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Estimation of whole body muscle, adipose/fat mass, validation in health and during weight loss

Development of prediction equations using MRI as reference method

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Abstract:

Background: Body composition is affected by diseases, and affects responses to medical treatments, dosage of medicines, etc., while an abnormal body composition contributes to the causation of many chronic diseases. While we have reliable biochemical tests for certain nutritional parameters of body composition, such as iron or iodine status, and we have harnessed nuclear physics to estimate the body's content of trace elements, the very basic quantification of body fat content and muscle mass remains highly problematic. Both body fat and muscle mass are vitally important, as they have opposing influences on chronic disease, but they have seldom been estimated as part of population health surveillance. Instead, most national surveys have merely reported BMI and waist, or sometimes the waist/hip ratio; these indices are convenient but do not have any specific biological meaning.

Anthropometry offers a practical and inexpensive method for muscle and fat estimation in clinical and epidemiological settings; however, its use is imperfect due to many limitations, such as a shortage of reference data, misuse of terminology, unclear assumptions, and the absence of properly validated anthropometric equations. To date, anthropometric methods are not sensitive enough to detect muscle and fat loss.

Aims: The aim of this thesis is to estimate Adipose/fat and muscle mass in health disease and during weight loss through; 1. evaluating and critiquing the literature, to identify the best-published prediction equations for adipose/fat and muscle mass estimation; 2. to derive and validate adipose tissue and muscle mass prediction equations; and 3.to evaluate the prediction equations along with anthropometric indices and the best equations retrieved from the literature in health, metabolic illness and during weight loss.

Methods: a Systematic review using Cochrane Review method was used for reviewing muscle mass estimation papers that used MRI as the reference method. Fat mass estimation papers were critically reviewed.

Mixed ethnic, age and body mass data that underwent whole body magnetic resonance imaging to quantify adipose tissue and muscle mass (dependent variable) and anthropometry (independent variable) were used in the derivation/validation analysis. Multiple regression and Bland-Altman plot were applied to evaluate the prediction equations. To determine how well the equations identify metabolic illness, English and Scottish health surveys were studied. Statistical analysis using multiple regression and binary logistic regression were applied to assess model fit and associations. Also, populations were divided into quintiles and relative risk was analysed.

Finally, the prediction equations were evaluated by applying them to a pilot study of 10 subjects who underwent whole-body MRI, anthropometric measurements and muscle strength before and after weight loss to determine how well the equations identify adipose/fat mass and muscle mass change.

Results: The estimation of fat mass has serious problems. Despite advances in technology and science, prediction equations for the estimation of fat mass depend on limited historical reference data and remain dependent upon assumptions that have not yet been properly validated for different population groups. Muscle mass does not have the same conceptual problems; however, its measurement is still problematic and reference data are scarce. The derivation and validation analysis in this thesis was satisfactory, compared to prediction equations in the literature they were similar or even better. Applying the prediction equations in metabolic illness and during weight loss presented an understanding on how well the equations identify metabolic illness showing significant associations with diabetes, hypertension, HbA1c and blood pressure. And moderate to high correlations with MRI-measured adipose tissue and muscle mass before and after weight loss.

Conclusion: Adipose tissue mass and to an extent muscle mass can now be estimated for many purposes as population or groups means. However, these equations must not be used for assessing fatness and categorising individuals. Further exploration in different populations and health surveys would be valuable.

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Publications Arising from this PhD Thesis

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AL-GINDAN, Y. Y., HANKEY, C. R., LESLIE, W., GOVAN, L. & LEAN, M. E. 2014b. Predicting muscle mass from anthropometry using magnetic resonance imaging as reference: a systematic review. Nutr Rev, 72, 113-26.*http://www.ncbi.nlm.nih.gov/pubmed/24447263*

AL-GINDAN, Y. Y., HANKEY, C., GOVAN, L., GALLAGHER, D., HEYMSFIELD, S. B. & LEAN, M. E. 2014a. Derivation and validation of simple equations to predict total muscle mass from simple anthropometric and demographic data. Am J Clin Nutr, 100, 1041-51. http://www.ncbi.nlm.nih.gov/pubmed/25240071

Al-Gindan YY, Hankey C, Govan L, Gallagher D, Heymsfield SB, Lean MEJ. 2014. Derivation and validation of simple anthropometric equations to predict adipose tissue fat mass, using MRI as reference method. *ICO* T8:S37.06 (Appendix 2)

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Author's Declaration

I hereby declare that I am the author of this thesis literature review, the study designs and statistical analysis with the guidance of my supervisors Professor Michael Lean, Dr Catherine Hankey and Dr Lindsay Govan.

Dr Wilma Leslie was the second reviewer in the systematic review (Chapter3). Professor Steven Heymsfield and Professor Dympna Gallagher contributed to the data collection studies presented in Chapters 4 and 5.

The BEYOND weight loss study (Chapter 6) was designed by George Thom, Naomi Brosnahan, and I, with the guidance of our supervisors. The study is divided to three work packages: I am the author and designer of work package 1 (body composition); George is the author and designer of work package 2 (metabolic adaptation and psychological experience during weight loss); and Naomi is the author and designer of work package 3 (binge eating disorder and weight loss maintenance). MRI measurements in work package 1 were led by Dr Giles Rodditi and analysed by Dr Gonzalez Marie, while strength measurements were measured by Dr Matthew Banger.

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Abbreviations

AA	African American
A	Asian
ATYY	Whole body adipose tissue predicted by (Al-Gindan et al., 2015)
BMI	Body Mass Index
C	Caucasian
CI	Confidence interval
CV	Coefficient of variation
DEXA	Dual-Energy X-ray Absorptiometry
FFM	Fat free mass
FM	Fat mass
H	Hispanic
MRI	Magnetic resonance imaging
OR	Odds ratio
PI	Prediction interval
RR	Relative risk
SEE	Standard error of the estimate
SM	Whole body skeletal muscle
SMYY	Whole body skeletal muscle predicted by (Al-Gindan et al., 2014a)
TAT	Total adipose tissue
TATM	Total adipose tissue mass
TATFM	Total Adipose tissue fat mass
TBF	Total body fat
WHR	Waist hip ratio
WHtR	Waist height ratio

Chapter 1

1 Literature review

1.1 Introduction

The study of body composition is a branch of human biology focusing on *in vivo* quantification of body components, their relationships, and change due to many factors, such as growth and disease (Wang et al., 1992).

The history of human body composition can be tracked back to roughly 440 BC, when Hippocrates suggested that the human body composition was blood, yellow bile, black bile and phlegm. The development of human body composition science was enriched by the development of other sciences, such as anatomy, histology, chemistry, physics, biochemistry and physiology (Wang et al., 1999).

Early stages of human body composition science focused on body components. Due to the absence of *in vivo* measurements, they used cadaver autopsy to acquire quantitative body composition data. In 1909 the first *in vivo* method for the estimation of body components by urinary creatinine excretion for the estimation of total body skeletal muscle mass was developed by Shaffer and Coleman (1909), while total body water was first estimated by Heveys and Hofer in 1934 using deuterium a stable isotope of hydrogen with a mass approximately twice that of the usual isotope (Wang et al., 1999). The largest referenced study to date is the Brussels cadaver analysis study (Clarys et al., 1984), which included 25 cadavers (12 men and 13 women). These were dissected, and a considerable amount of anthropometric date was collected (upper and lower limb: skin-fold thickness, circumferences), in addition to tissue masses (Clarys et al., 1984).

The first indirect method for human body composition estimation started in the laboratories of A.R. Behnke (Behnke and Welham, 1942), was the basics of hydrostatic weighing began. Keys and Brozek continued Behnke's research and suggested a more detailed densitometric method (Keys and Brozek, 1953).

In 1951 Sievert proposed that a radioactive isotope of the element potassium, ^{40}K, could be detected and quantified using radioactivity measurement and isotope

identification techniques. This was based on the knowledge that ^{40}K was present in all body tissues but at differing concentrations in muscle and adipose tissues (Wang et al., 1999). In 1961 Forbes estimated the fat and fat free mass from ^{40}K (Lukaski, 1987, Wang, 1999). As years went by, the discovery of the structure and physical properties of an atom and the law of thermodynamics developed non-invasive body composition methods, such as magnetic resonance imaging (MRI) (Siervo and Jebb, 2010).

Over the years' reference data has accumulated, although almost always these are based on a small number of subjects. The limited data is the basis of the assumptions that are used for the assessment of body composition in adults. In December 1963, the ICRP (international commission on radiological protection) committee requested the establishment of a task group to revise and develop the standard man concept, which was named the 'Reference Man'. The reference man is defined as a 20-30-year-old Caucasian, weighing 70 kg and 170 cm height, living (clothed) at an average ambient temperature of 10-20◦c (Snyder et al., 1975) **(Figure 1-2).**

The simplicity of the body mass index (BMI) in a body composition assessment does not take account of any differences in fat and muscle mass. New and old research revealed the connection between muscle/fat mass and chronic illness (Lang et al., 2015, Krotkiewski et al., 1983). The ratio of the waist to hip circumference (WHR) was investigated in the early 1980s, indicating that regional body composition estimated by the WHR showed strong associations with metabolic and cardiovascular complications (Kissebah et al., 1982). The estimation of muscle and fat mass are important to assess health, ageing and obesity.

This introductory chapter will briefly explain models and methods in the science of human body composition. Detailed methodologies for muscle and fat estimation are explained in subsequent chapters.

1.2 Body Composition Models

Constructing a model is related to the number of components used. Body weight is the key body measurement; representing in a single figure the sum of the weights of each body component. In a 2-compartment body composition model, it is assumed that there are only two components, usually body fat and the remainder, 'fat-free mass', thereby reducing body weight to two components. A 3-compartment model assumes that the body has only three compartments, usually fat, muscle and the remainder, which includes bone, skin, brain, blood, inner organs, etc., and is based on measurements of two separate components, usually fat and muscle, in addition to weight. More complicated multi-compartment models may include measurements or estimates of bone mass, blood volume, skin mass, organ sizes etc. (Heymsfield et al., 1990).

The classic 2-compartment model has been widely used (Behnke and Welham, 1942). It depends on separating the body weight components by the density of fat and fat free mass; fat mass density = 0.9007g/cc, and fat free mass density = 1.1000g/cc, assuming a constant proportion of fat free mass (water 73.8%, mineral 6.8% and protein 19.4%) (Behnke and Welham, 1942, Brozek et al., 1963). However, bone mineral mass, protein and water, are influenced by many factors, including age, gender, diet, level of exercise, and genetic factors, leading to systematic prediction errors in calculating both the fat and fat free mass when applied to populations with different constant values (Withers et al., 1998). The main methods segmenting body composition into fat mass and fat free mass are: underwater weighing; ^{40}K; and the evaluation of total body water. The distribution of fat-soluble dyes has been used in the past.

The emerging of 'black-box' methods, such as Dual-Energy X-ray Absorptiometry (DEXA) and bioelectrical impedance analysis (BIA), have facilitated the estimation of different body compartments leading to the development of three and four compartment models, which examine fat and muscle tissue, bone, etc. These methods are harnessing technology, and are probably less dependent upon technician expertise. Instead of making assumptions using the 'reference man', as

in the two compartment model, these more technological but indirect methods both depend on validation against measurements of body density, mineral and/or aqueous compartments (Siri, 1961, Lohman, 1986, Heymsfield et al., 1990, Shen et al., 2003a).

There have been efforts to compare two, three and/or four compartment models (Clasey et al., 1999, Withers et al., 1998). These studies are not conclusive, and the amount of error between the two, three and four compartment models are still under debate. One important consideration is that the 'black-box' methodologies are also based on assumptions and need validation with reference methods. However, there are no 'gold standards' or 'correct' methods for body composition.

The first attempt to organise more than 30 main body components into five different levels was by Wang et al. (1992). The five levels (atomic, molecular, cellular tissue system and whole body) have clearly defined components, providing a structural background for studying human body composition. Each level and its components are distinct and interact with each other at the same time (Figure 1-1).

The atomic level of organisation is based on elements that are the building blocks of the human body. Close to 98% of body weight comes from oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorous (Wang et al., 1992). In a reference man, oxygen alone is >60% of the total body weight (Snyder et al., 1975). Sulphur, potassium, sodium, chlorine, magnesium and residual atoms make up <2 % (Wang et al., 1992). For the last two decades, elements have been measured by neutron-activation techniques (Wang et al., 1992).

The most widely used body composition level is the molecular level, which is composed of water, lipid, protein, mineral, and carbohydrate, in the form of glycogen (Wang et al., 1992). Water is the most abundant compound, accounting for 60% of human body weight (Snyder et al., 1975) (Figure 1-2). Water is bound to a variety of chemical compounds, such as proteins, glycogen, and mixed with

electrolytes, and can be divided to intracellular and extracellular compartments (Heymsfield et al., 1997).

Proteins are made of amino acids and can be in the form of cytoskeletal proteins, plasma proteins, and complex nucleoproteins. There are different types of proteins, and as far as body composition is concerned, there are methods available to estimate total protein, muscle proteins and non-muscle proteins (Heymsfield et al., 1997). It is estimated that 15% of reference man's body weight comes from protein (Snyder et al., 1975).

Glycogen is the main storage form of carbohydrates, and is found in the cytoplasm. It is mainly found in the liver and muscle, which contain 2.2% and 1% wet-weight, respectively (Wang et al., 1992). Minerals (calcium, sodium, potassium, oxygen, phosphorous and chlorine) account for approximately 5% of body weight, and are divided into bone minerals and soft tissue minerals.

The most confusing element at the molecular level is lipid, leading to possible errors in miscalculations in constructing body composition models. This is mostly due to lipids being present within tissues and organs, as well as deposits (Wang et al., 1992). Confusions and assumptions related to lipid estimations will be discussed later in this chapter. In a healthy reference-man it is estimated that 18% of body weight comes from lipids.

According to the available variables, it is common to combine different components from the molecular level to come up with an equation for the development of body composition methods. The goal is to measure one compartment assuming a constant relationship to estimate another compartment (Heymsfield et al., 1997).

There are several methods for estimating molecular level components, such as underwater weighing, bioelectrical impedance, and DEXA.

The cellular level is composed of three main compartments; extracellular solids, extracellular fluids, and cell mass. These compartments can be measured by isotopic dilution methods and neutron activation analysis (Wang et al., 1992).

Body weight at the tissue level is organised into skeletal muscle mass, adipose tissue, bone, blood, and the remainder. Most of the information from the tissue level comes from cadaver studies, and there are only a few methods that can detect the major compartments at the tissue level, such as computerised tomography (CT) and MRI (Wang et al., 1992).

Finally, the whole body level concerning body shape, size, physical and exterior features, can be measured by stature, breadth and circumferences, skinfold thickness, body volume, weight, BMI, density, segment length, and body surface area. Variables at the whole body level are simpler to use, thus are better suited for use in large scale field work (Wang et al., 1992).

1.3 Body Composition Methods

Measurement of human body composition allows the estimation of excess and shortfalls of body components for the assessment of nutritional status. Various body composition methods and techniques exist, ranging from simple to complex, and cheap to expensive, and each have their strengths and limitations. It is important to identify the body compartment under review, the purpose of the assessment, and the availability of a measurement technique in order to select the best methodology.

There have been some attempts to classify or organise human body methodologies to five widely used levels (Figure 1-1) (Wang et al., 1992, Wang et al., 1995, Mitsiopoulos et al., 1998b), which adds a further degree of complexity. To keep it simple, classification will be according to purpose, reference methods, and field (prediction) methods.

There are important considerations in selecting a reference method for body composition research. Mainly, the body compartment to be measured, sample size,

study design and availability. Cadaver data provided the first reference data for human body composition (Clarys et al., 1984) and it is the basis of many assumptions and reference man data (Clarys et al., 1984).

Cadaver analyses provide a direct measurement of body compartments and were used for the validation of equations derived from indirect methods (Janssens et al., 1994, Mitsiopoulos et al., 1998b). There are two methods used in cadaver analysis; chemical to obtain fat, mineral and water content; and anatomical, a process involving the quick dissection of body compartments following death (Singh and Mehta, 2009). Despite the limited number and size of cadaver studies, they are the basis of most of the assumptions used in indirect methods and reference man. The Brussels study is the largest and most referenced, based on the analysis of 25 cadavers over five years (1978–1983) (Clarys et al., 1999). Due to the many limitations, including the small number of cadavers, ethical barriers, loss of body fluids and desiccation of tissues during dissection, challenging and time consuming methods, and a limited range of body sizes and gender, high cost and exposer to embalming chemicals which might cause health problems researchers have searched for other reference methods (Ackland et al., 2012). However, there is no perfect error-free reference method, as all depend on assumptions that are without robust validations.

Hydro-densitometry or underwater weighing (UWW), was established by (Behnke and Welham, 1942) using the famous principle of Archimedes: 'the volume of an object in water equals the volume of water displaced by the object'. Behnke proposed an inverse relation between body density and adiposity and developed the concept of a reference body consisting of fat mass and fat free mass assuming the fat free mass to be constant (Behnke and Welham, 1942).

The accuracy of hydrodensitometry is affected by many factors: 1. volume of air present in the lungs and gastrointestinal tract; 2. technician skills; 3. accuracy of the equipment and calibration; and 4. model and assumptions used (Vivian and Dale, 2004). UWW is currently not a preferred method due to its impracticality and advancements in imaging techniques.

Air displacement plethysmography uses pressure instead of water to estimate body volume and density. Early attempts in air displacement were unsuccessful, until Dempster and Aitkens (1995) developed the 'BOD Pod'. They used the relationship between pressure and volume to derive the body volume, including the measurement of body mass. This enabled them to calculate body density and thus body fat and fat free mass. The most important sources of error found were: 1. test conditions (resting condition, clothing, gas volume); 2. measured subject (excess body hair, body size); and 3. conversion formula percentage body fat (Wagner, 2004).

Water is the most abundant constituent of the human body, accounting for >60% of body weight (Malina, 1969). Most of the body water (73%) is present in fat free mass (Pace et al., 1947). Hydrometry is the measurement of body water using a dose of labelled water (deuterium, isotopes of hydrogen and tritium) (Lukaski, 1987), and it depends on many assumptions: 1. the even distribution of tracer only in body water; 2. the time of the tracer equilibrium; 3. distribution of tracer is equal throughout all water compartments; 4. no metabolism of the tracer during the equilibrium time; and 5. for the estimation of %BF, 73% of fat free mass is water.

Imaging techniques present a major advance in the science of body composition, as they are able to provide a direct visualisation and estimation of body compartments. CT and MRI measure body composition at the tissue level, and have been used for the validation of other body composition methods (Borkan et al., 1983, Sjostrom and Kvist, 1988, Salinari et al., 2002, Varady et al., 2007, Bridge et al., 2011, Al-Gindan et al., 2014b). Because of radiation exposure and cost, CT scanning is limited in body composition research. Ultrasonography, another imagining method, however is not widely used because of poor image quality (Fuller et al., 1994b, Gradmark et al., 2010). Finally, DEXA is considered as a reference method in many research studies (Ejlerskov et al., 2014, Rom et al., 2015).

Dual-Energy X-ray Absorptiometry (DEXA) is based on the measurement of the attenuation of the X-ray source with photon energies specific to bone, fat, and fat free mass (Andreoli et al., 2009). Despite it being a preferred method because of its relatively low cost and minimal need for training, there are major sources of error. DEXA is based on the assumptions that the attenuation of the X-ray is due to differences in densities and chemical composition that are constant for all individuals. In addition, it is susceptible to fat estimation error, due to variation in soft tissue hydration (Pietrobelli et al., 1998). It is difficult to validate DEXA because there is a lack of standardisation in the use of estimation models between different manufacturer's models and software versions (Wagner, 2004). Reviewing the literature, there are two main issues that need to be considered; 1. DEXA is not a reference method, although it has been used widely as a reference method for body composition field methods especially for the validation of BIA (Hofsteenge et al., 2015, Faria et al., 2014, Rech et al., 2008). DEXA is a method that is based on assumptions which themselves need to be validated. The second issue that should be noted when validating DEXA is that it measures appendicular lean soft tissue and fat mass, while MRI and CT measure muscle and adipose tissue volume. The difference between fat mass and adipose tissue mass will be discussed later in this chapter. There are published studies in the literature that compare adipose tissue to fat mass and skeletal muscle to lean tissue without converting one to the other, (Fields et al., 2015). The quantity of literature in validating DEXA with a gold standard reference method is limited, this is understandable due to the high cost of MRI and CT scans. UWW is a valid reference method to validate DEXA as both measures the same thing. DEXA accuracy in fat estimation in obese adults was validated against 4-compartment model using UWW for body density, deuterium dilution for total body water and DEXA for bone mineral mass. Data for Eight men and six women (22-54 years, 28.7-39.9kg/m^2) were investigated. While values for both methods had high correlations R^2 = 0.89, there was high intra-individual difference (-3.04%-4.01%BF). These differences suggested that the error was associated with tissue thickness, suggesting that the DEXA device was unable to accurately account for beam hardening (artefact) in obese subjects (LaForgia et al., 2009). There is an obvious gap in the literature when it comes to DEXA validation, and more studies are needed to clarify the area.

MRI uses a combination of pulses of radio wave energy and a strong magnetic field to produce a comprehensive image of the body and internal organs, relying on the interaction between the nuclei of the hydrogen (Lukaski, 1987). Hydrogen is used because of its abundance in the human body (fat, carbohydrates, and water), normally hydrogen protons spin randomly creating **a magnetic field**. An MRI system is composed of a primary magnetic field, gradient coils, radiofrequency coils (RF)- which are enclosed in a copper-lined examination room to avoid noise and interference- and the computer system. When a magnetic field is introduced, the protons will align either parallel or antiparallel to the external field. The parallel is the preferred alignment because it requires less energy, thus more align parallel, the excess of protons aligned parallel to the external magnetic field is called the magnetisation and it is orientated along the axis of the scanner (Abi Berger, 2002). The field strength of MRI scanners is generated by main magnet coils, most magnet fields range between 1.5 and 3.0 tesla. 1 tesla is approximately 20 000 times the earth magnetic field. The strength of the magnetic field can be changed electronically using electric coils. The gradient coil generates secondary magnetic fields over the primary fields producing **a linearly varying magnetic field gradient**, the arrangement of the gradient coil gives MRI the capacity to image directionally (x, y and z).

The RF coil transmits radiofrequency energy to and from the tissue of interest, disturbing the proton realignment with the static of magnetic field. Since the RF pulse pushes the protons against their nature, once the pulse is turned off the proton will realign with magnetic field releasing magnetic energy. The output signal is sent to the computer processor for complex mathematical manipulations to yield the image. Different tissues relax at different rates when the radiofrequency pulse is switched off. The time needed for protons to completely relax is measured in T1: time needed for the magnetisation longitudinal component to return to its resting state, and T2: is the time needed for the transversal component of the magnetisation to return to its equilibrium state. Different tissues have different relaxation time**s** making it easier to distinguish between them (Currie et al., 2013).

Different transmitted radio-frequency pulses could be used to emphasise specific tissues. Different molecules have different relaxation time, hydrogen bound to large molecules like lipids tumble very slow demonstrating low efficacy at T1 relaxation. On the other hand, free water has a small molecular size thus tumble too quick to be effective at T1 relaxation. Accordingly, both free water and lipid have relatively long T1 relaxation time. On the contrary, when water is partially bound to protein, tumbling rate is slowed, resulting in a much less T1 relaxation time than free water. Fat has a short T1 relaxation value because of the carbon bonds at the end of the fatty acid having frequencies closer to the Larmor frequency "how many times the proton presses per second". These differences can be highlighted by how quickly we put the RF pulses "repetition time TR "and how quickly we listen to the return signal "echo time TE "this process is referred to as the pulse sequence. T2 relaxation controls the decay of the magnetization in the transversal plane. In general, fluids have the longest T2 while water based tissues tend to have longer T2 values than fat-based tissue. Notice that T2 values are always shorter than T1 values in tissue and also that T1 and T2 relaxation processes are independent of each other. Due to the difference in the local environment of the protons, they have different T1 and T2 relaxation this is used in MRI as an advantage to generated images with different contrast. This is used to analyse various tissues components, such as the brain, spinal cord, breasts, joint and soft tissue pathology, and to find tumours, bleeding injuries, or even infections. Beyond different organs and tissue types, MRI allows the measurement of muscle mass and adipose tissue. In addition, it can be used to further investigate problems seen in other imaging techniques, like ultrasound, CT scans, and X-rays. Images from MRI can serve as reference values for the development of anthropometric reference values in all age groups (Lee et al., 2001). In order to do that, it is important to discriminate the tissue compartments and quantify their amount by segmentation techniques. The Gold standard for segmentation techniques is histology. Segmentation of images can be done manually, automated or both. Manual segmentation is time-consuming especially for larger scale studies and needs an expert operator identifying structure of interest. (Brunner et al., 2011, Karlsson et al., 2015, Positano et al., 2009). There are several popular image analysis software packages for segmenting MRI images such as; Slice-O-Matic

(Tomovision,Montreal, Canada), Analyze TM(Mayo clinic, Rochester, MN) and the public domain programs such as image J(National institute of health , Bethesda, MD). Each has its differences, the algorithm to identify and measure the anatomical structure, time efficiency, flexibility, ease of use and cost (Positano et al., 2009, Demerath et al., 2007).

MRI machines can only be operated by well-trained technologists, with the images produced requiring evaluation by radiologists and physicians of other specialties, together with a minimum duration of 30 minutes ranging to 120 min. In the UK the average cost/scan for an MRI ranges between £84 – £472, as estimated by the National Audit Office (NHS, 2010). Safety concerns result from risks due to hypothermia from the cold environment, burns resulting in wounds from contact with equipment cables, anaphylaxis from intravenous agents, and high noise levels from the machine resulting in hearing damage (Mary et al., 2002). In contrast to CT scans, MRIs do not use ionised radiation, making it a safe procedure for vulnerable groups, such children, and pregnant women, with low risks associated with repeated measurements made on the same subject. Its images show high levels of contrast between muscle, connective and adipose tissues, allowing whole limb and muscle visualisation (Heymsfield et al., 1995).

