
</tr>
</tbody>
</table>

BMC, Bone mineral content; LSC (95%), Least significant change at 95% confidence interval; LSC (80%), Least significant change at 80% confidence interval; RMS-SD, Root mean square standard deviation; RMS-CV, Root mean square coefficient of variance

Table 3.3.2 Precision of short-term repeat measurement of visceral adiposity (VAT) of 81 adult subjects (39 men, 42 women) acquired by iDXA

<table>
<thead>
<tr>
<th>VAT (g)</th>
<th>N</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>RMS-SD</th>
<th>RMS-CV%</th>
<th>LSC (95%)</th>
<th>LSC (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0g</td>
<td>81</td>
<td>473 (437)</td>
<td>0.1-1,963</td>
<td>47.0</td>
<td>27.9</td>
<td>130</td>
<td>85</td>
</tr>
<tr>
<td>0.1-400g</td>
<td>48</td>
<td>201 (107)</td>
<td>0.1-391</td>
<td>30.5</td>
<td>35.5</td>
<td>85</td>
<td>55</td>
</tr>
<tr>
<td>&gt;400g</td>
<td>33</td>
<td>872 (422)</td>
<td>400-1,963</td>
<td>64.0</td>
<td>8.4</td>
<td>177</td>
<td>116</td>
</tr>
</tbody>
</table>

LSC (95%), Least significant change at 95% confidence interval; LSC (80%), Least significant change at 80% confidence interval; RMS-SD, Root mean square standard deviation; RMS-CV, Root mean square coefficient of variance; VAT, Visceral adipose tissue
Precision error was also calculated for site-specific BMD, and is presented in Table 3.3.3, ranging from 0.85-1.63% CV across sites.

<table>
<thead>
<tr>
<th>BMD (g/cm²)</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>RMS-SD</th>
<th>RMS-CV%</th>
<th>LSC (95%)</th>
<th>LSC (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-L4 Spine</td>
<td>33</td>
<td>1.133 (0.008)</td>
<td>0.862-1.596</td>
<td>0.010</td>
<td>0.85</td>
<td>0.028</td>
<td>0.018</td>
</tr>
<tr>
<td>Total Femur</td>
<td>33</td>
<td>1.021 (0.007)</td>
<td>0.785-1.215</td>
<td>0.014</td>
<td>1.63</td>
<td>0.040</td>
<td>0.026</td>
</tr>
<tr>
<td>Fem Neck</td>
<td>33</td>
<td>0.962 (0.009)</td>
<td>0.735-1.164</td>
<td>0.011</td>
<td>1.13</td>
<td>0.029</td>
<td>0.019</td>
</tr>
</tbody>
</table>

BMD, Bone mineral density; LSC (95%), Least significant change at 95% confidence interval; LSC (80%), Least significant change at 80% confidence interval; RMS-SD, Root mean square standard deviation; RMS-CV, Root mean square coefficient of variance

3.3.4 Discussion

Precision errors in body composition measurement are commonly reported on a percentage basis, i.e. as a coefficient of variation (CV) of repeated measurements. However, the apparent comparability of percentage units can be misleading. Therefore, this study undertook a precision analysis based on ISCD recommendations, utilising error terms based on RMS-SD and CV, while also determining levels that represent significant change through calculation of the LSC in units of measurement.

Precision values for body composition parameters using iDXA are comparable with those previously reported. RMS-CV for whole body composition measurement on the iDXA has been reported to be 0.4-0.5% for whole body LTM, 0.7-1% for whole BFM and 0.5-0.6% for whole body BMC (Hind et al 2011, Rothney et al 2012, Toombs et al 2012). All values were below 1.2% and within the recommended minimum acceptable precision limits (3% for BFM and 2% for LTM) (Hangartner et al 2013). The precision of regional body composition measurement has been shown to be poorer than whole body measurements (RMS-CV 2-3%), particularly for fat mass of the arms which is likely owing to the placement of arms relative to torso leading to differences in the anatomical definition of the ROI (Rothney et al 2012). The importance of manual adjustment of ROIs in post-scan analysis should also be noted (Section 3.2.3), with reports of increased reproducibility in manual vs. automatic ROI placement (r=0.93 vs. 0.74) (Lohman et al 2009). Incorrect ROI
positioning can also alter whole body precision due to differences in algorithms used to define arm ROI versus leg and trunk ROI.

The regional distribution of adiposity is an area of clinical and research interest due to the relationship between visceral adiposity and metabolic syndrome (Kuk et al 2009, Chang et al 2012). Results of this study report the short-term repeat measurement precision of the new DXA-based VAT estimation tool. This analysis is clinically relevant for interpretation of a change in VAT through intervention. The precision error (RMS-SD) of CoreScan for repeat measurement of VAT was 47g. This precision error was associated with a high CV (27.9%). One previous study carried out repeat measurements in 32 obese women with VAT ranging from 404-2,297g and observed a precision value of 47.6g, with a CV of 5.1% (Rothney et al 2013). In the current study, analysis was split by VAT amount and 33 participants with VAT mass >400g were compared to Rothney et al (2013). Although comparable, the higher CV measured in this study (8.4%) may be a result of the lower mean VAT in our participants (872g vs. 1,110g), thus increasing the CV estimation. Due to the high CV observed in the study at values of VAT less than 400g, (35.5%), caution is warranted in assessing participants below this threshold. A limitation of this new software is the development of an algorithm that attempts to partition a component of the android fat mass into subcutaneous and visceral components, and derives a zero value for VAT. It may not be appropriate to carry out a precision assessment based on values of VAT that are virtually undetectable. Further validation is therefore required, to determine a lower limit of detection or lowest ‘true’ measurement using MRI or CT to confirm this finding. Until such time, it may be more appropriate to use categories of VAT (i.e. low to high), rather than absolutes, when deriving relationships between DXA-measured VAT and other variables of interest.

Precision in the measurement of BMD is required for diagnosis of disease (osteoporosis) and to monitor therapy. Bone is less susceptible to error from changes in hydration status such as soft tissue and is therefore only affected by technical error (i.e. subject/ROI positioning or variation in the machine). The minimum acceptable BMD precision for an individual technician is 1.9% (Lumbar Spine), 1.8% (Total Femur) and 2.5% (Femoral Neck) (Baim et al 2008). This study produced precision values of <1.6% at each site which is comparable to ISCD recommendations.
Least significant change (LSC) was calculated in this study to aid clinical decision-making for longitudinal measurements and to determine the smallest change deemed to be statistically significant at either the 95% or 80% CI level. Varying confidence limits may be appropriate under different clinical situations. For example, when identifying someone who has responded to therapy in a situation where response is expected, the required confidence may be somewhat less. However, in a situation where a change in a course of therapy is being considered, the clinician may require the 95% confidence in order to assess the intervention (Gluer et al 1999).

3.4 Conclusion

Dual-energy x-ray absorptiometry offers advantages over traditional body composition measurements, such as scan time (only 6-7 minutes for whole body assessment), clinical convenience, minimal radiation exposure, and measurement of whole and regional body composition. Although previous studies have reported typical error measurements of whole body and regional body composition, not all have not provided information on subject presentation, subject positioning, and scanning techniques.

The variation in measurement that can occur through both technical and biological deviation has been highlighted. In this study, checks have been made to ensure accurate scanning and minimisation of technical and biological variation through inclusion of a standardisation protocol for all testing (summarised in Table 3.4.1). Following these procedures a repeat scan precision of ≤3% measured in a representative sample of our subject population was attained (Table 3.3.1). Of concern is the detection of zero grams of VAT in this analysis, which is a non-physiological finding that may distort data interpretation in future analysis. Caution should be exercised in its use among populations that represent the extremes along the spectrum of fat mass, particularly low fat mass, due to high CV % values noted.

Thus, future studies are recommended to report their scanning and analysis protocol so that results can be interpreted appropriately. The DXA technology can be improved if the uncertainties associated with the precision and accuracy of DXA measurements are addressed. While a convenient and useful diagnostic tool for assessing both BMD and body
composition, comprehensive operator training in subject positioning and post-scan ROI positioning is recommended to reduce technical error and misinterpretation of results.
Chapter 4

Establishing metrics of adiposity to define the anthropometric phenotype: Is body mass index appropriate?
4.1 Introduction

Different levels of adiposity and energy stores in an individual are associated with altering states of malnutrition and obesity. Each has been shown to increase morbidity and mortality (Flegal et al 2013, Correia et al 2003); emphasising the requirement for accurate, non-invasive methods for assessing nutrition status. This requirement has been the impetus behind the custom use of body mass index (BMI) to measure body composition, forming the basis of the World Health Organisation’s classification of ‘underweight’, ‘normal’, ‘overweight’ and ‘obese’ (WHO 2000). Despite signs of stabilisation in some populations (Rokholm et al 2010), the changing trend in obesity (i.e. BMI>30kg/m\(^2\)) is apparent, with obesity prevalence rates in Ireland rising considerably since 1990, from 8 to 26% in men and from 13 to 21% in women (IUNA 2011). BMI is commonly relied on as the primary indicator of adiposity or obesity in epidemiologic studies and has been incorporated into clinical practice due to its ease of calculation from body mass and height (mass (kg)/height\(^2\) (m)). The underlying assumption of the use of BMI to define obesity is that at a given height, higher body mass is associated with increased adiposity. However, BMI is an imperfect measure of adiposity as it cannot distinguish body fat mass (BFM) from fat-free mass (FFM), two indicators which have contrasting biological influences on health.

The Health Service Executive (HSE) Framework for Action against Obesity (HSE 2012) has recently highlighted the need to enhance effectiveness in surveillance, research, monitoring and evaluation of obesity, as well as the need to develop a quality uniform approach to its detection and management. However, BMI may not be the most appropriate metric in this regard, despite its widespread use. A systematic review and meta-analysis carried out by Okorodudu et al (2010) to determine diagnostic performance, observed a pooled sensitivity of 0.50 and specificity of 0.90 of BMI to identify excessive body adiposity. These results indicate that a BMI ≥30kg/m\(^2\) is a highly specific predictor of excess adiposity. However, approximately half of individuals with high body fat percentage (BF%) are labelled as non-obese, which has clinical implications for diagnostic health. Therefore, although BMI has been invaluable for observation at a population level, it may not be the best determinant of adiposity and adiposity-related parameters of health. The development of technologies such as dual-energy x-ray absorptiometry (DXA) has allowed a move beyond traditional classification towards a more direct measure of adiposity.
A focus on BF% (BFM relative to total mass) as the criterion measure of adiposity has been proposed by many, including Gallagher et al (2000), who established healthy BF% ranges equivalent to BMI. Whilst useful, this approach is limited to working backwards from the proxy method (BMI) to the true measures of adiposity. Using subjects from universities in the UK, USA and Japan, age- and sex-specific formulae were developed by the authors for estimation of BF% from BMI. More recently, regression equations were developed by Heo et al (2012) using the US NHANES database. It is unknown whether these formulae are applicable to an Irish sample today. In addition, the expression of adiposity relative to total mass is under scrutiny, since a change in fat-free mass has the potential to alter BF%, regardless of a change in adiposity. To obviate such difficulties, height-normalised indices, namely a BFM index (BFM/height\(^2\) or BFMI) and a FFM index (FFM/height\(^2\) or FFMI), were proposed by van Itallie et al (1990).

BFMI and FFMI may offer the advantage of assessing static and dynamic nutrition status at the level of the individual, and in a population, during illness, dietary or nutrition intervention and ageing. This is particularly applicable with regard to the ageing population, as BMI and BF% may mask an increase in total BFM and a decrease in FFM when body mass remains stable. While some authors have constructed percentiles of these indices for incorporation into clinical and research practice (Kyle et al 2003, Kelly et al 2009), their application is limited by (i) a lack of awareness of the limitations of BMI and BF% in the accurate representation of adiposity; (ii) the cost and lack of availability of appropriate measurement tools.

While the total amount of adiposity is crucial to the definition of an anthropometric phenotype, the distribution of adiposity may be even more influential. An abdominal or android body fat distribution is more strongly linked to insulin resistance and metabolic syndrome than those with a peripheral gynoid fat distribution around the hips (Lee et al 2008). In particular, intra-abdominal or visceral adiposity within the android compartment is thought to release fatty acids into the portal circulation, where they may cause insulin resistance in the liver and subsequently in muscle (Despres & Lemieux 2006). The recent development of DXA software to measure visceral adiposity may increase the availability of an accurate measurement tool for body composition-related markers of health (Kaul et al 2012). However, it is yet unknown how visceral adipose models to total and android fat.
With the use of DXA as a reference measure of body fat mass (BFM), fat-free mass (FFM), android fat and visceral adipose tissue (VAT) in a sample of Irish adults aged 18-81 years, the aims of this study were as follows:

(i) To identify the most appropriate criterion metric of adiposity by examining the validity of BMI, BF% and BFMI;
(ii) To derive an age- and gender-specific algorithm for the field-based prediction of BFMI using BMI;
(iii) To examine the relationship of VAT with total and android adiposity in the determination of the most appropriate metric of regional adiposity.

4.2 Methods

4.2.1 Participants

A convenience sample of adults ≥18 years old were recruited from the faculty, student body and surrounding community of the University of Limerick, via email advertisement and word-of-mouth, to participate in the University of Limerick Body Composition Study (ULBC) between 2009 and 2013. Following written, informed consent (Appendix A), 1,606 adults (683 men, 923 women) aged 18-81 years took part in the study. Ethical approval was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18). To observe the influence of age on variables of interest, participants were categorised into one of three age groups: 18-29y, 30-49y and ≥50y. This was based on the following considerations: (i) total potassium (an index of skeletal muscle mass) and bone mineral density decline after 30y of age in both men and women (He et al. 2003, Looker et al. 2012); (ii) hormonal changes due to menopause occur approximately after age 50y in women; (iii) body mass begins to decrease after age 50y in men and women (Bemben et al. 1998).

4.2.2 Body Composition

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ v.14.1 software was used to capture whole body scans in all participants. A detailed
explanation of the pre-scan protocol is included in the standard operating procedure (SOP) in Section 3.2. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK) and body mass to the nearest 0.1 kg (Tanita MC-180MA Body Composition Analyser, Tanita UK Ltd.). Whole body DXA scans were used to measure body fat mass (BFM), fat-free mass (FFM), android and visceral fat (VAT) in all participants.

### 4.2.3 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.). Statistical significance (two-tailed) was set at \( p < 0.05 \) for all analyses. Kolmogorov-Smirnov and Levene's tests were conducted to assess whether variables were normally distributed and homoscedastic respectively. Mean values and standard deviations (SD), median and interquartile range (IQR) and min-max provide descriptive statistics. Spearman’s rho was used to determine the correlation coefficient between variables that were non-normally distributed. Models for predicting measures of adiposity were developed using multiple stepwise linear regression analysis with BMI, sex and age as independent variables. Indices of diagnostic performance used included sensitivity, defined as the probability that a person who actually has the condition of interest will have a positive test result; and specificity, defined as the probability that a person who does not have the condition of interest will have a negative test result.

### 4.3 Results

#### 4.3.1 Identifying an Appropriate Metric of Adiposity

Body composition characteristics of participants are described in Table 4.3.1. 35.9% (282 men, 294 women) of participants were classified as ‘overweight’ by BMI definition (>25 to <30 kg/m\(^2\)), with 10.1% (68 men, 94 women) classified as ‘obese’ (≥30 kg/m\(^2\)).

The partitioning of BMI into fat-free mass index (FFMI) and body fat mass index (BFMI) by normalising absolute FFM and BFM to height\(^2\) was hypothesised to offer an improved metric of body composition and nutrition status.
Table 4.3.1 Descriptive statistics of ULBC participants (n=1,606)

<table>
<thead>
<tr>
<th>Total (n=1,606)</th>
<th>Men (n=683)</th>
<th>Women (n=923)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td><strong>Median (IQR)</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>Age (y)</td>
<td>38.2 (17.1)</td>
<td>24.9 (18.1)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 (0.1)</td>
<td>1.70 (0.2)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>73.5 (13.8)</td>
<td>80.8 (14.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 (3.9)</td>
<td>25.1 (3.9)</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>21.2 (8.9)</td>
<td>16.6 (10.8)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28.8 (9.8)</td>
<td>20.6 (11.2)</td>
</tr>
<tr>
<td>BFM (kg/m²)</td>
<td>7.5 (3.5)</td>
<td>5.7 (2.6)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>52.4 (12.2)</td>
<td>8.6 (2.7)</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>17.8 (2.5)</td>
<td>20.0 (2.3)</td>
</tr>
</tbody>
</table>

Normally distributed (Kolmogorov-smirnov test p>0.05)

BFM, body fat mass; BFMI, body fat mass index; BMI, body mass index; FFM, fat free mass; FFMI, fat free mass index

The correlations between BMI and the components of body composition (i.e. adipose and fat-free mass) are presented in Table 4.3.2. BMI correlated significantly with BFMI (ρ=0.73-0.95, p<0.05) and to a lesser extent with FFMI (ρ=0.56-0.71, p<0.05) in all cases, regardless of sex or age group. The relationship between BMI and adiposity was stronger in women, while the relationship between BMI and FFMI was stronger in men. This shows that BMI is related to fat and fat-free mass, being unable to distinguish the two.

Table 4.3.2 Spearman rho (ρ) correlation of BMI with BFMI and FFMI in men and women by age

<table>
<thead>
<tr>
<th></th>
<th>BFMI</th>
<th>FFMI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-29y</td>
<td>0.83¹</td>
<td>0.68¹</td>
</tr>
<tr>
<td>30-49y</td>
<td>0.73</td>
<td>0.68</td>
</tr>
<tr>
<td>≥50y</td>
<td>0.81</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-29y</td>
<td>0.95¹</td>
<td>0.69¹</td>
</tr>
<tr>
<td>30-49y</td>
<td>0.83</td>
<td>0.64</td>
</tr>
<tr>
<td>≥50y</td>
<td>0.95</td>
<td>0.71</td>
</tr>
</tbody>
</table>

All correlations significant at the 2-tailed level (p<0.001) ¹Additionally adjusted for age
Additionally, the diagnostic ability of BMI to define excess adiposity was examined. While no established criteria to define excess adiposity exist currently, a BF% cut-off of 25% for men and 35% for women have been suggested by the American Association of Clinical Endocrinology/American College of Endocrinology (AACE/ACE 1998) and World Health Organisation (WHO 1995). By application of these criteria to the current data, BMI ≥30kg/m² was found to provide a specificity of 99% and a sensitivity of 24% to detect excess adiposity. This lack of sensitivity in highlighted by the variability of adiposity and lean mass for a given BMI category i.e. in participants with a BMI of between 18.5 and 25kg/m² (i.e. ‘normal’), BF% ranged from 5.2 to 39.1% and FFM ranged from 41.3 to 82.1kg in men, while in women, BF% ranged from 13.3 to 45.3% and FFM ranged from 31.2 to 61.2kg.

As a percentage of total body mass, adiposity defined as BF% may not be the most appropriate metric of adiposity due to assumption of a stable FFM component. This is supported by Figure 4.3.1 which represents the relationship between BF% and BFMI. A weaker relationship is observed between BF% and BFMI at higher values of adiposity and in older age (>50 years), where BF% plateaus as BFMI continues to increase.

![Figure 4.3.1](image)

**Figure 4.3.1** Scatter plot representing the relationship between BF% and BFMI in men and women by age. Cut-off for BF%-defined obesity (---)
Further support is provided in Figure 4.3.2, where BF% is observed to slightly over-estimate the % change in adiposity (+1%) with age from 50-59y to 60-69y due to a decrease in FFM. Conversely, BMI under-estimates the mean increase in adiposity (-1.4%) due to a decrease in FFM and subsequent body mass.

![Figure 4.3.2](image)

**Figure 4.3.2** Line chart representing the ability of various metrics to track an age-related change in adiposity

### 4.3.2 Generalised Equation for the Prediction of BFMI using BMI

The association between BMI and DXA-measured BF% was analysed using a linear regression model. BMI predicted 51% of the variance in BF% in men and 65% in women. Using gender and age as independent variables, a prediction equation for the estimation of BF% from BMI was generated, with an $R^2$ of 0.8 and standard error of estimate (SEE) of 4.4%. Past models based on American reference data (Gallagher *et al.* 2000, Heo *et al.* 2012) tended to overestimate BF% in an Irish cohort by 1.5-3.7%, highlighting the need for population-specific reference data. This analysis is presented in Appendix C. Since BFMI was deemed the most appropriate metric of adiposity, prediction equations for the estimation of BFMI were generated instead.

The relationship between BFMI and BMI is displayed in Figure 4.3.3. Taking BFMI as the new criterion measure of adiposity, a multiple linear regression model was developed to
predict BFMI using BMI, sex and age as independent variables (Equation 4.1). BMI predicted 60.7% of the variance in BFMI, followed by sex (27.2%) and age (1.6%) in this model (p<0.001).

$$BFMI = 0.693 \times BMI - 3.316 \times sex + 0.029 \times age - 9.704$$

(Equation 4.1)

where sex=0 for women and sex=1 for men. Multiple $R = 0.946$, $R^2$ of 0.896 and SEE of 1.1%:

4.3.3 Relationship of Visceral Adipose Tissue to Total and Android Adiposity

The regional distribution of adiposity was investigated through the measurement of adiposity in the android compartment, and within this, the intra-abdominal or visceral adipose tissue (VAT). 79 participants (5 men, 74 women) were excluded from this analysis on the basis of a negligible VAT measurement (<0g VAT) as described in Chapter 3. Median android fat was 1.25(±1.5) kg in men and 1.74(±1.4) kg in women, while median VAT was 388(±813) g in men and 325(±726) g in women.

Android fat modelled linearly to BFMI ($R^2=0.95$ in men, 0.91 in women) since it is dependent on body size (measured as 20% distance between the iliac crest and the mandible). To determine if VAT models similarly to BFMI and android fat, a scatter plot
(Figure 4.3.4) was constructed, using VAT as the dependent variable. The relationship between VAT and BFMI was linear, with an $R^2$ value of 0.74 (SEE=0.4) for men and 0.68 (SEE=0.3) for women. A stronger relationship was observed between VAT and android fat, with an $R^2$ value of 0.83 (SEE=0.3) for men and 0.79 (SEE=0.3) for women. Using equations derived from a sex-specific linear regression analysis, it was determined that for every 1kg/m$^2$ increase in BFMI, a 250g increase in VAT is predicted in men, with a 140g increase predicted in women. For every 1kg increase in android fat, a 610g increase in VAT is predicted in men, and a 460g increase in VAT is predicted in women.