1.3.1 Field Methods

Brozek and Keys (1956) highlighted the importance of accurate body composition measurements for the assessment of nutritional status. Subsequently, measurements, recommendations and reference data have emerged in the literature. Today, anthropometry is widely used to simply measure size, and for categorical descriptions of individuals at a population level.

Body weight (BW), body mass index (BMI), height, waist, hips, waist hip ratio WHR, waist height ratio (WHtR), limb circumferences and skin-fold thickness (SFT) are all quick, non-invasive, inexpensive anthropometric methods that do not require a high level of training. Therefore, they are collected extensively in clinical and epidemiological settings. There have been many attempts in the literature to combine simple anthropometric measurements into prediction equations for the

estimation of body density, % body fat, fat free mass, and muscle mass. This is a developing area that needs guidelines to standardise and optimise practice, and it forms the focus of the work reported in this thesis.

BIA is based on the difference in electrical conductivity of fat mass and fat-free mass (which contains water). The impedance of an electrical current (800μA; 50 KHz) passing between two electrodes is measured. Fat-free mass is a good conductor of an electric current due to its water content, whereas fat is a poor conductor. Hence, equations have been developed for the estimation of fat mass, total body water, and fat free mass (Vivian and Dale, 2004, Norgan, 2005). A key limitation is that BIA is dependent upon many assumptions, and some of have not been investigated (Heymsfield et al., 1997). Dehydration, eating, exercise, sweating, skin temperature, and stage of the menstrual cycle, BMI may affect measures. As with DEXA, there is no standardisation between BIA analysers produced by different manufacturers, with different models giving different results. No studies have convincingly shown BIA to be superior to anthropometry to estimate body fat. Many validation studies mistakenly use DEXA as the reference method(Yi-Chun et al., 2013, Verney, 2015 , Solomon, 2016) in validating BIA. On the other hand, few studies used CT and MRI for their validation (Lee, 2015, Varady et al., 2007) with small sample size, regional body measurement and the comparison was between total adipose tissue measured by MRI and fat mass measured by BIA. Despite reference methods for the validation of BIA have not been critically chosen, studies tend to show over or underestimation. BIA is a practical tool for clinical and epidemiological body composition assessment, however more validation studies are needed with the proper reference methods, correct conversion factors and population suitable prediction equations.

1.4 Assumptions and Rules in the Estimation of Fat/Adipose Tissue Mass

There is no ‘gold-standard’, i.e. true reference method, to quantify body fat or muscle mass. Modern high-resolution imaging, such as MRI and CT, has been accepted as the current reference method (Cornier et al., 2011), but confusion

and errors can arise because the word ‘fat’ is often poorly defined and may be used to refer to the organ (adipose tissue), as well as to the body’s lipid content present within other tissues (Shen et al., 2003a). Fat mass is of direct relevance to health and medical practice (Despres, 2012). Many methods, whose results can vary, are available for its estimation, and assumptions about terminology can lead to serious consequences for the anthropometric assessments of body composition used in clinical practice and epidemiology (Wang et al., 1992). Some of these ‘field methods’ are based on a form of imaging which quantifies adipose tissue volume, either CT (Tokunaga et al., 1983, Kvist et al., 1988b, Sjostrom and Kvist, 1988) or MRI (Barnard et al., 1996, Thomas et al., 1998, Muller et al., 2011). Others come from estimates of whole body lipid content, based on UWW or hydrometry as reference methods, and assume a two-compartment model. More complicated 4-compartment models include the quantification of bone mass, fat mass, total body water, minerals and protein (Withers et al., 1998).

Many technical methods, such as whole-body DEXA and BIA, have been promoted commercially, mainly for clinical use, but all need to be validated against one of the reference methods. Among these, whole-body DEXA is the most widely used in research, although it is expensive and time-consuming, and validation against CT-scan and MRI shows systemic bias (Glickman et al., 2004, Norgan, 2005, LaForgia et al., 2009, Lee and Kuk, 2013). Bioimpedance methods are cheap but may not perform better than simpler anthropometry in validations against reference methods (Coppini et al., 2005, Varady et al., 2007, Dehghan and Merchant, 2008, Hemmingsson et al., 2009, Caicedo-Eraso et al., 2013).

A common confusion in the English-language literature arises between the terms ‘fat’, ‘lipid’ and ‘adipose tissue’. Adipose tissue is a specialised loose connective tissue or organ that contains fat. Fat itself is triglyceride, the main subcategory of body lipid, (Wang et al., 1992, Shen et al., 2003a) and is present in variable amounts in several organs, as well as in adipose tissue.

There are three views of ‘body fat’ which need to be clearly distinguished during any scientific discussion: total adipose tissue, total body fat, and total adipose

tissue fat mass (Figure 1-4). These terms have different values but can become confused if all methods were assumed to measure the same thing (Staten et al., 1989, Ludescher et al., 2009, Thomas et al., 2013) (Table 1-1). For instance, the results of adipose tissue imaging by MRI or CT have been compared directly with those of reference methods which actually estimate total body fat, such as UWW and D_2O (McNeill et al., 1991, Sohlstrom et al., 1993, Tothill et al., 1996a, Ludescher et al., 2009), and both have been used to 'validate' different field methods (e.g. DEXA, BIA, and anthropometry) (Scherzer et al., 2008, Kullberg et al., 2009, Lee and Kuk, 2013, Pietilainen et al., 2013).

1.4.1 Proportion of Fat within Adipose Tissue

Adipose tissue contains vascular and connective tissue, as well as fat. Garrow (1975) reviewed 10 studies on the physical composition of adipose tissue published from 1958 to 1963. He concluded that the fat content of adipose tissue was on average 78.7% by weight in lean subjects, rising to 83.2% in the obese **(Figure 1-4).** These figures have been used in the literature (Fuller et al., 1994b, McNeill et al., 1991), and some papers have applied a rounded figure of 80% to both lean and obese subjects (Tothill et al., 1996a, Thomas et al., 1998, Shen et al., 2003b). A higher figure of 85% was used by Leroy-Willig et al. (1997) when referencing Brozek et al. (1963), whose rather theoretical work assumed a non-obese body composition (the 'reference body'), to which 'obesity tissue', of different fixed composition was added through weight gain and shed during weight loss.

The density of fat is 0.9007gm/ml (Brozek et al., 1963, Siri, 1993, Fidanza et al., 1953) (Figure 1-4). However, adipose tissue, through its content of vascular and connective tissue, is denser, and is usually assumed to be 0.92kg/L (Garrow, 1975). This figure has been widely used to convert adipose tissue volume from MRI and CT measurements to adipose tissue mass (Sohlstrom et al., 1993, Tothill et al., 1996a). However, some studies have used this figure inappropriately to convert adipose tissue volume to 'kg fat mass', assuming this to be the same as total body fat (Scherzer et al., 2008, Kullberg et al., 2009).

After the dissection of six cadavers, Martin et al. (1994) reported that adipose tissue density varied between 0.970g/ml in the leanest subject and 0.925g/ml in the fattest, with a mean of 0.95g/ml. They concluded that individuals can have a large range in the lipid fractions within adipose tissue (0.54 – 0.85) and reported a strong correlation ($r = 0.95$, $P<0.005$) between the calculated lipid fraction of adipose tissue and the 'percentage adiposity', calculated as 100 x the total adipose mass/body mass.

The precision and accuracy of MRI in the estimation of total adipose tissue was compared with estimates from cadaver dissections in two males and one female, aged 54 – 69 years, BMI 27, 31, 23 kg/m^2, whose death was due to cancers (Abate et al., 1994). The results of the two methods were closely matched for all measured adipose tissue compartments (intraperitoneal, retroperitoneal, and subcutaneous), with a mean difference in adipose tissue measurements between MRI and cadaver of 0.076kg (95%CI: +0.005 to +0.147 kg). Despite the small sample size, authors concluded that the limits of agreement evaluated from the mean and standard deviation (SD) of the difference between the two methods were -0.066 to +0.218 kg (Abate et al., 1994).

1.4.2 Studies of Multiple Methods

Some studies have compared a number of methods in one study. McNeill et al. (1991) conducted whole-body MRI in seven lean and seven obese women, together with several other body composition assessments; UWW, body water dilution, whole body ^{40}K, bioelectrical impedance, and skin-fold thicknesses. They reported that UWW had the lowest variability in terms of differences, and the greatest level of agreement with MRI, reporting a MRI/UWW ratio of 1.04 in obese and 1.11 in lean subjects.

The same research group using the same subjects studied the distributions of total and subcutaneous adipose tissue and their correlations (McNeill et al., 1991), while Fowler et al. (1991) used a truncated cone model to estimate total body adipose tissue from MRI. In both studies they converted adipose tissue to 'body fat' by

assuming that adipose tissue contains 78.3% lipid in lean and 83.2% in obese subjects by referencing Garrow (1975) (Table 1-1).

Similarly, Sohlstrom et al. (1993) measured adipose tissue by MRI and estimated total body fat using MRI, UWW and body water dilution (BWD), in 20 healthy women. When assessed by MRI (converting adipose tissue volume to weight x 0.9225 then multiplying by 0.8 to convert to weight of fat) the participants were found to contain 1.4 ± 2.9% less than UWW measured total body fat, while BWD yielded 4.7 ± 4.0%, so more TBF compared to UWW.

In a third study comparing MRI with UWW, Tothill et al. (1996a) compared measurements of regional and total fat using MRI, DEXA and UWW in 13 healthy women (BMI: 21-33kg/m^2), using the truncated cone model to calculate adipose tissue volume from MRI. They used the assumption that adipose tissue contains 80% fat, 2% protein, 18% water **(Figure 1-4),** with densities as described in **(Table 1-1).** They highlighted the fact that the head, feet, forearm and hands were not included in MRI measurements, thus some differences between MRI and DEXA arise. To estimate the amount of missing fat, regional analysis of DEXA scans was used. The mean proportion of the fat mass was 8%, corresponding to a difference of 3% for the total body mass. Different terms were used throughout the paper 'total body fat mass', 'total fat masses' or 'fat' in addition to 'adipose tissue'. The authors reported a poor agreement between the methods but high correlations. The mean value of fat as a percentage of the total body mass were noticeably different: MRI=23%, UWW = 28.6%, DEXA = 40.0%.

Kullberg et al. (2009) 'validated' whole body MRI measurements of adipose tissue using an established CT protocol (Chowdhury et al., 1994) as a reference method in ten Swedish obese sibling-pairs. In addition, whole body DEXA scanning was performed. The three methods were compared on a whole body and at the slice level. The term 'fat weights' as stated in the paper, were compared between the three models. They found strong correlations between MRI and CT whole body and adipose tissue volumes but DEXA underestimated 'fat weights' by 5.23±1.71kg compared to CT. In the method section the authors explained that total subject

weight and ‘fat weight’ were estimated using the density 0.923kg/dm^3, although it is not clear how this number was used. While CT and MRI scans measure adipose tissue, DEXA was assumed to represent the sum of all fat in the soft tissues, not exclusively adipose tissue, but DEXA probably only identifies adipose tissue (Goodpaster, 2002).

These examples have identified several discrepancies in reporting terms and in the assumptions made when attempting to quantify body fat content. Imaging methods measure total adipose tissue, while UWW, D_2O and DEXA estimate the lipid content, i.e. ‘total body fat’ (Goodpaster, 2002). They exclude fat in the hands, feet, head, etc., which Tothill et al. (1996a) estimated to comprise 8% of the total body fat. Imaging methods also ignore the fat within organs, such as liver, muscle and heart, ‘ectopic fat’ of particular relevance to metabolic diseases (Gaggini et al., 2015)

Method descriptions are sometimes insufficiently detailed and can be difficult to follow. It may be difficult to establish whether a conversion factor, e.g. 0.80, should be applied to the measured adipose tissue volume by multiplication or division. If we are estimating total adipose tissue fat mass then obviously we would divide, since total adipose tissue is larger than the lipid contained in adipose tissue. However, it may be assumed that the 80% conversion factor was applied to take account of fat in other parts of the body not included in imaging, which could in theory make the fat mass larger. Sohlstrom et al. (1993) reported the mathematics behind the conversions they used. They first multiplied the measured adipose tissue volume by 0.92 to estimate the adipose tissue mass, then they multiplied the total by 0.80 to convert adipose tissue mass to ‘fat mass’. A reader might easily interpret this description as estimating the total body fat mass, while in fact it would result in an estimate of total adipose tissue fat mass (i.e. the lipid content of adipose tissue), excluding that in the forearms, feet and head.

The way the word ‘fat’ is used in English, to refer both to triglyceride and to adipose tissue, probably underpins the confusion in the scientific literature on body composition measurements. Greater clarity is needed in the reporting of

measurements, using imaging or body-compartment methods, and to distinguish between estimates of adipose tissue volume, mass and lipid content, and whole body fat/lipid content.

1.5 Anthropometric Estimation of Muscle Mass

Muscle mass estimation does not present the same conceptual or language problems as adipose tissue/fat mass. Until CT and MRI scans became available there was no way to estimate muscle mass, except through studies on cadavers. It was estimated very crudely, and perhaps changes could be detected, from limb circumference and skin-fold thickness. However, muscle mass can now be measured by MRI, although the results are dependent upon the limited cadaver data. Muscle mass has been assessed as part of the fat free mass or lean body mass. Attempts to estimate muscle mass as a single variable have been limited.

Although they are not the same thing, the terms 'lean body mass' and 'fat free mass' have been used interchangeably (Hume, 1966). Fat free mass (FFM) includes muscle, bone, extracellular fluid and organs, while lean body mass (LBM), also called lean body weight, has the same constituents of FFM in addition to essential lipids in the central nervous system (CNS), cellular membranes and bone marrow (Janmahasatian et al., 2005). Due to the small fraction of essential lipid in the LBM (3% in men and 5% in women), FFM and LBM have been sometimes considered as synonyms. Since measuring LBM and FFM gives an estimate of muscle and bone together, there is no way to know if a deficiency occurs from a muscle deficiency (sarcopenia) or a bone deficiency (osteoporosis), or both. Anthropometric estimations of muscle mass will be discussed in detail in the next chapter.

1.6 Use of Prediction Equations in Body Composition

A combination of skin-fold thickness, limb, waist, hip circumferences, BMI, height, and/or BW can be used in multiple regression equations, in addition to age, gender and ethnicity, to predict body composition (body density, fat free mass and % body fat). There are two types of prediction equations: general and population specific. Population specific prediction equations are valid only for individuals with the

same physical characteristics (ethnicity, age, gender, BMI, clinical condition, physical activity) (Wilmore and Behnke, 1969, Wilmore and Behnke, 1970, Sinning, 1978), while generalised prediction equations have been developed for a heterogeneous population (Lee et al., 2000c) and these inevitably make even more assumptions. Giving the numerous published prediction equations, it is difficult to choose the best prediction equation that accurately estimates the body compartment of interest, as all incorporate various limitations.

1.7 Statistical Concepts in Developing Prediction Equations

To properly evaluate and/or derive and validate prediction equations, it is important to assess the validity and accuracy of the statistical methods used.

Body composition research uses regression analysis for the development of prediction equations. The aim of a regression is to predict a dependent variable (Y) from a non-dependent variable or predictor (x). Pearson's correlation coefficient quantifies a linear relationship between Y and X, R_{xy} = between -1.00 and 1.00, and a value of 0 indicates no correlation. A correlation measures the strength of the relationship between a dependent and independent variable (Wagner, 2004).

Multiple regression analysis uses two or more variables to predict the dependent variable. The goodness of fit (R^2), is the percentage of variation in Y that is explained by X_1, X_2, X_3……, the higher the R^2 value, the more variation is explained, in other words how close the fitted model is to the observed points. To identify the best combination of variables, we can use hierarchical regression or stepwise regression. Stepwise regression is based on statistical criteria, mainly the variable that increases the R^2 value more. In contrast, hierarchical regression is based on theoretical and logical considerations (Leigh, 1988).

To define the accuracy of a prediction equation, the standard error of the estimate (SEE) is used. The lower the SEE, the more accurate the equation (Lohman, 1992) (Table 1-2). For comparing models, the coefficient of variation

(CoV) is calculated, using the ratio of the SEE to the mean of the dependent variable.

Most body composition research uses Bland and Altman (1999a) analysis to evaluate the limits of agreement and confidence intervals between predicted and measured methods. Bland Altman plots the difference of the measured and predicted values against the mean. When data is normally distributed, we expect values to lie between ±2 SD from the mean difference, which defines the 95% confidence intervals (95% CI); the smaller the limits of agreement the better.

In clinical epidemiological studies researchers are concerned about the extent to which body compartments affect the occurrence of metabolic disease, i.e. the risk of diabetes with increased and decreased muscle mass, risk of hypertension with increased or decreased body fat. One of the most common statistical analyses is the odds ratio (OR), which is the ratio of the odds. This is calculated using the number of cases, with the outcome as the numerator and the number of cases without the outcome as the denominator (P/P-1). Overall, the OR can measure the effect of certain body compartments on the risk of having a disease. An OR of 1 indicates no difference between groups, an OR >1 indicates the rate of an event is increased, and the opposite for <1. The OR is given with 95% confidence intervals, which should not include 1, to be statistically significant, and P value should be ≤0.05 (Harris and Taylor, 2011).

1.8 Objectives and Research Questions

Objective A: To identify and evaluate simple methods to estimate adipose tissue mass/volume and muscle mass/volume in adults using MRI as the reference method.

Key research questions:

1. What are the published anthropometric prediction equations that use whole body MRI as a reference method in adults?

2. What is the quality of these prediction equations and the published paper?

3. What are the limitations that can be identified?

4. What is the best muscle mass/volume adipose tissue mass/volume and body fat prediction equation that can be used in adult epidemiological studies?

Objective B: To derive and validate anthropometric prediction equations to quantify whole-body skeletal muscle mass, whole body adipose tissue, and whole body adipose tissue fat mass in adults using MRI as the reference method.

Key research questions:

1. What is the best anthropometric variable as a predictor of adipose tissue mass and muscle mass in adults?

2. What is the best combination of anthropometric measurements to predict adipose tissue mass and muscle mass in adults?

3. Can the widely used variable race be substituted with simple anthropometric measurements?

4. How does the performance of the validated prediction equations compare to BMI?

Objective C: To explore how the new prediction equations, and other published prediction equations, may be applied in large national health surveys.

Key research questions:

1. What is the strength of the association between the prediction equations and diabetes and hypertension?

2. What is the correlation between the prediction equations and metabolic and health measures (HbA1c, systolic and diastolic blood pressure)?

3. How well do the prediction equations predict HbA1c, systolic/diastolic blood pressure, compared to existing prediction equations and anthropometric measurements?

4. How well do the prediction equations associate with diabetes and hypertension, compared to existing prediction equations and anthropometric measurements?

Objective D: To test our developed prediction equations, with reference to measured values from whole body MRI scans, in obese women during weight loss. Also, in the same clinical study, to investigate the relationship between estimated muscle/fat mass and functional muscle strength.

Key research questions:

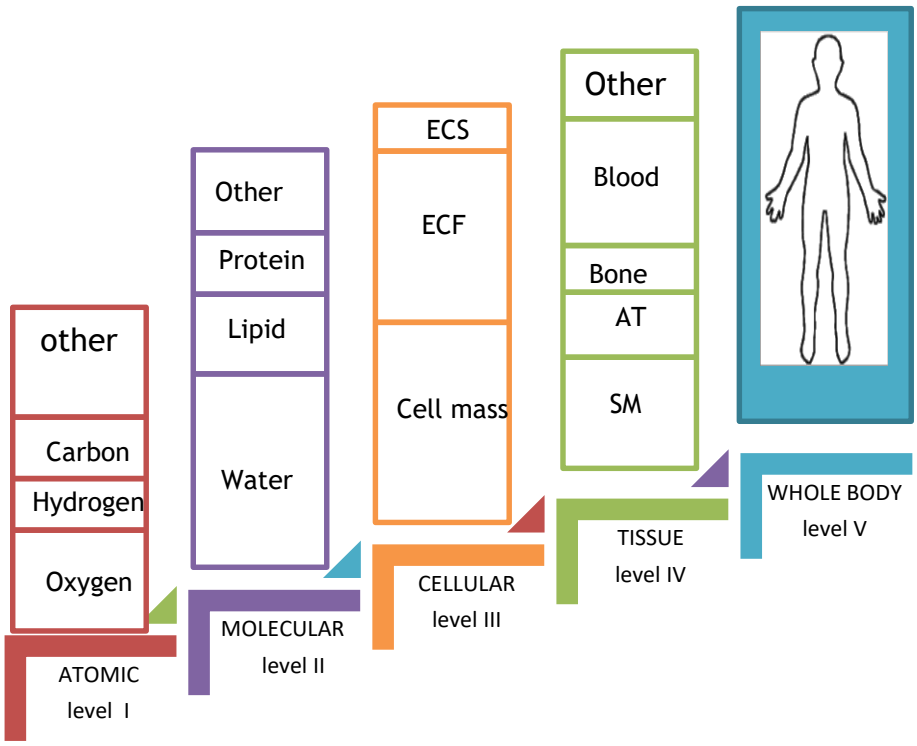
1. How do existing adipose tissue mass estimation equations perform in estimating whole-body adipose tissue mass in overweight and obese subjects before and after weight loss?

2. How do existing muscle mass estimation equations perform in estimating whole-body skeletal muscle mass in overweight and obese subjects before and after weight loss?

3. How does the ability of the equations to estimate changes in adipose tissue mass and muscle mass vary across a wide range of weight changes?

4. What are the associations of adipose tissue mass and muscle mass with measures of functional strength pre and post intervention?

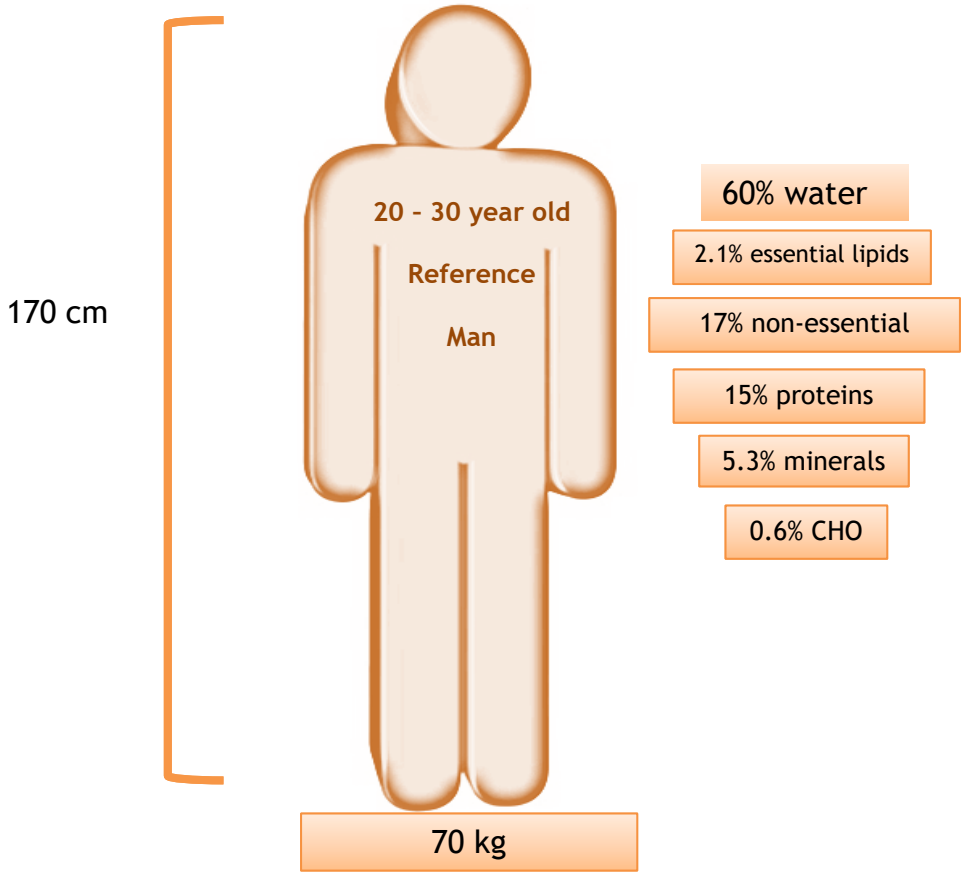
Figure 1-1: The five level model; atomic, molecular, cellular, tissue and whole body levels, and their components



Adapted from Wang et al. (1992)

ECS: extracellular solids, ECF: extracellular fluids, SM: skeletal muscle, AT: adipose tissue.

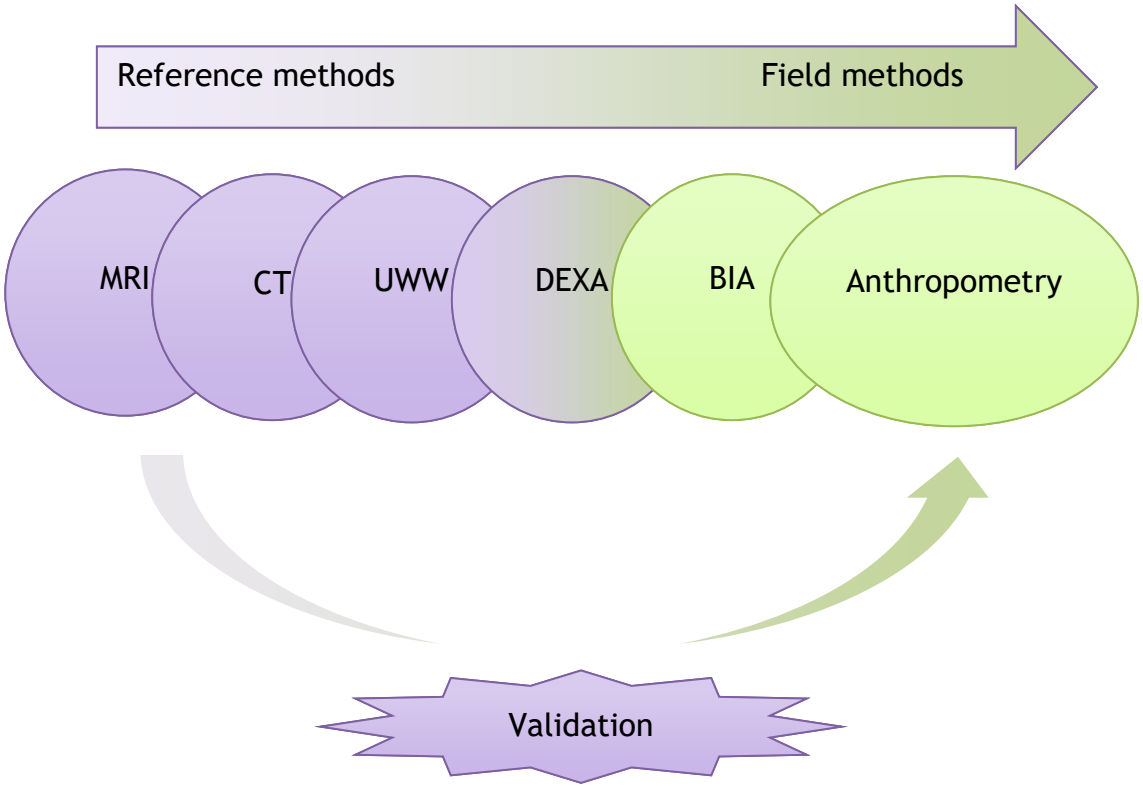
Figure 1-2: Body composition at the molecular level in a 70kg reference man



Based on Snyder et al. (1975)

CHO: carbohydrate, Reference Man: 20 -30 years, 70kg, 170 cm height

Figure 1-3: Commonly used human body composition reference and field methods



MRI: magnetic resonance imaging, CT: Computerised tomography, UWW: underwater weighing, DEXA: Dual-Energy X-ray Absorptiometry, BIA: bioelectrical impedance. In this thesis we will validate anthropometry using field method MRI. In the literature DEXA is used as a reference method and a field method.

Figure 1-4: Adipose tissue and fat mass densities across body compartments

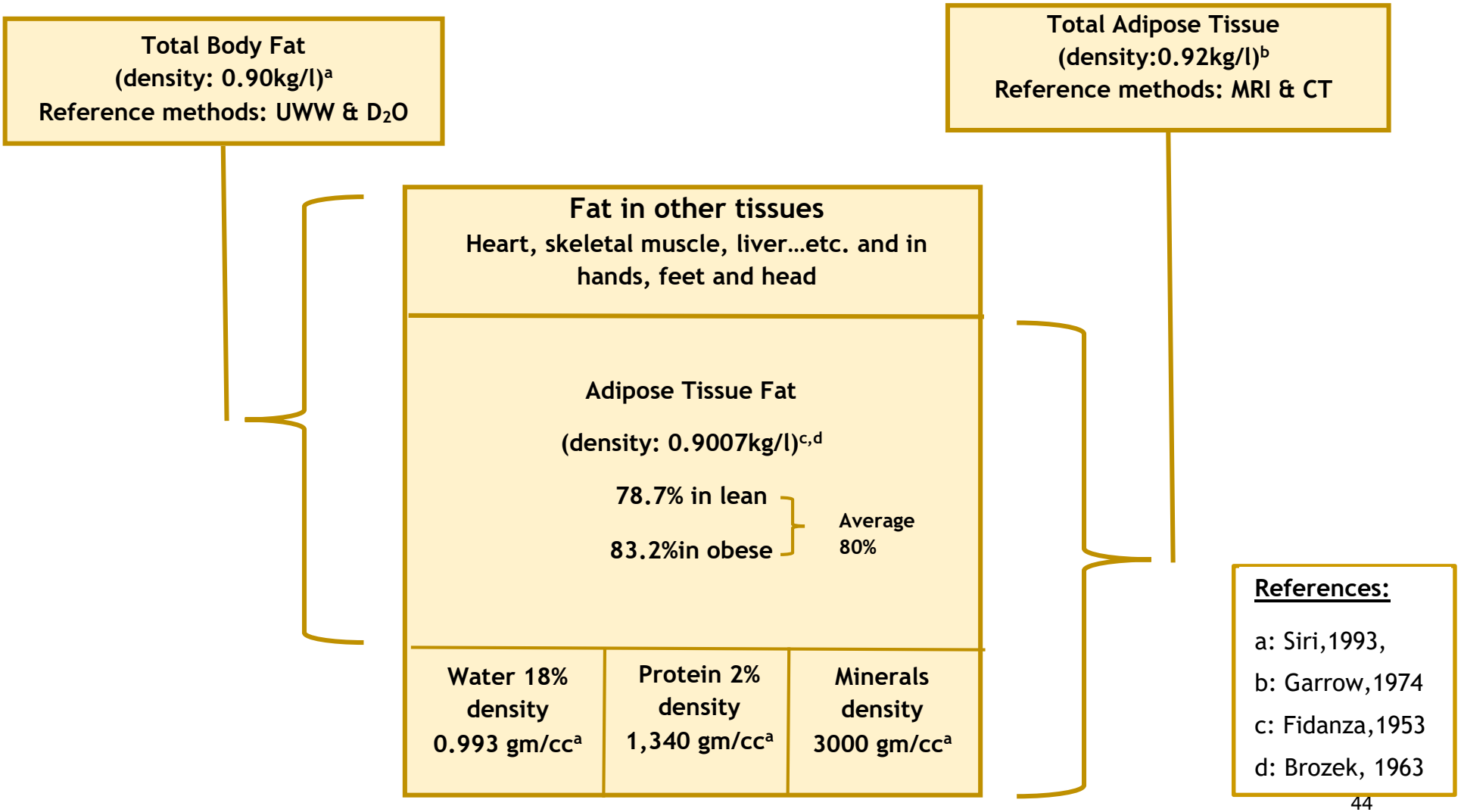


Table 1-1: Summary of the different terms used in the literature and assumptions made in their use

Reference	Title	Assumption 'quoted'
(McNeill et al., 1991)	Body fat in lean and overweight women estimated by six methods	'Estimates of total body adipose tissue volume were made using a truncated cone model (Sjostrom and Kvist 1988)and body fat was calculated assuming that adipose tissue contains 78.3 % lipid in lean subjects and 83.2 % in obese subjects (Garrow, 1975)'
(Fowler et al., 1991)	Total and subcutaneous adipose tissue in women: the measurement of distribution and accurate prediction of quantity by using MRI	'Volumes of adipose tissue were corrected to 78.3 percent lipid in lean women and 83.2 percent lipid in obese women. Tissue volumes based on the truncated cone model were used to calculate percent of tissue'
(Sohlstrom et al., 1993)	Adipose tissue distribution as assessed by MRI and total body fat by MRI, underwater weighing, and body-water dilution in healthy women	'MRI. Adipose tissue was assumed to contain 80% fat, 2% protein and 18% water (Garrow, 1975) with densities of 0.900, 1.34, and 0.999kg/l respectively (Siri, 1961), giving a density for adipose tissue of 0.9225kg/L. Thus volume of adipose tissue was converted to weight of fat by multiplying the volume by 0.9225x0.8'
(Abate et al., 1994)	Estimation of adipose tissue by MRI: validation against dissection in human cadavers	'Assuming that adipose tissue composed of 84.67% fat, 12.67% water and 2.66% proteins (Thomas, 1962). The density of adipose tissue was calculated to be 0.919 kg/l. Therefore, adipose tissue mass was calculated in kilograms for each 10-mm slice. The masses obtained for each slice were summed to calculate the total adipose tissue mass for each identified compartment (subcutaneous, interperitoneal, retroperitoneal)'
(Tothill et al., 1996b)	Comparison between fat measurements by dual-energy x-ray absorptiometry, underwater weighing and MRI in healthy women	'The MRI measurements did not include the head, feet forearm and hands, so this disparity has to be considered in making comparisons with the other techniques. Adipose tissue volumes were calculated using a truncated cone model. Adipose tissue was assumed to contain 80% fat, 2 % protein, 18 % water, with corresponding densities of 0.900, 1.34, 0.993kg/l, giving an adipose tissue density of 0.9225kg/l (Garrow, 1975)'

Reference	Title	Assumption 'quoted'
(Leroy-Willig et al., 1997)	Body composition determined with MR in patients with Duchenne Muscular Dystrophy, Spinal Muscular Atrophy, and Normal subjects	'Total adipose tissue and lean tissue volumes having been determined, the volumes were converted into masses according to literature values for composition and density of tissues. Fat mass was determined from adipose tissue volume, assuming the fraction of fat in adipose tissue to be 0.85 (Brozec, 1963). And a density of fat of 0.9007 (Fidanza, 1953). Then the whole body fat percentage was obtained from the fat mass and body mass'
(Scherzer et al., 2008)	Comparison of dual-energy x-ray absorptiometry and MRI measured adipose tissue depots in HIV-infected and control subjects.	'for comparison with DEXA, adipose tissue volume from MRI were multiplied by 0.9 kg/ L to convert to KG fat mass, because adipose tissue has a density of 0.9kg/L.'
(Kullberg et al., 2009)	Whole- body adipose tissue analysis: comparison of MRI, CT and dual-energy x-ray absorptiometry	'Subject weight and fat weight were estimated by the use of density values reported in the literature (AT, 0.923 kg dm^{-3})'
(Pietilainen et al., 2013)	Agreement of bioelectrical impedance with dual-energy X-ray absorptiometry and MRI to estimate changes in body fat, skeletal muscle and visceral fat during a 12-month weight loss intervention	'Viseral fat volumes were assessed with Sliceomatic and converted to fat weight using an adipose tissue density of 0.9196mg/ml'
(Lee and Kuk, 2013)	Changes in fat and skeletal muscle with exercise training in obese adolescents: comparison of whole-body MRI and dual energy X-ray absorptiometry	'Fat and skeletal muscle volume was converted to mass units (kg) by multiplying the volumes by the assumed constant density for adipose tissue (0.92 kg/l) and SM(1.04 kg/l)'

Published studies that used the terms, fat mass, adipose tissue, fat weight, lipid, fat. The conversion factors used and assumptions are included.

Table 1-2: Evaluation of standard error of the estimate

SEE %BF	SEE Db (g/cc)	SEE FFM (kg)	SEE FFM (kg)	Subjective Rating
Male and Female	Male and Female	Male	Female	
2	0.0045	2.0-2.5	1.5-1.8	ideal
2.5	0.0055	2.5	1.8	excellent
3	0.007	3	2.3	very good
3.5	0.008	3.5	2.8	good

Adapted from (Lohman, 1992)

Chapter 2

2 Systematic review

Predicting muscle mass from anthropometry, using magnetic resonance imaging (MRI) as reference: a systematic review

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2.1 **Abstract**

Background: Muscle is important for physical and metabolic health, but identification and management of low muscle mass, or sarcopenia is limited by lack of reliable simple approaches to assess muscle mass.

Aim: To identify and evaluate simple methods to quantify muscle mass/volume of adults.

Search methods: Systematic review, using Cochrane Review methods, of Medline (1946–2012), Embase (1974–2012), Web of Science (1898–2012), Pubmed (to 08/2012) and Cochrane Library (to 08/2012),

Selection criteria: Publications which included prediction equations (from anthropometric measurements) to estimate muscle mass (by MRI) in adults.

Data collection and analysis: Studies were checked for relevance by two reviewers independently. Methodological and critical quality assessment was completed using a critical review form and QUADAS.

Results: out of 257 papers identified from the primary search terms, 12 studies met the inclusion criteria. Most (10 studies) assessed only regional/limb muscle mass/volume: evidence relating regional/limb to whole-body muscle mass is weak, with just one MRI study showing strong relationships, R^2 =0.84-0.77 for men and women respectively. Only Lee et al, (2000) n=324, included over 70 subjects. Many (9 studies) used limb circumference adjusted for skinfold thickness, limiting their practical applications. Only two studies included validation in separate subject-samples. Bland-Altman plots showed reasonable agreement in one (Lee et al, 2000), and over-estimation in the other (Mathur et al, 2008).

Conclusion: Factors which predict muscle mass include sex, age, weight, height, waist/hips/thigh/calf/arm circumferences, and skinfold thicknesses. A simple equation including body weight, height, age, sex and race (Lee et al, 2000),

validated among obese subjects, had R^2 = 0.86–0.79 and reasonable agreements with MRI-measured whole-body muscle mass. This equation has yet to be validated in a separate population with different investigators, and it did not incorporate widely-available trunk/limb girths which offered valuable prediction in other studies.

2.2 **Introduction:**

Muscle is important for physical activity, thus social functioning, and also metabolic health. Measuring muscle mass is of interest for various reasons, such as evaluating the effects of weight loss or gain, the effect of disease on body composition, the training effect of physical activity, and to predict frailty and falls. The term 'sarcopenia' has been used for over two decades to describe loss of muscle mass, strength and/or quality (Morley et al., 2010). Low muscle mass or loss of muscle function have major effects on quality of life, contributes to frailty, and relates to chronic illnesses like diabetes and heart disease (Janssen et al., 2002, Nair, 2005). Low muscle mass may thus affect physical, mental and social aspects of health through a range of functional, nutritional, endocrine, metabolic consequences (Khamseh et al., 2013) of particular importance to ageing (Bauer and Sieber, 2008). Recognition of these clinical and public health consequences of sarcopenia (Lukasaki, 2005b, Rosenberg, 2011) has attracted some research interest in evaluating approaches to assess muscle mass, and its quality and functional capacity. However, progress is hampered by lack of agreed simple approaches to estimate muscle mass (Cooper et al., 2012, Lukasaki, 2005b) to provide unified criteria for diagnosis, clinical application and epidemiological practice (Janssen, 2011).

Sarcopenia can result from a primary disease of muscle, such as myasthenia, or secondary to a variety of primary causes, including metabolic diseases, inflammation, neurological diseases, and physical inactivity (as a result of any condition) as disuse atrophy (Visser and Schaap, 2011, Morley, 2008). Overweight and obesity, which now affect well over half of all adults in post-industrialized societies, are generally associated with increased muscle mass to support greater weights; however, the extent of muscle hypertrophy may be insufficient to maintain metabolic and physical capacities. A relatively low muscle mass in obese people "sarcopenic obesity" is difficult to identify clinically but appears to be increasing (Lean et al., 2013b) and presents limitations to mobility and function, promoting diabetes and cardiovascular disease (Stenholm et al., 2008).

2.2.1 Approaches to assess Sarcopenia

Before sophisticated technological methods were available, skeletal muscle was most commonly estimated from upper-arm (Jelliffe and Jelliffe, 1969) and/or lower-leg circumferences (Heymsfield et al., 1979), and from simple tests of strength or endurance, such as hand-grip, stair-climbing and chair-rise (Young et al., 1990, Newman et al., 1984). Measurement now includes imaging methods such as magnetic resonance imaging (MRI), dual energy absorptiometry (DEXA), computerized tomography (CT), bioelectrical impedance (BIA) (Heymsfield et al., 1997) and muscle metabolites; 3-methylehistidine and creatinine (Lukaski, 1997). Both DEXA and BIA have been used in some epidemiological settings (Fielding et al., 2011) but their accuracies for predicting MRI or CT-measured muscle mass vary (Lee et al., 2001), and they are not practical for routine clinical use. Whilst widely promoted, DEXA has many limitations: (1) muscle is not quantified directly and several crude assumptions are made to calculate muscle mass, (2) patients are still exposed to radiation, (3) there is potential interference from fluid, dehydration or oedema, (4) it is relatively costly, and (5) it needs skilled technicians (Lee et al., 2001, Lukasaki, 2005b).

While different criteria for sarcopenia have been proposed, no definition has been widely used, nor have diagnostic criteria been tested thoroughly to reach a consensus (Cooper et al., 2012). In a randomly selected sample of 199 subjects from the 1993-1995 New Mexico Elderly Health Survey (Baumgartner et al., 1998), skeletal muscle mass (SM) was determined as the total lean soft tissue of the arm and leg, estimated from DEXA. The best predictive equation, developed by step-wise regression, included weight, height, hip circumference, grip strength, gender (R^2 =0.91, SEE =1.58). Sarcopenia, defined as SM/(height)^2 below two standard deviations from mean in a young reference group, increased with age to 13-24% in >70-year-olds, and >50 % in 80-year-old subjects.

Bioelectrical impedance (BIA) has been used in many large-scale health surveys, for example in 4,504 adults aged over 60 years from NAHNES III, where sarcopenia, defined arbitrarily as a low muscle mass index (SM (from BIA)/body mass x 100 ≤ 1

or 2 standard deviation of young adult value), was associated with disability and functional impairment (Janssen et al., 2002). However, many factors introduce error and limit the value of BIA: (1) the size and type of electrodes and equipment calibration (2) need for multi-frequency signal, (3) effect of hydration, oedema, temperature variations and sweating on electrical impedance (Lukaski, 1997). BIA has not been shown superior to anthropometry for estimating body composition.

Moving towards consensus, the European Working Group on Sarcopenia in Older People (EWGSOP) suggests three measurable variables: mass, strength and/or physical performance (Cruz-Jentoft et al., 2010). Muscle strength can be measured by grip-strength and performance by the short physical performance battery, gait-speed, 6-minutes walking test and stair-climb test (Cruz-Jentoft et al., 2010). The International Sarcopenia Consensus Conference Working Group (ISCCWG) proposed including both muscle mass and physical function (Fielding et al., 2011). Approximating to muscle mass, they used the index (whole-body fat-free mass to height-squared), with cut points used in other epidemiological studies: ≤7.23 kg/m^2 for men and ≤5.67 kg/m^2 for women. They proposed functional capacity should be indicated by gait-speed. Janssen in (Janssen et al., 2004) recommended a quantitative definition based on the muscle mass index or ratio, derived by dividing appendicular SM by height^2. Individuals with ratios between -1 and -2 standard deviations of young controls of the same gender would be considered to have class I sarcopenia (men: 8.51–10.75 kg/m^2, women 5.76-6.75 kg/m^2). Individuals with ratios below -2 would be categorized as class 2 sarcopenia (men ≤8.51kg/m^2, women ≤5.75 kg/m^2).

MRI and CT-scanning are generally considered valid and reliable for quantification of muscle mass, based on validations against cadaver dissections (Mitsiopoulos et al., 1998b), firstly to derive simpler field measurements using techniques such as anthropometry, and secondly to validate the prediction equations in the variety of clinical and epidemiological settings where such measurements are needed (Chen et al., 2007, Mitsiopoulos et al., 1998b, Wang et al., 2000a, Elia et al., 2000). Even with modern imaging, problems remain; (1) limb muscle groups are separated

by non-muscular components and covered by variable amounts of fat (Housh et al., 1995b), (2) Obesity, aging and some illnesses lead to fat infiltration of muscle, complicating its identification and quantification (Goodpaster et al., 2004), (3) Sophisticated scanning instruments, and trained staff, are costly (Lee et al., 2001), (4) High quality reference data have not been defined for individuals of different sex, age, race and body fat content (Cruz-Jentoft and Morley, 2012), (5) There may still be confounding effects from smoking, nutrition, occupation, alcohol and physical activity (Doupe et al., 1997).

Efforts to economise by using a single cross-sectional MRI slice, or a limited number of slices, raises questions about three steps in the prediction of muscle mass:

- Does limited cross-sectional muscle area of limbs, represent the whole muscle mass of the region (limb) examined? if so how many slices are needed?

- Does regional muscle mass, eg of limbs, quantitatively reflect whole-body muscle mass?

- Which single or contiguous imaging (thigh, arm, or calf) best represents whole-body muscle mass or volume? No study appears to have drawn comparisons between the three limb areas, although thigh was most frequently used.

The validity of estimating thigh muscle volume (quadriceps) using a single MRI image was examined by (Morse et al., 2007) using upper leg volume in eighteen active young men. A single MRI scan taken at 60% of the femur length from the distal end of the femur estimated muscle volume with R^2 =0.90, SEE10%. Similarly, (Tothill and Stewart, 2002) showed strong correlation between a single mid-thigh MRI muscle area and thigh muscle volume from contiguous scans (R^2 =0.96, SEE =207cm^3) **(Table 2-1)**

Lee et al, (2004) related MRI measurement of thigh SM muscle to whole body SM and reported the correlation coefficients between a single MRI measurement or

multiple MRI images in 387 white men and women (Lee et al., 2004). The findings, perhaps unsurprisingly, indicated that a 7-slice estimate of thigh muscle mass had a higher correlation with whole body SM (R^2 = 0.84, SEE = 5.4% in men, R^2= 0.90, SEE = 5.1% in women) than muscle area on a single thigh image (R^2 = 0.77, SEE = 6.5% in men; R^2 = 0.79, SEE = 7.4% in women). However, both measurements showed sufficient correlation to provide useful prediction for many purposes (**Table 2-1**).

Accepting these limitations, we have used a systematic review approach to explore the published literature for simple equations based on anthropometry to predict the muscle mass of adults, as measured by MRI.

2.3 **Methods:**

2.3.1 Selection of studies:

A search strategy was conducted according to Cochrane Review criteria (Smidt et al., 2007) using the key words in (**Table 2-2)**. Mesh terms were used in Medline (1946–2012), Embase (1974–2012), Web of Science (1898–2012), Pubmed and the Cochrane Register of Clinical Trials (to 08/2012). Limits were human and adults. In the primary search, irrelevant articles were eliminated first using title and abstract. The remaining articles were read and eliminated if they did not meet the inclusion criteria. Reference lists of relevant papers were checked. Reference manager version 12 was used to manage articles.

Inclusion criteria: Articles reporting human studies of adults (>18 years) that (1) used MRI as a reference method to measure lean or muscle mass/ volume, and (2) used anthropometric measurements with prediction equations of lower or upper limb circumferences and/or skin-fold thickness.

Exclusion criteria: Studies that: 1) did not use prediction equations, 2) used reference methods other than MRI, 3) used comparator methods other than simple

anthropometric measurements commonly available in health surveys, eg DEXA, bioelectrical impedance (**Figure 2-1).**

2.3.2 Quality assessment:

Studies were checked for relevance by two reviewers Yasmin Algindan and Wilma Leslie independently using the same search strategy in **(Table 1-1),** both reviewers agreed on included studies. Technical and critical quality assessment was completed using a critical review form (Law et al., 2007) and QUADAS a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews (Whiting et al., 2004) (**Table 2-3, Table 2-4**)

2.4 Results:

257 studies were identified by the primary search terms (**Table 2-2**). After eliminating the duplicates (179), 78 studies were identified by title and abstract as potentially relevant. Of these, 12 studies met all inclusion criteria and were included in the review (**Figure 2-1**).

2.4.1 Quality Assessment:

Critical quality assessment: The purpose of the study was stated clearly in all papers (**Table 2-3 and Table 2-5**). All studies with the exception of one were cross-sectional. Nakamura et al, (2006) was a longitudinal study that lasted 3 years, with measurements made once annually (Nakamura et al., 2006). However, the time points used to compare reference and index methods were not specified. Sample size justification was not reported in all studies. Sample size was below 69 subjects in all studies except one (Lee et al., 2000b) with 324 subjects (Table 2-6). Reliability and validity were assessed in most studies (**Table 2-3**). Studies that used corrected for skin-fold thickness limb circumference, using the method of (Jelliffe and Jelliffe, 1969) for their prediction equation, were considered validation studies, given that this equation has been already validated (Heymsfield et al., 1982, Heymsfield et al., 1979). Limitations and biases were reported only in

3 studies (Chen et al., 2011b, Nakamura et al., 2006, Ross et al., 1994b). The Bland-Altman method to assess agreement and distributions of errors was used by (Lee et al., 2000b) and (Mathur et al., 2008). Overall there were no major concerns over the quality of papers included in this study, although all could be criticised (**Table 2-3**).

Technical quality assessment: (**Table 2-4**) several points of criticism emerged from the technical assessment of the included studies; (1) the period between performing the reference and index tests was not reported in most studies (9 studies). Since they were cross-sectional studies, it was assumed that measurements were made close together, but this information would increase confidence in the conclusions (**question 4, Table 2-4**), (2) None of the studies mentioned if the test results were analysed or interpreted with or without (ie blinded) knowledge of the other test (**questions 10, 11, Table 2-4**), (3) Nakamura et al, (2006) was the only study that mentioned withdrawals, as that study was longitudinal. All male subjects withdrew for varying reasons; so they focused their study on female subjects. (Baumgartner et al., 1992) used data from New Mexico Aging Process Study, and mentioned a drop-out rate of 5.8/year and death rate 4.9/year (**question 14, Table 2-4**), (4) Response rate was not mentioned in any of the studies, however selection criteria were clearly explained in all cases. Each study represented a specific age group of interest (**question 1, 2 Table 2-4**).

2.4.2 Magnetic resonance imaging (MRI):

All studies except two (Lee et al., 2000b, Ross et al., 1994b) attempted to use regional muscle mass as a marker of whole body muscle mass (**Table 2-7**). Ten studies used T1-weighed spine echo sequences (**Table 2-8**), which are optimal for representing anatomy (Goodpaster et al., 2004).

MRI machines ranged from 0.5 to 1.5 tesla: seven of the 12 studies used 1.5 tesla. One study (Bamman et al., 2000) used whole body imaging and 4.1 tesla spectroscopy. MRI scanning methodologies varied (**Table 2-8**): (1) The strength of the magnetic field varied between 0.5–1.5 tesla, affecting scanning time and thus

image quality: not all studies reported scanning times; reported scans were between 2 min and 90 minutes, the main difference being between whole body and single cross-sectional measurement (2) Considerable variation in repetition time and echo time, (3) Variation in number and thickness of images (5mm or 10 mm), (4) Gap thickness varied from 2.5-50 mm, (5) In most studies cross sectional areas were summed, converted to volumes and then muscle mass/volume calculated. Different software programmes were used for these calculations.

2.4.3 Anthropometry and subject characteristics

All studies, with the exception of 4 (Chen (Chen et al., 2011a, Nakamura et al., 2006, Ross et al., 1994b, Lee et al., 2000a) combined regional skinfold thicknesses and circumferences in their anthropometric explanatory variables (**Table 2-7**). Ten used thigh circumference, three used arm circumference (Tonson et al., 2008b, Lee et al., 2000b, Baumgartner et al., 1992) and three used calf circumference (Bamman et al., 2000, Lee et al., 2000a, Fuller et al., 1994a) (**Table 2-7**).