![Scatter plot of the relationship between VAT and BFMI (left) and VAT and android fat (right) in men and women aged 18-81 years (n=1,527).](image)

Due to age-related increases in VAT, analysis was repeated on participants grouped according to age category. The outcome of these various models is presented in Table 4.3.3. A stronger relationship was observed in older age, in conjunction with a greater increase in VAT for every unit increase in BFMI or android fat. For every 1kg/m$^2$ increase in BFMI, the amount of VAT almost doubled in men and women from youth to middle age (140 to 260g in men, 70 to 130g in women), with a minimal further increase predicted in older age (+40g in men, +10g in women). A similar trend was observed in the relationship between VAT and android fat, with more VAT per kg increase in android adiposity with
age. The greatest increase was observed from youth to maturation, with a smaller increase to older age.

<table>
<thead>
<tr>
<th>Age</th>
<th>18-29y</th>
<th>30-49y</th>
<th>≥50y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BFMI (kg/m^2)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.74</td>
<td>0.65</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>0.68</td>
<td>0.73</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Table 4.3.3 The relationship (R^2) of VAT with BFMI and android with the slope of the line representing the increase in VAT (g) for every unit increase in BFMI or android adiposity

<table>
<thead>
<tr>
<th>Age</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.74</td>
<td>0.68</td>
<td>0.83</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>140</td>
<td>610</td>
<td>460</td>
</tr>
<tr>
<td>18-29y</td>
<td>0.65</td>
<td>0.62</td>
<td>0.78</td>
<td>0.76</td>
</tr>
<tr>
<td>30-49y</td>
<td>0.79</td>
<td>0.73</td>
<td>0.90</td>
<td>0.82</td>
</tr>
<tr>
<td>≥50y</td>
<td>0.79</td>
<td>0.64</td>
<td>0.88</td>
<td>0.80</td>
</tr>
</tbody>
</table>

### 4.4 Discussion

The aim of this chapter was to determine the most appropriate metric to define ‘adiposity’ in body composition analysis. This was achieved through the exploration of the relationships between BMI and DXA-measured adiposity in a large convenience sample of participants with varying levels of adiposity. The purpose was to explore the potential to develop appropriate criterion for interpretation of adiposity, through the use of height-normalised indices. In this approach, it was also desirable to develop a generalised prediction equation incorporating BMI, age and sex to predict BFMI in a clinically-accessible manner. Finally, an exploration of the relationship between VAT and measures of total and abdominal fat was undertaken to determine if VAT relates to these variables.

#### 4.4.1 Identifying an Appropriate Metric of Adiposity

This study, involving a large convenience sample of Irish adults with a wide range of adiposity, demonstrates that BMI is limited in its ability to define adiposity due to the inability to distinguish between BFMI and FFMI. While BMI was highly correlated to BFMI (ρ=0.73-0.95), significant correlations were also observed between BMI and FFMI (ρ= 0.56-0.71). Higher correlates to BFMI were observed in women (p<0.001), which can be explained by the higher mass of fat tissue and lower mass of fat-free tissue in women vs. men as highlighted in Table 4.3.1. Additionally, BMI was also shown to display limited diagnostic performance to accurately predict BF%-defined obesity in men and women. This
may have clinical implications and indicates that excess adiposity is being under-diagnosed in many individuals. The use of BMI primarily as an index of adiposity is evidently inappropriate.

BF% is a metric of adiposity used by many (Gallagher et al 2000, WHO 2000). Although shown to be a better indicator of cardiometabolic health regardless of body mass (Romero-Corral et al 2010), BF% contains information about both components of body composition and cannot distinguish either to best effect. For example, high BF% could result from high BFM and/or low FFM. This is illustrated by the curvilinear association between BF% and BFMI in Figure 4.3.1. At the higher spectrum of BFMI, BF% plateaus at 40-50%. This finding would suggest that weight gain is not proportionally distributed to fat mass and fat-free mass. It is likely that the gravitational effect of carrying excess adipose tissue causes a concomitant increase in lean tissue, thus causing BF% to stabilise. Similarly, in individuals with very low BFM and low FFM e.g. anorexia nervosa, BF% may appear normal. Furthermore, the proportional distribution to fat mass and fat-free mass likely depends on age. In the elderly, a decrease in FFM may result in an increased BF%, despite no change in adiposity.

A potential method to overcome this bias is to adjust adiposity for stature, whereby increases or decreases in adiposity can be accurately tracked regardless of changes in total or fat-free mass. This was demonstrated by VanItallie et al (1990), where 12 weeks of semi-starvation was induced on 32 non-obese young men in the Minnesota study. As protein-calorie malnutrition became more severe, an increase in FFM% was observed due to a faster decrease in BFM than FFM. Also, the extent of the decrease in BF% was underestimated due to the concurrent decline in FFM. Thus, the calculation of BFMI and FFMI were more representative of the presence of malnutrition and were recommended to avoid the ambiguities frequently generated when components are reported as BMI, percentages of body mass and/or by absolute mass.

4.4.2 Generalised Equation for the Prediction of BFMI using BMI

The fundamental motives for the continued use of BMI as a measure of body composition by clinicians are its simplicity and extensive applicability. While DXA was used as the reference method in this current study to estimate indices of BFM and FFM, it is
acknowledged that such methods have limited availability clinically, posing a challenge to widespread incorporation of these indices as criterion measures of body composition. However, the use of DXA offered an opportunity to develop the first age-and sex-specific prediction equation for the simple calculation of BFMI using BMI. While past studies have reported a similar analysis to predict BF% (Gallagher et al 2000, Heo et al 2012), these predication equations have not shown transferability to an Irish phenotype (Appendix C), and are not recommended due to the known limitations of BF%. In this study, a new reference equation using BMI and incorporating age and sex as independent variables was developed to improve the prediction of BF%. BMI showed a better correlation with BFMI than BF%, and thus allowed development of highly representative ($R^2=0.896$, SEE=1.1%) prediction coefficients. This ‘BFMI calculator’ is convenient for public use or for healthcare professionals without access to measurement tools for body composition. Alternatively, where surrogate measures of BF% are available, conversion to BFMI can be carried out simply through the following equation:

$$BFMI = \frac{(BF\% \times Body\ Mass)/100}{(Height^2)}$$

Similarly, FFMI can be calculated using the inverse of BF% or BMI - BFMI. Various field methods of BF% measurement have been validated previously by this research group including BIA (Leahy et al 2012a), skinfolds (Leahy et al 2012b) and ultrasound (Leahy et al 2011c). These are all suitable alternatives to DXA that may offer superior accuracy to estimate adiposity than prediction equations using BMI. However, a comparison between these methods has not yet been undertaken.

Offering an improved representation of parameters of body composition, it is expected that BFMI and FFMI would have a better association with disease risk, morbidity and mortality. Wang et al (2009) and Liu et al (2013) have shown through DXA and BIA measurement respectively, that high BFMI was strongly associated with the presence of metabolic syndrome in Chinese men and women. Also, the adjusted odds ratios of the risk of metabolic syndrome were higher than that of BMI and BF%. However, similar studies relating to risk of metabolic syndrome are lacking in Caucasian populations. Using BIA, Bigaard et al (2004) observed that both high BFMI and low FFMI are independent predictors of all-cause mortality in a Danish follow-up study with 27,178 men and 29,875 women. Previously, a U-shaped association between BMI and mortality has been
extensively described (Calle et al 1999). It is the division of BMI into two components (BFMI and FFMI) that reveals a J-shaped and reverse J-shaped relationship respectively. Reference values or centiles of these indices have been reported in past research but applicability is limited due to differences in populations studied (American NHANES – Kelly et al 2009) and method of measurement used (BIA – Kyle et al 2001, Schutz et al 2002). Determination of ranges to define body composition-related disease (e.g. obesity, metabolic syndrome and sarcopenia) using DXA in a European or Irish sample is warranted.

4.4.3 Modelling of Visceral Adipose Tissue to Total and Android Fat

In order to complete the body composition phenotype, an indication of body fat distribution is necessary due to the health implications that are associated with android adiposity above and beyond that of total adiposity. Within this compartment, it is necessary to distinguish between visceral and subcutaneous adipose, since VAT reduction has been shown to induce greater beneficial effects on parameters of the metabolic syndrome than SAT reduction (Park & Lee 2005). The estimation of VAT through imaging methods is not commonly available in clinical or research settings. Therefore, this analysis aimed to determine how VAT models to more accessible metrics of adiposity i.e. BFMI and total android fat.

A strong relationship between VAT and BFMI was observed, with BFMI predicting 74% of the variance of VAT in men and 68% in women. Linear regression modelling determined that a 1kg/m² increase in BFMI equates to a 250g increase in VAT in men and a 140g increase in women. However, this model should be interpreted with caution since it can be seen from the scatter plot that a high BFMI does not necessarily equate to a high VAT (and vice versa) at the individual level. Similar observations were made between VAT and android fat although this was a stronger model of fit, with android fat predicting 83% of the variance of VAT in men and 79% in women. For every 1kg increase in android fat, a 610g VAT increase was predicted in men, and a 460g VAT increase in women. However, this relationship may be dependent on a genetic disposition to gain visceral over subcutaneous fat, as well as factors such as age, diet, physical activity and hormonal status.

This analysis was repeated to determine the effect of increasing age on the relationship between VAT and total and android adiposity. The allometric relationship changed in men
and women, indicating that more VAT was present per unit increase in BFMI and android fat in older participants. In all cases, the greatest change was observed from youth to maturation, with a minimal increase thereafter. One study (Hallgreen & Hall 2008) has reviewed the changes in VAT and absolute BFM through weight loss interventions to determine the mathematical relationship between a loss in visceral to total adiposity. This study revealed an allometric relationship ($R^2=0.73$) between changes of VAT and BFM that held true for both genders. However, age was not considered in this relationship. The constant $k$ was greater than 1 (i.e., $k = 1.3 \pm 0.1$) indicating the preferential loss of VAT versus BFM, independent of the fat loss method. This study also determined changes in VAT to be dependent on the initial ratio of VAT to BFM.

In conclusion, while the change in VAT is proportional to BFM during weight loss, this may not hold true for changes in VAT with growth and age. Since VAT is within a specific component of the body that is measured based on distance between the iliac crest and mandible, it is not as pertinent to adjust the absolute value, as is the case with total adiposity. However it may be useful to account for total, android or subcutaneous adipose tissue (SAT) in the expression of a health-related metric of regional adipose distribution since abdominal SAT is suggested to have a protective role on health indices. In the Framingham Heart Study (Porter et al 2009), cardiometabolic risk factor prevalence was increased in the highest tertile of CT-measured VAT in >3,000 participants. Within this high risk group, increasing SAT was associated with decreases in cardiometabolic risk factor prevalence despite increasing total abdominal adiposity. Despite this, expression of VAT as a ratio of total abdominal adiposity or SAT is limited when used in solitary. An individual with high quantities of both components may have the same ratio as an individual with low quantities of both components, while not necessarily carrying the same risk factors. Therefore, it may be worthwhile to investigate both absolute VAT and SAT or as a ratio to android fat to determine change, and take forward for cross-analysis with variables of health.

This study is strengthened by use of an accurate measurement tool and metric to measure adiposity, in addition to availability of a large dataset with which to test the hypotheses. The age distribution of participants may be a confounding factor in this study. This applies particularly with regards men, where recruitment was higher in 18-29 year olds (n=435) vs.
adults >50 years (n=121). By necessity, participants were a convenience sample and recruited by email and through word-of-mouth. The location of the study within a university resulted in a larger pool of younger participants available to recruit from. This may explain the lower measures of adiposity in comparison to the general population. Prevalence of ‘overweight’ was higher in the National Adult Nutrition Survey (NANS) than ULBC (43.8% vs. 35.9%), as was prevalence of ‘obesity’ (25.8% vs. 10.1%) (IUNA 2011). However, as demonstrated, BMI may not be an accurate representation of adiposity. The NANS survey also took measurements of BF% using bioelectrical impedance analysis (BIA), which, through comparison, was higher in men (23.3 vs. 20.6%) and marginally lower in women (33.9 vs. 34.1%). While similar, use of BIA is known to underestimate BF% in comparison to DXA (Leahy et al 2012a). Therefore, the validation of prediction equations is required on larger numbers to infer applicability to the general population.

4.5 Conclusion

BMI has been an invaluable tool in the endeavour to raise awareness of the growing epidemic of obesity worldwide over the past few decades. However, its use as an “approximation of total body fat” (NIH 2008) can lead to widespread misclassification of obesity, particularly at the individual patient level. Individuals classified as overweight do not always have high adiposity and similarly, those with a BMI in the normal ranges can, in reality, have a high proportion of their body mass made up of adipose tissue. Similarly, BF% fails to identify whether fluctuations in body mass are a result of changes in BFM and/or LTM, particularly in the ageing. Correct evaluation of these cases requires a move beyond traditional classification towards a more direct measure of both total and regional adiposity and fat free mass. In recognition of the limitations of BMI and BF%, recommendations are made for correcting composition for body stature as opposed to body mass. BFMI and FFMI eliminate differences in adiposity and muscularity due to height. This chapter has developed a generalised equation for the prediction of BFMI from BMI as a potential mechanism to allow incorporation of accurate measurement into clinical practice. In the assessment of regional adiposity, VAT is found to increase linearly with both total and android adiposity, but this relationship is dependent on age and gender. The identification of the most applicable body composition metrics has formed a basis for
appropriate classification of the anthropometric phenotype with the aim of being used to determine health outcome in future research.
Chapter 5

Defining reference man and woman in Irish young adults using indices of body fat and fat-free mass
5.1 Introduction

In order to classify individuals based on their body composition phenotype, appropriate reference ranges are required. The previous chapter defined a common approach for expression of adiposity and lean mass and identified the most suitable metrics for both population and individual use. Reference ranges that are population-specific are essential for comparison of body composition indices with ‘normal’ or ‘healthy’ ranges. The applicability of using only age-specific references without consideration of anthropometric, ethnic, or environmental differences is under question, along with the use of out-dated reference tables that no longer represent the typical young adult (YA) man and woman. The ISCD has recently described a need for more country-specific reference datasets to be developed for future research (Petak et al. 2013). Additionally, reference tables using inappropriate metrics of measurement such as body mass index (BMI) and body fat percentage (BF%) may provide misleading information.

As shown in Chapter 4 of this thesis, defining body composition and nutrition status based on indices of body fat (BFMI) rather than BMI or BF% offers an accurate representation of adiposity, regardless of changes in fat-free mass (FFM) (Van Itallie et al. 1990). It is clear that adiposity is not independent of age and sex, and therefore any metric of adiposity should not attempt classify based on such assumptions. However, development of appropriate reference ranges that classify excess adiposity using this metric is lacking. This is imperative since high BFMI has been linked with all-cause mortality, independent of body mass (Bigaard et al. 2004).

In growth references and standards based on international data or those from individual countries, Z-scores of +2 and -2, and/or certain percentiles (e.g. 5th, 95th), have often been chosen as cut points to classify problematic growth/nutritional status such as malnutrition or obesity (Wang & Chen 2012). In the determination of how reference data should be used, use of either Z-scores or centiles is considered appropriate by the ISCD (Petak et al. 2013). These criteria are based on statistical distribution rather than on the risks of health outcomes. The classification of “high risk” individuals and population groups should ideally be based on the evidence of increased risk for morbidity, mortality, or/and impaired function performance (WHO 1995). However, representative body composition data for this classification approach is not yet available.
The measurement of regional fat distribution or visceral adipose tissue (VAT) has been considered to be crucial in understanding the link between obesity and health risk. VAT is associated with increased risk for mortality above and beyond that associated with total adiposity (Chang et al 2012), is known to increase with age (Kuk et al 2009) and vary between populations (Lear et al 2007). Therefore, a reference measure for DXA-measured VAT in young Irish adults is required for comparison with different age groups and other nationalities. Furthermore, this chapter describes a VAT-to-Android (V/A) ratio, defined as VAT divided by the total android (abdominal) fat deposit, in order for a comparison to be made to subcutaneous android fat.

While much attention is currently given to measures of adiposity, less consideration is given to measurement of FFM (lean tissue mass (LTM) + bone mineral content (BMC)) in young adults. Knowledge of healthy YA reference values may be valuable as levels of physical inactivity continue to rise, particularly in adolescence (Nelson et al 2006). Long periods of inactivity may impact negatively on LTM development and achievement of potential peak muscle mass. In older age, the loss of LTM or sarcopenia is associated with lower strength and contributes to the development of functional limitations and disability (Sayer et al 2013). In order to define sarcopenia, an ethnicity and sex-specific YA reference comparison is required. Appendicular lean tissue mass index (ALTMI) is the addition of lean tissue in the appendages divided by stature\(^2\). Class I sarcopenia is defined as ALTMI one standard deviation below the YA median and Class II sarcopenia is defined as ALTMI two standard deviation below the YA median (Baumgartner et al 2008, Cruz-Jentoft et al 2010). This proposed classification currently provides a definition for sarcopenia but lacks population-specific reference ranges.

Although records of BMI-defined obesity are available, no reference measures of body fat mass (BFM), lean tissue mass (LTM) or its components for an Irish population currently exist. The changing phenotype requires an update of determinants which classify certain diseases states such as obesity and sarcopenia. Using a YA convenience sample of Irish 18-29 year old men and women, the aims of this study were as follows:

(i) To describe a YA body composition reference base using indices of DXA-derived body fat and lean mass
(ii) To generate criterion values for fat obesity and sarcopenia in this dataset based on a YA Z-score and centiles.

5.2 Methods

5.2.1 Participants

Young adults aged 18-29 years were recruited from the UL faculty, student body and surrounding community via email advertisement and word-of-mouth to participate in the University of Limerick Body Composition Study (ULBC). Following written, informed consent, 763 young adults (431 men, 332 women) took part in the study. Ethical approval was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18). A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ v.14.1 software was used to capture whole body scans in all participants. A detailed explanation of the pre-scan protocol is included in Section 3.2. DXA was used to assess body fat mass (BFM), lean tissue mass (LTM), and their components, and also bone mineral content (BMC) and total body bone mineral density (TBMD) to create young adult (YA) reference values for body composition.

5.2.2 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.). Statistical significance (two-tailed) was set at p<0.05 for all analyses. Kolmogorov-Smirnov and Levene’s tests were conducted to assess whether variables were normally distributed and homoscedastic respectively. Mean values and standard deviations (SD), median and interquartile range (IQR) and ranges are reported for descriptive statistics. An independent t-test or Mann-Whitney U test was used, where appropriate, to determine any significant differences in body composition variables between genders. Indices of diagnostic performance used included sensitivity, defined as the probability that a person who actually has the condition of interest will have a positive test result; and specificity, defined as the probability that a person who does not have the condition of interest will have a negative test result. YA Z-scores for BFMI, FFMI, LTMI and ALTMI were calculated as follows based on WHO recommendations:

\[
\text{Observed Value-YA Median Value} \\
\text{YA Standard Deviation}
\]

(WHO 1995)
5.3 Results

5.3.1 YA Reference Tables

Descriptive statistics for the YA reference man and woman are presented in Table 5.3.1. All body composition variables were significantly different according to gender (Independent t-test or Mann-Whitney U test p<0.001), with the exception of age (p=0.431).

Table 5.3.1 Young adult (YA) 18-29 year old reference man and woman (n=763)

<table>
<thead>
<tr>
<th></th>
<th>Reference YA Man (n=431)</th>
<th>Reference YA Woman (n=332)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median(IQR)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>22.6 (2.9)</td>
<td>21.8 (3.9)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 (0.06)</td>
<td>1.80 (0.09)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>80.4 (11.4)</td>
<td>78.8 (13.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 (2.9)</td>
<td>24.3 (3.1)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>65.4 (7.3)</td>
<td>65.0 (9.7)</td>
</tr>
<tr>
<td>LTM (kg)</td>
<td>62.0 (7.0)</td>
<td>61.5 (9.3)</td>
</tr>
<tr>
<td>FFM (kg/m²)</td>
<td>20.1 (1.7)</td>
<td>20.1 (2.3)</td>
</tr>
<tr>
<td>LTMI (kg/m²)</td>
<td>19.1 (1.6)</td>
<td>19.0 (2.1)</td>
</tr>
<tr>
<td>ALTM (kg)</td>
<td>30.5 (3.9)</td>
<td>30.1 (5.1)</td>
</tr>
<tr>
<td>ALTM (kg/m²)</td>
<td>9.4 (0.9)</td>
<td>9.3 (1.3)</td>
</tr>
<tr>
<td>BFMI (kg)</td>
<td>15.4 (7.0)</td>
<td>13.6 (7.5)</td>
</tr>
<tr>
<td>BFMI (kg/m²)</td>
<td>4.7 (2.1)</td>
<td>4.3 (2.3)</td>
</tr>
<tr>
<td>Body fat %</td>
<td>18.6 (6.1)</td>
<td>17.4 (8.1)</td>
</tr>
<tr>
<td>Android (kg)</td>
<td>1.1 (0.8)</td>
<td>0.9 (0.8)</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>361 (353)</td>
<td>263 (236)</td>
</tr>
<tr>
<td>V/A Ratio³</td>
<td>0.32 (0.2)</td>
<td>0.32 (0.2)</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>3.4 (0.4)</td>
<td>3.4 (0.6)</td>
</tr>
<tr>
<td>TBMD (g/cm²)</td>
<td>1.40 (0.1)</td>
<td>1.40 (0.1)</td>
</tr>
</tbody>
</table>

¹Normal distribution (Kolmogorov-Smirnov test p>0.05); ²Non-significantly different from YA man (Independent t-test or Mann-Whitney U-test p>0.05); n=700, 5 men and 62 women excluded based on LLOD of VAT (Section 3.3.)

ALTM, Appendicular lean tissue mass; ALTMi, Appendicular lean tissue mass index; BFMI, Body fat mass index; BMC, Bone mineral content; FFM, Fat-free mass; FFMI, Fat-free mass index; IQR, Interquartile range; SD, standard deviation; LTM, lean tissue mass; LTMI, lean tissue mass index; TBMD, total bone mineral density VAT; visceral adipose tissue; V/A Ratio, visceral/android ratio; YA, young adult
Men were taller (+0.14m) and heavier (+16kg) than women, with more lean tissue (+20kg) and bone tissue (+0.9kg). Women had higher total (+4.4kg) and abdominal fat mass (+0.2kg), but lower visceral fat (-0.2kg).