All studies included in this review recruited healthy adults except (Mathur et al., 2008), which included adults with chronic coronary obstructive pulmonary disease. That study validated the prediction equations developed by (Housh et al., 1995b), and results showed very low correlations R^2= 0.01–0.2 (**Table 2-6**), **(Table 2-9).**

It was difficult to assess variations in anthropometric prediction by age, because age varied widely between studies (range 18-92 years) and within samples, e.g. (Tothill and Stewart, 2002) had an age range between 23-49 years. Tonson et al (2008) validated Jones and Pearson equation in children and adolescents, as well as adults (**Table 2-6**), and reported that compared to MRI, anthropometry tends to overestimate muscle; the overestimation was higher in children and adolescents than adults (43.1%, 38.5% and 20.5% respectively).

Nakamura et al (2006), assessing thigh muscle mass, included underweight elderly subjects (BMI = 21.0 ± 3.7). The remaining studies did not use BMI as an inclusion criterion, hence BMI ranges were wide, from 18-39 kg/m^2 (**Table 2-6**), with the

exception of (Lee et al., 2000a), who cross-validated equations derived in non-obese subjects separately in obese (BMI>30kg/m2) and non-obese groups. In general, more bias was seen in the obese subjects than non-obese, using the skin-fold-corrected model (-0.36 ± 2.99, -0.27 ± 2.50 respectively) and the body weight and height model (-2.33 ± 3.31, -0.34 ± 2.73 respectively). This study concluded that the equation with limb skinfold circumference was more robust for use in obese subjects than the simple equation which included body-weight, height, sex, age, and race **(Table 2-9).**

2.4.4 Method reproducibility

Whether single or multiple observers were used was mentioned in most studies. A detailed method reproducibility explanation was given by (Fuller et al., 1999, Tothill and Stewart, 2002) (**Table 2-10**). In general, limb circumference had the least variability, and skinfold thickness involved the greatest variability (**Table 2-10**).

2.4.5 Prediction equations:

Prediction equations listed in **(Table 2-9).** There were 4 studies that used simple anthropometric approach, and nine studies which involved local skinfold thickness measurements and employed existing equations for the adjusted skin-fold thickness approach. The measurements found to offer useful prediction of whole-body muscle mass were: body weight, height, hip circumference, waist circumference, thigh circumference, age, sex and race.

2.4.6 Reporting results

Not all studies reported standard error of the estimate and/or % error. There was a wide range in the strength of correlations from very low (R^2 = 0.01) to very high (R^2= 0.95) **(Table 2-9).** Agreement between methods, and distribution of errors, using the Bland-Altman method (Bland and Altman, 1999a), were presented only by Mathur et al, (2008) (showing overestimation) and by Lee et al, (2000) showing

reasonably good agreements for measurements in very similar non-obese subject samples of the same population made by the same investigators, and somewhat less good agreement for a validation in obese subjects also in the same population and made by the same investigators (**Table 2-9).**

2.4.7 Validation studies

Validation is an important step in developing prediction equations for general use. The equations of Ross et al, (1994) and Chen et al, (2011) were practical and gave moderate to high correlations, however they were not validated, nor has agreement between methods been investigated. Ross et al, (1994) were assessing lean body mass and Chen et al assessed thigh muscle mass, not whole-body muscle.

Lee had divided their subjects into 3 subject samples. Non-obese subjects were randomised into Groups A and B. Group A (n = 122, non-obese) was used to develop the equation: (0.226 x body weight + 13.0 x height – 0.089 x age + 6.3 x sex + race – 11.0) R^2 = 0.85, SEE = 3.0 kg. This equation was cross-validated in group B (n = 122 non-obese): R^2 = 0.86 SEE = 2.6 kg. The final equation was developed with subjects from both A and B groups: (SM (kg) = 0.244 x body weight + 7.80 x height – 0.098 x age + 6.6 x sex + race – 3.3 R^2 = 0.86, p< 0.0001, SEE = 2.8 kg) this equation was then evaluated in the third group of obese subjects (n=80, 39 men). R^2 = 0.79, p< 0.0001, SEE = 3.0. However, the mean muscle mass of the obese group was significantly overestimated (~ 10%) and significant skewing was seen, with correlation between the difference of measured and predicted, and measured muscle mass (R^2 = 0.18, p<0.001). The significant correlation means that for lower values of SM the equation over-estimates SM but for larger values it underestimates. The same method was used for their skin-fold circumference model which gave higher correlations especially for the obese subjects **(Table 2-9).**

2.5 **Discussion:**

Total body mass and its major constituents (total body fat, muscle mass, etc) vary with age, sex, race and lifestyle, but can in principle be correlated with height, weight, and with circumferences, from which prediction equations can be derived that will to some extent account for variations by factors such as race and lifestyle which can be hard to define. There are many advantages of using anthropometric measurements (simple, quick, safe, non-invasive, cheap, needs only low skill levels, give immediate results), however it is essential to make anthropometry practical, sensitive and specific for the quantification of muscle mass (Lee et al., 2001). Anthropometry is susceptible to errors, depending on the assumptions and specific characteristics of derivation and validation population (which mainly introduce bias) and on observer error in measurements (Knapik et al., 1996, Forbes et al., 1988) which may be random or systematic.

As long ago as 1921, Mateiga measured circumferences of forearm, upper-arm, calf and thigh corrected for skinfold thickness, in order to estimate whole-body muscle mass anthropometrically and derived a value muscle limb radius. The value was squared, multiplied by height and a constant of 6.5. This equation was not validated by Mateiga or later investigators. However this work was expanded by (Doupe et al., 1997) and (Martin et al., 1990) and whole body anthropometric SM prediction models were developed in the Brussels Cadaver Study of 12 elderly men (Lukaski, 1997, Lee et al., 2000a): equation (1) SM (g) = height x (0.0553 x (corrected for skin-fold thickness thigh girth)^2 + 0.0987 x (uncorrected for skin-fold thickness forearm girth(cm))^2 +0.0331 x (corrected for skin-fold thickness calf girth)^2 – 2445) R^2 = 0.97, SEE = 1.53kg. Equation (2) SM = height x (0.031 x (modified upper thigh girth) ^{2} + 0.064 x (corrected for skin-fold thickness calf girth) ^{2} + 0.089 x (corrected for skin-fold thickness arm girth) ^{2})-3006. These equations were either never validated or based on small sample size and proved too complicated for wide application.

In adults most skeletal muscle is found in the lower limb (30%), with lesser amounts in the upper limb, head and trunk (Lukasaki, 2005b). In an honours thesis published in UniSA Research Archives, (Hellmanns, 2011) measured whole body muscle mass using contiguous MRI scans in healthy adults, and estimated the torso muscle mass to be 11.43±3.39 kg, upper appendicular muscle mass 3.27±1.23 kg, lower appendicular muscle mass 10.45±3.27 kg, with summed whole-body muscle mass 25.14±7.11kg. Recently the thigh has received most interest as a predictor of whole-body muscle mass (Chen et al., 2011b, Mathur et al., 2008). Thigh muscles are major determinants of total muscular physical activity, and quadriceps and hamstrings are the most powerful muscles of humans (Tothill and Stewart, 2002). In addition to their volume/mass, they can be assessed by measuring maximal power production in anaerobic capacity tests, and related to functional capacity and independence at older age (Winter and Brookes, 1991, Tothill and Stewart, 2002).

Calf circumference has also been considered, but it showed only moderate correlation (R^2=0.48) with regional MRI-muscle mass (Bamman et al., 2000). Lee et al, (2000) found skinfold-corrected arm circumference to have a higher correlation (R^2=0.77) than skinfold-corrected thigh and calf circumferences (R^2 =0.61 and 0.67 respectively). Rolland et al (2003) reported R^2 = 0.40 for the correlation between calf circumference and appendicular muscle mass, using DEXA, in elderly women. Hip circumference, which is closely related to gluteal muscle mass, has received relatively little interest for whole-body muscle mass estimation, despite suggestions that it may be relevant based on associations with chronic diseases (Han et al., 1998, Lissner et al., 2001b).

The present review sought all previous studies that used MRI, as the most accurate method for measurement of muscle mass, and which derived and/or validated predictive equations from anthropometry. Two anthropometric approaches were identified in the literature: (1) simple anthropometric measurements that included limb circumferences, weight, height, age, sex and/or race, (2) circumferences and corresponding skinfold thickness to correct for subcutaneous fat, ie. mid-arm

circumference and triceps skinfold, mid-thigh circumference and thigh skinfold, mid-calf circumference and calf skinfold thickness (Lee et al., 2000a). The equation adopted (limb muscle mass = limb circumference – π x skinfold thickness), and employed extensively in chronic disease and protein energy malnutrition studies (Martin et al., 1990), depends on several simplifying assumptions. First, that skinfold measurement by callipers gives an estimation of the thickness of superficial adipose tissue. Second, for estimation of muscle mass, fat and bone are a negligible or a constant proportion of the non-superficial adipose tissue. Third, the limb is cylindrical and the superficial adipose tissue structures form an annulus. Finally, limited measurement sites can be used to predict muscle volume (Tothill and Stewart, 2002). These assumptions are relatively crude and introduce error. Knapik et al, (1996) suggested that errors in predicting MRI measurements from thigh muscle area reflect underestimation of fat and skin by excessive skinfold calliper tension, and over-estimation of total thigh area due to the assumption that the thigh is cylindrical. Taking skinfold thickness measurements is time-consuming, requires undressing which compromises practicality for large-scale survey work, and can increase measurement errors **(Table 2-10).** Another approach in regional muscle mass estimation was introduced by Jones and Pearson in 1969, who proposed dividing leg volume into six segments of a truncated cone. Their equation was applied in 32 young men and 15 women, with validation against a water displacement method. and showed correlation coefficients for the total leg muscle mass 0.98 in men and 0.99 in women. Tonson et al, (2008) used the same method for estimate forearm lean (muscle + bone) volume. Compared with MRI, correlations were as high as 0.90.

Amongst the studies which used simpler measurements without skinfolds, body weight was included in all except for (Nakamura et al., 2006), with different combinations of age, height, sex, race, hip circumference, waist circumference and/or thigh circumference, and correlations remained relatively high, ranging between R^2 =0.86–0.62 (Ross et al., 1994b, Chen et al., 2011b, Lee et al., 2000b) **(Table 2-7).** Only one study explored hip circumference (Ross et al., 1994b)

however that study predicted lean tissue mass including bone, SM and organs, rather than muscle mass. The relationship of hip circumference to body composition is complicated by the dominating effect of obesity on all circumferential measurements, but outside the context of obesity, hip circumference does have a close relationship to the gluteal muscles. It has been hypothesized that the strong association of chronic illnesses with high waist/hip ratio may reflect small hip circumference (indicating reduced muscle mass), rather than greater waist (indicating increased body fat) (Han et al., 1998, Seidell et al., 2001b, Lissner et al., 2001b, Snijder et al., 2003).

The limitations of the studies in the present review constrain the use of anthropometric prediction equations to estimate muscle mass. It was surprising to find only two studies that used whole-body MRI scans for the development of prediction equations (Lee et al., 2000b, Ross et al., 1994b). All other studies used regional muscle mass as a marker of whole body muscle mass. Many studies did not consider gender differences and used the same equation for both genders. Previous research has reported that women have 25% less muscle mass than men (Ross et al., 1994b).

Another approach found by this review was to distinguish between the muscle groups of the thigh, to avoid including non-muscle tissue like adipose, nerves, vessels and fascia in the cross sectional area measurement (Housh et al., 1995b). However, validation of this equation in patients with COPD and healthy elderly subjects (Mathur et al., 2008) found very low correlations with MRI measurements **(Table 2-9).**

It is clear that whole body muscle mass can in principle be predicted from simple anthropometric measures, but the existing literature does not provide a method which can be applied generally with confidence, and variable methodological approaches present problems. The small sample sizes in studies included in the present review, which limit study power, are understandable, as MRI is considered the best reference method, its high cost restricts its use. Heterogeneity between

relatively small studies (measurement of different limbs, whole body versus regional, total thigh versus components of thigh, circumferences versus skin-fold thickness, different age groups, different ethnicities, made it difficult to directly make comparisons between equations or to form a unified conclusion. Bland-Altman analysis is considered the best method to assess agreement and distribution of errors between two methods (Bland and Altman, 1999a) but was used in only 2 studies in the present review (Mathur et al., 2008, Lee et al., 2000b). All other studies depended on linear correlations alone to assess the accuracy of prediction equations. Using prediction equations to monitor change in muscle mass across time, or with interventions, has not been explored: although one study was longitudinal (Nakamura et al., 2006), its main purpose was not muscle mass estimation, but longitudinal assessment of nutritional status of elderly women (n=16) in a nursing home over a period of three years. They used only simple linear regression from thigh circumference (MRI thigh muscle volume (cm^3) = 21xthigh circumference (cm) + 979). Thigh circumferences decreased significantly during the three years' observation period $p<0.05$. However, correlation between the two measurements, thigh circumference and MRI thigh muscle volume using the previously mentioned equation was very low (R^2 =0.12).

Measuring skeletal muscle mass is of growing importance both clinically and epidemiologically. There are many published papers on deriving and validating anthropometric prediction equations to estimate whole body or regional skeletal muscle mass. It is important to critically evaluate the quality of the research already completed. The choice of a reference method is the main obstacle, looking at the studies that derived and validated anthropometric prediction equations which were not included in this systematic review. There are studies that used DEXA as the reference method (Pereira et al., 2013) (Sanada et al., 2006). As described earlier some MRI studies used regional MRI measurements to count for whole body MRI as the reference method. On the other hand, many studies in the literature used BIA as the field method (Pietilainen et al., 2013, Bosaeus et al., 2014). These studies were not included in the systematic review, due to the limitations of BIA already discussed in chapter 1. Not much research has been

published since the publication of this systematic review. Encouraging signs that there is research interest in this area is reflected by the citation of this paper seven times.

2.6 **Conclusion:**

This systematic review identified only two studies that developed prediction equations for whole-body muscle mass or lean body mass, as measured by MRI, from simple anthropometric measures. Several studies have generated anthropometric prediction equations for regional/limb muscle mass, which mostly include limb circumferences and local skin-fold thickness, but evidence is insufficient that limb muscle mass can be used to estimate whole-body muscle mass.

Studies differed in participant characteristics, BMI, age, gender, and measurement methodology, and do not provide firm enough evidence to propose an anthropometric method which could be applied routinely as a reliable indicator to estimate muscle mass or to diagnose sarcopenia. However, some of the regression equations show promise and demand further investigation, particularly through validation in separate populations and assessment of changes across time and during illness. The variables included in the simpler anthropometric approaches (body weight, height, age, sex, race and limb circumferences) are readily available widely in population health surveys, with R^2 of the best published equations ranging from 0.62-0.86. Though the practical equations retrieved from this systematic review were encouraging, especially that of (Lee et al., 2000b), the validations conducted have not involved separate populations using measurements by independent investigators, and the equations do not take advantage of trunk or limb girths which are widely available in health surveys and which other studies have shown to offer valuable prediction of muscle mass.

Developing a simple, clinically-friendly definition of sarcopenia with unified criteria for its diagnosis would help its detection and management (Janssen, 2011). Early identification of muscle loss, to target management, could be of great

clinical benefit to combat frailty and falls, to improve quality of life with chronic illnesses and with aging.

Authors responsibilities:

Yasmin Algindan: designed the systematic review, systematically searched for relevance of papers and contributed to the manuscript with the guidance of her supervisors Michael Lean, Catherine Hankey and Lindsay Govan; Wilma Leslie: was the second independent reviewer searching papers for relevance and also reviewed the manuscript prior to submission.

Table 2-1: MRI images

Study	Sample size	Sex	Age	BMI	MRI	Scan time	Area/comparison	Regional	
								Single image	**Multiple images**
Regional versus whole body:									
Lee, 2004	190 197	M W	18-84 18-88	29.1±4.3 28.7±5.5	1.5 T	30 min	Thigh: single images + multiple images versus whole body	1-cm thick M: R^2 = 0.77, SEE = 6.5% W: R^2 = 0.79, SEE = 7.4%	31-cm thick, 7 images M: R^2 = 0.84, SEE = 5.4% W: R^2 = 0.90, SEE = 5.1%
Continuous versus discontinuous images:									
Morse, 2007	18	M	23.9±3.4	65-81kg	0.2 T		Thigh: 3 site single images versus 11 contiguous transverse MRI scans	40% of femur length R^2 = 0.84, SEE = 26.8±5.2% 50% of femur length R^2 = 0.93, SEE = 12.5±5.4% 60% of femur length R^2 = 0.90, SEE = 9.9±5.7%	
Mathur, 2008	18 22	M W	56 -78	24.3±2.2	1.5T		Muscle CSA versus muscle volume	†30 % quadriceps: R^2 = 0.85 80% hamstring: R^2 = 0.56 50 % adductor: R^2 = 0.59	
Tothill, 2002	8 2	M W	23 -49 24 - 39	24.3±1.4 19.6±0.4	1 T		Torso to feet: single and 5 images compared to all images	Single mid-thigh R^2 = 0.96, SEE = 207 cm^3	5 slice:R^2 = 1.00, SEE = 42cm^3

Regional versus whole body and Continuous versus discontinuous images, † highest correlation in each muscle group reported in this table.

Table 2-2: Key words used to search the published literature

Search term	Medline	Pub med	Embase	Web of Science	Cochrane Library
Sarcopenia & Anthrop* & MRI	1	1	5	5	0
"muscle mass" & Anthrop*& MRI	14	22	31	15	1
"muscle volume" & Anthrop* &MRI	14	18	14	13	0
"mid-arm circumference" & MRI	1	1	1	0	0
"arm circumference" & MRI	7	9	14	8	2
"mid-thigh circumference" & MRI	0	0	0	0	0
"thigh circumference" & MRI	8	11	8	9	4
"mid-calf circumference" & MRI	0	0	0	0	0
"calf circumference" & MRI	4	5	5	3	3
Total	49	67	78	53	10

MRI: magnetic resonance imaging

Figure 2-1: flow chart of selection of studies,

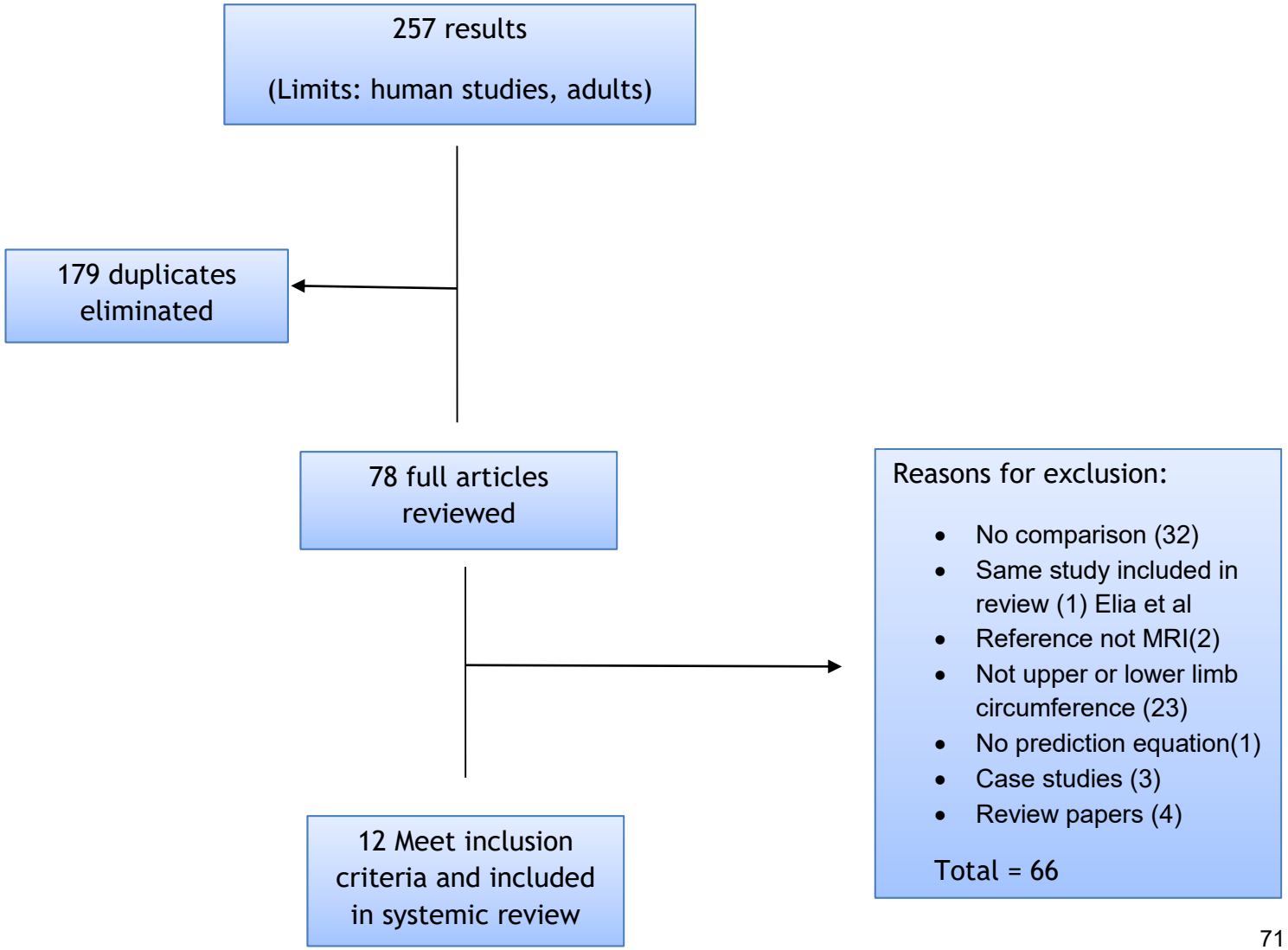

Table 2-3: Quality Assessment (1. Critical Review)

		Chen, 2011	Mathur, 2008	Tonson , 2008	Nakamura, 2006	Tothill, 2002	Lee, 2000	Bamman, 2000	Fuller, 1999	Knapik, 1996	Ross, 1994	Housh, 1994	Baumgartner, 1992
Study purpose		√	√	√	*√	√	√	*√	√	√	*√	√	√
Literature reviewed		√	√	√	√	√	√	√	√	√	√	√	√
Design	**(cross-sectional)**	√	√	√		√	√	√	√	√	√	√	√
	Longitudinal				√								
Sample	**Described**	√	√	√	√	√	√	√	√	√	√	√	√
	Justified												
Outcomes	**Reliability**	√	√			√	√	√	√	√	√	√	
	Validated		√	√	√	√	√	√	√	√		√	√
Prediction equation described		√	√	√	√	√	√	√	√	√	√	√	√
Results reported in statistical significance		√	√	√	√	√	√	√	√	√	√	√	√
Limitations and bias reported		√			√						√		
Appropriate conclusion		√	√	√	√	√	√	√	√	√	√	√	√

*, developing or validating prediction equation not main purpose of study (check table 2-5 for aim of each study)

Table 2-4: Quality assessment (2. Technical assessment, QUADAS)

	Chen, 2011	Mathur, 2008	Tonson, 2008	Nakamura, 2006	Tothill, 2002 **	Lee, 2000	Bamman, 2000	Fuller, 1999	Knapik, 1996	Ross, 1994	Housh, 1994	Baumgartner, 1992
1.Was the spectrum of participant's representative of the patients who will receive the test in practice?	√	√	√	√	√	√	√	√	√	√	√	√
2.Were selection criteria clearly described?	√	√	√	√	√	√	√	√	√	√	√	√
3.Was the reference standard likely to classify the target condition correctly?	√	√	√	√	√	√	√	√	√	√	√	√
4.Was the period between performance of the reference standard and the index test short enough to be reasonably sure that the target condition did not change between the two tests?	~	~	~	~	√	~	~	√	√	~	~	~
5.Did the whole selection of the sample receive verification using the reference standard?	√	√	√	√	√	√	√	√	√	√	√	√
6.Did participants receive the same reference standard regardless of the index test result?	√	√	√	√	√	√	√	√	√	√	√	√
7.Was the reference standard independent of the index test? (that is, the index test did not form part of the reference standard)	√	√	√	√	√	√	√	√	√	√	√	√

8.Was the execution of the index test described in sufficient detail to permit its replication?	√	√	√	√	√	√	√	√	√	√	√	√
9.Was the execution of the reference standard described in sufficient detail to permit its replication?	√	√	√	√	√	√	√	√	√	√	√	√
10.Were the index test results interpreted without knowledge of the results of the reference standard?	~	~	~	~	~	~	~	~	~	~	~	~
11.Were the reference standard results interpreted without knowledge of the results of the index test?	~	~	~	~	~	~	~	~	~	~	~	~
12.Were the same clinical data available when the test results were interpreted as would be available when the test is used in practice?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13.Were un-interpretable, indeterminate or intermediate test results reported?	~	~	~	~	~	~	~	~	~	~	~	~
14.Were withdrawals from the study explained?	N/A	N/A	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

** not all participants underwent anthropometric measurements; N/A: not applicable; ~: not reported; √: reported.

Table 2-5: Purpose of included studies

	Author ,year	Aim
1	Chen, 2011	To correlate MRI measured thigh muscle volume with anthropometric measurements and physical functions in Taiwanese elderly subjects
2	Mathur,2008	To examine the relation between volume and muscle cross-sectional area Using MRI and to compare anthropometric estimations and MRI measured mid-thigh cross-sectional area
3	Tonson,2008	To determine the relationship between muscle size and maximum isometric strength during maturation. They quantified the potential measurement bias produced by anthropometric measurement of local muscle volume compared to MRI. Also they determined the difference between muscle size estimated from volume and muscle cross-sectional area measurements in a paediatric population
4	Nakamura,2006	To analyse the nutritional status of Japanese elderly living in a nursing home longitudinally over 3 years by serum albumin, anthropometry, and muscle and fat volumes estimated by MRI.
5	Tothill, 2002	To predict thigh adipose tissue and muscle volumes from anthropometry, to assess the validity of the method by examining the various components of the measurements and the assumptions involved using MRI as reference method.
6	Fuller, 2000	To assess the reproducibility and validity of muscle and adipose tissue volume in thigh and calf predicted using BIA, compared with anthropometry against estimates of MRI.
7	Lee, 2000	To develop and cross-validate anthropometric SM prediction models in healthy adults using MRI as reference method.
8	Bamman, 2000	To verify whether anthropometry or DEXA estimates of muscle size are valid predictors of plantar flexor maximum voluntary contraction strength and could be used in lieu of more sophisticated methods (MRI). Also they compared the association between maximum voluntary contraction an three MRI measured muscle size: anatomical, physiological and cross-sectional and muscle volume
9	Knapik, 1996	To develop and validate an anthropometric estimation of thigh muscle cross-sectional area for men and women.
10	Ross, 1994	To compare regional and total lean tissue, adipose tissue distribution measured by MRI in obese android men and women
11	Housh, 1995	To derive skin-fold and circumference equations for estimating anatomical CSA of hamstring, quadriceps and total thigh muscles; and to use cross-validation procedures to examine the accuracy of the predicted equations by comparing the CSA values to values from MRI.
12	Baumgartner, 1992	To determine the correlation between anthropometric measurements of subcutaneous adipose tissue and muscle + bone of the mid-upper arm and mid-thigh and actual areas estimated by MRI in a sample of elderly women and men . also to estimate the amount of over-estimation, if any, of the anthropometric method and the relationship of these errors with indicators of level of adiposity

Table 2-6: Subject characteristics of included studies

	Author ,year	Gender	Age (years)	subjects	BMI or weight
1	Chen et al, 2011	W M	76 ± 6.0	36 33	24.5 ± 3.0 kg/m^2
2	Mathur et al ,2008	W/M healthy W/M COPD	56 - 78	11/9 11/9	24.3 ± 2.2 kg/m^2 26.6 ± 4.7 kg/m^2
3	Tonson et al ,2008	M (boys, adolescent, men)	11.3 ± 0.8 13.3 ± 1.4 35.4 ± 6.4	14 16 16	16.4 ± 1.0 kg/m^2 18.3 ± 3.7 kg/m^2 22.7 ± 2.5 kg/m^2
4	Nakamura et al ,2006	W	<60 >60	9 7	20.5 ± 4.3 kg/m^2 21.0 ± 3.7 kg/m^2
5	Tothill et al, 2002	W M	23 – 49*	10 9	19.6 ± 0.4 – 29.5 ± 4.8 kg/m^2 24.3 ± 1.4 – 34.0 ± 1.5 kg/m^2
6	Fuller et al, 1999	W M	41 – 60 43 - 62	8 8	25.1 ± 5.4 kg/m^2 28.6 ± 5.4 kg/m^2
7	Lee et al , 2000	W M W M	41 ± 15 38 ± 12 43 ± 10 42 ± 13	109 135 41 39	23.8±3.4 kg/m^2 25.2±3.1 kg/m^2 34.8±3.5 kg/m^2 33.8±2.7 kg/m^2
8	Bamman et al, 2000	W (trained) W (untrained)	34 ± 5 36 ± 8	7 32	55.6 ± 5.0 kg 67.6 ± 8.8 kg
9	Knapik et al, 1996	W (trained) M (trained)	21 ± 2.3 25.2 ± 5.5	9 9	59.6 ± 7.0 kg 81.6 ± 7.0 kg
10	Ross et al, 1994	W M	35.9 ± 7.8 39.1 ± 10.5	40 17	33.4 ± 5.5 kg/m^2 32.0 ± 3.6 kg/m^2
11	Housh et al, 1995	M	25 ± 5	43	81.1 ± 12.8 kg
12	Baumgartner et al, 1992	W M	80.5 ± 6.2 77.0 ± 3.8	17 8	23.3 ± 3.8 kg/m^2 26.8 ± 3.6 kg/m^2

BMI: body mass index; COPD: chronic obstructive pulmonary disease; M: men; W: women.

Table 2-7: anthropometric and MRI measurements

Author, Year	Derivation	Validation	Circumferences					SFT	Wt	Ht	Age	Sex	Race	MRI		
			Arm	Thigh	calf	hip	Waist							Region	Image	Muscle/lean
Chen, 2011	√			√			√		√		√	√	*	Thigh	Continuous slices	muscle(cm^3)
Mathur, 2008		√		√				√						Thigh	Mid-thigh CSA	muscle(cm^3)
Tonson, 2008		√	√					√						Arm	Continuous (5mm thickness, 10mm gap)and cross-sectional of the highest area measured.	muscle(cm^3)
Nakamura, 2006	√			√									*	Thigh	Continuous cross sectional scans at intervals of 1 cm	muscle(cm^3)
Tothill, 2002		√		√				√						Torso to feet	Institution 1: Continuous (10 mm thick, gap of 2mm), Institution 2(10 mm thick, gap of 50mm)	muscle(cm^3)
Lee, 2000	√	√	√	√	√			√	√	√	√	√	√	WB	Continuous (10mm thick, 40mm gap)	muscle(kg)
Bamman, 2000		‡			√			√						calf	Continuous (5mm thick, 10mm gap) Image with anatomical largest cross-section was used	muscle(cm^2)
Fuller, 1999		√		√	√			√						leg	Continuous (1cm slices at 5cm intervals)	muscle (cm^3)
Knapik, 1996	√	√		√				√						Thigh	Cross- sectional image	Lean(cm^3)
Ross, 1994	√			√		√	√		√					WB	Continuous (10 mm thick every 50 mm)	Lean (L)
Housh, 1995	√	√		√				√						Thigh	Cross-sectional image	Muscle(cm^2)
Baumgartner, 1992		√	√	√				√						Arm, thigh	Cross-sectional images	Lean(cm^2)

*all subjects Asian; ‡muscle; +bone; CSA: cross-sectional area; lean: muscle + bone; WB: whole body; Ht: height; WT: weight; SFT: skin fold thickness.

Table 2-8: MRI characteristics of included studies

	Author ,year	MRI tesla	Axial images	Matrix (pixel)	Repetition – time(ms)	Echo-time(ms)	Scan time	Field of view	Gap thickness	Slice thickness(mm)
1	Chen et al, 2011	1.5	T1-Weighed	512x384x1	136	4.8	20min	380x285	2.5mm	5
2	Mathur et al,2008	1.5	T1-Weighed	512x384	650	8	-------	40cm^2	2 – 2.5 cm	5
3	Tonson et al,2008	1.5	T1-Weighed	512x512	490	12	122 s	200 mm	10mm	5
4	Nakamura et al,2006	0.5	T1-Weighed	-------	530	15	-------	-------	-------	-------
5	Tothill et al , 2002*	1	T1-Weighed	128x256	570	15	-------	500x500mm	2mm	10
		0.95	T1-Weighed	-------	1150	12	-------	-------	50mm	10
6	Fuller et al, 1999	0.5	T1-Weighed	256x192x2	TE/TR = 17	-------	-------	48x36cm	-------	10
7	Lee et al, 2000*	1.5	T1-Weighed	-------	210	17	25 min	-------	40	10
8	Bamman et al, 2000 ‡	4.1	-------	-------	1000	14.5	-------	256	10mm	5
9	Knapik et al, 1996	1.4	T1-Weighed	-------	200	22	5 min	-------	-------	-------
10	Ross et al, 1994†	1.5	T1-Weighed	-------	500/210	20/15	1hr/30m	-------	50mm	10
11	Housh et al, 1995	1.5	-------	-------	600	20ms	-------	-------	-------	-------
12	Baumgartner et al, 1992	1.5	T1-Weighed	256x256	1500	Te/ti=20	-------	20 – 60cm	-------	10

*Scans were done in two different centres; † MRI was upgraded; ‡ spectroscopy; ms; millisecond; mm; millimetre; cm: centimetre; -------: not reported; TE/TI: echo time/inversion time; TE/TR: echo time/repetition time.

Table 2-9: prediction equations included in the systematic review

	Author ,year	Prediction equation	R^2	SEE	% error
Simple anthropometric prediction equations:					
10	Ross et al, 1994	M: Lean tissue (L) = 0.990 x BW(kg) – 0.542 X waist (cm) – 0.881 x thigh (cm) + 73.12 W: Lean tissue (L) = 0.501 x BW(kg) – 0.379 x hip (cm) + 43.01	D:0.89 D:0.62	2.1L 2.8L	3.6% 6.5%
7	Lee et al, 2000	SM (kg) = 0.244 x BW (kg) + 7.80 x Ht (m) – 0.098 x age (years) + 6.6 x sex + race – 3.3	D:0.86 V:0.79	2.8kg 3.0kg	------- -------
4	Nakamura et al,2006	Thigh muscle volume (cm^3) = 21 x thigh (cm) + 979	D:0.12	-------	-------
1	Chen et al, 2011	M: SM (cm^3) = 7168.8 – 52.1 x age(years) + 96.5 x BW(kg) – 67.4 x waist(cm) + 47.3 thigh(cm) W: SM(cm^3) = 1719.3 – 29.9 x age(years) + 53.5 x BW(kg) + 39.8 x thigh(cm) C: SM(cm^3) = 4226.3 – 42.5 x age(years) – 955.7 x gender(1men, 2women) +45.9 x BW(kg) + 60.0 x thigh (cm)	D:0.68 D:0.62 D:0.74	608.1cm^3 496.0cm^3 581.6cm^3	------- ------- -------
Corrected for skin-fold thickness limb circumference prediction equations:					
2	Mathur et al, 2008	Quadriceps muscle CSA = (2.52 x mid-thigh(cm)) – (1.25 x anterior thigh skin-fold(mm)) – 45.13 Hamstring muscle CSA = (1.08 x mid-thigh(cm)) – (0.64 x anterior thigh skin-fold(mm)) – 22.69	V:0.057 V:0.078	------- -------	------- -------
3	Tonson et al, 2008*	Volume 1 = 1/3h (area of wrist +(area of wrist x area of mid-forearm)$^{0.5}$ + area of mid-forearm Volume 2 = 1/3h(area of mid-forearm + (area of mid-forearm x area of elbow)$^{0.5}$ +area of elbow, Volume = volume 1 + volume2	V:0.90	-------	†20.5%
	Author ,year	**Prediction equation**	**R^2**	**SEE**	**% error**
7	Lee et al, 2000	SM (L) = Ht x (0.00744 x CAG^2 + 0.00088 x CTG^2 + 0.00441 x CCG^2) + 2.4 x sex -0.048 x age + race +7.8	D:0.91 V:0.83	2.2kg 2.9kg	------- -------

5	Tothill et al, 2002	Area of the lean tissue: *AL = (circumference of inner tissue - 2 π * thickness of superficial adipose)2 /4 π* Lean volume = $(A_1 + A_2 + (A_1 \times A_2)^{0.5}) \times Ht/3$	V:0.95	288cm^3	30%
6	Fuller et al, 1999	Muscle CSA (cm^2) = (girth^2/4 x 3.14) – (girth x SFT/2) - 6∞	VT:0.35 VC:0.69	------- -------	40% 22%
8	Bamman et al, 2000ǂ	Right calf skin-fold thickness and maximum circumference	V:0.45	-------	-------
9	Knapik et al, 1996	Thigh muscle CSA=0.649 x ((thigh circumference/π – fat plus skin thickness)^2 – (0.3. bone)^2)	0.92	10.1 cm^2	22%CSA
11	Housh et al, 1995	Quadriceps muscle CSA = (2.52 x mid-thigh(cm)) – (1.25 x anterior thigh skin-fold(mm)) – 45.13 Hamstring muscle CSA = (1.08 x mid-thigh(cm)) – (0.64 x anterior thigh skin-fold(mm)) – 22.69 Total thigh muscle CSA = (4.68 X mid-thigh(cm)) – (2.09 x anterior thigh skin fold (mm)) – 80.99	D,V:0.72, 0.64 D,V:0.52, 0.29 D,V:0.74, 0.77	5.4, 7.3 3.2, 3.7 9.6, 12.5	------- ------- -------
12	Baumgartner et al, 1992	Muscle plus bone area = (limb circumference- πskin-fold thickness/2)^{2}4π	VT:0.43 VC:0.69	------- -------	41.5% 46.8%

* Jones and Pearson method; ∞for thigh and calf bone with its constituents' marrow assumed 6cm^2; ǂ this method was developed by Gurney et al in 16 men 1973; M: men; W: women; C: men and women; CSA: cross sectional area; CAG: corrected arm girth; CTG: corrected thigh girth; SFT skin fold thickness; Ht: height; SM: skeletal muscle; D: derivation, V: validation, VT: validation thigh, VC: validation calf, A_1 and A_2: area at the top and bottom of section, -------: not reported

Table 2-10: Reliability and observers error

Author ,year		Anthropometry					MRI				
		# subjects	# of measurement	# of observers	Intra-observer*	Inter-observer	# subjects	# of measurement	# of observers	Intra-observer*	Inter-observer
1	Chen, 2011		averaged	----------	----------	----------		----------	1	<1.0%	----------
2	Mathur,2008		3	3.2% at 5cm, 4.2% at 10cm†					2		0.4%
3	Tonson,2008		----------	----------	----------	----------		----------	----------	----------	----------
4	Nakamura,2006		----------	2	----------	----------		----------	----------	----------	----------
5	Tothill, 2002		2	1	0.9% thigh girth, 3.5% thigh skinfold	0.6-0.8% thigh girth, 4% thigh skinfold‡		1	2		2.2% muscle∝
6	Fuller, 1999			4 (different range of experience)	Thigh: girth 0.6, SFT 2.5, CSA 1.7 Calf: girth 0.5,SFT 8.2, CSA 3.2	Thigh: girth 1.2, SFT 27.5, CSA3.5. Calf: girth 1.2,SFT 19.6, CSA8.2			2 (one experienced one not)	0.08% experienced, 0.63% less experienced	0.11%
7	Lee, 2000		3	>1	**		3 men, 3 women	1 series of 7 images	2	0.34±1.1%	1.8±0.6% 2.0±1.2% ‡
8	Bamman, 2000		2	2							
9	Knapik, 1996	18	3	2		Thigh girth: 1.2 men, 4.2 women. SFT: 29.7men, 25.4 women¶	18		3		
10	Ross, 1994										
11	Housh, 1994	43		1	Reliability r>0.90		15			r>0.96	

12	Baumgartner, 1992	25	↑					2	2	>0.98	

*coefficient of variation of repeat assessment of the same image,† average % error between thigh circumference measured by MRI and tape,‡ comparable measurements from (Carter and Ackland,1994),∝Coefficient variation, ǂ inter-laboratory difference since measurements were taken in two different labs, **matched (Lohman,1988) Anthropometric standardization manual, ¶ in this table between ratters variation on day two reported only, more details in article.[3] Knapik study for MRI measurements only between day and subject was calculated, no between observer reported, ↑Technical error for anthropometric measurements <0.6cm for upper arm and mid-thigh girths, <1.2 mm for triceps and mid-thigh skinfolds.

Chapter 3

3 Derivation and validation “muscle mass”

Derivation and validation of simple equations to predict total muscle mass from simple anthropometric and demographic data

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3.1 **Abstract**

Background: Muscle mass reflects and influences health status. Its reliable estimation would be of value for epidemiology.

Objectives: To derive and validate anthropometric prediction equations to quantify whole-body skeletal muscle mass (SM) in adults.

Design: *Derivation-sample*: 423 subjects (227 women) aged 18–81 years; BMI I5.9–40.8 (kg/m^2). *Validation-sample*: 197 subjects (105 women), aged 19–83 years; BMI 15.7–36.4 (kg/m^2). Both samples were of mixed ethnic/racial groups. All underwent whole-body magnetic resonance imaging to quantify SM (dependent variable for multiple regressions) and anthropometry (independent variables).

Results: Two prediction equations with high practicality and optimal derivation-correlations with SM were further investigated to assess agreement and bias using Bland-Altman plots, and validated in separate datasets: Including race as a variable increased R^2 by only 0.1% in men and 8% in women

Men: SM(kg) = 39.5 + 0.665 body-weight(kg) - 0.185 waist(cm) - 0.418 hips(cm) - 0.08 age(years) (derivation: R^2= 0.76, SEE=2.7kg; validation: R^2= 0.79, SEE=2.7kg). Bland-Altman plots demonstrate moderate agreement in both derivation and validation analyses.

Women: SM(kg) = 2.89 + 0.255 body-weight(kg) - 0.175 hips(cm) - 0.038 age(years) + 0.118 height(cm) (derivation: R^2= 0.58, SEE=2.2kg; validation: R^2= 0.59, SEE=2.1kg). Bland-Altman plot showed negative slope, indicating a tendency to overestimate SM among women with smaller, and underestimate among those with larger, muscle masses.

Conclusion: Anthropometry predicts SM better in men than women. Equations including hip-circumference showed agreement between methods, with predictive power similar to that of BMI to predict fat-mass, with potential for applications in groups, epidemiology and survey settings.

3.2 **Introduction**

As well as its obvious roles in posture control and capacity for movement, skeletal muscle has important metabolic functions, all influence health and wellbeing. Variations in muscle mass and its functional capacity thus affect physical security and metabolic factors related to cardiovascular risk (Atlantis et al., 2009). Skeletal muscle mass (SM) can vary for several reasons. It usually reaches a peak in early adult life, with differences between individuals of presumed genetic origin as well as training effects, and declines with advancing age (Visser, 2013). Reductions in SM, with loss of physical and metabolic functions, occur through local injury, denervation, systemic disease and chronic inflammation, and as a result of aging combined with a sedentary lifestyle.

Despite widespread recognition that low SM mass and strength, or 'sarcopenia', has major clinical and epidemiological importance, its diagnosis, and thus research into its clinical and public health consequences, is hampered by the lack of any agreed simple method to assess SM (Buffa et al., 2011, Lukasaki, 2005a). Both muscle mass and strength are two important aspects of sarcopenia (Cruz-Jentoft et al., 2010, Fielding et al., 2011), but these components need to be measured or estimated reliably and unified in agreed criteria, maintaining both sensitivity and practicality.

Total body mass and its major constituents (total body fat, SM, etc) can be measured accurately by modern imaging methods and then correlated with height, weight, and circumferences for field use. Anthropometric measurements have many advantages, (simple, quick, safe, non-invasive, cheap, need only low skill levels, give immediate results), provided they are shown to be sensitive and specific predictors (Lee et al., 2001). We have previously developed anthropometric prediction equations for total body fat content, which were found to depend strongly on waist circumference (Lean et al., 1995): this work gave rise to key diagnostic criteria for metabolic syndrome (Han et al., 1995). An agreement on a method to quantify muscle mass, in addition to muscle strength, would be a step towards better identification of sarcopenia, at least at a

population level. Our systematic review (Al-Gindan et al., 2014b) identified only one published anthropometric method to estimate whole-body muscle mass as measured by MRI (Lee et al., 2000a), they developed two equations that were both cross-validated by the same investigators in a separate subject-group from the same population. One used height, gender, race, limb circumferences (arm, thigh and calf) and skin-fold thicknesses. The need for skin-fold measurement limits practical application. The second equation used more widely available simpler anthropometric variables: SM (kg) = 0.244 × BW(kg) + 7.80 × Ht(m) - 0.098 × age(years) + 6.6 × sex + race - 3.3. High correlations (R^2 = 0.86, P< 0.0001, and SEE = 2.8kg) were seen in the derivation study in non-obese subjects, and in their validation among obese subjects (R^2 = 0.79, P < 0.0001, SEE = 3.0kg) (Lee et al., 2000a). However, the term for race is specific to US categories and these strong correlations may have exaggerated the predictive value for individuals as the equations included the wide ranges afforded by combining the sexes.

The aim of the present study was to derive, and to evaluate for possible use in clinical and/or epidemiological settings, prediction equations for SM estimation, using simple anthropometric variables and whole body MRI as the reference method. We validated the derived prediction equations, and also the previously published SM prediction equation of (Lee et al., 2000a) in an independently measured sample.

3.3 **Materials and Methods**

Data included in the derivation and validation studies were collected from adult subjects in whom the same measurements had been made by different investigators, in studies conducted at New York Obesity Nutrition Research Center's Body Composition Unit, St. Luke-Roosevelt Hospital, New York. For both anthropometric and MRI measurements, readers were blinded. Race/ethnicity was determined by self-report and included declaration of race/ethnicity for parents and grandparents. Variables were created for four race/ethnicity categories: Caucasian (C), African American (AA), Hispanic (H), and Asian (A). All studies obtained written informed consent and were approved by the Institutional Review

Board of St. Luke's-Roosevelt Hospital (Heymsfield et al., 2007a, He et al., 2009a, Bosy-Westphal et al., 2013b).

3.3.1 Subjects

***Derivation study sample*:** Subjects 423 (227 women), aged18–81 years, BMI I5.9–40.8 (kg/m^2) participated in several related studies between 2000 and 2004 (Heymsfield et al., 2007a). Subjects were classified as having no known or diagnosed diabetes, cancer, heart disease, or any health conditions that would affect body composition or fat distribution; they were ambulatory, weight-stable (less than 2 kg weight change in previous 6 months) adults who underwent testing that included a whole-body MRI scan. Four subjects were excluded from this sample because of technically poor or incomplete MRI scans.

Validation study sample: Data sets from two previous studies (He et al., 2009a, Bosy-Westphal et al., 2013b) were combined, giving a total of 197 subjects (105 women, 92 men). Subjects were recruited (Study 1: 2001 to 2004 (He et al., 2009a), Study 2: 2011 (Bosy-Westphal et al., 2013b) through advertisements in local newspapers, internet, and on flyers posted in the local community. A body mass index (BMI) (in kg/m^2) upper limit of 37 was set to accommodate the MRI scanner capacity limitations. Participants were required to be ambulatory non-smokers, free of medical conditions or metabolic characteristics (abnormal thyroid or cortisol concentrations) that could affect the variables under investigation, weight stable (<2 kg change within past 6 months), and not regularly engaging in vigorous exercise. The subjects varied in age (18–83 years) and BMI (15.7–36.4kg/m^2) **(Table 3-1).** This final sample, carefully checked to ensure that there was no duplication of subjects between the derivation and validation samples, or between the two validation samples, was used in validating our derived equations and those of (Lee et al., 2000a).

3.3.2 Methods

Magnetic resonance imaging (MRI): All data were collected in the same laboratory by an analysis team (n = 3) for derivation and validation samples, for the 2011 study a single MRI analyst performed the measurements, therefore the MRI methods used are identical for both studies. Total-body skeletal muscle volume was measured using whole-body multi-slice MRI. Subjects were placed on a 1.5-T scanner (6X Horizon; General Electric, Milwaukee, WI) platform with their arms extended above their heads. Images were created using a T1-weighted spin-echo sequence with a 210-ms repetition time and an echo time of 17 ms. The intervertebral space between the fourth and fifth lumbar vertebrae (L4—L5) was set as the point of origin for all scans. Transverse images (10 mm slice thickness) were then obtained across the entire body, with between-slice gaps of 40 mm. Each whole-body scan thus included ≈30–40 cross-sectional images. Images were analyzed by using SLICEOMATIC software (TomoVision Inc, Montreal, Canada) for segmentation and calculation of cross-sectional tissue areas. Total-body skeletal muscle volume estimates were converted to mass using an assumed density of 1.04 kg/L for skeletal muscle. The technical error for repeated readings of the same adult whole-body scans by the same analyst of MRI-derived skeletal muscle volume is small with coefficient of variation considered similar to that of CT- scanning at 1.4% (Mitsiopoulos et al., 1998a). The intra-class correlation coefficient between analyses for total-body MRI-derived skeletal muscle from the same adult subjects was 0.99. For the validation samples, the technical error for 3 repeated readings of the same scan by the same observer for the MRI-derived SM was 1.9% (Song et al., 2004a).

Anthropometric measurements: Three technicians were trained in body composition laboratory, they obtained all anthropometric data. Body weight was measured to the nearest 0.1 kg using a balance beam scale (Weight Tronix, New York, NY) with the subject wearing a hospital gown. A wall-mounted stadiometer (Holtain, Crosswell, Wales) was used to measure standing height to the nearest 0.1cm. Anthropometric circumferences were obtained using a heavy-duty inelastic plastic fiber tape measure (Gulick II Tape Measure, Fischer Scientific): waist was

measured midpoint between the lowest rib and the upper border of the iliac crest (Wang et al., 2003b); hips at the level of the pubic symphysis and the greatest gluteal protuberance; mid-arm at the mid-point between the lateral tip of the acromion and the most distal point on the olecranon; mid-thigh at the mid-point between the inguinal crease and the proximal border of the patella; and the maximum girth of the calf.

3.3.3 Statistical analyses

All statistical analyses were carried out using Minitab ® 15.1.30.0. Datasets had all previously been checked and cleaned for errors of data entry, but were explored to confirm that all data-ranges were plausible. Multiple linear regressions generated equations, separately for males and females, to predict whole body SM mass measured by MRI. Eight anthropometric variables were considered of interest, on grounds of practicality for routine clinical and epidemiological work: age, weight, height, and circumferences (hip, waist, thigh, arm and calf). Forward and backward stepwise regression analysis was accomplished (alpha to enter 0.15, alpha to remove 0.15) using the eight variables. Analyses were carried out using four sets of variables (age, body-weight, height, hip and waist), (age, body-weight, height, hip, waist and mid-thigh), (age, body-weight, height, hip, waist, mid-thigh and mid-arm) and (age, body-weight, height, hip, waist, mid-thigh, mid-arm and mid-calf) the highest R^2 value of each set of stepwise regression was used for further investigation. Bland-Altman plots were used to explore distributions of errors (Bland and Altman, 1999b). The best of the equations obtained from the derivation sample were then applied in a separate validation sample, for external validation. Bland-Altman plots were also created in the validation sample, using the predicted SM values and observed values of SM from MRI, to determine levels of agreement between predicted and true MRI estimates of SM. To investigate the effect of adding the variable 'Race' (as applied in mixed US populations) to the equation, we used the derivation study sample, with the addition of the variable (Race) to our best derived equations for both men and women. Given that we have a categorical value (Race), ANCOVA general linear model was used. To compare between

models, we used coefficient of variation (CV). CV was calculated as the ratio of the standard error of the estimate (SEE) to the mean of the dependent variable (%).

3.4 **Results**

Subject characteristics, including the MRI, anthropometric data and % of each racial group, are shown on **(Table 3-1)**.