5.3.2 *Reference Measure of Adiposity and Re-Classification of ‘Obesity’*

Whole-body adiposity was normalised to height$^2$ to calculate body fat mass index (BFMI). The sex-specific centile distributions for BFMI and VAT are presented in *Table 5.3.2*.

<table>
<thead>
<tr>
<th>Centile</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BFMI Men (kg/m$^2$)</strong></td>
<td>431</td>
<td>2.4</td>
<td>2.6</td>
<td>3.2</td>
<td>4.3</td>
<td>5.5</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>BFMI Women (kg/m$^2$)</strong></td>
<td>332</td>
<td>4.0</td>
<td>4.9</td>
<td>5.6</td>
<td>6.7</td>
<td>8.2</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>VAT Men (g)</strong></td>
<td>431</td>
<td>52</td>
<td>100</td>
<td>175</td>
<td>264</td>
<td>420</td>
<td>705</td>
</tr>
<tr>
<td><strong>VAT Women (g)</strong></td>
<td>332</td>
<td>3</td>
<td>8</td>
<td>28</td>
<td>89</td>
<td>200</td>
<td>394</td>
</tr>
</tbody>
</table>

BFMI, body fat mass index; VAT, visceral adipose tissue

BFMI was used to re-classify ‘obesity’ based on median man and woman due to the non-normal distribution of body fat variables. Using a Z-score approach, ‘underfat’ was defined as BFMI one standard deviation below the YA Z-score. ‘Overfat’ was defined as BFMI one standard deviation above the YA Z-score and ‘fat obese’ was defined as BFMI two standard deviations above the YA Z-score (*Table 5.3.3*).

<table>
<thead>
<tr>
<th>Centile</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Underfat (kg/m$^2$)</strong></td>
<td>2.2-4.2</td>
<td>4.3-6.6</td>
</tr>
<tr>
<td><strong>Normal (kg/m$^2$)</strong></td>
<td>4.3-6.3</td>
<td>6.7-9.0</td>
</tr>
<tr>
<td><strong>Overfat (kg/m$^2$)</strong></td>
<td>6.4-8.4</td>
<td>9.1-11.4</td>
</tr>
<tr>
<td><strong>Fat Obese (kg/m$^2$)</strong></td>
<td>≥8.5</td>
<td>≥11.5</td>
</tr>
</tbody>
</table>

BFMI, body fat mass index; YA, young adult
Using these criteria, 11% of men and 12% of women were classified as ‘overfat’, with 6.4% of men and 4.5% of women classified as ‘fat obese’. Analysed by BMI, 31.7% of men and 19.6% of women classified as ‘overweight’ and 5.3% of men and 2.7% of women classified as ‘obese’. Figure 5.3.1 shows the number of potential misclassifications of obesity by BMI when compared to BFMI in adults. Comparing classification criteria for excess adiposity, a BMI $\geq 30$kg/m$^2$ had 99% specificity and 58% sensitivity to define ‘fat obese’ by BFMI in these young adults.

![Figure 5.3.1](image)

**Figure 5.3.1** Distribution of BFMI classification within BMI classification in young adults (n=763)

### 5.3.3 Reference Measure of Lean Tissue Mass

Fat-free, whole body and appendicular lean tissue mass was expressed relative to height$^2$ to generate a YA fat-free mass index (FFMI), lean tissue mass index (LTMI) and appendicular lean tissue mass index (ALTMI). Table 5.3.4 presents the sex-specific centile distributions for these metrics of lean mass.
Table 5.3.4 Lean mass (FFMI, LTMI and ALTMI) centile distributions for young adult men and women

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFMI Men (kg/m^2)</td>
<td>431</td>
<td>17.4</td>
<td>18.1</td>
<td>19.1</td>
<td>20.1</td>
<td>21.3</td>
<td>22.3</td>
<td>23.1</td>
</tr>
<tr>
<td>FFMI Women (kg/m^2)</td>
<td>332</td>
<td>13.9</td>
<td>14.2</td>
<td>15.1</td>
<td>15.9</td>
<td>17.0</td>
<td>17.8</td>
<td>18.3</td>
</tr>
<tr>
<td>LTMI Men (kg/m^2)</td>
<td>431</td>
<td>16.4</td>
<td>17.1</td>
<td>18.1</td>
<td>19.0</td>
<td>20.2</td>
<td>21.1</td>
<td>22.0</td>
</tr>
<tr>
<td>LTMI Women (kg/m^2)</td>
<td>332</td>
<td>13.0</td>
<td>13.4</td>
<td>14.2</td>
<td>15.0</td>
<td>16.1</td>
<td>16.8</td>
<td>17.2</td>
</tr>
<tr>
<td>ALTMI Men (kg/m^2)</td>
<td>431</td>
<td>7.9</td>
<td>8.3</td>
<td>8.8</td>
<td>9.3</td>
<td>10.0</td>
<td>10.6</td>
<td>10.9</td>
</tr>
<tr>
<td>ALTMI Women (kg/m^2)</td>
<td>332</td>
<td>5.7</td>
<td>6.0</td>
<td>6.4</td>
<td>7.0</td>
<td>7.4</td>
<td>7.9</td>
<td>8.2</td>
</tr>
</tbody>
</table>

ALTMI, appendicular lean tissue mass index; FFMI, fat free mass index; LTMI, lean tissue mass index

Table 5.3.5 describes the values for FFMI, LTMI and ALTMI that define class I sarcopenia and class II sarcopenia based on standard deviations from the YA median. Distribution of ALTMI and prevalence of sarcopenia in YA men and women is displayed in Figure 5.3.2.

Table 5.3.5 ALTMI, LTMI and FFMI reference ranges derived from YA Z-scores in men and women (n=763)

<table>
<thead>
<tr>
<th></th>
<th>Men (kg/m^2)</th>
<th>Women (kg/m^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALTMI</td>
<td>LTMI</td>
</tr>
<tr>
<td>Class I Sarcopenia</td>
<td>7.7-8.5</td>
<td>16.0-17.5</td>
</tr>
<tr>
<td>Class II Sarcopenia</td>
<td>≤7.6</td>
<td>≤15.9</td>
</tr>
</tbody>
</table>

ALTMI, appendicular lean tissue mass index; FFMI, fat free mass index; LTMI, lean tissue mass index; YA, young adult
5.4 Discussion

This chapter defines an updated ‘typical’ anthropometric phenotype of the Irish young adult man and woman established in a convenience sample of young adults based primarily in a university community. In comparison to a representative sample of the Irish population as investigated by the Irish Universities Nutrition Alliance (IUNA), this cohort of young adults may be deemed to under-represent those classified as ‘overweight’ or ‘obese’ by BMI in the general population. The National Adult Nutrition Survey (NANS; IUNA 2011) selected a population sample based on demographics, age, gender and social class. 32.4% of young adults (n=531) were classified as ‘overweight’ compared to 26.5% of young adults in the ULBC cohort. Prevalence of ‘obesity’ was also lower in this study (13.2 vs. 4.2%). This discrepancy may partially be explained by differences in age groups, with IUNA analysing data based on 18-35 year olds, resulting in an increased prevalence of obesity due to higher age range. Due to location in vicinity of a university renowned for its sporting campus, it is reasonable to postulate that the ULBC cohort may be more physically active and educated on healthy lifestyle compared to the normal population. While mean BMI was higher in NANS (25.8 vs. 24.7 kg/m² in men; 24.8 vs. 23.2 kg/m² in women), BF% as measured by bioelectrical impedance analysis (BIA) was remarkably similar (19.2
vs. 18.6% in men; 31 vs. 30% in women). Although this may suggest that BMI offers an inaccurate comparison of body composition, it is worth noting that BIA tends to underestimate BF% compared to reference measures such as DXA (Leahy et al 2012a). This is, however, the first study to define the young Irish adult through DXA-based body composition measures of adiposity, lean tissue and bone health.

Comparison in this cohort was also made with international, DXA-based studies such as the US National Health and Nutrition Examination Survey (NHANES) 1999-2004 with data on 2,183 white men and 2,018 white women aged 20-39 years (Li et al 2009, Kelly et al 2009), an Italian cross-sectional study of 25 men and 25 women aged 18-30 years (Bazzocchi et al 2012) and a New Mexico cross-sectional study on 107 men and 122 women aged 18-40 years (Baumgartner et al 1998). Irish young adults were found to have less mean BFM than their American counterparts in men (-8.3kg) and women (-9kg), but more compared to Italian men (-1.8kg) and women (+0.8kg). While BFM varied greatly between American and European young adults, FFM was similar in both populations, ranging from 63.1-65.4kg in men and 44.5-44.6 in women, with slightly lower values recorded in Italians (58.5 and 40.8kg). Higher values of ALTM were recorded in Irish young adults compared to Italians and New Mexicans (+3.2-3.9kg in men, +1.7-2.2kg in women). Data from Bazzocchi et al (2012) is the only other research to date with reference values of DXA-measured VAT. Results were very similar to that measured in Irish adults, with more VAT measured in Italian men (+83g) and less in Italian women (-14g). Mean total bone mineral density (TBMD) was observed to be higher in Irish young adults than in Americans (1.40 vs. 1.21g/cm$^2$ in men; 1.22 vs. 1.11 g/cm$^2$ in women) and Italians (1.26 g/cm$^2$ in men; 1.09 g/cm$^2$ in women). Site-specific BMD measurement (lumbar spine and dual femur) was not available in this cohort. Since these sites are required for diagnosis of low BMD or osteoporosis (Melton et al 2005), reference values for BMD are not discussed any further.

While certain similarities in lean tissue measurement were noted between Irish and American/European cohorts, larger discrepancies were observed in measures of BFM between countries of origin. This finding confirms the requirement for ethnic-specific reference measures to define ‘normal’ or ‘healthy’.
5.4.1 Reference Measure of Adiposity

In the previous chapter, BFM divided by stature\(^2\) (BFMI) was proposed as an improved reference measure for adiposity. Adiposity is presented in the form of centiles, or the percentage of observations that falls below the value of a variable. Identification of individuals in the lower centiles (e.g. 5\(^{\text{th}}\) centile) is often labelled as ‘underweight’, while the higher centiles (e.g. 90\(^{\text{th}}\) and 95\(^{\text{th}}\) centiles) are labelled as ‘overweight’ and ‘obese’ respectively when analysed in terms of paediatric BMI growth curves (Wang & Chen 2012).

Classification criteria for excess adiposity was also categorised based on the YA median Z-score. A BFMI greater than one standard deviation from the Z-score was defined as ‘overfat’ (11.5\% of the population), while a BFMI greater than two standard deviations from the Z-score was defined as ‘fat obese’ (5.5\% of the population). This approach is similar to that used to define osteoporosis based on YA bone mineral density Z-scores (Kanis et al 1994) and sarcopenia based on YA ALTMI Z-scores (Baumgartner et al 1998), but differs from other approaches to define a reference for BFMI. Kyle et al (2003) described BFMI ranges that corresponded to BMI classifications in Swiss adults (2,986 men, 2,649 women) aged 15-98 years. Although measures of BFM were quantified using BIA, BFMI criteria for ‘obesity’ were similar to this study (8.3 vs. 8.5kg/m\(^2\) in men and 11.8 vs. 11.5kg/m\(^2\) in women). However, the largest discrepancies were observed in the classification of ‘normal’ adiposity, with ranges defined by Kyle lower than those defined in the ULBC cohort through the Z-score method (1.8 to 5.2 kg/m\(^2\) vs. 4.3 to 6.4 kg/m\(^2\) and 3.9 to 8.2 kg/m\(^2\) vs. 6.7 to 9.1 kg/m\(^2\) in men and women respectively). These differences may be explained with the use of the BIA method as a measurement tool, combined with the limitation of working backwards from the proxy method (BMI) to define ranges.

Similarly, Kelly et al (2009) developed classifications for BFMI using the NHANES 1999-2004 DXA-based dataset, by selecting values that matched the population prevalence of the WHO BMI classifications in young adults at age 25. While there were 235 men and 323 women in the 20-25 year age bracket, the authors did not disclose how many participants were used to develop the reference values.

Coin et al (2008) defined reference values based on centiles of BFMI in 20-29 year old Italian adults. This study was limited by lower numbers, with 89 men and 104 women used
to develop centiles. The sample size would need to be approximately 200 subjects to enable percentiles to be calculated from the 5th to the 95th percentile (WHO 1995). A comparison between DXA-measured BFMI classification ranges using different methodologies is presented in summarised in Table 5.4.1. Additionally, centile tables of BFMI for the ULBC cohort at different age groups are displayed in Appendix D of this thesis.

Table 5.4.1 A comparison of DXA-measured BFMI (kg/m²) classification ranges using different methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>n</th>
<th>Under-fat</th>
<th>Normal</th>
<th>Over-fat</th>
<th>Fat Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULBC</td>
<td>Z-score</td>
<td>431</td>
<td>2.4</td>
<td>4.3</td>
<td>6.4</td>
<td>9.5</td>
</tr>
<tr>
<td>Kelly</td>
<td>BMI</td>
<td>&lt;235</td>
<td>2.3</td>
<td>3.0</td>
<td>6.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Coin⁸</td>
<td>Centiles</td>
<td>89</td>
<td>2.3</td>
<td>3.7</td>
<td>6.2</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>332</td>
<td>4.3</td>
<td>6.7</td>
<td>9.1</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;323</td>
<td>4.0</td>
<td>5.0</td>
<td>9.0</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105</td>
<td>4.0</td>
<td>6.4</td>
<td>10.8</td>
<td>11.5</td>
</tr>
</tbody>
</table>

⁸5th, 50th, 90th and 95th centiles displayed

Although this cohort may under-represent higher values of BMI in comparison to an Irish reference population, ‘normal’ or median values of adiposity appear to be in line with that of other studies. In the determination of how reference data should be used, the ISCD have recommended that use of either Z-scores or centiles are appropriate, as long as methods are used to adjust for non-normality (Petak et al 2013). While different methods for deriving ranges in BFMI have shown similar results, Z-scores are the preferred approach in anthropometry (WHO, 1995) and are more useful in capturing the severity of extreme values, and in assessing longitudinal change. However, whether these ranges represent appropriate indices of health and mortality remains to be investigated.

Analysis of the ULBC data to determine the ability of BMI to correctly identify those with excess adiposity as measured by BFMI was also examined. A cut-off of ≥30kg/m² (i.e. ‘obese’) had excellent specificity (99%) to determine those who are not ‘fat obese’ as defined by BFMI. However BMI showed poor sensitivity and only classified 58% of adults with high BFMI-defined adiposity as ‘obese’. This confirms that excess adiposity is being under-diagnosed in many individuals through a reliance on BMI as the defining factor.

Development of a new classification system for adiposity will have implications if it is to be integrated and applied clinically. Using BFMI as a reference will result in more individuals classified as ‘fat obese’ compared to those identified as ‘obese’ using BMI. This may lead to earlier detection of excess adiposity than would normally occur and initiate a
more timely opportunity to intervene or elicit a lifestyle change. While measurement through the DXA method can be costly and limited to hospitals or research institutions, simpler and cost-effective field-based methods to measure BF% have been validated for the Irish population (Leahy et al 2012). Combined with measures of body mass and height allows a simple method of classifying excess adiposity using BFMI calculation.

5.4.2 Reference Measure of Lean Tissue Mass

Classification of sarcopenia or muscle deficiency was first described by Baumgartner et al (1998), using a reference group of 229 American white men and women aged 18-40 years. Criterion values for sarcopenia, based on ALTMI more than two standard deviations below the mean of the reference population was very similar to the reference ranges found in the present study in both men (7.3 vs. 7.6kg/m²) and women (5.5 vs. 5.4kg/m²). This definition of sarcopenia was significantly associated with self-reported physical disability in men and women, independent of covariates such as ethnicity, age, morbidity, obesity, income, and health behaviours. In more recent years, Newman et al (2003) developed ranges based on the lowest 20th centile ALTMI of American 70-79 year olds in the Health ABC Study (1,435 men, 1,549 women). These ranges were again similar (7.2kg/m² in men, 5.7kg/m² in women) to those developed in this study, and were incorporated by the International Working Group on Sarcopenia as part of consensus definition (Fielding et al 2011).

Criteria for diagnosis as set out by the European Working Group on Sarcopenia in Older People (EWGSOP) must include evidence of low muscle mass (criterion 1) plus a measure of low muscle strength or physical performance (criterion 2 or 3) (Cruz-Jentoft et al 2010). These criterion can be used to diagnose sarcopenia or estimate prevalence of sarcopenia in an Irish population. Whilst large differences were found between American and European mean indices of fat, mean FFM appears to be similar between populations, as confirmed by similar ranges calculated for ALTMI. Therefore, reference measures as described by Fielding et al (2011) can be used interchangeably in an Irish population. The LTMI or FFMI ranges defined in this study may be of benefit in practice where values for appendicular lean mass are not available or measurable. Estimation of body fat through
field measures such as anthropometry will allow derivation of a FFMI, and a surrogate definition of sarcopenia can be made.

While this study is the first of its kind to attempt to characterise the typical young Irish adult in relation to accurate metrics of body composition, there are limitations associated with data collection. Distribution of age was based around the lower end of the spectrum, with a mean age of 22 amongst male and female participants. While participants were apparently healthy at the time of the scan, physical activity, co-morbidities or history of chronic disease information was not collected. Suggested ranges of BFMI are deemed appropriate for use but may not be definitive- future work is needed on large datasets to determine whether these indices are appropriate health-related markers. Use of the YA as a ‘healthy’ or ‘normal’ reference range is appropriate for measures of lean mass due to the decrease in lean mass with age (sarcopenia), although it is currently unclear whether indices of ALTM vs. FFM vs. LTM offer the most accurate representation of function. Use of the YA reference range for measures of adiposity at all age groups may come under scrutiny since although BFM has been shown to increase with age; high levels of adiposity is certainly not dependent of old age. However, median BFMI in a YA may offer a more realistic interpretation of ‘healthy’ rather than ‘normal’, as would be seen in age-matched Z-scores. Therefore, for future analysis of ‘fat obesity’ in adults ≥30 years, use of the BFMI T-score is recommended i.e. comparison to the reference BFMI and standard deviation from a young adult as described in this chapter.

5.5 Conclusion

Population-specific reference values allow distinct classification an individual based on their anthropometric phenotype. A young adult reference base allows comparison across different ethnicities and the change associated with ageing. Specific to an Irish adult convenience sample, reference ranges of BFMI based on YA Z-score are presented, offering classification of the individual as ‘underfat’, ‘normal’, ‘overfat’ or ‘fat obese’ based on deviation from the median. Additionally, criterion reference ranges for the classification of sarcopenia based on ALTMI are presented. These ranges will offer a more
accurate interpretation of body composition ranges and may contribute to identifying deviations that lead to health-risk and disability in the future.
Chapter 6

*Health-related changes in body composition associated with ageing*
6.1 A cross-sectional analysis in of body composition changes with ageing in an Irish convenience sample

6.1.1 Introduction

Ageing is a process that conveys negative effects on almost every facet of human body composition. These changes occur at the cellular, tissue and whole body level that may culminate in disease states such as obesity, sarcopenia and osteoporosis. Reported prevalence of these disorders is not only highly dependent on ethnicity and environment (Kelly et al 2009), but also the metric of measurement. Body mass index (BMI) is the most popular tool used for body composition assessment due to simplicity of application and low cost. Dual-energy x-ray absorptiometry (DXA) provides an improved assessment due to its ability to segment into a 3-component model of body fat mass (BFM), lean tissue mass (LTM) and bone mineral content (BMC). Currently, no reference data is available for DXA-based measures of body composition across the lifespan in Ireland.

In Ireland, prevalence of BMI-defined obesity has been observed to increase with age from 12.9% and 13.4% of young adult men and women respectively, to 42.1% and 30.9% of men and women aged 51-64 years (IUNA 2011). As the incidence of obesity continues to increase, the need for accurate measurement of adiposity to allow appropriate diagnosis and treatment is warranted. Using a more direct measure of adiposity, international DXA-based studies have tracked an increase in BFM throughout the lifespan, with a peak at age 50-70 years, and a plateau or slight decline thereafter (Kelly et al 2009, Coin et al 2008, Kuk et al 2009). The use of a body fat mass index (BFMI) as a criterion measure of adiposity offers superior sensitivity over BMI in tracking changes in adiposity with age (Kelly et al 2009) and may therefore offer the most appropriate reference at both the individual and population level as demonstrated in Chapter 4 and 5. In addition to the increase in total adiposity, ageing is related to changes in regional adipose distribution, with an increase in visceral adiposity (VAT) observed in both men and women (Kuk et al 2009). Evidence to date indicates that VAT is the important link between obesity and metabolic syndrome (glucose intolerance, hypertension, dyslipidemia, and insulin resistance), even among those of normal weight (Goodpastor et al 2005). The measurement of VAT using DXA is a novel approach that has shown precision and accuracy (Kaul et al 2012, Rothney et al 2013a), but requires age-and sex-specific population or reference data before clinical incorporation can commence.
Body composition-related health risks in ageing cannot be evaluated simply by body fatness and fat distribution, but also by the loss of lean mass. The progressive loss of skeletal LTM in the appendages (ALTM) must be considered, since this is the primary portion of skeletal muscle involved in ambulation and physical activities (Gallagher *et al* 1997). Termed ‘sarcopenia’, the concomitant decrease in muscle quality and strength that accompanies the decline in LTM leads to significant implications for patient functionality, quality of life and subsequent morbidity (Sayer *et al* 2013). LTM has been observed to peak between 30-40 years and decline thereafter, with the most substantial decreases occurring after age 70 (Kelly *et al* 2009). While sarcopenia is usually not diagnosed until later in life, identification of the age of decline in an Irish population may lead to detection of opportune intervals to intervene in order to maintain peak LTM and offset the age-related deterioration in functionality.