Derivation Study: Linear regressions of each variable against MRI whole body SM are shown in (**Table 3-2**). The correlations of single variables with MRI SM were stronger for men than for women against almost all variables, except for height and waist. Body weight was the variable with the highest correlation with MRI SM (R^2 = 0.54 and 0.39) for men and women, respectively.

Stepwise regressions of all variable combinations showed greater correlations for men than for women (R^2=0.76, 0.58 respectively). The single best equation for both men and women was used for further analysis on the basis of correlation strengths (**Table 3-3**, **Table 3-4),** for further evaluation. In men, mid-arm circumference was a stronger predictor than in women and the opposite was true for mid-thigh circumference. Height was a stronger predictor for women than men. All the most powerful prediction equations included hips circumference as a significant independent variable.

Male equation: In the derivation analysis **(Figure** 3-1**A)** agreement between methods was assessed in relation to MRI-measured vs predicted estimates, with a strong correlation R^2 = 0.76, SEE 2.7kg, CV = 8% and significant slope (p-value < 0.001) (for values of 25kg and 35kg, CVs = 11% and 8% respectively). The addition of the race (**Figure 4-1C)** variable using ANCOVA general linear model did not advantage agreement or correlation: R^2 increased by only 1.2%, both SEE and CV remained the same and there was a significant slope.

Distributions of errors were evaluated from Bland-Altman plots (**Figure** 3-1**B and Figure** 3-1**D)**, a significant negative relation exists (P = 0.001). Width of the 95%

prediction intervals (Figure 1B), was 10.3kg (95% PI: -4.2, 6.1) for a fitted value of 25kg, and 10.3kg (95% PI: -5.6, 4.7) for a fitted value of 35kg.

Validation of our derived men equation **(Figure** 3-1**E)** showed higher correlation R^2 = 0.79, SEE = 2.7kg, CV = 9% and significant slopes (P < 0.001) than the derivation analysis, also adding race **(Figure** 3-1**G)** did not increase value of the equation. Bland-Altman plots **(Figure** 3-1**F and H)** were better compared to the derivation analysis with no relation between mean and difference (P = 0.285 and 0.289). Significant constant bias was observed for equations without and with race (limits: 7.1, -3.7 and 6.7, -4.1 respectively), the 1-sample t-test was significant (P = 0.000), with limits of agreement calculated using SD. Using the same data to validate (Lee et al., 2000a) equation **(Figure** 3-1**I and Figure** 3-1**J)**, correlations were lower R^2 = 0.75 and SEE was higher at 2.9kg, CV remained the same, with significant slope (P<0.001). Bland-Altman plot showed clear negative relation (P < 0.001), indicating an overestimation for those in the lower range of muscle mass and underestimation for those with larger values of muscle mass.

Female equation: In general, women **(Figure** 3-2**A and Figure** 3-2**C)** had lower correlations and SEE than men. In the derivation analysis R^2 was 0.58, SEE =2.2kg, CV = 11%, and significant slope (p < 0.001) (for values of 15kg and 25kg, CVs = 15% and 9% respectively). Adding race to the equation increased R^2 to 0.61 and SEE and CV decreased by 0.1kg and 1% respectively, and with significant slopes (p<0.001). Bland-Altman plots **(Figure** 3-2**B and Figure** 3-2**D)** showed negative relationships (P < 0.001) for the equation with and without race. For women with SM in the lower range, the prediction equations tended to overestimate SM, while they underestimated SM for women in the higher range. For example, the prediction equation overestimates by 8kg (95% PI: -2.5, 5.5), for an average woman whose total muscle mass is 15kg, while it underestimates by 8kg (95% PI: -5.5, 2.5), for an average woman whose total muscle mass is 25kg**.**

As in men, higher R^2 values for both equations with and without race **(Figure** 3-2**E and Figure** 3-2**G)** were seen after validation (R^2= 0.67 and 0.59 respectively) while SEE decreased to 2.1kg and CV remained the same (11, 10%) in both equations

(significant slopes were still observed p-value<0.01) in Bland Altman plots **(Figure** 3-2**F and Figure** 3-2**H).** In women validation analysis showed

a clear negative relationship (P = 0.000) (for values of 15kg and 25kg, CVs = 15% and 9% respectively). Validation with Lee et al equation (Foster et al., 1984) **(Figure** 3-2**I)**, using the same validation sample we used on our equations, showed lower correlations than our equation with race and higher than our equation without race (R^2 = 0.63). Nevertheless, our validations of the Lee equations' SEE and CV were almost the same (SEE = 2.1kg, 1.9kg, 2.2kg) and (CV = 10%, 10% and11%) for validation of Lee et al (significant slopes p-value<0.001), as our equations with and without race, respectively. However, there was no negative relation in the Bland-Altman plot **(Figure** 3-2**J)** (P = 0.236) and mean difference was 2.6 kg in the validation of Lee et al equation (95% limits of agreement: 7.2, -2). The 1-sample t-test was significant, with limits of agreement calculated using SD.

In order to improve the agreement between our predictions and the MRI measurements for women we performed 2 forms of calibration. Firstly, as suggested by Bland-Altman (Bland and Altman, 1999b), logarithmic transformations of both the predicted measure and MRI measure were plotted in the Bland-Altman plot (figure not included). However, this did not account for the negative relation between mean and difference in the Bland-Altman plot. Secondly, we performed a calibration of our equations. This involved regressing the predicted values against the MRI values and adjusting the new equation to remove the negative relation seen in the Bland-Altman plots for the derivation analysis. The calibration was successful in the Bland-Altman plot for the derivation analysis, where the negative relation between mean and difference of the measures no longer existed. However, using the calibrated equation did not account for the negative relationship in the Bland-Altman plot for the validation dataset. Therefore, for women with SM in the lower part of the range, the prediction equations tend to overestimate SM by 8kg on average, while they underestimate SM for women at the higher end of the range by 8kg on average.

Combining men and women, Lee et al equation (Lee et al., 2000a) **(Figure** 3-2**K)** increased R^2 to 0.85, SEE = 2.6kg. Bland-Altman plots **(Figure** 3-2**L)** did not have a significant slope (P = 0.229)**,** although there was evidence of slight bias: mean difference 2.77 and 95% limits of agreement (8, -2.4).

3.5 **Discussion**

The aim of this study was to develop and validate simple equations for estimation of SM, sufficiently practical for use in epidemiological settings. Although CT scanning was used in the past (Kvist et al., 1988a), MRI is now established as the preferred reference method to measure SM (Heymsfield et al., 1997). DXA (Dual-energy X-ray Absorptiometry) scanning has been used as a screening tool for low muscle mass (Goodman et al., 2013). However, it is a relatively expensive method, only an indirect estimate of MRI measurement, and impractical for whole-body muscle-mass estimation for large-scale health surveys or routine clinical work.

As whole-body MRI is time-consuming and expensive, a number of studies have focussed on single limbs and produced anthropometric prediction equations for regional muscle volumes based on single slices of MRI for limb-muscle areas (Knapik et al., 1996, Baumgartner et al., 1992, Housh et al., 1995a). Although single-slice based estimates may relate to physical function and possibly identify malnutrition, regional muscle masses have not been established to relate in a direct way to whole-body muscle mass. Lee et al, compared MRI-measured regional and whole-body muscle mass and found that skeletal muscle values obtained from a single scan of the thigh region was a reasonably good indicator of whole body muscle mass (R^2 = 0.77, 0.79; SEE = 7.4,5.4%) in men and women, respectively. Using seven consecutive images of the thigh region was only marginally better (R^2 = 84%, 90%; SEE = 5.4, 5.1). No existing study provides a method to convert limb SM to whole-body SM, although one study has demonstrated correlations between regional and whole body SM using MRI measurements (Lee et al., 2004). In attempts to improve the prediction of regional muscle mass, several studies have adjusted limb circumferences with the overlying skinfold thickness (Fuller et al., 1999, Tonson et al., 2008a, Knapik et al., 1996,

Lee et al., 2000a). Incorporating skinfold thickness appears to improve estimation of regional muscle mass, for example in the context of malnutrition, and thus potentially the prediction of whole-body muscle mass. However, skinfold measurement takes time, requires training and may introduce high individual variability (Martin et al., 1992, Tothill and Stewart, 2002). Skinfold thickness measurements are not made routinely in most population health surveys.

We have explored the potential to use the type of anthropometric measurements made routinely in large-scale health surveys, to predict SM as measured by MRI. There appears to have been only one published study where this was attempted, by Lee et al (Lee et al., 2000a). Our validation of Lee et al's equations concur with the original publication, which showed R^2 = 0.75, SEE = 2.3 in men and 0.63, 2.2 in women (R^2 = 0.86 and SEE = 2.8kg for both men and women). Combining men and women, as (Lee et al, 2000) did, will increase the number of adults studied and also the range in body composition. This increased R^2 to 0.85 but without improving the prediction of individual SM, as shown by the errors on Bland-Altman plots **(Figure** 3-2**K and L).** The standard deviation of 3.4 kg indicates the spread of measurements SM for females, which is less than that for males at 5.5kg **(Table 3-1).** The combined group **(Figure** 3-1**I and Figure** 3-2**I and Figure** 3-2**K)** standard deviation was 8.9kg. The relative proportion of variation (i.e. the R^2 value) explained by the prediction equation is greater in males (0.75) and the combined group (0.85) than that for females (0.63). This does not necessarily mean the prediction equation was less accurate for females than for males, it may simply reflect that the relative amount of variation that could be explained in females was less.

The validations of our new equation gave higher R^2 and SEE values for men compared to our validation of the Lee et al equation. On the other hand, in women, our validation of Lee et al equation **(Table 3.4)** gave higher R^2 and SEE than our new equation**.** Our new equations included only anthropometry, whereas Lee et al used race as a variable. Incorporating a term for race increased R^2 of our validations by only 0.1% in men and 7.9% in women, indicating that most of the variance associated with race was accounted for by simple anthropometric

measurements especially in men. Attributing race to individuals in mixed populations can be potentially difficult and misleading, so there is a practical advantage for equations that do not require this term.

Our equations all used simple measurements, which can be made quickly with modest training in epidemiological settings. Indeed, these measurements are already being made routinely in most national population health surveys. A consistent finding in the equations **(Table 3.4)** we examined is that the prediction of SM was substantially less accurate for women than for men. This was also the case for the published equations to predict lean body mass (Ross et al., 1994a) and SM (Lee et al., 2000a). This gender difference probably reflects the much smaller muscle mass of women, and a greater range in variability in other tissues, particularly fat mass. Ross et al, 1994 found body weight and hip circumferences contributed strongly to predictions of MRI whole body measured lean tissue in obese android women. In men a combination of thigh and waist circumferences and body weight gave the strongest prediction. Among our derived equations from stepwise regression, those with the highest correlations with SM (R^2 = 0.73- 0.76 for men and R^2 = 0.54-0.58 for women) all included hip circumference as a variable**.** This finding supports previous reports suggesting that variance in hip circumference may reflect differences in muscle mass (Han et al., 1998, Lissner et al., 2001a) thereby explaining some of the health associations of 'waist/hip ratio'. Waist/hip ratio is not a useful indicator of total body fat or fat distribution (Tothill et al., 1996b, Burton and Lean, 2013) but it does predict type 2 diabetes, insulin resistance and coronary heart disease in cross-sectional studies (Seidell et al., 2001a). The explanation may, therefore, be that reduced SM through illness or inactivity (e.g. in people developing type-2 diabetes) results in a lower hip circumference and thus a greater waist/hip ratio, rather than a greater body fat content with increased waist.

The present study allows a degree of confidence, greater for men than for women, for estimation of total body muscle mass from simple measures that can be collected in analysis surveys **(Table 3.4).** Our equations showed moderate to high correlations with MRI-measured whole body SM, and moderate SEE and CV.

However, there are limitations to the study. From our published systematic review (Al-Gindan et al., 2014b), SEE was within the ranges seen in other SM anthropometric prediction studies, but we found few studies that used simple anthropometric measurements to estimate total body muscle mass using whole body MRI as the reference method, which limited our capacity to compare equations.

In general, our equation seemed to be more sensitive in men, and the (Lee et al., 2000a) equation was more sensitive for women. There is a significant negative relationship between the mean difference and the average value in the Bland-Altman plots in women. The negative relationship crosses over the zero line, meaning the mean difference will be pulled more towards zero (i.e. lower values are positive while upper values are negative) suggesting a better agreement than actually exists. The samples for derivation and validation studies were drawn in different years 2000-2004 for the derivation study subjects and (2001-2011) for the validation study subjects, which give some confidence for the validity of the equations when applied in other groups. However, wider application must be made with caution, as subject numbers are always restricted in studies using whole-body MRI, and our population samples were also of mixed racial types in North America. Confirmation is needed that our prediction equations do not give rise to systematic errors if applied to groups of subjects with restricted ranges of ages, or BMI, or of a single racial type. In particular, it is possible that the different body compositions of some Asian and pacific groups will demand specific prediction equations for muscle mass, as they do for body fat (Wen et al., 2011, Chen et al., 2011a). The difference in R^2 from adding ethnicity to our prediction equations was minimal, so for general use in mixed populations, where an individual's ethnicity is often mixed and hard to verify, we favour using a simpler equation without ethnicity.

Our samples included few subjects who were obese or severely obese, in particular elderly-obese, among whom relative paucity of muscle (sarcopenic obesity) is an emerging health concern (Han et al., 2011). Data to confirm anthropometric estimation of muscle mass in obese and elderly groups are therefore needed. A

limitation that could not be avoided was age and BMI distribution of the population analysed. The derivation data had 12% women with age >65 years and 22% BMI ≥30, in men 5% were above >65 years and 10% ≥ 30 BMI. In the validation analysis 9.5% women were >65 years of age and 6.6% men. BMI 14% men and 13% women had BMI above 30. Thus, we are unable to come to a confident conclusion on how the equations work on older people and for sarcopenia obesity.

It will also be valuable to establish the effects of factors such as illness and weight change on the reliability of anthropometric SM estimation in future longitudinal studies. Finally, while our equations have been validated and appear to offer value for epidemiology and in groups, their predictive power is insufficient for clinical use or among individuals. The R^2 values for predicting muscle mass in this study are similar to those models that use BMI to predict fat mass (Lean et al., 1996).

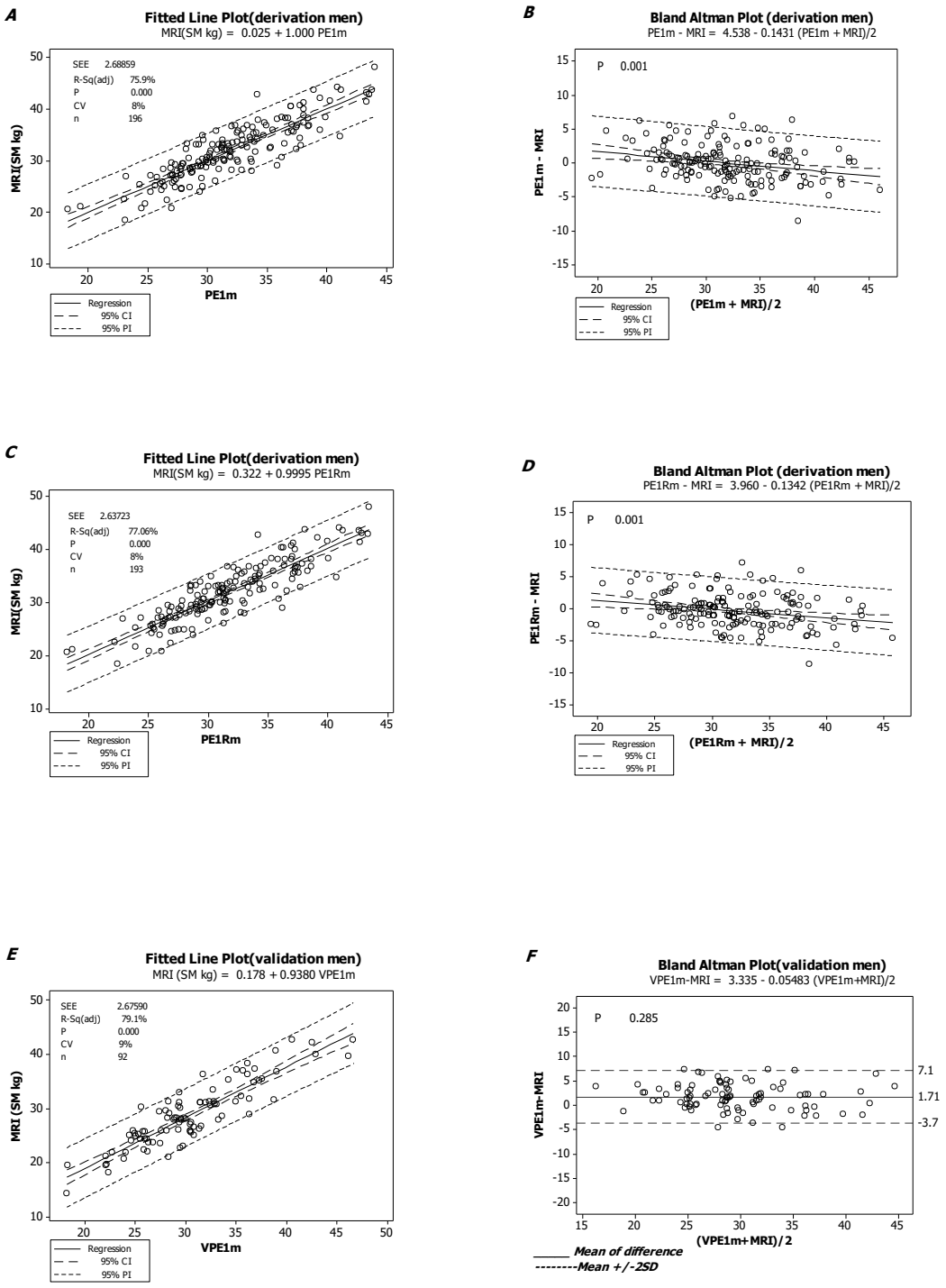
3.6 **Conclusions**

Anthropometric prediction equations for whole-body muscle mass were derived and externally validated using separate populations. Predictions have greater predictive power and less error for men than for women. Predictive equations with the greatest R^2 included hip circumference which emerged as a consistent predictor of SM. Two equations (including for men: body weight, waist, hip and age; women: body weight, hip, age and height) have been identified as offering high practicality. They lack predictive power for use in individuals or for clinical purposes, but have sufficient accuracy for use to estimate skeletal muscle mass in groups and for research and survey purposes within mixed populations, without need to adjust for race.

Authors responsibilities:

Yasmin Algindan designed the study, carried out the data analysis and contributed to the manuscript with the guidance of her supervisors Michael Lean, Catherine Hankey and Lindsay Govan. Steven Heymsfield and Dympna Gallagher contributed to the data collection. All authors critically revised the final manuscript.

Figure 3-1: derivation and validation of men equation



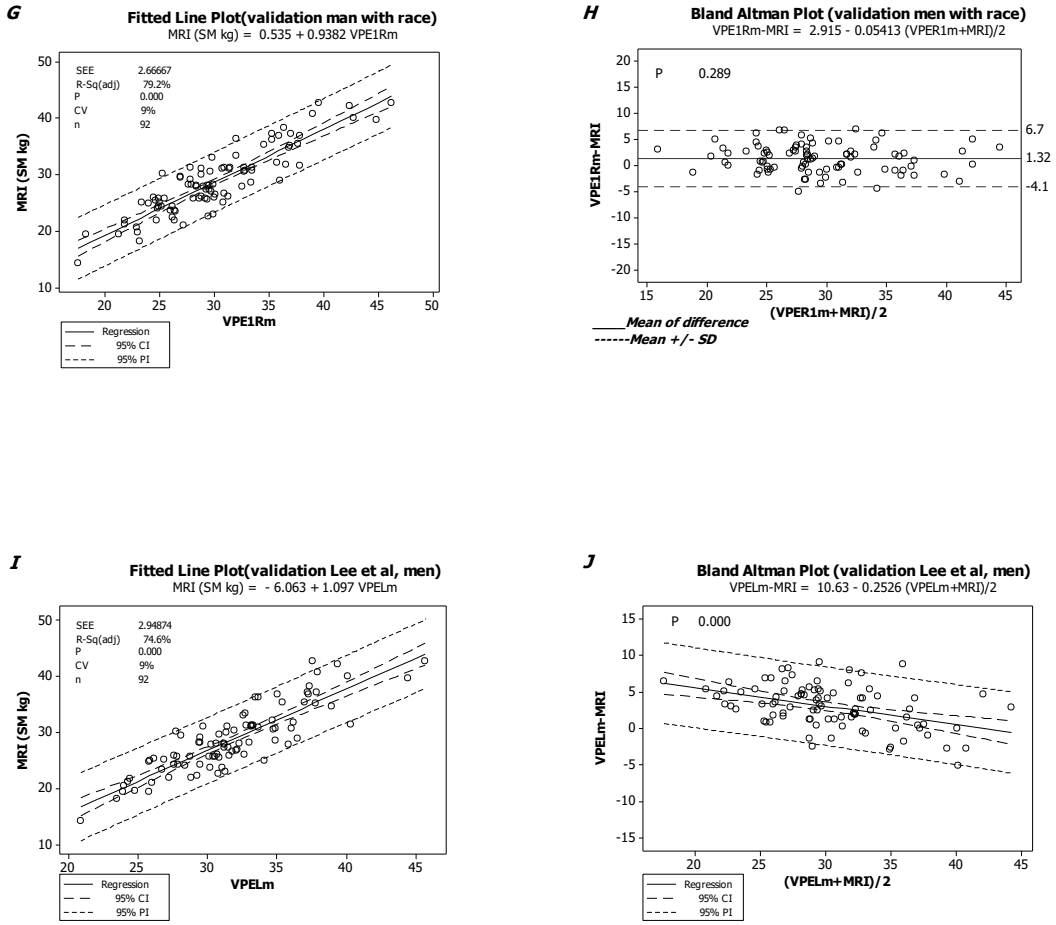
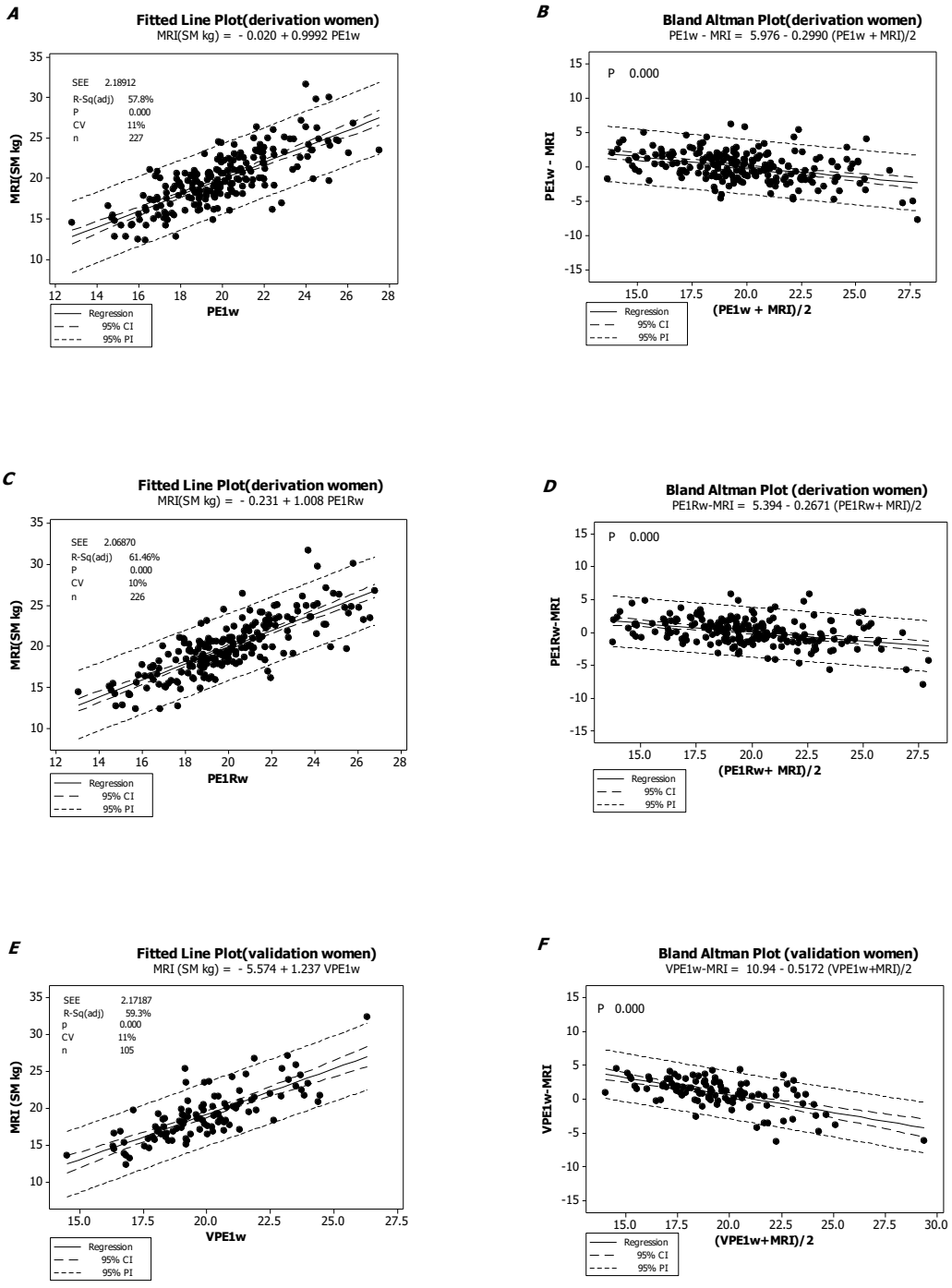


Figure 3-1**(men):** Column 1 (A, C, E, G, I) are scatterplots of MRI measured SM (y-axis) against estimated SM from prediction equations, while column 2 (B, D, F, H, J) are Bland-Altman plots of difference between predicted and MRI-measured SM (y axis) against their mean (x axis). Plots (A, B) and (C, D) represent results from the derivation of our equation without race (PE1m) and with race (PE1Rm), respectively. Plots (E, F) and (G, H) represent results from the validation of our equation without race (VPE1m) and with race (VPE1Rm). Plots (I, J) represent validation of Lee at al equation (VPELm). For the plots with no significant slope, Bland-Altman plots show the mean difference with limits of agreement around the mean difference a test for bias (mean difference significantly different from 0) using the one-sample t-test. For the plots with significant slope, Bland-Altman plots show the PI around the regression line. P-values represent a test of significance of the slope.

Figure 3-2: derivation and validation of women equations



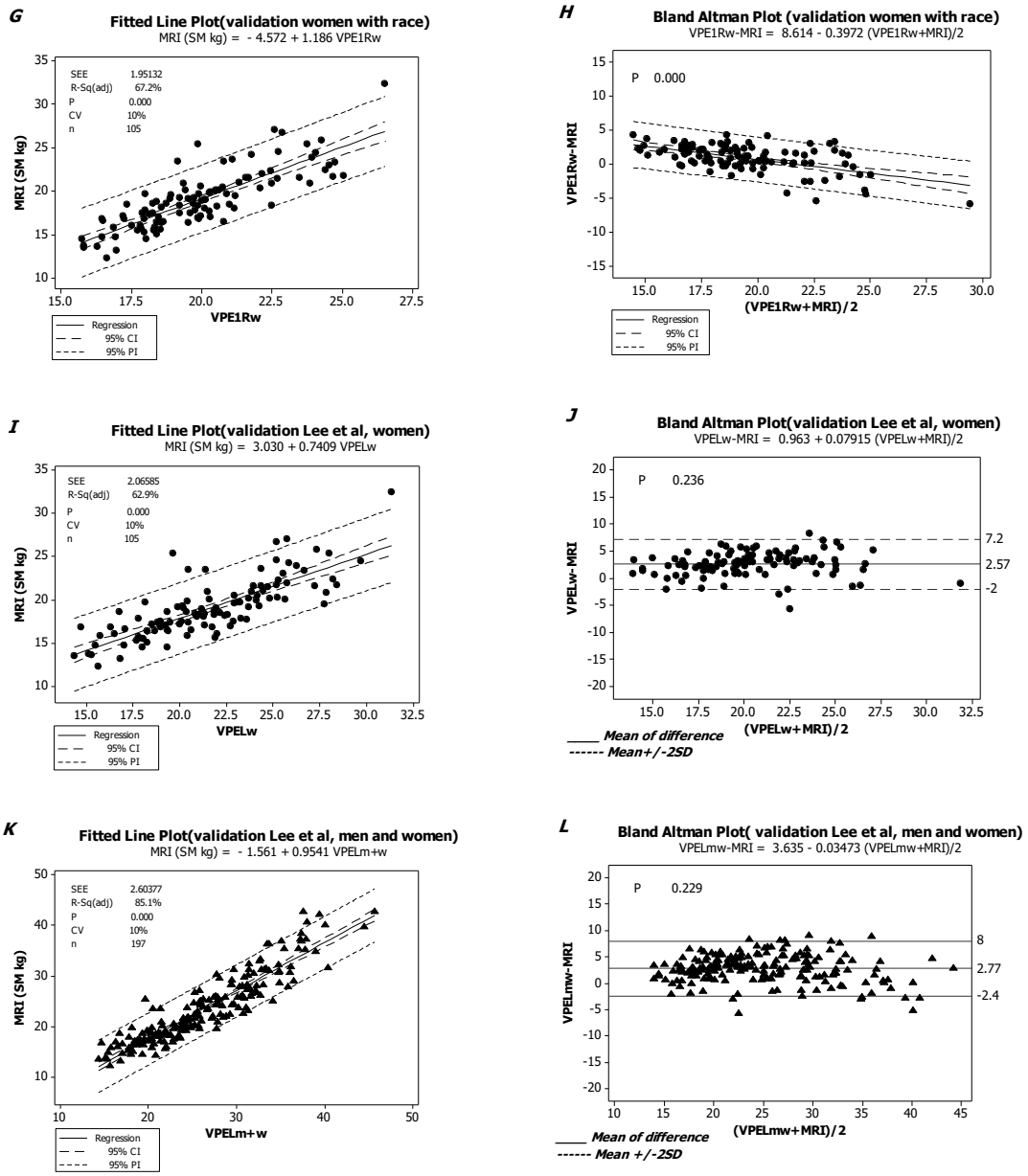


Figure 3-2**(women):** Column 1 (A, C, E, G, I, K) are scatterplots of MRI measured SM (y-axis) against estimated SM from prediction equations, while column 2 (B, D, F, H, J, L) are Bland-Altman plots of difference between predicted and MRI-measured SM (y axis) against their mean (x axis). Plots (A, B) and (C, D) represent results from the derivation of our equation without race (PE1w) and with race (PE1Rw), respectively. Plots (E, F) and (G, H) represent results from the validation of our equation without race (VPE1w) and with race (VPE1Rw). Plots (I, J) represent validation of Lee at al equation for women (VPELw) and plots (K, L) represent validation of Lee at al equation for men and women combined. For the plots with no significant slope, Bland-Altman plots show the mean difference with limits of agreement around the mean difference a test for bias (mean difference significantly different from 0) using the one-sample t-test. For the plots with significant slope, Bland-Altman plots show the PI around the regression line. P-values represent a test of significance of the slope.

Table 3-1: Subject characteristics and variables used in this study,

	Derivation Sample		Validation Sample	
	Men (n = 196)	Women (n = 227)	Men (n = 92)	Women (n =105)
Age(y)	39.2 ± 13.9	44.4 ± 16.2	43.3 ± 15.9	44.1 ± 16.4
Body weight(kg)	79.8 ± 12.7	67.1 ± 15.1	77.4 ± 14.1	66.3 ± 10.7
Height (cm)	176.0 ± 6.8	162.0 ± 7.2	174.5 ± 7.3	162.2 ± 5.9
BMI (kg/m^2)	25.4 ± 3.7	25.6 ± 5.5	25.4 ± 4.0	25.2 ± 4.1
MRI SM (kg)	31.8 ± 5.5	19.7 ± 3.4	28.8 ± 5.8	20.0 ± 3.4
MRI fat(kg)	18.4± 7.9	25.6±12.4	----------	----------
Hip circumference(cm)	99.7 ± 7.5	101.2 ± 11.9	97.5 ± 7.9	99.6 ± 8.5
Waist circumference(cm)	87.6 ± 10.7	80.1 ± 13.4	88.3 ± 12.1	82.0 ± 11.1
Caucasian (%)	41.4	42.9	36.6	33.6
African American (%)	29	31.9	26.9	36.4
Hispanic (%)	15	14.5	16.1	14.5
Asian (%)	14.5	11.1	20.4	15.5

Measurements reported as mean ± standard deviation, ---- MRI fat mass not measured in validation studies, BMI: body mass index (kg/m^2), MRI: magnetic resonance imaging, SM: skeletal muscle mass

Table 3-2: Explained variance (R^2) from linear regressions

Variable	Men (R^2)%	Women (R^2)%
Age	5.2	1.9
Body weight	53.9	38.8
Height	22.3	30.4
BMI	31.4	18.8
Race [1]	15.7	15.3
Waist circumference	11.6	16.7
Hip circumference	27.9	22.6
Mid-arm circumference	51.6	25.1
Mid-thigh circumference	36.9	27.7
Mid-calf circumference	44.5	13.2

Explained variance (R^2) in MRI whole body skeletal muscle mass, from linear regressions in the derivation study.
[1]Race included four categories as defined among the US population, Caucasians, African-American, Asian, and Hispanic

Table 3-3: Prediction equations derived from stepwise regression

	Prediction Equations: Men	R^2 (%) no race	R^2(%) including race
PE1m[1]	MRI (SM) = 39.5 + 0.665 body weight (kg) - 0.185 waist (cm) - 0.418 hip (cm) - 0.0805 age (yrs)	76	77.1[2]
PE2m	MRI (SM) = 38.4 + 0.581 body weight (kg) - 0.194 waist (cm) - 0.387 hip (cm) - 0.0738 age (yrs) - 0.0222 height (cm) + 0.279 mid-arm (cm)	75.8	76.2
PE3m	MRI (SM) = 5.4 + 0.355 body weight (kg) - 0.406 hip (cm) - 0.108 age (yrs) + 0.0998 height (cm)+ 0.410 mid-arm (cm)+ 0.299 mid-calf (cm)	75.6	76.0
PE4m	MRI (SM) = 40.0 + 0.710 body weight (kg) - 0.394 hip (cm) - 0.294 waist (cm)	73.0	74.1
	Prediction Equations: Women	**R^2 (%) no race**	**R^2(%) race**
PE1w[3]	MRI (SM) = 2.89 + 0.255 body weight (kg) - 0.175 hip (cm) - 0.0384 age (yrs) + 0.118 height (cm)	58	61.5[4]
PE2w	MRI (SM) = - 1.51 + 0.219 body weight (kg) - 0.217 hip (cm) - 0.0252 age (yrs) + 0.136 height (cm) + 0.133 mid-thigh (cm)	57.5	60.0
PE3w	MRI (SM) = - 4.33 + 0.214 body weight (kg) - 0.231 hip (cm) + 0.153 height (cm) + 0.148 mid-thigh (cm)	56.3	58.0

1: prediction equation chosen for further analysis, 2: Prediction equation: MRI = 38.809 + 0.62855 body weight (kg) - 0.17843 waist (cm) - 0.38782 hip(cm) - 0.08351 age(yrs) -1.13176 (Asian) + 0.56004 (African American) + 0.21902 (Hispanic) - 0.3527 (Caucasian). PE1m: prediction equation 1 for men. PE2m: prediction equation 2 for men. PE3m: prediction equation 3 for men. PE4m: prediction equation 4 for men.

3: prediction equation chosen for further analysis, 4: Prediction equation: MRI = 3.995 +0.22249 body weight (kg) -0.15890 hip(cm) -0.045317 age(yrs)+ 0.11523 height (cm)-0.79309 (Asian) + 1.34820 (African American) -0.50311 (Hispanic) + 0.052003(Caucasian). PE1w: prediction equation 1 for women. PE2w: prediction equation 2 for women. PE3w: prediction equation 3 for women.

Table 3-4: Derivation and validation analysis summary

		Men				Women			
	Equation	**R^2**	**SEE(kg)**	**CV for mean (%)**	**Mean difference (kg)±SD***	**R^2**	**SEE(kg)**	**CV for mean (%)**	**Mean difference (kg)±SD***
Derivation	**Prediction equation 1**	0.76	2.7	8	NA	0.58	2.2	11	NA
	Prediction equation 1 with race	0.77	2.6	8	NA	0.61	2.1	10	NA
Validation	**Prediction equation 1**	0.79	2.7	9	1.7 (2.7)	0.59	2.2	11	NA
	Prediction equation 1 with race	0.79	2.7	9	1.3 (2.7)	0.67	2.0	10	NA
	Prediction equation Lee (11)	0.75	2.9	9	NA	0.63	2.1	10	2.6 (2.3)
	Men and women combined								
	Prediction equation Lee (11) men + women	0.85	2.6	10	2.8 (2.6)				

Derivation and validation analysis summary of present prediction equations and summary of validation of Lee et al (11) prediction equations for men and women separate and combined; **R^2**: correlation evaluating the variability explained by the model; **SEE**: Standard error of the estimate measuring how different the raw data from the prediction line; sum of square error; **CV**: coefficient of variation the ratio of the standard error of the estimate (SEE) to the mean of the dependant variable and measures the relative closeness of the prediction to the actual value; **Mean difference ± SD***: mean difference between SM values as predicted using the equation and observed MRI values for SM ± standard deviation; equations with slope (significant relation between mean and difference) are (NA) not applicable

Chapter 4

4 Derivation and validation total adipose tissue/fat

Derivation and validation of simple anthropometric equations to predict adipose tissue mass and total fat mass with MRI as reference method

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4.1 **Abstract**

Background: The reference body composition measurement method at an organ level is now MRI. Practical estimations of total adipose tissue mass (TATM), total adipose tissue fat mass (TATFM) and of total body fat (TBF) are valuable for epidemiology, but validated prediction equations based on whole body MRI are not currently available.

Objectives: Derivation and validation of new anthropometric equations to estimate MRI-measured TATM /TATFM/TBF, and comparison with existing prediction equations based on older methods.

Design: Derivation-sample: n=416 (222 women), aged 18–88 years; BMI 15.9-40.8 (kg/m2). Validation-sample: n=204 (110 women), aged 18–86 years; BMI 15.7-36.4 (kg/m2). Both samples included mixed ethnic/racial groups. All underwent whole-body MRI to quantify TATM (dependent variable) and anthropometry (independent variables). Prediction equations developed using stepwise multiple regression were further investigated for agreement and bias, before validation in separate datasets.

Results: Simplest equations with optimal R^2 and Bland-Altman plots demonstrating good agreement without bias in validation analyses;

Men: TATM (kg) = 0.198 weight (kg) + 0.478 waist (cm) - 0.147 height (cm) - 12.8

(Validation: R^2 =0.79, CV=20%, SEE=3.8 kg).

Women: TATM (kg) = 0.789 weight (kg) + 0.0786 age (y) - 0.342 height (cm) + 24.5

(Validation: R^2 =0.84, CV=13%, SEE=3.0 kg).

Adding a ‘race’ variable did not add predictive power.

Published anthropometric prediction equations, based on MRI and CT-scans, correlated strongly with MRI-measured TATM: (R2=0.70 – 0.82). Estimated TATFM, assuming TATFM = TATM x 0.8, correlated well with published prediction equations for TBF based on UWW (R2= 0.70 – 0.80), with a mean bias of (2.5-4.89) which was corrected with log transformation in most equations.

Conclusion: New equations, using simple anthropometric measurements, estimated MRI-measured TATM with high correlation and good agreement across a wide range of fatness, and provide predictions of TATFM comparable with TBF equations based on underwater weighing.

4.2 **Introduction**

In clinical and epidemiological settings, where practicality and low cost are dominant issues and using a reference measurement method is not possible, reliable practical methods are needed to estimate body composition. Anthropometry has been used widely for many years as a simple method to assess body composition, and specifically total body fat content, which relates importantly to metabolic disease risks. Body mass index (BMI), skin-fold thicknesses, limb and trunk circumference, dual-energy X-ray absorptiometry (DEXA) and bio-impedance methods have all been used in clinical settings and epidemiological surveys. Each of these methods has strengths and limitations, but all need to be calibrated against a reference method. In the past this was most commonly densitometry, using underwater weighing (UWW), or CT imaging, but magnetic resonance imaging (MRI) is now the preferred reference method for body composition measurements at organ level (Heymsfield et al., 1997).

It is important to recognise that MRI can only quantify body fat from measurement of adipose tissue not including head, feet, hand and forearm, in addition total body fat includes fat within other organs such as muscle and liver. It is conventionally assumed that adipose tissue comprises 78.3% total body fat in lean subjects and 83.2% in obese (Garrow, 1975) (Figure 1-4).

Estimates of total adipose tissue from MRI and CT scans have been compared with methods designed to estimate total body fat such as UWW and DEXA (McNeill et al., 1991, Sohlstrom et al., 1993, Kullberg et al., 2009). These studies used the well-established assumptions that adipose tissue has a density of 0.92kg/L and contains 80% fat (Shen et al., 2003a, Garrow, 1975) **(Figure 1-4).**

Using densitometry measurements to estimate total body fat as % body weight, Deurenberg et al (Deurenberg et al., 1991b) used BMI, age and sex as variables to predict fat mass among 1229 men and women with wide range of age (7 – 83 years) and BMI (13.9 – 49 kg/m^2). Anthropometric estimates of % body fat in adults had high correlations with UWW measured total body fat (R^2 = 0.80) that were supported by cross-validations (Deurenberg et al., 1991b). However, this study

combined men and women, possibly therefore giving misleadingly high correlations, and it assessed bias by showing the relation among difference between observed and predicted against observed total body fat measurement. This assessment method could show an association when there is none (Bland and Altman, 1995). A better analysis method, as described by Bland and Altman, is to plot the difference against the average of observed and predicted (Bland and Altman, 1995).

In 1996, Lean et al developed regression equations from simple anthropometric measurements to predict total body fat calculated from body density measured by underwater weighing in 84 women and 63 men. The best simple prediction equations, with least bias, and validated in a separate population sample were from waist circumference adjusted for age (R^2 0.69 for men, 0.75 for women). This study also validated (for the first time) the widely-used skin-fold measurement predictions of total body fat published by Durnin and Womersley in 1974, and found that waist circumference provided almost identical predictive power (Lean et al., 1996).

As well as predicting total body fat, waist circumference performed well in predicting total adipose volume in men, Ross et al, (1992) investigated the relationship between anthropometric variables and MRI measured total adipose tissue volume in 27 healthy men. The combination of waist circumference and waist: hip ratio explained 91% of variation in total adipose tissue volume. Nevertheless, this equation was not cross validated nor was agreement between methods investigated.

Kvist et al, (1988) developed whole-body adipose tissue predictive equations from whole body computed tomography (CT) in 17 men and 10 women. After cross-validation in 7 men and 9 women, total adipose tissue volume was best predicted by weight and height with standard error of difference ≤ 11%. The very small sample size is a serious limitation in this study; also women with ulcerative colitis were pooled with healthy women, then allocated to derivation and cross-validation

group. There was no assessment of agreement or biases (Bland and Altman, 1999a).

The present study was therefore designed to derive new prediction equations, using simple anthropometric variables to estimate TATM from MRI measurements, and to validate them in an independent sample. We also compared the most widely used existing prediction equations for total body fat, estimated from underwater weighing with estimates based on our MRI measurements of adipose tissue.

4.3 **Materials and Methods**

Data included in the derivation and validation studies were collected from adult subjects in whom the same measurements had been made by different investigators, in studies conducted at New York Obesity Nutrition Research Center's Body Composition Unit, St. Luke-Roosevelt Hospital, New York. For both anthropometric and MRI measurements, readers were blinded (subject's names and anthropometric data were anonymized). Race/ethnicity was determined by self-report and included declaration of race/ethnicity for parents and grandparents. Variables were created for four race/ethnicity categories: Caucasian (C), African American (AA), Hispanic (H), and Asian (A).

All studies obtained written informed consent and were approved by the Institutional Review Board of St. Luke's-Roosevelt Hospital (Heymsfield et al., 2007b, He et al., 2009b, Bosy-Westphal et al., 2013a).

4.3.1 Subjects

Derivation study sample: A total of 416 (222 women) subjects aged 18–88 years; BMI 15.9–40.8 (kg/m^2) participated in several related studies between 2000 and 2004 (Heymsfield et al., 2007b). Subjects were classified as having no known or diagnosed diabetes, cancer, heart disease, or any health conditions that would affect body composition or fat distribution. All were ambulatory, weight-stable (less than 2 kg weight change in previous 6 months) adults, who underwent

investigations that included anthropometry and whole-body MRI scanning. Four subjects were excluded from this sample because of technically poor or incomplete MRI scans.

Validation study sample: Data sets from two previous studies (He et al., 2009b, Bosy-Westphal et al., 2013a) were combined, giving a total of 204 subjects (110 women, 94 men). Subjects were recruited (Study 1: 2001 to 2004(He et al., 2009b), Study 2: 2011(Bosy-Westphal et al., 2013a)) through advertisements in local newspapers, internet, and on flyers posted in the local community. A body mass index (kg/m^2) upper limit of 37 was set to accommodate the MRI scanner limitations. Participants were required to be ambulatory non-smokers, free of medical conditions or metabolic characteristics (abnormal thyroid or cortisol concentrations) that could affect the variables under investigation, weight stable (<2 kg change within past 6 months), and not regularly engaging in vigorous exercise. The subjects varied in age (18–86 years) and BMI (15.7–36.4kg/m^2) **(Table 4-1**). This final sample was carefully checked to ensure that there was no duplication of subjects between the derivation and validation samples. The validation sample was used to validate the new derived equations and the existing equations of Lean et al (Lean et al., 1996), Deurenberg et al (Deurenberg et al., 1991b), Kvist et al (Kvist et al., 1988a) and Ross et al (Ross et al., 1992).

4.3.2 Methods

Magnetic resonance imaging (MRI): All data were collected in the same laboratory by an analysis team (n = 3) for derivation and validation samples. For the 2011 study a single MRI analyst performed the measurements. A 1.5 tesla MRI scanner (6x HORIZON; General Electric, Milwaukee) was used for both studies (Song et al., 2004b). Whole-body MRI was carried out to identify and quantify total body and regional adipose tissue (Shen et al., 2003b). The procedure involved acquisition of approximately 40axial images, of 10mm thickness at 40mm intervals throughout the whole body (Song et al., 2004b). Cross-sectional images were analysed for subcutaneous adipose tissue, visceral adipose tissue, inter-muscular adipose tissue, total adipose tissue by three trained observers with the use of VECT image analysis software (Slice-O-Matic, Montreal, Canada), and total volumes were calculated as

reported by Shen (Shen et al., 2003a). Intra-class correlation coefficients (CIs) for agreement among multiple readers were subcutaneous adipose tissue 0.99 (0.81–1.0), and visceral adipose tissue 0.95 (0.58–0.99) (Song et al., 2004b)

Anthropometric measurements: Three technicians, who were trained in the body composition laboratory, reported all anthropometric data. Body weight was measured to the nearest 0.1 kg using a balance beam scale (Weight Tronix, New York, NY) with the subject wearing a hospital gown. A wall-mounted stadiometer (Holtain, Crosswell, Wales) was used to measure standing height to the nearest 0.1cm. Anthropometric circumferences were obtained using a heavy-duty inelastic plastic fiber tape measure (Gulick II Tape Measure): waist was measured as the midpoint between the lowest rib and the upper border of the iliac crest(Wang et al., 2003a); hips at the level of the pubic symphysis and the greatest gluteal protuberance; mid-arm at the mid-point between the lateral tip of the acromion and the most distal point on the olecranon; mid-thigh at the mid-point between the inguinal crease and the proximal border of the patella; and the maximum girth of the calf). The procedures outlined for anthropometric measurement sites and training were as outline in the "Anthropometric Standardization Reference Manual" (Martorell, 1988)

4.3.3 Statistical analysis

Datasets had all previously been checked and cleaned for errors of data entry, but were initially explored to confirm that all data ranges were plausible.

4.3.3.1 Assumptions for computations

Total Adipose Tissue Mass: Measured whole-body adipose tissue volume, reported in litres, was converted to kilograms by multiplying volume of tissue by the reference density of adipose tissue: (0.92 g/L) (Garrow, 1975).

Total adipose tissue fat mass: Determined assuming the proportion by weight of the lipid fraction in adipose tissue to be 0.80 (Sohlstrom et al., 1993, Wang et al., 2003a, Snyder et al.).

4.3.3.2 Prediction equation development

Multiple linear regressions generated equations, separately for males and females, to predict TATM measured by MRI. Eight anthropometric variables were considered of interest, on grounds of practicality for routine clinical and epidemiological work: age, weight, height, and circumferences (hip, waist, thigh, arm and calf). Forward and backward stepwise regression analysis was performed (alpha to enter 0.15, alpha to remove 0.15) using the eight variables. The highest R^2 value of each set of step wise regression was investigated further. Bland-Altman plots were used to explore distributions of errors (Bland and Altman, 1999a). Log transformation was used to resolve skewness of the sample and investigate any relation in the Bland Altman plot, by plotting the difference between the natural logarithm of MRI-measured TATM and the natural logarithm of predicted TATM, against natural logarithm of the mean of the MRI-measured and predicted TATM (Bland and Altman, 1999a). To investigate the effect of adding the variable 'Race' (as applied in mixed US populations) to the equation, we used the derivation study sample, with the addition of the variable (Race) to our best derived equations for both men and women. Given that we have a categorical value (Race), ANCOVA general linear model was used.

The best derived prediction equations were validated using linear regression against whole body MRI measurements in a separate validation sample. Bland Altman plots were also created in the validation sample to determine levels of agreement between predicted and true MRI adipose tissue mass.

Standard error of the estimate (SEE) was used to define the accuracy of prediction equations. Judgement is based on comparison with similar published equations (Deurenberg et al., 1991b, Lean et al., 1996, Ross et al., 1992, Kvist et al., 1988a). To compare models, coefficient of variation (CV) was calculated as the ratio of the standard error of the estimate to the mean of the dependant variable. To investigate limits of agreements between MRI-estimated TATM and prediction equations, 95% confidence interval (95% CI) was used.

4.3.3.3 Association with other adipose tissue & body fat equations:

Two total adipose tissue prediction equations one was developed using CT scan (Kvist et al., 1988a) and the other used MRI (Ross et al., 1992), these equations were compared with our TATM derived prediction equation.

Two total body fat UWW derived prediction equations (Deurenberg et al., 1991b, Lean et al., 1996) were compared with our derived TATM after converting it to TATFM using the following equation: [TATFM = TATM x 0.80].

All statistical analyses were carried out using Minitab ® 16.2.0.0.

4.4 Results

Subject characteristics are shown in **(Table 4-1).** Linear regression of single variables against MRI-measured TATM **(Table 4-2)** showed generally stronger correlations for women than for men, except mid-calf circumference and waist/hip ratio.

4.4.1 Equations for TATM:

4.4.1.1 Derivation of prediction equations for TATM

Best equation for men (P1TATM): The best variables after step-wise regression in men were body weight, waist and hip. Multiple regression gave high correlations R^2 = 0.82, SEE 3.4kg and CV 18% with narrow95% CI (17.5, 18.5) for an 18 kg adipose tissue measurement, yet 95% PI was high (11.2, 24.8) **(Figure 4-1A).** Bland Altman plots showed negative relation P = 0.004 **(Figure 4-1B),** however after log transformation this relationship was no longer present P = 0.728, and the negative slope no longer exists **(Figure 4-5A).** Limits of agreement based on 1-sample t test were (95%CI=-0.03, 0.04). Adding race as a variable to the new equation did not affect the results for men (data not included).