Sarcopenia can co-occur with an increase in adiposity, a condition termed ‘sarcopenic obesity’, which has become a concern due to the double metabolic burden derived from the cumulative risk of both. The presence of both conditions together has been shown to carry 2-3 times the risk of self-reported disability compared to either condition alone (Baumgartner *et al* 2004). Typically classified using ranges of ALTMI and BF%, a more accurate definition may be to incorporate a classification of adiposity using BFMI. Prevalence rates in Ireland are unknown, but an increasingly obese elderly population will undoubtedly present a growing financial burden on the healthcare system.

The assessment of body composition change with ageing is required since deviations from the norm are related to health status, functional performance and disease. While the previous chapter has established a young adult reference base for measures of adiposity and lean tissue, this chapter will determine the effects of age on these parameters. The aims of this study were as follows:

(i) To construct a body composition profile of a convenience sample of Irish adults by age and gender using appropriate metrics i.e. BFMI, VAT and ALTMI;

(ii) To use these metrics to assist in the classification of health–related disorders of body composition associated with ageing, and establish prevalence of obesity, sarcopenia and sarcopenic obesity within this population using updated classification criteria.
6.1.2 Methods

6.1.2.1 Study Design and Participants

A convenience sample of adults aged ≥18 years old were recruited from the UL faculty, student body and surrounding community via email advertisement and word-of-mouth to participate in the University of Limerick Body Composition Study (ULBC) between 2009 and 2013. Ethical approval was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18). Following written, informed consent, a Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ v.14.1 software was used to capture whole body scans. A detailed explanation of the pre-scan protocol is included in Section 3.2. This study involved a retrospective cross-sectional analysis of whole body scans by gender and age band. Three age bands were chosen to represent change in body composition through stages of growth, maturation and decline (18-29 years, 30-49 years and ≥50 years) as per Chapter 4. A total of 1,606 participants were included in the final analysis. DXA was used to assess body fat mass (BFM), lean tissue mass (LTM), and their components, along with bone mineral content (BMC) and total body bone density (TBMD) to map body compositional change in an Irish convenience sample by age.

6.1.2.2 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.) and LMSChartMaker (LMS) Light Version 2.43 (Pan & Cole 2011). Statistical significance (two-tailed) was set at p<0.05 for all analyses. Kolmogorov-Smirnov and Levene’s tests were conducted to assess whether variables were normally distributed and homoscedastic respectively. Based on these tests, the majority of body composition variables were non-normally distributed with the exception of those highlighted in Table 6.1.1-2. Mean values and standard deviations (SD), median and interquartile range (IQR) and ranges are reported for descriptive statistics. An independent t-test or Mann-Whitney U test was used, where appropriate, to determine any significant differences in body composition variables between genders and between age groups. LMS was used to generate centile curves for BFMI, VAT and ALTMI due to the non-normally distributed data. The independent measure (age) is divided into groups and a power transformation is applied which extends one tail of the distribution and contracts the other, thus eliminating skewness (Kelly et al 2009). An obesity, sarcopenia and sarcopenic obesity classification scheme based on BFMI
and ALTMI respectively were calculated using young adult (YA) Z-scores (median and standard deviation) as per Chapter 3.

6.1.3 Results

A total of 1,606 participants (683 men; 923 women) were recruited between the ages of 18 and 81 years. BFMI Z-scores classified 2.6% as ‘underfat’, 57.9% as ‘normal’, 21.6% as ‘overfat’ and 17.6% as ‘fat obese’. Body composition characteristics for measures of adiposity by gender and age band are presented in Table 6.1.1 and Figures 6.1.1-2. BMI increased with age in both men and women, with significant increases observed in men from youth to maturation (+1.6kg/m$^2$), which slowed from maturation to decline (+1.3kg/m$^2$) (p≤0.003). In women, the increase from youth to maturation (+0.9kg/m$^2$), was not as substantial as the increase observed at age ≥50y (+2.3kg/m$^2$) (p≤0.005). BFM in women was significantly higher than men at age 18-29y and age ≥50y only (p≤0.029). Similar to BMI, the greatest increase in BFM for men was observed at age 30-49y (+5.2kg, p<0.001), while the greatest increase in women was from age 50y (+6.0kg). BFMI was significantly higher in women at all age groups (p<0.001) and is observed to increase significantly with age in men and women at each age band (≤0.027). This trend was analysed further by age decade using the LMS method in Figure 6.1.2. Here, BFMI can be seen to plateau by age 60 years, particularly in men. Displayed as a percentage of body mass, BF% showed an increase from youth through maturation and old age in men (+5.4%, +5.3%; p<0.001). However, in women, a non-significant +1% increase was observed from youth to maturation (p=0.06), and a significant +7.3% increase was detected from maturation through to old age.

In observation of regional fat distribution, android fat was found to be significantly higher in young women (p<0.001), but was higher in men thereafter (p<0.001) Similar to total BFM, the greatest increase in android fat in men was at age 30-49y (+820g, p<0.001). However, the greatest increase in VAT was observed at age ≥50 years in men, and surpassed the increase in android fat (+970 vs. +790g, p<0.001). In women, the greatest increase in VAT and android fat was at age ≥50 years (+574 vs. +910g, p<0.001). VAT did not rise in proportion to android fat, as can be seen from the VAT/Android (V/A) ratio. Men were observed to carry significantly more VAT for android fat at all ages (p<0.001). After age 50 years, more than half of male android fat was composed of VAT (0.63±0.2).
Table 6.1.1 Descriptive Statistics for measurement of adipose variables by DXA in total men (n=683) and women (n=923) and by age band

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td>Range</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>32.0 (14.6)</td>
<td>24.9 (18.1)</td>
<td>18.0-69.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.9 (0.1)</td>
<td>1.79 (0.1)</td>
<td>1.57-2.01</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>82.5 (11.7)</td>
<td>80.8 (14.7)</td>
<td>53.0-135.4</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>18.1 (8.2)</td>
<td>16.6 (10.8)</td>
<td>3.7-54.9</td>
</tr>
<tr>
<td>BFMIZ</td>
<td>5.7 (2.6)</td>
<td>6.2 (3.5)</td>
<td>1.2-17.1</td>
</tr>
<tr>
<td>BFMIZ Z-score</td>
<td>0.7 (1.2)</td>
<td>0.4 (1.7)</td>
<td>-1.1-6.1</td>
</tr>
<tr>
<td>Body fat %</td>
<td>34.3 (7.6)</td>
<td>34.1 (10.8)</td>
<td>13.3-58.2</td>
</tr>
<tr>
<td>Android fat (kg)</td>
<td>1.59 (1.1)</td>
<td>1.25 (1.5)</td>
<td>0.15-6.66</td>
</tr>
<tr>
<td>VAT² (g)</td>
<td>708 (744)</td>
<td>386 (786)</td>
<td>0.4-9.30</td>
</tr>
<tr>
<td>V/A Ratio²</td>
<td>0.39 (0.2)</td>
<td>0.38 (0.3)</td>
<td>0.0-0.92</td>
</tr>
</tbody>
</table>

1 Normally distributed (Kolmogorov-Smirnov p>0.05) *n=1,527 (678 men, 849 women), 79 excluded based on VAT LLOD

BFM, body fat mass; BFMZ, body fat mass index; BMI, body mass index; IQR, interquartile range; SD, standard deviation; VAT, visceral adipose tissue; V/A Ratio, visceral/android fat ratio

<0.001
Descriptive statistics displaying changes in lean and bone tissue with age are presented in Table 6.1.2 and Figure 6.1.3. 272 (17%) individuals were categorised as ‘class I sarcopenia’ (ALTMI Z-score -1 to -1.99), with 34 (2%) categorised as ‘class II sarcopenia’ (ALTMI Z-score <-2). LTM was significantly different between men and women across all age bands (p<0.001). In men, LTM increased slightly at age 30-49y (+0.3kg, p=0.708), but declined significantly at age ≥50 years (-3.7kg; p<0.001). ALTMI tracked these changes accordingly, with the largest decline again observed in old age (p<0.001). In women, total LTM also declined at ≥50 years, but not to the same extent as men (-2.3kg, p<0.001). The smoothed reference curves in Figure 6.1.3 show that ALTMI declines at a much faster rate in men than women, beginning at age 30 years and continuing to age 70 years.
TBMD were significantly higher in men compared to women (p<0.001). Both variables declined with age, with a significant decline occurring in men at age 30-49y (p=0.004) and in men and women at age ≥50 years (p<0.001).

Table 6.1.2 Descriptive Statistics for lean and bone measurement by DXA in total men (♂; n=683) and women (♀; n=923) and by age band

<table>
<thead>
<tr>
<th>Variable</th>
<th>SD (kg)</th>
<th>Mean (kg/m²)</th>
<th>Range (kg/m²)</th>
<th>p (age)</th>
<th>Median (IQR)</th>
<th>p (age)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFM (♂)</td>
<td>64.7 (7.5)</td>
<td>20.1 (1.7)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>64.3 (9.6)</td>
<td>&lt;0.001</td>
<td>65.0 (9.7)</td>
</tr>
<tr>
<td>(kg)</td>
<td>43.4 (5.0)</td>
<td>16.1 (1.4)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>42.9 (6.6)</td>
<td>&lt;0.001</td>
<td>43.9 (6.9)</td>
</tr>
<tr>
<td>LTM (♂)</td>
<td>61.3 (7.1)</td>
<td>19.0 (1.7)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>60.9 (9.1)</td>
<td>&lt;0.001</td>
<td>61.6 (9.4)</td>
</tr>
<tr>
<td>(kg)</td>
<td>41.0 (4.7)</td>
<td>15.2 (1.4)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>40.5 (6.3)</td>
<td>&lt;0.001</td>
<td>41.4 (6.5)</td>
</tr>
<tr>
<td>ALTM (♂)</td>
<td>30.0 (4.0)</td>
<td>19.0 (1.7)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>29.7 (5.1)</td>
<td>&lt;0.001</td>
<td>30.1 (5.1)</td>
</tr>
<tr>
<td>(kg)</td>
<td>18.6 (2.6)</td>
<td>15.2 (1.4)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>18.2 (3.3)</td>
<td>&lt;0.001</td>
<td>19.2 (3.6)</td>
</tr>
<tr>
<td>FFMI (♂)</td>
<td>9.3 (1.0)</td>
<td>6.9 (0.8)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>9.3 (1.3)</td>
<td>&lt;0.001</td>
<td>9.3 (1.3)</td>
</tr>
<tr>
<td>(kg/m²)</td>
<td>6.9 (0.8)</td>
<td>6.8 (1.0)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>4.9 (10.0)</td>
<td>&lt;0.001</td>
<td>7.0 (1.0)</td>
</tr>
<tr>
<td>ALTIMZ (♂)</td>
<td>0.0 (1.1)</td>
<td>-0.1 (1.4)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>0.1 (1.4)</td>
<td>&lt;0.001</td>
<td>0.1 (1.4)</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.0 (1.1)</td>
<td>-0.2 (1.2)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>-2.6 (4.8)</td>
<td>&lt;0.001</td>
<td>-2.6 (4.8)</td>
</tr>
<tr>
<td>BMC (♂)</td>
<td>3.4 (0.4)</td>
<td>2.4 (0.3)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>3.3 (0.6)</td>
<td>&lt;0.001</td>
<td>3.4 (0.6)</td>
</tr>
<tr>
<td>(kg)</td>
<td>2.4 (0.3)</td>
<td>2.4 (0.5)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>2.1 (5.2)</td>
<td>&lt;0.001</td>
<td>2.5 (0.4)</td>
</tr>
<tr>
<td>TBMD (♂)</td>
<td>1.375 (0.1)</td>
<td>1.179 (0.1)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>1.382 (0.2)</td>
<td>&lt;0.001</td>
<td>1.401 (0.1)</td>
</tr>
<tr>
<td>(g/cm²)</td>
<td>1.179 (0.1)</td>
<td>1.182 (0.1)</td>
<td>1.0-17.9</td>
<td>&lt;0.001</td>
<td>0.79 (1.5)</td>
<td>&lt;0.001</td>
<td>1.225 (0.1)</td>
</tr>
</tbody>
</table>

1Normally distributed (Kolmogorov-Smirnov p>0.05) ALTM, appendicular lean tissue mass; ALTIM, appendicular lean tissue mass index; BMC, bone mineral content; FFM, fat-free mass; FFMI, fat-free mass index; IQR, interquartile range; LTM, lean tissue mass; LTMI, lean tissue mass index; SD, standard deviation; TBMD, total bone mineral density

Figure 6.1.3 Box plot representing the change in ALTMi (left) and by 3rd, 50th and 97th centile (right) in men (smooth line) and women (broken line) by age
Sarcopenic obesity was defined as an ALTMI Z-score $\leq -2$ combined with a BFMI Z-score $\geq 2$. Of the 34 participants described as ‘class II sarcopenic’, three men were also classified as ‘fat obese’ and therefore categorised as having ‘sarcopenic obesity’. A further 53 participants (24 men; 29 women) were defined as both ‘class I sarcopenic’ and ‘overfat’. Figure 6.1.4 and 6.1.5 show the relationship between ALTMI and BFMI Z-scores by age.

**Figure 6.1.4** Categorisation of sarcopenia vs. fat obesity defined by ALTMI and BFMI Z-scores in men (n=683)

**Figure 6.1.5** Categorisation of sarcopenia vs. fat obesity defined by ALTMI and BFMI Z-scores in women (n=923)
6.1.4 Discussion

Age-related changes in body composition, dependent on region and variation from the norm, have varying effects on morbidity, disability, and health status. These patterns are difficult to identify using conventional anthropometric measures, such as BMI. This study aimed to quantify the age-and sex-specific changes in body composition using DXA in a cross-sectional analysis of Irish adults from age 18-81 years. This analysis was anticipated to better define the Irish phenotype, and provide a ‘normal’ reference standard for health-related body composition parameters through age. The ageing process is analysed in terms of three main developments that affect functional outcome: (i) the progressive increase in BFM and re-distribution of fat (VAT) (fat obesity); (ii) the progressive decline in LTM (sarcopenia); (iii) the loss of BMD (osteopenia and osteoporosis).

A progressive increase in all measures of adiposity was shown with increasing age in men and women. Centile curves in Figure 6.1.2 show that median BFMI tends to plateau at ~55 years in men and after ~65 years in women which corresponded to values of 7.9 and 10.5 kg/m\(^2\) respectively. In past studies, the age of peak fat mass has been shown to differ between race and ethnicity, varying between age 50 and 79 years in Caucasian men and women, dependent on nationality and method of measurement (Kuk et al 2009). The American NHANES population, with DXA records available for participants aged 20-85 years, has shown an increase in BFMI until approximately age 65 years with a decline thereafter (Kelly et al 2009). Peak median values of BFMI were 8.8kg/m\(^2\) in men and 12.1kg/m\(^2\) in women, and were observed to be much higher than this Irish sample. A cross-sectional Italian reference database (n=1,866; age 20-80 years) observed BFMIs similar to this study at an older age (70-80 years), with peak median values of 7.0kg/m\(^2\) in men and 9.6kg/m\(^2\) in women (Coin et al 2008). However, these values were not significantly different from the previous decade suggesting BFMI had reached a plateau. Similarly, in a Spanish DXA reference group (n=1,113; age 0-80 years), absolute BFM and BF% increased until age 70 years and declined thereafter in men and women (Hench et al 2008). However, the authors did not calculate BFMI for their participants. In the present investigation, the BFMI Z-score also increased progressively with age, indicating that median man and woman over the age of 50 years are classified as ‘overfat’.
Abdominal adiposity is an important metric of body composition since the re-distribution of fat from gynoid or subcutaneous regions to android or visceral depots appears to be a better indicator to predict morbidity and mortality in older populations (Chang et al 2012). Being metabolically distinct from subcutaneous fat, VAT secretes several inflammatory hormones and cytokines, increasing the risk of cardiovascular disease by promoting insulin resistance and low-level chronic inflammation (Goodpastor et al 2006). While clearly relevant, the accurate measurement of VAT represents a challenging goal in clinical practice. The latest advancement in DXA technology has allowed estimation of VAT in whole-body scanning, and has shown accuracy against gold standard techniques such as CT (GE CoreScan™; Kaul et al 2012). This thesis is one of the first to explore this measurement technique across a large database of varying age and adiposity. Irish adults had increased VAT with age, with the most prominent increase in men and women observed after age 40 years (Figure 6.1.2). VAT increased by a factor of 2.5 at age 30-49y (+402g, p<0.001) and again at age ≥50y (+970g; p<0.001) in men. In women, VAT was significantly lower than men at all ages (p<0.001), and increased by a factor of 2.2 at age 30-49y (+69g, p<0.001) and by a factor of 5.5 at age ≥50y (+574g, p<0.001). The V/A ratio was consistently higher in men (p<0.001), but increased significantly in women at age 50+ (p<0.001), indicating that the proportion of android fat comprising of VAT increased from 14 to 31%. This rapid preferential gain in VAT over subcutaneous fat in women is likely attributed to the hormonal changes associated with menopause in women at this age (Kuk et al 2009). This result draws parallels with a longitudinal study by Franklin et al (2009), who found that menopause has an influence on relative visceral fat distribution rather than absolute, despite an increase in both. These trends were similar to the only comparative study to use CoreScan™, in healthy Italians aged 18-70 years with 25 men and 25 women in each of 5 age bands (Bazzocchi et al 2012). An overall reduction in android fat in women was observed, coinciding with an increase in VAT at age 61 years. Early indications have shown DXA VAT to be significantly associated with increased odds of hypertension, impaired fasting glucose, metabolic syndrome, and type II diabetes (p < 0.001) in adults with a mean age of 56 years and mean BMI of 26 kg/m² (Rothney et al 2013b). However, it is still unknown which cut-offs of VAT or V/A ratio represent health risk.

An objective of this study was to characterise the age- and sex-related variations in lean tissue mass and its components i.e. appendicular lean tissue mass (ALTM) as an index of
stature squared as described by Baumgartner et al (1998). In both men and women, LTM did not change significantly at age 30-49y (p≥0.708), but declined significantly at the ≥50y age band. Women appeared to preserve lean mass better than men, with an absolute difference of -3.7kg (-2.9kg ALTM) in men compared to -2.3kg (-1.7kg ALTM) in women from peak mass to age ≥50 years. This equated to a similar relative loss of 6% (-9.4% ALTM) in men and 5.5% (-8.9% ALTM) in women. Change represented by centiles in Figure 6.1.3 shows that ALTMI begins to decline at age 30 years in both men and women. This decline in lean mass with age has been well documented in past cross-sectional studies (Kelly et al 2009, Shaw et al 2007, Coin et al 2008). Declines in ALTMI in this study were very similar to that in NHANES. In men, peak median scores were attained at age 40 years (9.1 kg/m²) with similar marked decreases after age 50 (8.8kg/m²) (Kelly et al 2009). This is comparable to data from ULBC at the same intervals (9.3kg/m² to 8.9kg/m²). In women, peak ALTMI was the same in both studies (7.0kg/m²), declining to 6.7 kg/m² in NHANES and 6.6 kg/m² in the ULBC cohort.

Prevalence rates of sarcopenia in Ireland do not currently exist. Based on ALTMI definition alone, 17% (98 men; 147 women) of this cohort were categorised as ‘class I sarcopenic’, with 2% (18 men; 13 women) categorised as ‘class II sarcopenic’. Goodman et al (2013) analysed prevalence of ‘class I sarcopenia’ in the NHANES dataset of adults ≥65 years, reporting 40% of men and 38% of women with an ALTMI more than one standard deviation below the young adult. Prevalence of ‘class I sarcopenia’ in ULBC adults ≥65 years was lower, present in 20% of men and 14% of women. Since the current study was community-based with a low number of participants >65 years of age (n=107 with a median age of 67.6 years), it is likely that population rates are higher. However, this is the first study to estimate prevalence of sarcopenia in Ireland using population-specific reference criteria. Further research is required to analyse lean tissue in participants >80 years and particularly those in residential care, including an analysis of parameters of muscle strength or physical performance (Cruz-Jencreft et al 2010) before a more complete representation can be shown.

It has been hypothesized that the combination of high BFM and low LTM may act synergistically so that older individuals with both may have greater mobility limitations compared with having either condition alone (Dufour et al 2013). ‘Sarcopenic obesity’,
defined by Baumgartner (2000) is a combination of sarcopenia, with a BF% >28% in men and >38% in women. In a cross-sectional analysis of older adults >60 years, sarcopenic obesity was more strongly associated with disability than either body composition type alone. The odds ratio for two or more self-reported physical disabilities was reported as 8.72 for sarcopenic obesity in men compared with 3.78 for sarcopenia and 1.34 for obesity. The corresponding odds ratios in women were 11.98, 2.96, and 2.15, respectively, making it a worthwhile classification. The specific prevalence of sarcopenic obesity increased from 2% in those 60 to 69 years of age to 10% in those over 80 years.

In this study, a new definition of sarcopenic obesity was developed, based on population-specific reference scores for lean tissue and adiposity i.e. ALTMI Z-score ≤-2, combined with BFMI Z-score ≥2. Use of these criteria may offer a more accurate representation of sarcopenic obesity due to the limitations of BF% in the measurement of adiposity, particularly in the elderly as described previously in Chapter 4. Figures 6.1.4-5 define the phenotype of this Irish convenience sample based on these scores. Although only three individuals from this sample fall in to this defined category, almost 20% of those categorised as ‘class I sarcopenia’ also had a BFMI Z-score >1, classifying them as ‘overfat’ or ‘fat obese’ also. This is a worrying trend considering the low median age of older participants that are therefore likely to continue to lose LTM, putting them at risk for development of sarcopenic obesity in the future, and the negative consequences that coincide with it.

Since both low ALTM (Orsatti et al 2011) and high BFM and VAT (Katzmarzyk et al 2011) have been linked with poor bone health, the combination of sarcopenia and obesity may contribute to the development of osteoporosis. Since impaired insulin signalling has been shown to effect osteoblast function and bone formation (Fuzele et al 2010), increased lipid metabolites due to fat infiltration in skeletal muscle may contribute to low BMD (Kim et al 2013). In this study, TBMD reached a plateau at age 30-49 years in men and women, with a significant decline after age 50 years in women likely due to post-menopausal hormonal changes in availability of oestrogen. Measurement at whole body regions tends to underestimate osteoporosis as assessed by site-specific regions such as the lumbar spine, femoral neck and radius (Melton et al 2005). Therefore, complex interactions between the three combinations of ‘disordered’ body composition i.e. lean, fat and bone, require future study with analysis of site-specific BMD measurement.
While LTM in this study was analysed by DXA, use of CT to measure fatty infiltration into LTM has also been identified as a potential contributor to the declining strength and muscle quality associated with sarcopenia (Marcus et al 2012), and may be an attractive area of further research. Although large in number, this study is limited by uneven distribution of participants, particularly in men. Analysis was generally conducted according to three age groups to confer confidence in the data and observed changes. An increase in participant numbers in the future may allow the age-related change in body composition to be analysed by decade, allowing a more detailed interpretation of the age of peak lean, fat and bone mass. Further recruitment is needed to determine if these changes observed in body composition are representative of the Irish population. Moreover, the effect of the secular trend should be considered since new generations have different life-styles, eating habits and levels of physical activity from their elders. While cross-sectional trends are observed in this study, a longitudinal DXA analysis in Irish people is warranted before true inferences can be established.

6.1.5 Conclusion

The effect of ageing on body composition in Irish men and women is primarily defined by increases in adiposity and decreases in lean tissue. Describing the Irish phenotype by age will help to identify a ‘malleable range’ whereby intervention can minimise decline in functional status and maintain healthy ageing. Middle-age (age 30-49y) appears to be the age where the greatest changes occur in terms of increases in adiposity and decreases in lean mass. The age of peak BFMI is observed to be higher in women (~65y) vs. men (~55y), while the increase in VAT continues to increase past age 70 years. The loss of lean mass, as represented by ALTMI, was greater in men (-6%) than women (-5.5%) from peak mass to age 50y. However, the decline after age 60 years was observed to be greater in men. With knowledge of these changes as ‘typical’, comes an opportunity to develop a nationwide educational appeal to ‘keep healthy people healthy’. Prevalence of obesity, sarcopenia and sarcopenic obesity using appropriate metrics i.e. BFMI, VAT and ALTMI has been defined in this Irish convenience sample and may help to accurately identify those ‘at risk’ of age and body composition-related disease in the future.
6.2 Annual change in body composition associated with ageing in older adults aged 50-70 years

6.2.1 Introduction

Tracking age-related changes in body composition has become an increasingly prevalent area of research due to the consequences of excessive deviations from ‘normal’ on health. High or low indices of adiposity, lean and bone compartments are associated with risk factors for a variety of chronic diseases from middle to old age, such as obesity, sarcopenia and osteoporosis. Longitudinal observations offer the ability to follow individuals over time, thus eliminating the bias of secular trends and cohort differences. In addition, interventions are being developed that have been shown to positively influence the natural trajectory of body composition changes in older age i.e. through lifestyle, physical activity, nutrition or pharmaceutical interventions, that require appropriate reference data to quantify the decline in health observed with ageing.

Dual-energy X-ray absorptiometry (DXA) is increasingly recognised as a criterion measurement tool for the assessment of body composition, and has been utilised in both short-term interventions and longer longitudinal observations such as the Health, Ageing and Body Composition Study (Health ABC) (Goodpastor et al 2006). However, longitudinal assessments of body composition using DXA have rarely focused on regional measures of adiposity i.e. android and visceral adipose tissue (VAT) and changes with age. While cross-sectional increases in abdominal distribution of fat have been shown in the past and in the previous chapter (Chapter 6.1), trends observed may be due to the general increased prevalence of obesity over time (Flegal et al 2012), rather than as a consequence of age.

Osteoporosis and sarcopenia may represent the advanced stages of progressive, age-related body composition changes. Disability, functional limitations, falls, immobility and fracture are all linked to declines in lean tissue mass (LTM) and bone mineral density (BMD) (Sayer et al 2013, Gannon et al 2008). Rates of decline in fat-free mass (FFM) has been reported as between -0.06 to -0.25 kg/y in American men and women (Guo et al 1999, Visser et al 2003, Jackson et al 2012). The annual decline in BMD is reported as between -
0.87 and -3.12% in women and between -0.09 and -0.38% in men at the hip and lumbar spine (Hannon et al 2000, Zhai et al 2008, Sheu et al 2011). The annual change in body composition in Irish elderly participants is unknown.

The aim of this study was to track the annual change in body composition associated with ageing in 50-70 year old Irish men and women. Undertaking a longitudinal DXA analysis of Irish free-living older adults before and after a 1-yr follow-up, the aims of this chapter were as follows:

(i) To quantify any change in adiposity, lean mass and their components;
(ii) To quantify any change in measures of total and site-specific BMD;
(iii) To determine the effect of gender on these changes

6.2.2 Methodology

6.2.2.1 Study Design and Participants

A convenience sample of adults aged ≥50 years old were recruited from the UL faculty, student body and surrounding community via email advertisement and word-of-mouth to participate in the University of Limerick Body Composition Study (ULBC) between 2009 and 2013. Ethical approval was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18). This study involved a longitudinal analysis of whole body and BMD scans after 1-yr follow-up in older adult participants. Although no intervention was elicited, participants were free-living in the 1-yr interim and were not restricted to changes in dietary or lifestyle habits. A total of 66 participants were included in the final analysis.

6.2.2.2 Body Composition

Following written, informed consent, a Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with enCORE™ v.14.1 software was used to capture whole body scans and site-specific BMD scans at the spine (L1-L4), total hip and femoral neck. A detailed explanation of the pre-scan protocol and measurement method is included in the standard operating procedure in Section 3.2. DXA was used to assess the 1-yr change in BFM, LTM,
appendicular LTM (ALTM) and their indices, along with changes in android fat, VAT and BMD.

6.2.2.3 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.) Statistical significance (two-tailed) was set at p<0.05 for all analyses. Kolmogorov-Smirnov or Shapiro-wilk and Levene’s tests were conducted to assess whether variables were normally distributed and homoscedastic respectively. Obesity and sarcopenia were classified based on BFMI and ALTMI calculated using young adult (YA) Z-scores (median and standard deviation) as described in Chapter 5. A repeated measures ANOVA with gender as a between-subjects factor was used to determine the effect of time on the variables of interest. Measurement error was calculated as the least significant change (LSC) at the 95% confidence level for each anatomical region and was determined by a repeat scan analysis in Chapter 3.

6.2.3 Results

6.2.3.1 1-year Change in Adiposity and Muscle

The average time to follow-up was 12.1 (±1.5) months from baseline. Baseline descriptive statistics of participants who underwent whole-body DXA scans are presented in Table 6.2.1. Participants were deemed representative of the ULBC ageing cohort (age 50-70 years; n=538) due to similarities in mean BFMI (8.6 vs. 8.1 kg/m² in men; 10.5 vs. 10.4kg/m² in women) and ALTM (9.3 vs. 8.9kg/m² in men; 6.9 vs. 6.8kg/m² in women). Based on Z-scores of BFMI, 19 (28.8%) were classified as 'overfat' and 21 (31.8%) were classified as 'fat obese'. 9 individuals (13.6%) were classified as 'class I sarcopenia', while none had 'class II sarcopenia', defined by Z-scores of ALTMI.
<table>
<thead>
<tr>
<th></th>
<th>Total (n=66)</th>
<th>Men (n=16)</th>
<th>Women (n=50)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>59.4 (5.6)</td>
<td>59.8 (10)</td>
<td>58.8 (6.3)</td>
<td>59.8 (11.5)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 (0.1)</td>
<td>1.64 (0.1)</td>
<td>1.75 (0.0)</td>
<td>1.75 (0.1)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>74.8 (15.2)</td>
<td>71.3 (18.8)</td>
<td>88.9 (10.3)</td>
<td>87.4 (15.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 (5.2)</td>
<td>26.3 (4.8)</td>
<td>29.0 (4.0)</td>
<td>28.9 (6.2)</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>27.1 (9.5)</td>
<td>24.5 (9.6)</td>
<td>26.2 (7.3)</td>
<td>24.1 (12.1)</td>
</tr>
<tr>
<td>BF (%)</td>
<td>35.9 (7.0)</td>
<td>35.8 (9.0)</td>
<td>29.1 (9.1)</td>
<td>27.8 (9.1)</td>
</tr>
<tr>
<td>BFMI (kg/m²)</td>
<td>10.0 (3.8)</td>
<td>9.2 (3.5)</td>
<td>8.6 (2.7)</td>
<td>7.8 (4.0)</td>
</tr>
<tr>
<td>Android (kg)</td>
<td>2.50 (1.2)</td>
<td>2.22 (1.0)</td>
<td>2.81 (1.1)</td>
<td>2.47 (1.6)</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>1,043 (809)</td>
<td>913 (841)</td>
<td>1,740 (983)</td>
<td>1,451 (1,053)</td>
</tr>
<tr>
<td>V/A ratio</td>
<td>0.38 (0.2)</td>
<td>0.37 (0.2)</td>
<td>0.60 (0.1)</td>
<td>0.59 (0.2)</td>
</tr>
<tr>
<td>LTM (kg)</td>
<td>42.1 (9.3)</td>
<td>40.7 (12.2)</td>
<td>59.7 (4.8)</td>
<td>58.9 (5.4)</td>
</tr>
<tr>
<td>ALTM (kg)</td>
<td>20.6 (5.1)</td>
<td>18.6 (6.7)</td>
<td>28.6 (2.7)</td>
<td>28.6 (2.6)</td>
</tr>
<tr>
<td>ALTMI (kg/m²)</td>
<td>7.5 (1.3)</td>
<td>7.0 (1.9)</td>
<td>9.3 (0.9)</td>
<td>9.4 (1.8)</td>
</tr>
</tbody>
</table>

*Normally distributed (p>0.05) ALTM, appendicular lean tissue mass; ALTMI, appendicular lean tissue mass index; BFM, body fat mass; BFMI, body fat mass index; BF%, body fat percent; BMI, body mass index; IQR, interquartile range; LTM, lean tissue mass; SD, standard deviation; VAT, visceral adipose tissue; V/A ratio, visceral fat/android fat ratio

Changes in body composition after 1-yr follow-up are presented in Table 6.2.2. A non-significant decrease in total BFM (-0.4%) and BFMI (-0.9%) was observed after including gender as a between-subject factor (p=0.180 and 0.103 respectively). Time had a significant effect on android fat mass (p=0.029), whereby a mean increase of 31.4% was observed. An increase was also observed in the visceral component (+18.9%), but this change was not statistically significant (p=0.777). LTM declined by 1% at follow-up, a change that was approaching significance after adjustment for gender (p=0.062). Without adjustment for gender, this decline was significant (p=0.009). Non-significant declines were also observed in ALTM and ALTMI (p=0.849 and 0.167 respectively).
Table 6.2.2 1-yr change (Δ) in body composition variables in men and women (n=66) using repeated measures ANOVA with gender as a between-subject factor

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men Δ (SD)</th>
<th>Women Δ (SD)</th>
<th>All Δ (SD)</th>
<th>% Δ</th>
<th>Range</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFM (g)</td>
<td>-676 (1,639)</td>
<td>-37 (1,889)</td>
<td>-192 (2,218)</td>
<td>-0.4%</td>
<td>-4,580-7,610</td>
<td>0.180</td>
</tr>
<tr>
<td>BFMI (kg/m²)</td>
<td>-0.28 (0.6)</td>
<td>-0.06 (0.7)</td>
<td>-0.11 (0.7)</td>
<td>-0.9%</td>
<td>-1,59-2.79</td>
<td>0.103</td>
</tr>
<tr>
<td>Android (g)</td>
<td>+76 (539)</td>
<td>+618 (1,197)</td>
<td>+486 (1,096)</td>
<td>+31.4%</td>
<td>-1,050-4,230</td>
<td>0.029</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>-39 (257)</td>
<td>+60 (268)</td>
<td>+36 (266)</td>
<td>+18.9%</td>
<td>-431-1,317</td>
<td>0.777</td>
</tr>
<tr>
<td>LTM (g)</td>
<td>-210 (1,745)</td>
<td>-484 (1,085)</td>
<td>-418 (1,267)</td>
<td>-1.0%</td>
<td>-3,370-3,530</td>
<td>0.062</td>
</tr>
<tr>
<td>ALTM (g)</td>
<td>+34 (997)</td>
<td>-72 (569)</td>
<td>-46 (689)</td>
<td>-0.3%</td>
<td>-1,890-1,730</td>
<td>0.849</td>
</tr>
<tr>
<td>ALTMI (kg/m²)</td>
<td>-0.04 (0.4)</td>
<td>-0.05 (0.3)</td>
<td>-0.06 (0.3)</td>
<td>-0.8%</td>
<td>-0.81-0.52</td>
<td>0.167</td>
</tr>
</tbody>
</table>

1Including gender as a between-subject factor. ALTM, appendicular lean tissue mass; ALTMI, appendicular lean tissue mass index; BFM, body fat mass; BFMI, body fat mass index; LTM, lean tissue mass; SD, standard deviation; VAT, visceral adipose tissue

6.2.3.2 1-year Change in Bone Mineral Density

Repeated measures for site-specific BMD were available for 58 participants (L1-L4 Spine) and 57 participants (Dual Femur) respectively. Baseline descriptives for TBMD, L1-L4 spine, total hip and femoral neck BMD are reported in Table 6.2.4. Men had significantly higher BMD than women in all sites (p≤0.05). Prevalence of osteopenia in this sample was 40.7% and prevalence of osteoporosis was 6.8%.

Table 6.2.3 Baseline descriptive statistics for measures of BMD by DXA in men and women (n=66)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n=66) Mean (SD)</th>
<th>Total (n=66) Range</th>
<th>Men (n=16) Mean (SD)</th>
<th>Men (n=16) Range</th>
<th>Women (n=50) Mean (SD)</th>
<th>Women (n=50) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBMD (g/cm²)</td>
<td>1.178² (0.1)</td>
<td>0.84-1.62</td>
<td>1.315² (0.1)</td>
<td>1.04-1.62</td>
<td>1.134² (0.1)</td>
<td>0.84-1.43</td>
</tr>
<tr>
<td>Spine BMD²</td>
<td>1.116² (0.2)</td>
<td>0.80-1.42</td>
<td>1.198² (0.2)</td>
<td>0.90-1.34</td>
<td>1.096² (0.2)</td>
<td>0.8-1.42</td>
</tr>
<tr>
<td>Total Hip³</td>
<td>0.990³ (0.1)</td>
<td>0.75-1.31</td>
<td>1.085³ (0.1)</td>
<td>0.82-1.28</td>
<td>0.967³ (0.1)</td>
<td>0.75-1.31</td>
</tr>
<tr>
<td>Femoral Neck³</td>
<td>0.928³ (0.1)</td>
<td>0.72-1.24</td>
<td>0.994³ (0.1)</td>
<td>0.77-1.13</td>
<td>0.912³ (0.1)</td>
<td>0.72-1.24</td>
</tr>
</tbody>
</table>

1Normally distributed (p>0.05); ²n=58 (11 men, 47 women); ³n=57 (11 men, 46 women) TBMD, total bone mineral density
Changes in BMD after 1-yr follow-up are presented in Table 6.2.5. No significant changes occurred in any site, with or without the influence of gender \((p \geq 0.292)\). Slight increases were observed in men in all sites except the spine. In women, declines were observed in both hip sites. Combined, the largest decline was in the femoral neck (-0.5%).

<table>
<thead>
<tr>
<th>Table 6.2.4 1-yr change (Δ) in BMD variables in men and women ((n=66)) using repeated measures ANOVA with gender as a between-subject factor</th>
<th>Men Δ (SD)</th>
<th>Women Δ (SD)</th>
<th>All Δ (SD)</th>
<th>% Δ</th>
<th>Range</th>
<th>(p^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBMD ((g/cm^2))</td>
<td>+0.005 (0.02)</td>
<td>+0.001 (0.02)</td>
<td>+0.002 (0.02)</td>
<td>+0.2%</td>
<td>-0.06-0.04</td>
<td>0.292</td>
</tr>
<tr>
<td>Spine ((g/cm^2))</td>
<td>-0.005 (0.03)</td>
<td>+0.000 (0.03)</td>
<td>-0.001 (0.03)</td>
<td>-0.1%</td>
<td>-0.07-0.07</td>
<td>0.717</td>
</tr>
<tr>
<td>Total Hip ((g/cm^2))</td>
<td>+0.006 (0.01)</td>
<td>-0.003 (0.02)</td>
<td>-0.001 (0.02)</td>
<td>-0.1%</td>
<td>-0.04-0.03</td>
<td>0.511</td>
</tr>
<tr>
<td>Femoral Neck ((g/cm^2))</td>
<td>+0.001 (0.03)</td>
<td>-0.006 (0.03)</td>
<td>-0.005 (0.03)</td>
<td>-0.5%</td>
<td>-0.06-0.06</td>
<td>0.559</td>
</tr>
</tbody>
</table>

\(^1\)Including gender as a between-subject factor. TBMD, total bone mineral density

6.2.4 Discussion

This study was undertaken to determine the change in body composition that occur in Irish older adults aged 50-70 years after a 1-year follow-up. As a confirmatory analysis to the cross-sectional trends observed in Chapter 6.1, it was hypothesised that an increase in adiposity, combined with a decline in measures of lean mass and bone density would be observed. The aim of this investigation was to determine to what extent these changes take place after one year and to analyse whether gender can explain these changes.

6.2.4.1 1-year Change in Adiposity and Muscle

Changes to whole body measurements of fat and lean mass were generally small \((\leq 1\%)\) but variability of change was high. Since a strict pre-scan protocol was in place (Section 3.2) to limit technical and short-term biological variation, changes measured are likely due to inter-individual variability, except where differences were below known values of least significant change (LSC) at the 95% confidence interval level. A non-significant decrease
in BFM was observed (-0.4%; p=0.18), that was higher in men (-676g) than women (-37g). Overall change in BFM (-192g) was lower than the LSC for measures of total BFM (583g), and may therefore be below the minimum detectable change of DXA scanning. While the decline in BFM in men aged 50-70y is comparable to the cross-sectional analysis in Chapter 6.1, other longitudinal studies have reported an increase in BFM. In a slightly younger age group (40-66y) with serial measurements of body composition using underwater weighing over 20 years, the Fels Longitudinal study described a ~370g increase in men and a ~410g increase in women per annum (Guo *et al* 1999). Similarly in an older cohort (70-79y) of the Health ABC study, Visser *et al* (2003) observed a +107g increase in men after one year using DXA. However in women, an overall decrease in BFM (-165g) was reported. Variations in this study may be attributed to the difference in the population under study. The Aerobics Center Longitudinal Study (ACLS) with multiple body composition determinations (hydrostatic weighing and skinfold analysis) on 7,265 American men measured an increase in BFM from age 20 years that plateaued and began to decline after age 80 years (Jackson *et al* 2012). While this plateau may occur earlier in Irish individuals, it is more likely that the current study was not powered adequately to determine a true change in BFM.

Android fat mass increased over one year by +486g (+31.4%; p=0.029), and to a greater extent in women (+618g) than men (+76g). This change in women exceeded the LSC of 139g for the android compartment. Since total BFM did not increase, this observation would suggest a re-distribution of fat mass occurs with age, with a shift from the extremities to a more central distribution of adiposity. Within this compartment, changes to subcutaneous and visceral mass can occur independently. Although an increase of +18.9% was observed in VAT, this change was not significant (p=0.777) and did not exceed the LSC of 130g. This is the first study of its kind to measure the longitudinal change in regional fat distribution in Caucasians by DXA and therefore no direct comparisons can be made. However, ‘gold standard’ methods such as MRI have been utilised by Franklin *et al* (2009), who reported an increase in total abdominal (+35%), subcutaneous (+32%) and visceral (+44%) fat in women between pre-menopause and post-menopause 8 years later (p<0.05). Hormone replacement therapy has been found to both prevent VAT gain in a 12-month longitudinal study (Sumino *et al* 2003), and not alter VAT gain over a 2-yr period (Sites *et al* 2001) in postmenopausal women. Thus, it is yet unclear if loss of oestrogen is
the culprit for this trend in women. In elderly Caucasian men, longitudinal data is lacking on measures of abdominal or visceral adiposity using imaging techniques. Using anthropometry, Hughes et al (2004) measured a 10-yr change in 54 men and 75 women aged 60±7.8 y at baseline and found a significant increase in waist circumference and waist-to-hip ratio only in women (p<0.001). A decrease in hip circumference was found only in men (p<0.05), suggesting a decrease in subcutaneous fat.

Development of chronic diseases associated with ageing can be attributed in part to increased adiposity. However, it is the regional measures of fat deposition that have been more specifically linked to metabolic and cardiovascular disease, including insulin resistance, hypertension, dislipidemia, and coronary artery disease, that develop with advancing age (Kuk et al 2009, Enzi et al 1986). Longitudinal studies including accurate measures of regional adiposity in subjects older than 50 years are rare and report limited data. While no significant change in VAT was observed in this sample after one year (p=0.777), it is suggested that significant increases may take longer to occur in a small compartment. Although this current study will advance the knowledge and understanding of longitudinal changes to abdominal and VAT with age, it is expected that research will continue to improve in this field, as the availability of accurate measurement techniques, the prevalence of obesity and the ageing population grows.

Ageing is associated with a decrease in body mass, largely attributable to a decline in LTM (Newman et al 2005). A -418g (-1%) loss in LTM was observed over the duration of one year in adults aged 50-70y. This decline was greater in women (-484g) than men (-210g) and was not significant when the influence of gender was included (p=0.062). Total loss was lower than the LSC for LTM (719g) and therefore within the range of measurement error. However, these results compare favourably and exceed changes reported in larger longitudinal studies. The Fels Longitudinal Study observed that fat-free mass (FFM) declined at a linear rate of -70g/year for men and -110g/year in women aged 40–66 years (Guo et al 1999). Similarly, Jackson et al (2012) used a quadratic model to define the rate of decline of FFM as -60g/year in men aged 20+ years. These changes are quite small in comparison to the current study due to differences in study design. The annual change reported was the mean annual change over a number of years rather than a 1-yr follow-up and therefore may be under-representative of 50-70 year olds. In a similar design to the current study, a 1-yr follow-up was carried out by Visser et al (2003), but in an older age
group (70-79 year olds) in the Health ABC Study. A greater annual decline in men (-246g; -0.4%) compared to women (-52g; -0.1%) was observed in contrast to the current study. This rapid decline in men compared to women is expected to be due to the higher baseline LTM in men and may be more reflective of a true trend due to a higher sample size (n=2,040 vs. 16).

The decline in LTM or FFM with age is suggested to be highly influenced by a loss in LTM from the appendices (ALTM) (Gallagher et al 2000). A combined non-significant loss of -46g (-0.3%) was detected in men and women after one year (p=0.849), representing a decrease in women (-72g) and an increase in men (+34g). This change was below the LSC for ALTM (692g). Other studies have found a greater annual loss of ALTM in elderly men. Men from the Health ABC study lost -103g (-0.4%) of ALTM compared to a gain of +14g (+0.2%) in women (Visser et al 2003), while Gallagher et al (2000) reported an annual loss of ~-200g in men compared to ~-100g in women over age 60 years. It is likely that rates of decline in LTM and ALTM would be greater if this sample included participants older than 70 years of age. Prevalence of type I sarcopenia was 13.6%, while none from the sample were type II sarcopenic. If the rate of decline in ALTMI were to continue as projected, (-0.06 per annum), the prevalence of type I would double in the next 5 years (28.8%), while the prevalence of type II would increase to 3% of the sample. At the cellular level, sarcopenia stems from prolonged periods of net negative protein balance, brought about by dampened rates of muscle protein synthesis, elevated rates of muscle protein breakdown or a combination of the two. Maintaining an active physical lifestyle and an appropriate nutrient intake promotes an anabolic response in muscle and should offset the decline associated with disuse (Breen & Philips 2013). Further longitudinal studies are required to test this hypothesis in an Irish population.

6.2.4.2 1-year Change in Bone Mineral Density

Rate of bone loss in this study was minimal (-0.1 to -0.5%; p>0.511) and mean change did not reach LSC for BMD in any site (0.028-0.04g/cm²). Greatest change was observed in the femoral neck site (-0.5%), with a greater loss in men observed in the spine and a greater loss in women observed in the hip. The US population-based Framingham Osteoporosis Study with repeat BMD examinations over 4 years on 765 men and women aged 67-95 years reported larger annual losses in BMD than this study (Hannon et al 2000). In the
femoral neck site, loss of BMD per annum was -0.9% in women and -0.4% in men, while loss in the lumbar spine site was -1.1% in women and -0.8% in men. In postmenopausal women only, an even greater bone loss has been observed by Zhai et al (2008), in a 15-yr longitudinal study on 955 women aged 45-68 years from the UK. Authors reported a linear decline in femoral neck BMD (-1.7% per annum) and a quadratic decline in the spine (-3.1% per annum), which slowed by 0.02% per squared age increase. This highlights the influence of menopause on bone loss in women. Bone resorption, as assessed by biochemical markers, increases by 90% at menopause, whereas bone formation markers increase by only 45% (Garnero et al 1996). This imbalance leads to accelerated bone loss due to the decrease in oestrogen post-menopause. Age-related bone loss in both women and in men appears driven by changes in sex steroid (oestrogen and/or testosterone) production or availability and by secondary hyperparathyroidism (Khosla et al 1997, Khosla et al 2005). Risk factors such as calcium and vitamin D deficiency, reduced peak bone mass, family history, PA, smoking, alcohol consumption and weight loss (Hannan et al 2000, Cummins et al 2013) have a role to play and should be accounted for in future longitudinal studies to determine true rate of loss in Irish individuals.

Differences between studies may be due to variation in measurement technique, age, ethnicity and power. Strengths of this study include reporting on LSC to determine a true change in variables measured. While criteria for determination of measurement error for BMD is often reported, precision error for whole body DXA scanning is less well established, and rarely reported in longitudinal studies. A limitation of this study is the small study sample, particularly in men. Also, while participants were independent and free-living, no survey was administered to provide data on physical activity, nutrition, chronic disease, use of medication or menopausal status. These may be key elements that influence degree of change, independent of age.

6.2.5 Conclusion

This study supports the hypothesis that loss of LTM and BMD occurs with advancing age in elderly men and women, even in independently living subjects. This is the first study to report the longitudinal change in regional adiposity using DXA. Increases were observed in android fat (+31.4%; p=0.029) with age, despite a decrease in total fat (-0.4%; p=0.18).
These observations indicate a dynamic remodelling of body composition as Irish older adults advance in age. Lean mass declined by -1% (p=0.062) in the whole body and by -0.3% in the appendages (p=0.849), while the change in BMD was also non-significant (-0.1 to -0.5%; p>0.511). The influence of gender on these changes was minimal, with women experiencing greater losses in LTM and BMD, and a larger increase in android fat over one year, which may be related to hormonal changes associated with menopause. These have potential implications for future intervention studies that aim to offset body composition- and age-related decline in health through physical activity, nutrition and lifestyle, or medication.
Chapter 7

*The effect of adiposity on total and site-specific bone mineral density*
7.1 Introduction

Excess adiposity is a state that has detrimental effects on almost every facet of human health, most notably diseases such as diabetes, coronary heart disease, stroke, cancer, and osteoarthritis, increasing risk of all-cause mortality (Prospective Studies Collaboration, 2009). However, the extent to which adiposity influences bone health is a relationship that has not yet been fully elucidated. The previous chapter examined the changes in body composition that occur with age using accurate estimates of total or regional adiposity and bone mineral density. The following chapter will attempt to determine how inter-relationships between these variables may impact on bone health, a valid focus as the prevalence of obesity in the ageing population continues to rise (IUNA 2011).

Osteoporosis, characterised by low bone mineral density (BMD) and an increased susceptibility to fracture, constitutes a second large and growing public health concern due to impact on quality of life and mortality (Johnell et al 2004). BMD is influenced by a number of genetic and environmental factors, with body mass demonstrated as one of the important determinants. Higher body mass imparts greater physical loading to bone, which induces an anabolic effect on the bone remodelling process (Reid et al 1992, De Laet et al 2005, Dytfeld et al 2011). However, the relative effect of the components of body mass (lean tissue mass (LTM) and body fat mass (BFM)) on BMD should always be clarified. While LTM has consistently shown a positive influence on bone through the weight-bearing and muscle-mediated effect of physical exercise, the influence of adiposity on bone independent of its loading effect may stem from the production of hormones and adipokines by adipocytes (e.g. oestrogen, leptin, adiponectin, resistin, interleukins) or the secretion of bone-active hormones from the pancreas (e.g. insulin, amylin and preptin) (Reid et al 2008). Higher circulating concentrations of pro-inflammatory cytokines may also have a role to play in the regulation of bone turnover (Mundy 2007). Whether excess adiposity has a positive or negative effect on bone through these pathways remains to be clarified.

Several discrepancies can be found in the literature as to the influence of adiposity on bone in young vs. old, male vs. female, pre-menopausal vs. post-menopausal and in different ethnicities. Additionally, controversy remains regarding to the correct statistical model to use when addressing this relationship. In order to adjust for the mechanical weight-bearing effect of BFM, several recent studies (Hsu et al 2006, Zhao et al 2007) have included body mass as
a covariate in regression models to determine the non-weight-bearing effect of BFM on bone. Such studies have shown that BFM has a deleterious effect on bone, only after adjustment for body mass. Reid (2010) has argued that these analyses are confounded by the collinearity between the variables studied, and therefore has produced misleading results. However, it can be appropriate to adjust for factors on the potential casual pathway between adiposity and bone in order to test potential mechanisms, providing formal statistical tests for the influence of multicollinearity are carried out.

It is also suggested that the function of adipose tissues may depend on the location of fat deposition, e.g. visceral or subcutaneous. This is supported by the increased risk of cardiovascular disease (CVD), diabetes, and mortality with elevated visceral adipose tissue (VAT) as opposed to subcutaneous adipose tissue (SAT) (Goodpastor et al 2005) and the link between VAT and many facets of the metabolic syndrome: glucose intolerance, hypertension, dyslipidemia, and insulin resistance (Kang et al 2011). Gilsanz et al (2009) showed CT-measured SAT to be beneficial to bone structure and strength in premenopausal women, with the visceral component having a negative influence. However, other investigations have shown both VAT and SAT to have a negative influence on total BMD in men and women of varying age and ethnicity (Katzmarzyk et al 2011). Thus, abdominal fat distribution, rather than overall adiposity, is considered to be important in the understanding of how adiposity affects bone health. However, the relative effect of each component (VAT vs. SAT) is not yet certain.

Although obesity and osteoporosis both become more prevalent with advancing age, it is clear that complex relationships exist between the two. Apart from variation in statistical model used, disparity between studies is likely related to differences in definition of adiposity and bone health. Past models may be limited by the lack of correction for body size. As demonstrated in Chapter 4, the use of body fat mass index (BFMI) offers a more accurate representation of adiposity over absolute values or values relative to body mass. It also offers the advantage of having a lower correlation with body mass than BFM, thereby reducing the risk of multicollinearity. Additionally, this is the first study to investigate this relationship using DXA-measured VAT, a measurement tool that has shown strong validity compared to CT (Kaul et al 2012). Bone health as expressed by total BMD (TBMD) or site-specific BMD may be affected to different extents due to the different compositional structure. The lumbar spine contains more trabecular bone which may be more susceptible to
bone loss (Eastell 1998). TBMD is predominantly represented by cortical bone, thinning of which may also contribute to increased fracture risk (Holzer et al 2009). To investigate the relationship of total and abdominal adiposity and bone health, this study sought to:

(i) Determine the influence of BFMI, VAT and SAT on total bone mineral density (TBMD) in Irish adults aged 18-81 years, according to age and gender;

(ii) Determine the influence of BFMI, VAT and SAT on site-specific BMD at the lumbar spine and dual proximal femur in Irish older adults aged ≥50 years, according to gender.

7.2 Methodology

7.2.1 Study Design and Participants

Data for this study were collected from 1,606 adults aged 18-81 years, all of whom were participants in the University of Limerick Body Composition Study (ULBC) as described previously in Chapter 3.2. Participants were recruited from the UL faculty, student body and surrounding community via email advertisement and word-of-mouth. Ethical approval for this study was granted by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC 09/18).

The present investigation is a cross-sectional analysis of baseline data for participants who underwent whole-body and/or bone mineral density dual-energy x-ray absorptiometry (DXA) scans between 2009 and 2013. Site-specific BMD measurements at the lumbar spine and proximal femur was obtained in all participants aged ≥50 years from the beginning of 2011 and were not available for participants less than 50 years of age. In cases where participants had multiple baseline measurements, the earliest complete record was used. 79 participants (5 men, 74 women) were excluded from the final analysis on the basis of a negligible VAT measurement (<0g VAT) as described in Chapter 3.3.

7.2.2 Body Composition

Following written, informed consent, a Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) was used to capture whole body and site-specific BMD scans at the spine (L1-L4) and proximal femur (total hip and femoral neck). For proximal femur BMD scans,
the mean of right and left hip and femoral neck was taken unless a hip replacement was present on one side. A detailed explanation of this pre-scan protocol and analysis is included in the standard operating procedure in Section 3. EnCORE™ v.14.1 software was used to assess BFM, LTM, TBMD and site-specific BMD, with CoreScan™ software used to analyse VAT and SAT.

7.2.3 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.) Statistical significance (two-tailed) was set at p<0.05 for all analyses. Kolmogorov-Smirnov and Levene’s tests were conducted to assess whether variables were normally distributed and homoscedastic respectively. Gender differences were examined using independent sample t-tests or Mann-Whitney U tests.

A simple correlation analysis was conducted to assess the relationship between adiposity and BMD and to determine any potential highly correlated covariates for the regression analysis. Multiple linear regression models were run using a 'stepwise' approach to determine the \( R^2 \) change attributable to adiposity alone. Additional variables that were deemed to influence bone health were included in the model i.e. body mass, age and gender. Total body mass was used as an independent variable in all models to account for the weight-bearing effect of excess adiposity on bone. Lean mass was not used as a covariate as it does not fully account for the weight-bearing effect. Analysis was repeated to determine any difference in the relationship according to gender and age group (18-29y; 30-49y; 50+y). To assess abdominal adiposity, both VAT and SAT were included in models to determine the influence of one while accounting for the other. For all linear models, the assumptions of normality, linearity and homogeneity were checked by examining plots of residuals compared with predicted values and normal probability plots of residuals. On the basis of this, VAT and SAT were transformed to the square root (sqrtVAT and sqrtSAT). This normalised the distribution to a greater extent than log transformation. Variance inflation factor (VIF) analysis was carried out to ensure no influence of multi collinearity existed in the regression analysis. The model was accepted if VIF<10 (Belsley et al 1980).
Chapter 7

7.3 Results

The analysis of the adiposity-bone relationship in this study is divided into two sections - 7.3.1: The influence of adiposity on TBMD across all age groups (n=1,527); and 7.3.2: The influence of adiposity on site-specific BMD in older adults aged ≥50 years (n=480). Within each section, adiposity is represented firstly by BFMI, and secondly, by abdominal VAT and SAT.

7.3.1 The Association between Adiposity and Total Bone Mineral Density

Descriptive statistics of 1,527 adults (678 men, 849 women) that were included in the analysis of TBMD are displayed in Table 7.3.1. Mean BFMI and SAT was higher in women (+3.4kg/m², +533g; p<0.001), while levels of VAT were higher in men (+181g; p<0.001).

Table 7.3.1 Descriptive statistics of all participants with whole body DXA body composition analysis

<table>
<thead>
<tr>
<th>Total (n=1,527)</th>
<th>Men (n=678)</th>
<th>Women (n=849)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td><strong>Median (IQR)</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>Age (y)</td>
<td>38.9 (17.2)</td>
<td>32.7 (33.7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 (0.1)</td>
<td>1.70 (0.2)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>74.2 (13.7)</td>
<td>72.8 (18.1)</td>
</tr>
<tr>
<td>BFMI (kg/m²)</td>
<td>7.6 (3.5)</td>
<td>7.1 (4.6)</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>613 (658)</td>
<td>355 (724)</td>
</tr>
<tr>
<td>SAT (g)</td>
<td>1,178 (651)</td>
<td>1,108 (651)</td>
</tr>
<tr>
<td>TBMD (g/cm²)</td>
<td>1.266 (0.2)</td>
<td>1.263 (0.2)</td>
</tr>
</tbody>
</table>

Normally distributed (p>0.05); BFMI, body fat mass index; SAT, subcutaneous adipose tissue; TBMD, total bone mineral density; VAT, visceral adipose tissue

Adiposity correlated negatively with TBMD. This correlation was significant for BFMI (ρ=-0.405; p<0.001) and SAT (ρ=-0.256; p<0.001), but not VAT (ρ=-0.004; p=0.889). Poor to moderate significant relationships were observed for correlation of covariates selected for inclusion in regression models i.e. body mass vs. BFMI, VAT, SAT and VAT vs. SAT (ρ=0.160 to 0.576; p<0.001), indicating a requirement for VIF analysis in the regression models.
Table 7.3.2 Spearman correlation coefficients (ρ) between variables of interest in men and women (n=1,527)

<table>
<thead>
<tr>
<th></th>
<th>Body Mass</th>
<th>BFMI</th>
<th>VAT</th>
<th>SAT</th>
<th>TBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass</td>
<td>-</td>
<td>0.160*</td>
<td>0.576*</td>
<td>0.240*</td>
<td>0.582*</td>
</tr>
<tr>
<td>BFMI</td>
<td>0.160*</td>
<td>-</td>
<td>0.613*</td>
<td>0.894*</td>
<td>-0.405*</td>
</tr>
<tr>
<td>VAT</td>
<td>0.576*</td>
<td>0.613*</td>
<td>-</td>
<td>0.481*</td>
<td>-0.004</td>
</tr>
<tr>
<td>SAT</td>
<td>0.240*</td>
<td>0.894*</td>
<td>0.481*</td>
<td>-</td>
<td>-0.256*</td>
</tr>
<tr>
<td>TBMD</td>
<td>0.582*</td>
<td>-0.405*</td>
<td>-0.004</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significant at the <0.05 level. BFMI, body fat mass index; SAT, subcutaneous adipose tissue; TBMD, total bone mineral density; VAT, visceral adipose tissue.

To analyse the influence of adiposity on TBMD while adjusting for the potential effects of covariates, a linear regression analysis was carried out. Independent linear regression analysis was conducted by gender and by age group for BFMI. Results of the linear regression analysing the influence of BFMI on TBMD is presented in Table 7.3.3.

Table 7.3.3 Linear regression analysis measuring the influence of adiposity (BFMI) on TBMD in 1,527 adults

<table>
<thead>
<tr>
<th>Model</th>
<th>Total (n=1,527)</th>
<th>Men (n=678)</th>
<th>Women (n=849)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²%</td>
<td>β</td>
<td>R²% BFMI</td>
</tr>
<tr>
<td>1 (Total)</td>
<td>63.8</td>
<td>-0.402</td>
<td>4.0</td>
</tr>
<tr>
<td>2 (18-29)</td>
<td>59.9</td>
<td>-0.426</td>
<td>17.3</td>
</tr>
<tr>
<td>3 (30-49)</td>
<td>48.9</td>
<td>-0.335</td>
<td>9.6</td>
</tr>
<tr>
<td>4 (50+)</td>
<td>41.2</td>
<td>-0.416</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Dependent variable: TBMD
Models 1-4 include body mass, age and gender as covariates
R²%, R² of the model including covariates; R²% BFMI, R² attributable to adiposity within the model; β, standardised coefficient for adiposity; BFMI, body fat mass index; TBMD, total bone mineral density.

Model 1 described the total cohort and included BFMI, body mass, age and gender as covariates. Where the model was split according to sex, gender was removed as a covariate. Models 2, 3 and 4 were divided by age group (18-29y, 30-49y and 50+y) and analysis was repeated according to gender, using body mass and age as covariates. The effect of BFMI including covariates predicted 63.8% of the variance in TBMD which decreased as the model was split by categorical variable. The influence of adiposity alone on TBMD (R² BFMI) was small, ranging from 3.4 to 17.3%. However, this influence was consistently negative (β=-0.394 to -0.546; p≤0.003). Adiposity had a stronger influence in men vs. women (BFMI R²=8.9 vs. 6.0%), and in younger compared to older age groups (BFMI R²=17.3 vs. 11.6%). The influence of BFMI appeared to decrease with age and varied by gender. At age 18-29y, the influence was stronger in women vs. men (10.8 vs. 13.3%). From
age 30-49y the association was stronger in men (12.8 vs. 7.1%), and again at age 50y (6.7 vs. 3.4%). The standard error of the estimate (SEE) was below 0.1% for all models. VIF was <10 in all models (1.9 to 4.2), confirming no presence of multicollinearity.

This analysis was subsequently repeated substituting BFMI for VAT and SAT as independent variables (Table 7.3.4).

Both VAT and SAT had a negative influence on TBMD ($\beta=-0.15$ to -0.60; $p<0.05$), regardless of gender and age. In the total cohort, VAT had a stronger influence than SAT in men (5.2 vs. 2.7%) and SAT had a stronger influence than VAT in women (2.2 vs. 0.7%) At 18-29y, SAT had the strongest negative influence in men (7.2 vs. 1.6%) and women (9.6 vs. 1.4%). However, from age 30+ years, VAT was the strongest predictor in all models and SAT was not significant when divided according to gender. VIF was <10 in all models including VAT and SAT as independent variables (1.4 to 4.1), confirming no presence of multicollinearity.

7.3.2 The Association of Adiposity and Site-Specific Bone Mineral Density

To determine if the adiposity-bone relationship was different depending on bone site, an analysis of the spine, femoral neck and total hip was carried out respectively. Descriptive statistics of 480 older adults (95 men, 385 women) that were included in the analysis of site-
specific BMD at the spine and proximal femur are displayed in Table 7.3.5. One man was excluded from spine BMD analysis due to a metal implant in situ. Three women and one man were excluded from proximal femur BMD analysis due to bilateral hip replacements in situ.

| Table 7.3.5 Descriptive statistics of all participants with whole body and site-specific BMD DXA body composition analysis |
|-----------------|-----------------|-----------------|-----------------|
|                  | **Total (n=480)** | **Men (n=95)**  | **Women (n=385)** |
| **Age (y)**      | Mean (5.8)       | Median (9.1)    | Mean (5.8)       |
| **Height (m)**   | 1.64 (0.1)       | 1.63 (0.1)      | 1.76 (0.1)       |
| **Mass (kg)**    | 72.5 (13.7)      | 70.2 (18.2)     | 86.2 (11.6)      |
| **BFMI (kg/m²)** | 10.0 (3.3)       | 9.5 (3.8)       | 8.4 (2.5)        |
| **VAT (g)**      | 1,008 (752)      | 853 (977)       | 1,773 (847)      |
| **SAT (g)**      | 1,426 (617)      | 1,329 (740)     | 1,002 (379)      |
| **Spine² (g/cm²)** | 1.128 (0.2)    | 1.112 (0.2)     | 1.202 (0.2)      |
| **FN³ (g/cm²)**  | 0.916 (0.1)      | 0.915 (0.2)     | 0.963 (0.1)      |
| **TH³ (g/cm²)**  | 0.978 (0.1)      | 0.977 (0.2)     | 1.047 (0.1)      |

¹Normally distributed (p>0.05); ²Spine analysis n=479 due to metal implant in situ; ³Proximal femur analysis n=476 due to hip replacements in situ; BFMI, body fat mass index; FN; Femoral neck bone mineral density; SAT, subcutaneous visceral adipose; Spine, Spine bone mineral density; TH, Total hip bone mineral density; VAT, visceral adipose tissue.

Linear regression was carried out using BMD at the spine (Model 1), femoral neck (Model 2) and total hip (Model 3) as individual dependent variables. The influence of BFMI after adjustment for covariates on these sites is presented in Table 7.3.6. Body mass and age are used as covariates in all cases with gender removed as a covariate when analysis was split according to men and women.
BFMI had a significant negative influence on spine BMD in the total group (β=-0.126; p=0.011) and in women only (β=-0.346; p=0.001). This association was also observed for the women-only model at the femoral neck site (β=-0.306; p=0.002). However, the extent of the influence of BFMI within these models was quite small ($R^2$ change = 1.2-3.5%). The association between BFMI and BMD at the total hip site was non-significant for all models (p=0.180 to 0.846).

The analysis of site-specific BMD was subsequently repeated substituting BFMI for VAT and SAT as independent variables (Table 7.3.7).
7.4 Discussion

The aim of this chapter was to analyse how adiposity influences total and/or site-specific BMD in Irish adults across a wide age (18-81 years) and obesity range (BFMI 1.2-27.7kg/m$^2$). Since adipose tissue has been reported to differ in its effect on health by total amount and regional distribution, adiposity was analysed based on both BFMI and abdominal adiposity (VAT and SAT) as measured by DXA. It was hypothesised that adiposity would have a negative influence on BMD after accounting for the weight-bearing effect of total body mass, and that the amount of VAT would have the strongest negative influence on both total and site-specific BMD.

In this cross-sectional analysis, correlations revealed that higher values of BFMI, VAT and SAT were associated with reduced bone health. Since BMD has been shown to differ according to age and gender (Chapter 6), and contribute to risk of osteoporosis and fracture (Kanis et al 2002), regression analysis which included age and gender as covariates was important to determine the effect of adiposity alone on bone health. Positive associations between adiposity and bone are hypothesised to be primarily due to a weight-bearing osteogenic effect. To account for this interaction, total body mass was also included as a covariate.

BFMI was inversely associated with TBMD after adjustment for covariates (age, gender and body mass). Standardised β coefficients ranged from -0.335 to -0.502 with $R^2$ values between 4.0 and 17.3% (p<0.001). This study is the first to explore the association between measures of total adiposity, regardless of expression (BFM, BF% or BFMI), and TBMD using body mass as a covariate. Previous studies have investigated this relationship without adjusting for body mass, and have found BFM to exert a positive influence on bone (Chen et al 1997, Wang et al 2005, Fu et al 2011, Park et al 2012). One recent study has found a negative association between BFM and TBMD in older Spanish men aged 65-92 years after adjusting for age, height, physical activity and lean mass ($\beta=-0.265$; p=0.029) (Gomez-Cabello et al 2013), with a non-significant negative association found in older women ($\beta=-0.046$; p=0.644).

In the assessment of abdominal adiposity, the most appropriate metric to assess the potential influence of VAT on bone health is still unknown. In the identification of risk factors for cardiometabolic disease, VAT has been shown to have a detrimental influence, while SAT
has been shown to be beneficial (Porter et al 2009). To account for these differences, both VAT and SAT were included in the regression model to determine the effect of one relative to the other. In contrast to their effect on cardiometabolic disease, both VAT and SAT were found to have a negative influence on TBMD in this study (β=-0.15 to -0.60; p<0.05). Overall, SAT was found to have a stronger influence than VAT (1.9 vs. 1.0%). However, this varied according to gender, with men predominantly influenced by VAT and women predominantly influenced by SAT, likely due to the higher quantity of VAT relative to SAT in men. This association also varies according to age, with SAT exerting a stronger influence compared to VAT in young adults (14.7 vs. 0.6%). However, as absolute VAT and the ratio of VAT within the android compartment increases with age (as reported in Chapter 6), this data would suggest that its influence on TBMD also increases. In men and women aged >30 years, SAT no longer has a significant influence on TBMD, while the effect of VAT is varied (1.5 to 17.1%), but highly significant (p<0.001).

Using CT as a measurement tool for adiposity, one other study has measured the association between VAT and SAT on TBMD in a large cohort of American Caucasian and African-American men and women of varying age (18-74 years) (Katzmarzyk et al 2012). After adjustment for age, lean mass, gender and race, both VAT and SAT were found to exert a negative influence on TBMD (p<0.05). Similar to this study, when participants were divided according to gender, the negative influence of VAT in their respective models was stronger in men vs. women (1.4 vs. 0.8%), while the influence of SAT was significant in women (p=0.04) and non-significant in men (p=0.38). However, no separate analysis according to age was carried out.

These results suggest that adiposity has an effect on bone beyond its weight bearing influence. There are a number of other potential pathways by which adiposity can influence bone. The regulation of insulin, impaired in those with excessive adiposity and T2DM, has a complex relationship with bone, emphasised by the increased BMD, but reduced bone strength and high risk of fracture found in those with the disease (Strotmeyer et al 2004). Studies of adipocyte function have revealed that adipose tissue is not just an inert organ for energy storage, but is also considered an active endocrine organ which modulates energy homeostasis. Fat induced alterations in hormonal factors, cytokines and inflammatory factors may play a pivotal role in bone turnover. Adipokines such as leptin and adiponectin have been shown to regulate bone metabolism but the direction of this action is still unclear.
Leptin, directly proportional to the amount of adiposity in the body, has been found to exert a positive and negative effect on bone dependent on the mode of action (central or peripheral administration and dosage) (Gimble et al 2011). Adiponectin, an adipokine that is inversely related to adiposity, has yielded contradictory results in in-vitro studies investigating its effect on bone cells (Williams et al 2009), while clinical studies have shown a consistent inverse relationship between circulating adiponectin concentrations and BMD (Richards et al 2007). VAT, in particular, is associated with increased levels of pro-inflammatory cytokines such as TNF-α and IL-6, both of which increase bone resorption and promote osteoporosis and fracture risk (Cauley et al 2007). Since this regional deposition of fat mass has been a stronger predictor of health risk than total fat mass (Goodpaster et al 2005), it was hypothesised that VAT would have more of an influence on bone than SAT in this study. However, it would appear that VAT is not any more detrimental than SAT in its effect on bone health, at least until age 30 years.

A stronger association between adiposity and TBMD was found in men compared to women, and in younger vs. older adults. It has been well established that gender differences in body fat distribution exist, with men and oestrogen-deficient postmenopausal women tending to accumulate more VAT, and premenopausal women tending to have more SAT (Lovejoy et al 2009), which is reflected in the stronger influence of VAT on bone in men and in older age. Furthermore, the relative contribution of adipose tissue to produce oestrogen, which is one of the most decisive factors for BMD, is not equal across gender, age or site. Oestrogen is produced by adipocytes, with subcutaneous pre-adipocytes having higher aromatase activity in comparison to visceral adipocytes (McTernan et al 2002). The adipose tissue of premenopausal women is a relatively minor source of oestrogen, whereas in men and postmenopausal women, aromatization of oestrogen precursors in adipose tissue is the main source of oestrogen (Reid et al 1992). Therefore, in post-menopausal women, the role of adipose tissue may offer both negative (through the action of adipokines and pro-inflammatory cytokines) and, to a lesser extent, protective effects (through production of oestrogen), explaining the lower negative effect of adiposity observed in older women.

In this study, the association between adiposity and site-specific BMD in the elderly was weaker than its association with TBMD. In women BFMI had a significant negative association with BMD at the spine (β=-0.346; p=0.001) and femoral neck sites (β=-0.306; p=0.002) with an $R^2$ change of 2 to 3.5%. However, the effect of BFMI was non-significant.
in men and at the total hip site in all models. These results compare to Gomez-Cabello et al. (2013) who observed a non-significant association between BFM and BMD in Spanish elderly men ($\beta$=-0.03 to -0.19; p>0.12) and women ($\beta$=0.00 to 0.05; p>0.50) at the spine, femoral neck and total hip sites. However, these models were adjusted for total lean mass rather than total body mass. A study by Yoo et al. (2012), found a non-significant influence of BF% on spine BMD in Korean men and post-menopausal women even after adjustment for total body mass ($\beta$=0.06 and -0.14, p>0.16). In premenopausal women, a significant negative association was observed ($\beta$=-0.38, p<0.01). However, when VAT was analysed, no result was significant. This is similar to the current study where no significant observations between VAT and site-specific BMD in an elderly sample were found. Since the influence of VAT on TBMD was found to be stronger in younger vs. older participants, it is possible that a significant influence would be observed in a younger cohort. However, site-specific BMD measurement was not available for those <50 years and requires further exploration. In a study by Choi et al. (2010), significant negative associations ($\beta$=-0.13 to -0.30; p<0.05) were found between VAT and spine, femoral neck and total hip BMD in Korean men and women aged 20-83 years, while SAT was observed to have a significant influence in spine BMD in men only ($\beta$=-0.15; p=0.04). While body mass and age were included as covariates in this relationship, analysis was not divided by young vs. old. However, one study in young (15-25 years) women has found VAT to exert a negative influence and SAT to exert a positive influence on femoral bone using CT (Gilsanz et al. 2009).

A different fat-bone relationship was observed in this study depending on whole-body or site-specific BMD scan. TBMD was seen to be more strongly influenced by adiposity in men and younger adults, while site-specific BMD in older adults was affected by adiposity in women only. This may be synonymous with the structure and function of bone at different sites. TBMD disproportionately assesses cortical bone compared to the specific metaphyseal scanning sites that contain more cancellous or trabecular bone (Melton et al. 2005). In particular, the lumbar spine site is rich in metabolically active trabecular bone and is therefore considered the optimum site for measuring change in BMD, particularly in postmenopausal women (Eastell 1998). However, the negative effect of adiposity is proposed to be systemic, affecting the whole body, and use of this measure eliminates effects of differences in mechanical loading across the weight-bearing sites (i.e. spine and proximal
femur). It is suggested from this study that fat deposition may affect cortical bone more than trabecular bone, particularly in men. However, further clarification of this relationship is needed through use of peripheral quantitative CT (pQCT) scanning of volumetric BMD (vBMD) to quantify bone quality, as well as strength.

Such research has recently been carried out by Sukumar et al (2011), who investigated the effect of BFM on vBMD at the distal tibia in women. BFM was found to have a significant negative influence on cortical vBMD ($\beta=-0.43$, $p<0.001$) and a significant positive influence on trabecular vBMD ($\beta=0.41$, $p<0.001$). When split according to menopausal status, adiposity had a greater influence in premenopausal (65%) vs. postmenopausal (35%) women. Using CT measures of VAT, Gilsanz et al (2009) also found a negative association in cortical bone of young women. In obese men, Bredella et al (2012) observed VAT and bone marrow fat to have a negative association with bone microarchitecture (total cortical and trabecular vBMD and cortical area) and mechanical properties (i.e. failure load and stiffness) at the distal radius. A review by Dimitri et al (2012) has suggested that adiposity is protective against vertebral and hip fractures, but may increase the risk of ankle and humerus fractures due to observed increased cortical porosity. It would appear that trabecular bone is highly influenced by factors such as oestrogen and physical activity, while cortical bone is influenced negatively by the parathyroid hormone (PTH) and positively by vitamin D (25OHD) (Sukumar et al 2011). Adiposity may influence cortical bone in this manner since it is associated with lower circulating concentrations of 25OHD attributed to increased sequestration in the adipose tissue (Wortsman et al 2000). Adiposity is also associated with increased PTH levels, independent of 25OHD (Bolland et al 2007), which results in an increase in bone resorption to normalise serum calcium levels (Frost et al 2010). This may explain why adiposity tends to have a negative influence on cortical bone more than trabecular sites. However, since osteoporotic fractures are caused by both cortical thinning and trabecular bone loss, maintenance of both is a priority.

This study is strengthened by the variation in covariates available for analysis (e.g. gender, age, bone and adipose site), allowing a more detailed analysis of potential interactions. This study is limited by an unequal number of male to female participants, particularly for analysis of site-specific BMD. It is possible that an equal number of male participants would determine an adiposity-bone relationship with statistical significance. However, while statistical significance was observed for many parameters, whether this reaches biological
significance by contributing to the risk of fracture remains to be investigated. Also, there are a potential number of covariates not available for analysis in this study i.e. menopausal status, physical activity, calcium and vitamin D intake etc. that may have further influenced the modelling. Results of this study may also be confounded by the measurement tool, since this is the first study to use DXA estimates of VAT, of which only one validation study currently exists (Kaul et al 2012). Although DXA is the benchmark tool for diagnosis of osteoporosis, the use of secondary outcome measures such as pQCT or bone biomarkers may give more insight into this complex relationship. The use of DXA imaging may also be confounded in those with high abdominal fat content, where the tissue boundary between bone and soft tissue becomes distorted, resulting in an increased measurement of soft tissue composition (Kim et al 2012). Therefore, BMD readings for individuals who are overfat may be falsely high, possibly leading to an underestimation of the negative influence of adiposity on bone.

The adverse adipose-bone association found in this study lends further support to the growing amount of data indicating that the amount and allocation of adipose tissue in the body is an important predictor of disease risk. This has clinical implications, since fat-reducing medical interventions or lifestyle modifications may be favourable for both osteoporosis and obesity. Since body mass is an important positive predictor of BMD, any recommendations for fat loss should be accompanied with methods to maintain or increase lean mass. However, this cross-sectional analysis does not show a cause and effect relationship. In order to investigate the true relationship, a longitudinal study is warranted to investigate the effect a change in total and abdominal fat mass would have on bone, independent of body mass.

### 7.5 Conclusion

Osteoporosis and obesity have a pathophysiological linkage; both fat and bone cells originate from a common mesenchymal stem cell within the bone marrow, a relationship influenced by many factors that may ultimately determine the balance between adipose and bone tissue. This study has shown that two new metrics of adiposity; body fat expressed as an index of height$^2$ (BFMI) and abdominal adipose deposition (VAT and SAT) as measured by DXA, have a significant negative influence on whole body BMD, a relationship that
holds stronger in men vs. women and in younger vs. older adults. The relative effect of visceral vs. subcutaneous deposits of adiposity appears to be dependent on the total amount of each, with VAT having more of an impact in men and older adults. In site-specific BMD measurement in the elderly, adiposity may have less of an influence, possibly due to a stronger influence from other risk factors (e.g. loss of oestrogen, physical activity, diet), and the difference between cortical vs. trabecular change. A greater understanding of the physiological and functional differences of visceral and subcutaneous adipocytes and how they interact with biomarkers of bone could likely lead to novel therapeutic approaches for obesity and osteoporosis, and dispel the common theory that fat mass is beneficial for bone.
Chapter 8

*Thesis Discussion*
8.1 **Summary and Recommendations for Future Research**

This thesis documents a detailed profile of DXA-measured body composition in a convenience sample of 1,606 Irish adults across a wide age range (18 to 81 years). Interrogation of these data afforded the opportunity to define the anthropometric phenotype based on accurate metrics of body composition. The key findings of this analysis are summarised as follows; despite widespread use, BMI and BF% do not appropriately represent adiposity and the changes in adiposity associated with age and intervention. Adiposity expressed as body fat mass index (BFMI; body fat mass/height$^2$) is a more valid representation of adiposity adjusted for body size that is clinically measureable and independent of changes in fat-free mass. This thesis defined criteria for classification of ‘underfat’, ‘normal’, ‘overfat’ and ‘fat obese’ in an Irish population based on the ‘normal’ phenotype or the young adult median BFMI. Analysing changes associated with age, an increase and re-distribution of adiposity from subcutaneous to visceral compartments was observed, combined with declines in ALTMI and BMD from age 30 years in Irish men and women. This excess adiposity may contribute to the risk of osteoporosis, as BFMI, VAT and SAT have all been shown to negatively influence BMD. These results allow direct comparison with different ethnicities and nationalities, with the ultimate aim of providing enough knowledge for accurate clinical classification of health risk, identification of appropriate intervals to elicit change and ultimately avert the decline in health status associated with preventable lifestyle conditions.

8.1.1 **Health-Related Measurement and Expression of Adiposity**

The principal recurring theme of this thesis is adiposity, and how it may be detrimental to health in the form of muscle and bone health (sarcopenia and osteoporosis). Therefore, an examination of how adiposity is currently measured and defined was necessary. As a crude measure of adiposity, BMI has many limitations as a diagnostic indicator of obesity at the individual level, stemming from its inability to distinguish adiposity from FFM. In this large convenience sample, BMI was observed to correlate positively with BFM ($\rho=0.73$-$0.95$, $p<0.05$) and also with FFMI ($\rho=0.56$-$0.71$, $p<0.05$), being therefore incapable of differentiating the two components. This becomes a concern in instances where individuals have higher or lower FFM for their height e.g. due to illness, age or athleticism. A reform of current practice is evidently required using an accessible, accurate measure of adiposity.
However, the most appropriate metric of adiposity is also questioned. The use of BF% has been suggested and is widely used (Gallagher et al 2000). However, at higher levels of adiposity, BF% was observed to plateau due to concomitant increases in FFM. Also observed was an increase in BF% in old age, regardless of changes in adiposity, due to loss of FFM. Thus, correction of BFM for stature is deemed the most valid metric since changes to adiposity are detected independent of changes to FFM. Caution is therefore warranted in the interpretation of past studies that have used BMI or BF% to represent adiposity.

A recent position statement by the ISCD identified a lack of consensus regarding categorical thresholds to define adiposity-related obesity (Petak et al 2013). In Chapter 4, classification criteria to define excess adiposity based on BFMI were calculated using a young adult reference. One standard deviation from the young adult median was defined as ‘overfat’ (>6.4 kg/m$^2$ in men; >9.1kg/m$^2$ in women), while two standard deviations from the young adult median was defined as ‘fat obese’ (>8.5 kg/m$^2$ in men; >11.5kg/m$^2$ in women). This classification method is the preferred method for use in anthropometry (WHO 1995), and is similar to that applied for diagnosis of osteoporosis in bone health. It is considered a suitable approach for bone health since BMD in a young adult is assumed to be ‘healthy’, and deviations due to ageing are associated with fracture risk (Kanis 2002). In terms of adiposity, ‘healthy’ levels are not well defined, questioning the most appropriate reference base to use. Chapter 6.1 has shown BFMI to increase with ageing, and therefore the young adult was chosen as the best representation of ‘ideal’ or ‘normal’. However, these criteria are not definitive as they have not yet been prospectively validated against markers of health i.e. cardiovascular disease, metabolic syndrome and ultimately mortality.

It is likely that a reform of classification approach would change the current understanding of how obesity impacts on health. The most recent systematic review (Flegal et al 2013) of the association between BMI and all-cause mortality from analysis of 97 studies reported that a BMI classification of ‘overweight’ was associated with significantly lower all-cause mortality than ‘normal weight’. Careful extrapolation of studies of this kind is necessary, since ‘overweight’ status may be a consequence of a high FFMI, which is also associated with low mortality. This segregation was examined by Bigaard et al (2004) using BIA measurements of BFMI and FFMI on >57,000 Danish participants. This prospective analysis revealed that low FFMI and high BFMI on opposite sides are responsible for the peaks in mortality and traditional U-shaped relationship observed between BMI and
mortality. This study highlights the importance of segregating BMI into its components but is limited by use of BIA as a measurement tool (Leahy et al 2012) and narrow age range (50-64y). The new criterion for classification of adiposity devised in this thesis requires a similar exploratory investigation.

In the examination of the influence of adiposity on health, it was imperative to identify the specific visceral component of adiposity, due to its recognition as an important health determinant and prognostic indicator for disease risk. Since the measurement of VAT is a relatively new technological advance to DXA scanning, Chapter 3 aimed to determine the precision error of such a measurement tool in sample of 87 participants with varying adiposity levels. This analysis required removal of 6 of the 87 datasets due to a reported VAT estimation below 0g (displayed as zero), that were judged as ‘non-physiological’. In the larger cohort (n=1,606), 79 participants were excluded based on the criterion of non-detectable VAT. Additional research is required to determine the true lower limit of detection based on direct comparison to CT or MRI. However, for clinical use, it is likely that DXA will be used for detection of high levels of visceral adiposity in those deemed at risk, whereby this software has been shown to offer better precision (8.4% in this study, vs. 5.1% in Kaul et al (2012) for VAT>400g).

In the assessment of health risk, the expression of an appropriate metric of abdominal or visceral adiposity may require correction for total android or subcutaneous mass. However, this decision ultimately lies in the conclusion to whether abdominal subcutaneous adiposity is protective, damaging or indifferent to health; an answer which is uncertain for all health indices. SAT has been found to have a positive influence on cardiometabolic disease (Porter et al 2009), but in Chapter 7, was found to have a negative influence on bone health. Therefore, it may be necessary to observe both components in isolation. Since the measurement of VAT by DXA is already dependent on the height of the individual, adjustment for body size may not be required. Health risk may depend on the absolute amount of metabolically active tissue, rather than as a proportion of total android fat.

Future research in the area of adiposity classification requires a large scale prospective study to confirm or amend the proposed criteria for prediction of increased or decreased health risk. Coupling DXA measurements of total and regional adiposity with biomarkers for certain pre-requisites or presence of disease states such as cardiovascular disease,
Chapter 8

diabetes and metabolic syndrome is necessary for confirmatory analysis. From there, the approach can be extrapolated to interrogate larger international databases e.g. NHANES to determine if population-specific cut-offs are required before promotion and incorporation into clinical practice can commence.

8.1.2 Age-Related Changes to the Anthropometric Phenotype Associated with Health

Changes to the anthropometric phenotype with advancing age can have negative consequences for function, disability and cardiometabolic health. The wide age range of participants measured in this study allowed for the first cross-sectional profile of these changes to be established in an Irish population through each age decade. BFMI was observed to increase with age and plateau at age ~55 years in men and age ~65 years in women. Peak BFMI in Irish adults was found to be considerably lower than an American reference population (7.9 vs. 8.8 kg/m² in men; 10.5 vs. 12.5 kg/m² in women) (Kelly et al 2009), thus highlighting the necessity of population-specific normative reference values. VAT, conversely, did not plateau with age, increasing from 263g to 1,635g in men and from 57g to 700g in women from youth (18-30y) to old age (50+y) (p<0.001).

Lean mass decline with ageing was assessed using an index of lean mass in the appendages (ALTMI) as a diagnostic indicator for sarcopenia (Baumgartner et al 1998). ALTMI was observed to decrease from the age of 30y in both men and women, with significant losses occurring only after age 50y (-0.4 in men, -0.3kg/m² in women; p<0.001). For the first time, the prevalence of sarcopenia in an Irish sample, based on a population-specific young adult reference was assessed. In those ≥65 years of age (n=107), 24% were diagnosed as ‘class I sarcopenic’ (ALTMI Z-score -1 to -1.99) with 6% diagnosed as ‘class II sarcopenic’ (ALTMI Z-score <-2). These prevalence rates are similar to NHANES reference data of adults ≥65 years of age (Goodman et al 2013), with prevalence rates of 21% and 9% respectively. While population-specific reference data is essential for measurement of adiposity, less variation is observed between Irish and American populations for the estimation of lean mass. Therefore, classification criteria for sarcopenia may be interchangeable worldwide.

Age-related changes in muscle quality, determined by the interaction between adiposity and lean mass, may also contribute to loss of physical function in old age. Novel to this thesis
was a new definition of ‘sarcopenic obesity’ to classify individuals with a combination of low ALTMI (class II sarcopenia) and high BFMI (fat obesity). Prevalence rates in this population were 0.5% of adults >50 years, with a further 9.6% who were ‘overfat’ with class I sarcopenia. Classification within this phenotype was deemed to be associated with a much higher risk of disability than either condition alone, since muscular fat infiltration increases with age and is associated with metabolic conditions, including insulin resistance and diabetes (Schafer et al 2010), and has also been shown to negatively affect muscle strength in sarcopenia (Visser et al 2005). Previous classification has been reported using ALTMI and a limited measure of adiposity (BF%) (Baumgartner et al 2000).

Although this is the first study to estimate prevalence rates of sarcopenia in Ireland, further data is needed on adults over age 70 years, combined with measures of functional strength, disability and performance to allow a full representation of sarcopenia and sarcopenic obesity to be made (Cruz-Jentoft et al 2010). Similar studies amongst different ethnicities are required before a worldwide consensus on definitions can be established.

### 8.1.3 The Association of Adiposity and Bone Mineral Density

BFMI was used to investigate if adiposity has a negative influence on total and site-specific BMD, since it was deemed to offer an improved representation of adiposity over metrics used in past research i.e. total BFM, BF% and BMI. Using body mass as a covariate, BFMI was observed to exert a negative influence ($\beta=-0.020$ to -0.546) on BMD, which was significant at the whole body, but not in the site-specific regions i.e. spine, proximal femur for most models. This may be explained by the differing effect of adiposity on weight-bearing vs. non-weight-bearing sites and on the cortical (predominant in whole TBMD) vs. trabecular (predominant in site-specific BMD) components of bone. Studies using CT have found BFM to influence positively ($\beta=0.41; p<0.001$) on trabecular bone and negatively ($\beta=-0.43; p<0.001$) on cortical bone (Sukumar et al 2011), which aligns with results in this study. Similar results were observed in the analysis of regional adiposity, whereby both VAT and SAT had a negative influence ($\beta=-0.150$ to -0.600; $p<0.001$) on TBMD, which was non-significant for site-specific BMD. Both components of the android region were observed in conjunction and while SAT exerted a stronger influence in younger adults ($R^2=14.7$ vs. 0.6%), the influence of VAT vs. SAT was more detrimental in men ($R^2=5.2$ vs. 2.7%) and older adults ($R^2=1.5$ vs. 0.0%), in line with the higher quantities of VAT
observed in these groups. While VAT consistently looks to be harmful for all markers of health, the role of SAT as a ‘protective’ depot of adiposity for cardiovascular health (Porter et al 2009) does not appear to extend to bone.

Identification of a causal effect is limited in cross-sectional analysis. Future research needs to assess the outcome of a fat loss intervention on BMD. Additionally, use of CT and bone biomarkers as an outcome measure in conjunction with DXA would be valuable in future studies for a more sensitive indicator of bone health and metabolism.

**8.2 Clinical Implications**

Although a challenging task, it is anticipated that replacement of BMI with BFMI would prevent misclassification of ‘fat obesity’ and provide a more accurate diagnosis of adiposity-related health risk both at the population and individual level. This would have clinical implications for the worldwide incidence and prevalence of obesity since more people would be classified as ‘high risk’. While the prevalence of ‘overweight’ in this sample was higher than ‘overfat’ (36% vs. 22%), those classified as ‘obese’ by BMI underestimated those classified as ‘fat obese’ by BFMI (10% vs. 18%). This confirms the suggestion that the global epidemic of obesity has, in fact, been underestimated (Yusef et al 2005). Taking into consideration the potential under-representation of adiposity in this sample compared to NANS (IUNA 2011), the prevalence of ‘fat obesity’ is likely even higher in the whole population. This is a worrying trend given the impact of obesity on Ireland’s leading causes of non-communicable disease and premature death i.e. circulatory disease, cancer and ischaemic heart disease (WHO 2011). In 2005, it was estimated that about 2,000 premature deaths in Ireland were attributed to obesity and that these deaths could be costing the state around €4 billion a year (DOHC 2005).

A change in classification of adiposity is not expected to affect those already obese or severely obese as diagnosed by BMI, as BMI is highly specific (99%). However, it will identify those with apparently healthy BMI in the intermediary ranges (18.5-25kg/m²) i.e. normal-weight with high BFMI that are at health risk. In these cases, early identification may elicit an appropriate lifestyle intervention to prevent further increase in adiposity and a potential future burden on the healthcare system. Also identified are individuals in the
‘overweight’ category (25-30kg/m²) that may be advised on ‘weight loss’ strategies, despite healthy levels of adiposity. This occurs more frequently in sporting scenarios where athletes struggle to meet weight categories, often due to increased lean mass. The BFMI classification system offers a more appropriate judgement for clinicians at a ‘who to treat’ level, and highlights the necessity of careful use of language in terms of ‘fat loss’, rather than ‘weight loss’.

Given the well-described continued expansion of older populations in society, concerns over the availability of health care resources to counteract age-related disease are well founded. While the negative effects of sarcopenia and osteoporosis (low muscle strength, disability, fractures etc.) tend not to begin until old age (≥65 years), the decline in lean mass and bone health that leads to these disease states begins from age 30 years. Therefore, healthy ageing likely requires lifelong healthy lifestyle choices since achievement of peak muscle and bone mass is also a contributing factor. However, investigations into nutrient and exercise interventions to prevent further adiposity gain, muscle wasting and bone loss in the elderly are of great importance, particularly in terms of self-empowerment and self-efficacy (Baumgartner et al 2000, Bauman et al 2012). The 1-year longitudinal change in body composition parameters in an elderly population has been described in Chapter 6.2, which has potential value as a comparison for future studies that aim to offset body composition- and age-related decline in health through lifestyle-related interventions. This thesis recommends that sarcopenia should become more prominent in programmes of health promotion for healthy ageing, due to the levels of low muscle strength and disability associated with it (Sayer et al 2013). It is not a well-established health concern as of yet. However, a clinical measure of lean mass in the form of a fat-free mass index (FFMI) has been provided in this thesis, with corresponding criteria for diagnosis of muscle loss or sarcopenia for those without access to imaging methods.

Since adiposity is recognised as a contributing factor for the development of sarcopenia and osteoporosis, this phenotype may be more detrimental over others in the progression to frailty (Freid et al 2001) and potential of future falls and debilitating fractures (Verschueren et al 2013). Also, the identification of adiposity as a risk factor for reduced bone health suggests that adiposity-reducing medical interventions or lifestyle modifications may be favourable for sarcopenia, osteoporosis and obesity. It would appear that in order to reduce or prevent the bone loss associated with loss of adiposity, total body mass should be
maintained. This leads to the question of whether health professionals should be prescribing fat loss without a concomitant recommendation to increase muscle mass through resistance exercise programmes and/or nutritional advice.

8.3 Potential Implementation Strategies

Although the limitations are well-recognised in the literature, clinical use of BMI continues due to an apparent lack of knowledge translation. Dissemination to a wider audience is necessary for the advancement of this approach in the assessment of adiposity and integration of the BFMI measurement into routine practice. Cost and simplicity of measurement are two important factors that have resulted in the longevity of BMI as a measure of adiposity. Therefore, any new metric to replace BMI will require similar qualities, combined with a widespread promotion to educate both clinicians and the public on health risks associated with adiposity, as opposed to weight gain. Surrogate or field measurement tools such as anthropometry, ultrasound and BIA have all shown potential as accurate estimates of total BFM or BF% (Leahy et al 2012). Conversion of BF% to BFMI using body mass and height measurement can be carried out with relative ease. These tools are becoming more prevalent in nutrition and dietary practice but may require a certain degree of training. For less specialised multi-disciplinary clinicians that wish to assess nutrition e.g. general practitioners, physiotherapists and other clinical therapists, a generalised prediction equation has been developed that will calculate BFMI based on BMI, age and gender, without requirement of specialised equipment and/or training. This equation will predict BFMI to an accuracy of 1%, based on a convenience sample of Irish adults. It is likely that population-specific equations are required based on differences observed in adiposity between this Irish cohort and American cohorts e.g. NHANES.

The current epidemic of obesity has evolved over a relatively short time scale, approximately two to three decades. The human and economic burden of this epidemic provides sufficient evidence on the consequences of our failure to manage this societal challenge to-date and implore the question – who is responsible? Knowledge translation to all health professionals to encourage promotion of a healthy lifestyle to every patient is vital, since excess adiposity has been linked with such a wide spectrum of preventable conditions. Examples of just some of these conditions include disease states such as
diabetes, CVD, stroke, cancer and respiratory disorders (Wang et al 2011), musculoskeletal conditions such as osteoarthritis (Bliddal et al 2011), tendon pathology (Gaida et al 2010) and lower back pain (Shiri et al 2010), and also depression and stress (Stunkard et al 2003). As shown in this thesis, sarcopenia and osteoporosis may also be exacerbated by excess adiposity. Physiotherapists are uniquely positioned to take the lead in health promotion and prevention of lifestyle conditions, address many of their causes, as well as manage these conditions (Dean 2009). However, while 97% of physiotherapists agree that they have a role to play in health promotion with clients who are overweight or obese, only 43% agree that this role includes the assessment of adiposity (You et al 2012).

With a greater shift towards primary care and prevention of lifestyle conditions, successful initiation and implementation of change for physiotherapy practice has been suggested to require effort on professional, clinical, educational and research levels (O’Donoghue & Dean 2010). However, this paradigm can be applied to all health professionals in the promotion of accurate assessment and non-invasive management of excess adiposity. The professional body can ensure that appropriate professional development and education courses are available to practitioners (e.g. anthropometry, cognitive behavioural techniques). At the practice level, the clinician has a responsibility to acquire a better understanding of health and well-being and translate public health knowledge into practice. Both entry-level and continuing-education health professional programmes should include assessment of adiposity as part of the objective examination, and tackle any preconceptions or attitudes towards roles to ensure comprehensive patient care. Finally, research is required on how to translate such a health behaviour change from the societal to individual levels across all practice settings.

Widespread dissemination of this research area is required to reinforce the measurement of ‘body fat’ opposed to ‘body weight’ and BFMI opposed to BMI as the criterion measure of adiposity. Successful knowledge transfer will likely need to occur first to health professionals reinforced with lobbying to improve practice standards, and second, to the public in the form of a government-backed promotional campaign. In an era where healthcare cutbacks are rife, prevention of disease is key. The appropriate assessment of body composition is therefore a priority to identify health risk in the fight against the worldwide increase in non-communicable disease.
Bibliography


clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women', *Osteoporos Int*, 18(8), 1033-46.


neck bone mineral density loss in postmenopausal women', *BMC Musculoskelet Disorder*, 12, 225.


Appendices
Appendix A : Participant Information Sheet, Consent Form and Pre-Scan Protocol

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. Two different techniques, a DEXA scan and bioelectrical impedance 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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UL BODY COMPOSITION STUDY
(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, fat mass and bone density will be measured using 2 different techniques: bioelectrical impedance 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 15-20 minutes to complete.

You should attend wearing a loose fitting ‘T’-shirt or Polo top and shorts without metal or zips if possible

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 applied (it is so low you will not feel any sensation resulting from this) and the resistance of your body (impedance) is measured.

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 8 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 bone density, 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 the researchers conducting the study:

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<tr>
<td>086-0220375</td>
<td><a href="mailto:bodycompinfo@ul.ie">bodycompinfo@ul.ie</a></td>
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Should you any questions for the principal researcher, Professor Jakeman, he may be contacted by phone 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
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.

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
Please, refrain from any form of organised training or exercise session of greater than 20 minutes for a period of 24h before your appointment.

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

Before your appointment

What to do and when to do it

3 hours before
No further food intake

1 hour before
Drink 500ml water

10 minutes 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 us as soon as possible.

Phone: 086-0220375
Email: bodycompinfo@ul.ie
Appendix B: Additional information for participants undergoing repeat bone mineral density scan for precision analysis (Baim et al 2008)

Precision Assessment Information for Patients

To find out if there has been a change in your bone density, a recent bone density test is compared with a previous test. For an accurate comparison, we must know when the change is greater than the normal day-to-day fluctuation in the measurement itself. This is done by doing mathematical calculations on repeat bone density measurements of the same person made on the same day. This is called a “precision assessment.” You have been asked to participate in a precision assessment. You will have your bone density measured again at the spine and hip. After the first scan you will need to get off the table and then back on for the additional scan(s).

The Xray exposure involved in this is exceedingly small, typically less than the normal radiation all of us are exposed to on a daily basis. Nevertheless, you should not participate if you think you might be pregnant. Participation is up to you. If you do not wish to participate, it will have no effect on your future participation in research projects in this department. Please ask your doctor or nurse if you have any questions or if you do not understand why you have been asked to participate.
Appendix C: Generalised equation for the prediction of BF% using BMI

The association between BMI and DXA-measured BF% is illustrated in Figure C.1 using a linear regression model. BMI predicted 51% of the variance in BF% in men and 65% in women. This relationship varied minimally with age, i.e. young adult (18-29y; \( R^2 = 44\% \) in men, 51\% in women); middle age (30-49y; \( R^2 = 45\% \) in men, 69\% in women); and older adult (50+y; \( R^2 = 43\% \) in men, 66\% in women).

![Figure C.1. Scatter plot of BMI vs. BF% in 683 men (left) and 923 women (right). BMI cut-offs at 18.5, 25 and 30kg/m\(^2\) representing ‘underweight’, ‘normal’, ‘overweight’ and ‘obese’ (--).](image)

A multiple linear regression model using BMI, sex and age as independent variables generated the following equation for the prediction of BF\% (Equation C.1):

\[
BF\% = -12.379 \times \text{sex} + 1.297 \times \text{BMI} + 0.127 \times \text{age} -3.545 \\
(Equation C.1)
\]

where sex=0 for women and sex=1 for men.

Multiple \( R = 0.895, R^2 = 0.800 \) with standard error of estimate (SEE) = 4.4\%. Sex was the most significant predictor of BF\% in this model, predicting 41.9\% of the variance, followed by BMI (34.3\%) and age (3.9\%) (p<0.001). Due to the curvilinear association observed between BMI and BF\% in older women with higher values of BF\%, regression models using 1/BMI were also analysed to attempt to linearise the model. However, use of 1/BMI as an independent variable did not improve the relationship in men (\( R^2 = 0.491 \)), and only slightly improved the relationship in women (\( R^2 = 0.680 \)).
Previously regressed equations from Gallagher et al (2000) (Equation C.2) and Heo et al (2012) (Equation C.3) are presented below:

\[ BF\% = 76.0 - 1097.8 \left( \frac{1}{\text{BMI}} \right) - 20.6 \left( \text{sex} \right) + 0.053 \left( \text{age} \right) + 95.0 \left( \text{Asian} \right) \left( \frac{1}{\text{BMI}} \right) - 0.044 \left( \text{Asian} \right) \left( \text{age} \right) + 154 \left( \text{sex} \right) \left( \frac{1}{\text{BMI}} \right) + 0.034 \left( \text{sex} \right) \left( \text{age} \right) \]

(Equation C.2)

where sex = 0 for women and =1 for men; Asian = 0 for other races and =1 for Asians; multiple R = 0.90 and SEE = 4.31%.

\[ BF\% = \beta_0 + \beta_1 \left( \frac{1}{\text{BMI}} \right) \]

(Equation C.3)

where in men, \( \beta_0 = 0.51 \) to 0.56 and \( \beta_1 = -6.42 \) to -7.7 dependent on age and race-ethnicity. In women, \( \beta_0 = 0.62 \) to 0.65 and \( \beta_1 = -5.64 \) to -7.16 dependent on age and race-ethnicity.

BF\% was subsequently predicted using Equations C.1-C.3 and compared to DXA-measured BF\% in this cohort of participants. The association between derived and actual BF\% was similar across all three equations, with \( R^2 \) values of 0.800, 0.795 and 0.790 for ULBC, Gallagher et al (2000) and Heo et al (2012) equations respectively. Using a Bland-Altman analysis to compare prediction models, no difference was found between derived BF\% using Equation C.1 from this study and DXA measurement (bias -0.02%, 95% LoA of -8.6 to 8.6%; \( p = 0.841 \)) (Figure C.2.). Gallagher-predicted BF\% significantly (\( p < 0.001 \)) overestimated DXA measurement with a bias of +1.5% and 95% limits of agreement (LoA) of -7.2 to 10.2%. BF\% predicted by the Heo equation significantly (\( p < 0.001 \)) overestimated DXA measurement with a bias of +3.7% and 95% LoA of -5.5 to 12.9% (Figure C.3.). A summary of these results are presented in Table C.3.
Figure C.1. Bland-Altman plot describing the difference between DXA-measured BF% and BF% predicted from BMI through a linear regression model (Equation 4.1) in men and women aged 18-81y (n=1,606) Mean difference (—) and 95% limits of agreement (---)

Figure C.3. Bland-Altman plot describing the difference between DXA-measured BF% and BF% predicted from BMI through the Gallagher equation (left) and Heo equation (right) in men and women aged 18-81y (n=1,606) Mean difference (—) and 95% limits of agreement (---)

Table C.3. Summary of generalised equations from ULBC, Gallagher et al (2000) and Heo et al (2012) for the prediction of BF% from BMI compared to DXA derived values of BF%

<table>
<thead>
<tr>
<th>Equation</th>
<th>Author</th>
<th>$R^2$ (SEE)</th>
<th>DXA vs. Predicted ($R^2$)</th>
<th>Bias</th>
<th>95% LOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation 4.3.1</td>
<td>ULBC</td>
<td>0.800 (4.4%)</td>
<td>0.800</td>
<td>-0.02%</td>
<td>-8.6 to 8.6%</td>
</tr>
<tr>
<td>Equation 4.3.2</td>
<td>Gallagher et al</td>
<td>0.810 (4.3%)</td>
<td>0.795</td>
<td>+1.52%</td>
<td>-7.2 to 10.2%</td>
</tr>
<tr>
<td>Equation 4.3.3</td>
<td>Heo et al</td>
<td>0.54 to 0.79$^1$</td>
<td>0.790</td>
<td>+3.71%</td>
<td>-5.5 to 12.9%</td>
</tr>
</tbody>
</table>

$^1$R$^2$ dependent on gender, age and ethnicity, no SEE reported. SEE, standard error of estimate; LOA, limits of agreement

Adjusting for sex only, BMI represented 51% and 65% of the variance in BF% in men and women respectively, thus questioning the use of BMI as a reference measure of adiposity in
this cohort. It is clear from the relationship between BMI and BF% that adiposity is not independent of age and sex, and therefore any metric of adiposity should not attempt classify based on such assumptions. In this study, a new reference equation using BMI and incorporating age and sex as independent variables was developed to improve the prediction of BF%. The purpose of this analysis was to determine the validity of past equations in an Irish cohort. Two studies have developed regression equations based on DXA in 417 white American and British participants (Gallagher et al 2000) and 1,285 white American participants (Heo et al 2012) respectively. Both studies found a non-linear relationship between BMI and BF%, with a curvilinear relationship apparent at higher BMI values (~>35kg/m²), where BF% tended to plateau. Gallagher and Heo transformed BMI to 1/BMI in order to linearize the data and improve the prediction outcome. A curvilinear relationship was also observed in older women with higher BF% in this cohort; however neither fitting transformation to 1/BMI nor fitting the data using a quadratic function significantly improved the model. The present study applied the Gallagher et al (2000), Heo et al (2012) and current prediction equations to the ULBC cohort to demonstrate their validity across different nationalities and study populations. Both the Gallagher et al (2000) and Heo et al (2012) equations offered an improvement in the prediction of BF% over BMI alone (R²=79-79.5%), though Bland-Altman analysis found the Gallagher et al (2000) equation to overestimate BF% by 1.5% and Heo et al (2012) to overestimate BF% by 3.7%.

The prediction equation developed in this study allows for age-, sex- and ethnicity-specific prediction of BF%. In establishing an Irish anthropometric phenotype, it is imperative to recognise that adiposity is not independent of ethnicity or country of origin. In the study conducted by Gallagher et al (2000) on a convenience sample of subjects from 3 countries, the majority of the Caucasian sample used to generate equations comprised of subjects from the USA (n=475), and the UK (n=196). Comparing a representative sample of the population from the USA (NHANES – Flegal et al 2012, Li et al 2009) to a representative sample from Ireland (IUNA 2011), American adults have a higher prevalence of obesity (35.7 vs. 25.8%) as defined by BMI, and a higher mean BF% in men (28.1 vs. 23.3%) and women (40.0 vs. 33.9%) as demonstrated in the bias to overestimate BF% between 1.5 and 3.7% in this study. The equation developed in this study (Equation 4.1) for the indirect estimation of BF% using BMI offers improved accuracy (bias -0.02%) and linear fit (R²=0.8) that is specific to an Irish population. However, clinical use of this equation is not recommended, since BF% as a representation of adiposity has known limitations.
Appendix D: Centiles of BFMI, VAT and ALTMI by age and gender

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
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<tbody>
<tr>
<td>18-81 years</td>
<td>683</td>
<td>2.47</td>
<td>2.82</td>
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<td>9.41</td>
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BFMI, body fat mass index

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
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<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
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<tr>
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<td>923</td>
<td>4.45</td>
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<td>12.16</td>
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BFMI, body fat mass index
### Men

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
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<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
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<th>95th</th>
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<tbody>
<tr>
<td>18-81 years</td>
<td>678</td>
<td>80</td>
<td>126</td>
<td>216</td>
<td>388</td>
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<td>1,833</td>
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<td>426</td>
<td>52</td>
<td>100</td>
<td>175</td>
<td>264</td>
<td>420</td>
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<tr>
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<td>197</td>
<td>261</td>
<td>405</td>
<td>663</td>
<td>1,340</td>
<td>2,139</td>
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<tr>
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<td>488</td>
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<td>1,613</td>
<td>2,128</td>
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VAT, visceral adipose tissue

### Women

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<th>75th</th>
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<tr>
<td>18-81 years</td>
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<td>30-49 years</td>
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<td>703</td>
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VAT, visceral adipose tissue
### Men

<table>
<thead>
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<th>Age</th>
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<th>25th</th>
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<th>75th</th>
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<tr>
<td>18-81 years</td>
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ALTMI, appendicular lean tissue mass index

### Women

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
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<th>10th</th>
<th>25th</th>
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ALTMI, appendicular lean tissue mass index