Simplest prediction equation in men (P2TATM): The simplest prediction equation for TATM, providing a high predictive power in men included body weight, waist

and height. In Bland Altman skewness was resolved after log transformation **(Figure 4-5B).** There was no statistical difference between the best and simplest equation in men. **(Figure 4-1C, Figure 4-1D), (Table 4-3).**

Best equation for women (P1TATM): From step-wise regression, age, body weight, height and hip circumference gave best correlations with TATM. As in men, multiple regression gave high correlation R^2 = 0.89, with moderate SEE 4.2kg and CV 16%, 95% CI did not vary much with fatness, from (95% CI:19.3, 20.6) at the lower end 20kg TATM, however 95% PI was high (11.6, 28.4). At the upper end of adipose tissue mass 35kg TATM (95% CI: 34.2, 35.7 and 95%PI: 26.6, 43.4) **(Figure 4-2A)**. Bland Altman plots showed a mean difference between predicted and measured close to zero (-0.002), **(Figure 4-2B).** There was a slight negative correlation P = 0.013, no longer present after log transformation P = 0.804 **(Figure 4-5C)**. Limits of agreement based on 1-sample t test were (95%CI = -0.021, 0.029). Adding the race variable did not change results (data not included).

Simplest prediction equation in women (P2TATM): The simplest equation in women included body-weight, age and height. Predictive power was almost identical to the best equation: $R^2$0.88, SEE 4.2kg and CV 16% (**Figure 4-2C**) **(Table 4-4**) skewness was resolved after log transformation and 95% CI was the same as P1TATM in women **(Figure 4-2D, Figure 4-5D).**

4.4.1.2 Validation of derived equations to predict TATM

Validation of our best equations gave high correlations for both men and women (0.80 and 0.84 respectively). SEE for men was slightly higher than women (3.0kg versus 3.7kg) **(Figure 4-3A and Figure 4-4A).** CV decreased by 4% in women and increased by 2% in men. Limits of agreement in men was (95%CI: 24.7, 26.7 and 95%PI: 18.3, 33.1) for women (95%CI: 24.1, 25.3and 95%PI: 18.6, 30.7). Bland Altman plot for women showed a slight positive relation P = 0.04, which was resolved after log transformation P = 0.08 **(Figure 4-4B, Figure 4-5E).** No skewness was seen in Bland Altman plots for men, so there was no need for transformation (P = 0.67). There was a slight mean underestimation (-1.3kg) compared to MRI measured TATM in men.95% CI of the log transformed equations in women based

on 1-sample t test were (95%CI = -0.023, 0.032), in men limits of agreement based on 1-sample t test without log transformation were (95%CI:-2.4, -0.5) **(Figure 4-3B and Figure 4-4B).**

Validation of the simplest equations, without the variable hip, equations also showed very similar results to the best equation for both men and women **(Figure 4-3C, 4-3D, 4-4C, 4-4D)**. In men mean underestimated by -0.57kg. In women sample was skewed, after log transformation bias was no longer present **(Figure 4-5 F**).

Validation of published equations to predict TATM of (Kvist et al., 1988a) (P-Kvist)

The prediction equations originally derived and validated by Kvist et al, 1988 using CT scan and based on a mixed group of healthy adults and patients with ulcerative colitis were:

Men: total adipose tissue (L) = 1.36 weight/height - 42.0 (R^2= 0.93)

Women: total adipose tissue (L) = 1.61 weight/height – 38.3 (R^2= 0.96)

Correlations with our estimates based on the MRI measurements were greater in women than men (R^2 = 0.82, 0.70 respectively, however SEE was high (4.6 and 3.2 in men and women respectively) limits of agreement (95%CI: 24.5, 27.1 and 95%PI: 16.6, 34.9). The CV was higher in men than women (27% versus 12%) (**Figure 4-3M & Figure 4-4M).** Bland Altman plots revealed significant biases, with significant positive relationships between differences and the average of observed and predicted in women, which could not be corrected by log transformations. From the Bland Altman plot limits of agreement in 18kg fat men (95%CI: -3.9, -1.8 and 95%PI: -12.9, 7.1), in 30kg fat men limits of agreement were (95%CI: -3.3, 0.3 and 95%PI: -11.6, 8.7).

These results indicate substantial bias and error (**Figure 4-3N and Figure 4-4N).**

Validation of published equation (TATM) of (Ross et al., 1992) (P-Ross, men)

Ross et al derived a prediction equation from 27 healthy men, using MRI-measured adipose tissue total adipose tissue (L) = 1.003 x waist circumference – 56.475 x waist hip ratio – 21.364 (d: R^2 = 0.91). Our validation of this equation showed good correlations and reasonable agreement R^2 = 0.81, SEE = 3.6 and CV =24%. There was no relationship in Bland Altman plots to indicate bias, but there was a consistent underestimation: mean difference -5. Limits of agreement using 1-sample t test (95%CI: -5.5, -4.0) 95%PI from Bland Altman plots (-12.02, 2.57) **(Figure 4-3O, Figure 4-3P).**

4.4.2 Equations to estimate TATFM

To estimate TATFM, total adipose tissue mass (TATM) was converted to total adipose tissue fat mass using the factor 0.8(Sohlstrom et al., 1993, Wang et al., 2003a, Snyder et al.) TATFM = TATM × 0.8.

Men (P1TATFM, P2TATFM): correlations were high in both equations with and without hip circumference (R^2 = 0.82 and 0.79) for P1TATFM and P2TATFM respectively, SEE and CV (2.8, 3.0) and (18%, 20%) (**Figure 4-1E and Figure 4-1G**). The width of 95% CI was 1.4 in both P1TATFM and P2TATFMand the width of 95%PI was 11.9 and 12.2 respectively, for an 18kg measurement of TATFM. In Bland Altman plots mean difference was close to zero (-0.12 and 0.00) with slight bias (P = 0.003, 0.001) that was corrected by log transformation (P = 0.603, 0.137) respectively **(Figure 4-1F and Figure 4-1H and Figure 4-5G, Figure 4-5H).** Validation of men TATFM equations showed high correlations for both equations with and without hip circumference (R^2 = 0.80, 0.79 respectively) and SEE (3.0) and CV (20%) for both equations. Limits of agreement of 18kg measured TATFM (95%CI: 18.1, 19.5 and 95%PI: 12.9, 24.8) in P1TATFM and (95%CI: 17.5, 18.9 and 95%PI: 12.1, 24.3) in P2TATFM. No bias seen in Bland Altman plots, limits of agreement based on 1-sample t test are (95%CI= -1.75, -0.51) and (95%CI = -1.10, 0.17) respectively **(Figure 4-3E, F, G, H**).

Women (P1TATFM, P2TATFM): No difference in correlation, SEE and CV between best and simplest TATFM prediction equation **(Figure 4-2E and G).** Bland Altman plots showed slight bias (P = 0.013, 0.008) resolved by log transformation **(Figure 4-2F and H and Figure 4-5 I and J).** Our validation of women's adipose tissue fat mass equations showed high correlations (R^2 = 0.84) and good SEE (2.4, 2.5) and CV (12%, 13%) no bias in Bland Altman plots (95%CI: -0.5, 0.6 and 95%PI: -5.1, 5.2). Limits of agreement based on 1-sample t test were (95%CI = -0.30, 0.70) and (95%CI = -0.13, 0.89) in P1TATFM and P2TATFM respectively.

Association with published equations to predict total body fat of (Lean et al., 1996) (P-Lean))

Estimates of TATFM from MRI were compared with the simplest prediction equations for total body fat, derived and validated using underwater weighing by (Lean et al., 1996).

Men total body fat (%) = 0.567 waist (cm) + 0.101 age (y) – 31.8 (d: R2=0.78, v: R^2=0.69).

Women total body fat (%) = 0.439 waist (cm) + 0.221 age (y) -9.4 (d: R2= 0.70, v: R^2=0.75).

Correlations were higher in men (R^2 = 0.80) than women (R^2= 0.76), and SEE was 3.0 kg for both men and women **(Figure 4-3 I, Figure 4-4 I).**

Assuming that TATM comprises 80%fat we estimated TATFM by multiplying TATM by 0.80, and related them with Lean et al, estimates of total body fat, which showed mean overestimation of TATFM by 2.53kg in men and 4.89kg in women **(Figure 4-3J, Figure 4-4J)**. There was some skewing (P= 0.000), Bland Altman plots showed relationships between mean and difference, which persisted after log transformation (P <0.001) in men, but not in women (P = 0.188 after log transformation) **(Figure 4-5K)** 95%PI: -0.09, 0.56. Limits of agreement based on 1-sample t test in women was (95%CI = 0.197, 0.259). In men with lower values of

TATFM (18kg) 95%CI = (1.12, 3.56), and men in higher values of TATM (35kg) 95%CI = (5.68, 9.45).

Association with published equations to predict total body fat of (Deurenberg et al., 1991b) (P-Deurenberg)

Published equations from (Deurenberg et al., 1991b) were

total body fat (%) = 1.20 x BMI + 0.23 x age(y) – 10.8 x gender– 5.4. (1 for men, 0 for women)

The Deurenberg equation was more reliable in women than men in terms of R^2 (0.78 versus 0.70), SEE (2.9 kg versus 3.6 kg) and CV (12% versus 19%) **(Figure 4-3K and Figure 4-4K)** limits of agreement based on 95%PI: 7.6, 22.0 and 9.7, 21.2 for men and women respectively. Our derived estimates of TATFM showed mean overestimation of TATFM as predicted by Deurenberg et al, by 3.52 in men and 4.13 in women. There was some skewing in the Bland Altman plots revealing a minor negative relation in both men and women (P=0.04), (P <0.01) **(Figure 4-3L, Figure 4-4L)**, which was removed by log transformation **(Figure 4-5 L & M).** Limits of agreement based on one sample t test were (95%CI = 0.15, 0.27 and 0.16, 0.22) in men and women, respectively.

4.5 **Discussion**

BMI is still the most popular method for classifying fatness and thinness, despite its rather weak correlation with body fat content (especially in men), and its failure to distinguish fat mass from muscle mass; which have opposite implications for health and well-being. Body fat is better estimated using the sum of four skin-fold thickness measurements (Durnin and Womersley, 1974), but this method requires training and has a poor record for inter-observer variability (Wang et al., 2000b, Al-Gindan et al., 2014b). As a single-measure, waist circumference alone is simpler, and the most reliable circumference measurement, which gives similar prediction of total body fat to skin-fold measurements (Lean et al., 1995, Han et al., 1995). These methods were all based on estimation of total body fat from

underwater weighing using the two-compartment model. Adipose tissue, which is conventionally assumed to contain 80% of total body fat (Shen et al., 2003a), can be directly and accurately measured using modern imaging methods. Equations have been published using anthropometry based on CT scanning in a very small study (Kvist et al., 1988a) but MRI has been considered the reference for adipose tissue measurement for many years now, and anthropometric prediction equations have not previously been validated against whole body MRI.

We have explored the use of simple anthropometric measurements that are made routinely in health surveys to predict TATM as estimated by MRI as the reference method. We assessed new equations against four previously published methods based on different reference methods, (Lean et al., 1996) and (Deurenberg et al., 1991b) for total body fat based on underwater weighing (Kvist et al., 1988a) for TATM based on CT and (Ross et al., 1992) for TATM based on MRI but never validated **(Table 4-3, Table 4-4).** The different reference methods have never been directly compared in the same subjects, but the published anthropometric equations derived and validated using these different methods all gave broadly similar results when applied to MRI measurements in the present study. The new MRI-derived equations had similar R^2 but showed less errors and biases than the existing published methods. For published TATM equations in men, both Kvist and Ross equations underestimated TATM by -2.86 and -4.72 respectively. In women Kvist et al equation overestimated TATM by 3.08. As for total body fat equations for both Lean et al and Deurenberg et al, equations overestimated TATFM in men showing better results after log transformation in women.

The equations provide sufficient prediction of TATM in both sexes for many epidemiological purposes, with R^2 better than BMI, but there are inevitably limitations. Women showed consistently stronger correlations between anthropometry and TATM than men **(Table 4-2).** This may be expected as women have greater fat masses than men, and greater variation between individuals which allow higher R^2 values. The converse applies for prediction equations to estimate whole body muscle mass, where men have consistently higher correlations with

anthropometry (Al-Gindan et al., 2014a). For women, the main predictor of TATM was body weight while for men it was waist circumference **(Table 4-2).**

Comparisons of the new equations with those of (Lean et al., 1996) and (Ross et al., 1992), which both included waist circumference, showed higher correlations for men (Table 5.3). On the other hand, the Deurenberg et al (1990) and Kvist et al(1988) equations, which used BMI and weight/height respectively as variables, both showed higher correlations in women than men **(Table 4-4).**

The relationship between BMI and body fat has been studied extensively (Gallagher et al., 1996, Forbes, 1987). In our samples, BMI showed reasonable correlation in linear regression with measured TATM in women (R^2 = 0.82) but only moderate correlation in men (R^2 =0.66). When BMI was added into step-wise regression it did not appear as significant within the best equations **(Table 4-3).** Similarly, using equations based on BMI, (Deurenberg et al., 1991b), found only moderate correlations with estimated TATFM (0.70 – 0.78).

Waist: hip ratio has shown conflicting results in its relations to metabolic illness and to measured adipose tissue (Burton et al., 2012). Ross et al, (1992) reported that waist: hip ratio correlated strongly with MRI-measured total adipose tissue, but R^2 was only 72%. Adding waist circumference increased correlation substantially, to explain 91% of variance. The same was seen in our data set. After stepwise regression the best variables for men included waist, waist: hip ratio and body weight R^2 = 0.82, SEE = 3.4 (figures not included), compared to our equations no significant difference was seen, in terms of practicality we decided to use equations without waist: hip ratio, In the present study, waist: hip ratio was not a significant predictor of TATM in women (linear regression R^2= 0.025).

The best equations for both men and women included hip circumference. The additional predictive power from including hips was relatively small, probably because hip circumference is more strongly related to gluteal muscle mass outside extreme obesity.

Since measurement of hip circumferences requires removal of some clothing, and is less often performed in large health surveys, we evaluated prediction of MRI-TATM and TATFM from simpler, more practical, measures: age, height, weight and waist. From these variables, the best prediction of TATM in men was from body-weight, waist and height, and in women, body-weight, height and age. These equations performed well in validation analyses, with very similar predictive power to our 'best' derived equations **(Table 4-3).**

Log transformations of the data were needed in most of our Bland Altman plots to account for significant relationships between mean difference and average, due to a combination of non-constant variation and skewness in the predicted and MRI measurements. This would be expected since there were fewer subjects in the samples who had large fat masses than with low or average fat mass measurements.

It was perhaps surprising that age did not appear as a significant variable in the best prediction equations for men. This could be due to the relatively small number of adults aged over 60 years (12 subjects) in our derivation sample, but in general the use of physical measures with high prediction accounted for differences related to aging. The same applied to the race variable, which is valuable as defining race but problematic in mixed populations.

4.6 **Strength and limitations of the present study**

Our study included a larger number of subjects than previous studies to develop anthropometric prediction equations, which allowed a more robust analysis of agreements and biases. Our data were from diverse samples in terms of age and of racial groups. It was reassuring that adding a term for racial group into our model did not add predictive value, indicating that simple anthropometric measures accounted for inter-racial differences in body composition. Other predictive equations which do not include body circumferences needed a term for race to be included, which presents practical difficulties for ascertainment, particularly in mixed-race populations. Ethnicity, usually self-attributed, is even trickier, (Gallagher et al., 1996) studied a cohort of 706 adults using a 4-

compartment body composition model to estimate total body fat as a percentage of body weight. They concluded that BMI is sex and age dependent when used as an indicator of body fat, but that BMI is independent of ethnicity in Caucasian and African American adults. It is important for our validation studies that the measurements were all made following an identical protocol to the derivation studies. Ideally these measures would have been made by completely independent investigators, and perhaps using different equipment, in order to confirm transferability of the method. However, the similarities in predictions of MRI-measured TATM and TATFM with the previously published equations using different methods allow confidence that our methods are likely to be reliable when applied in different settings.

The relatively low number of aging adults in our derivation sample may have introduced bias; ideally number of subjects over 60 would be higher.

It is important to recognize that adipose tissue fat mass measured by MRI correlates with "fat" as estimated by two-component methods like DEXA or UWW, but they estimate different targets. In our analysis we used two assumptions that have been used extensively in literature (Garrow, 1975). To convert total adipose tissue in volume to mass in kg we multiplied by 0.92 and to convert TATM to TATFM we multiplied TATM by 0.8 **(Figure 1-4).** These assumptions incur limitations, particularly using a single factor for all subjects for the fat content of adipose tissue: this is likely to vary with degree of fatness. MRI does not capture small fat depots, below the level of resolution, within muscles, liver etc, hands, feet and head are commonly excluded from whole body MRI. Thus in our analyses, total body fat derived from UWW correlated strongly with total adipose tissue fat mass measured by MRI (R2 = 0.70 - 0.80), but there were differences between them, rising with fatness. Bland Altman plots **(Figure 4-3 J, L and Figure 4-4 J, L)** show considerable variability, but the difference between these estimates was about 1kg for men, 2 kg for women for an average thin individual with 15kg TATFM, and 5-7kg for an average obese individual with 37 kg TATFM.

We could not interpret the effects of illness, physical disability or extremes of age within our dataset. Further validation would be advisable for the equations to be used in these conditions.

4.7 **Conclusion**

New equations, using simple anthropometric measurements and without need for a race variable, estimated MRI-measured TATM with higher correlations and better agreements than existing equations. The new equations for TATM, with standard conversions to estimate total body fat, generated broadly similar figures to published anthropometric equations for total body fat. The degree of individual variation, as with previous prediction equations, implies that they should not be used for clinical or diagnostic purposes, but they have value for use among groups and populations, and estimate body fat substantially better (modestly greater R^2) than BMI alone.

Authors responsibilities:

Yasmin Algindan designed the study, carried out the data analysis and contributed to the manuscript with the guidance of her supervisors Michael Lean, Catherine Hankey and Lindsay Govan. Steven Heymsfield and Dympna Gallagher contributed to the data collection. All authors critically revised the final manuscript.

Figure 4-1: Derivation men

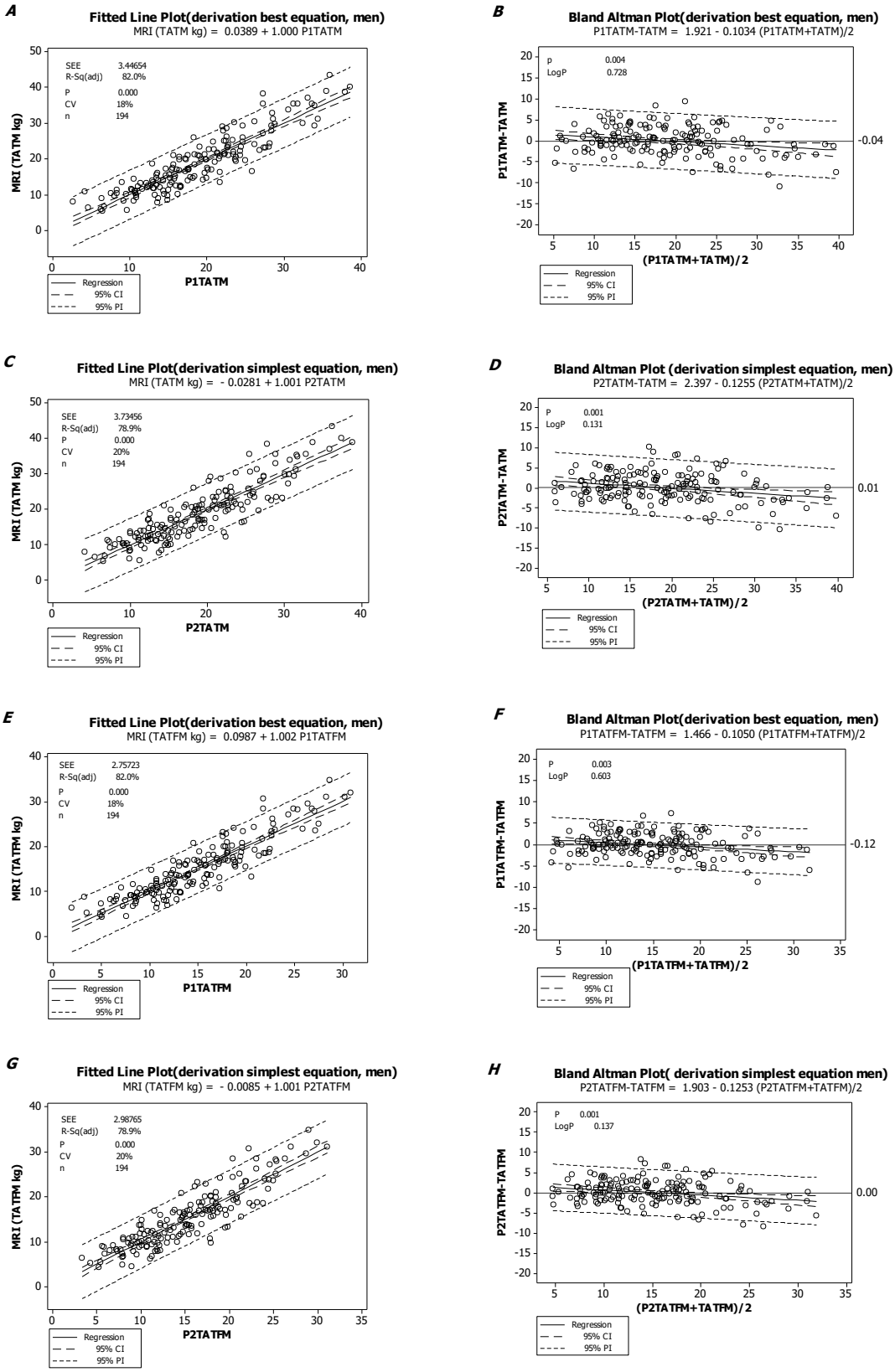


Figure 4-1 (derivation analysis men): Column 1 (A, C) are scatter-plots of MRI measured TATM (x-axis) against estimated TATM from prediction equations, while column 2 (B, D) are Bland Altman plots of difference between predicted and MRI-measured TATM (y axis) against their mean (x axis).

Column 1 (E, G) are scatter-plots of MRI measured TATFM (x-axis) against estimated TATFM from prediction equations, while column 2 (F, H) are Bland Altman plots of difference between predicted and MRI-measured TATFM (y axis) against their mean (x axis). Lines represent mean difference of 0, regression and 95% confidence and prediction intervals.

Figure 4-2: derivation women

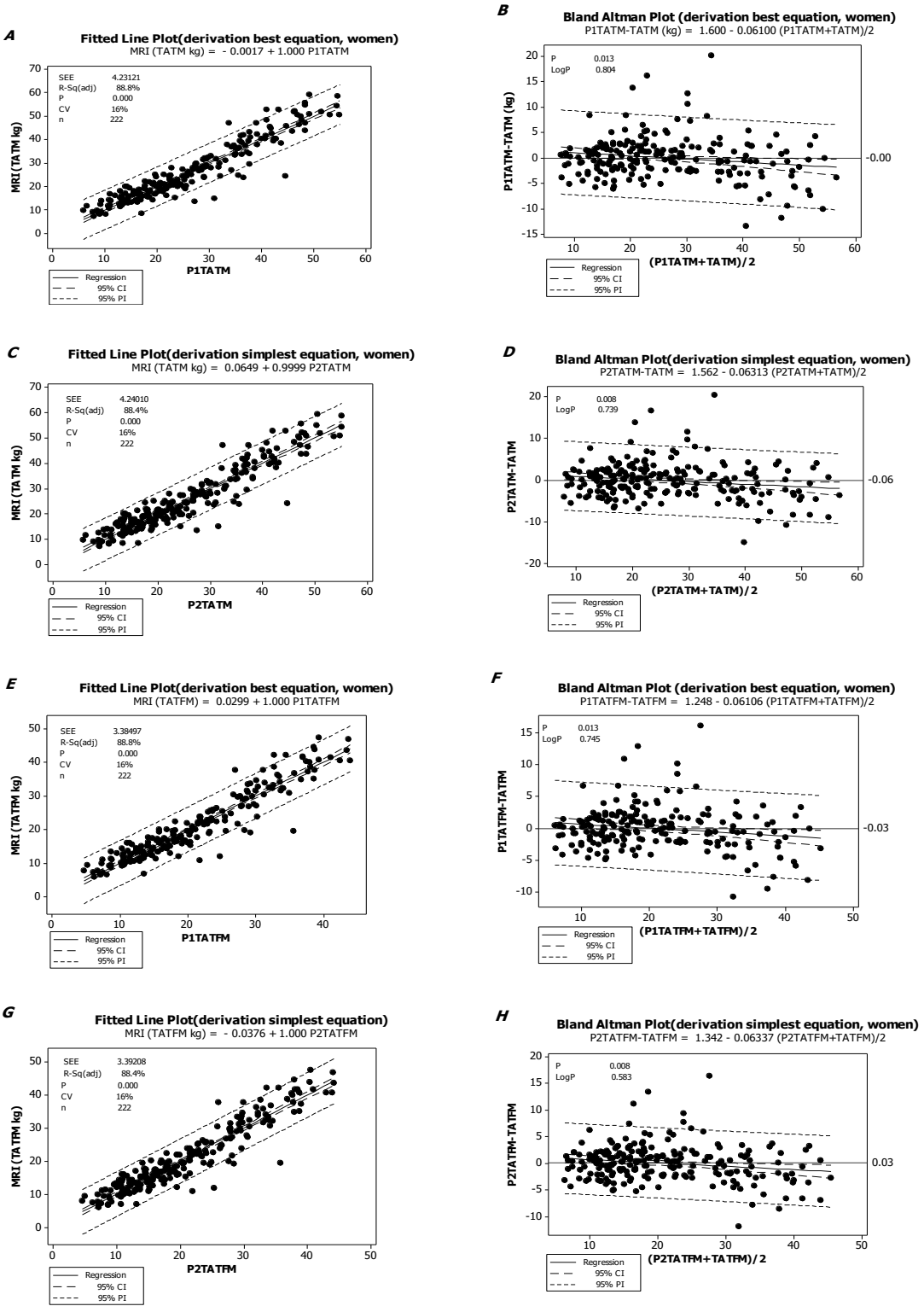
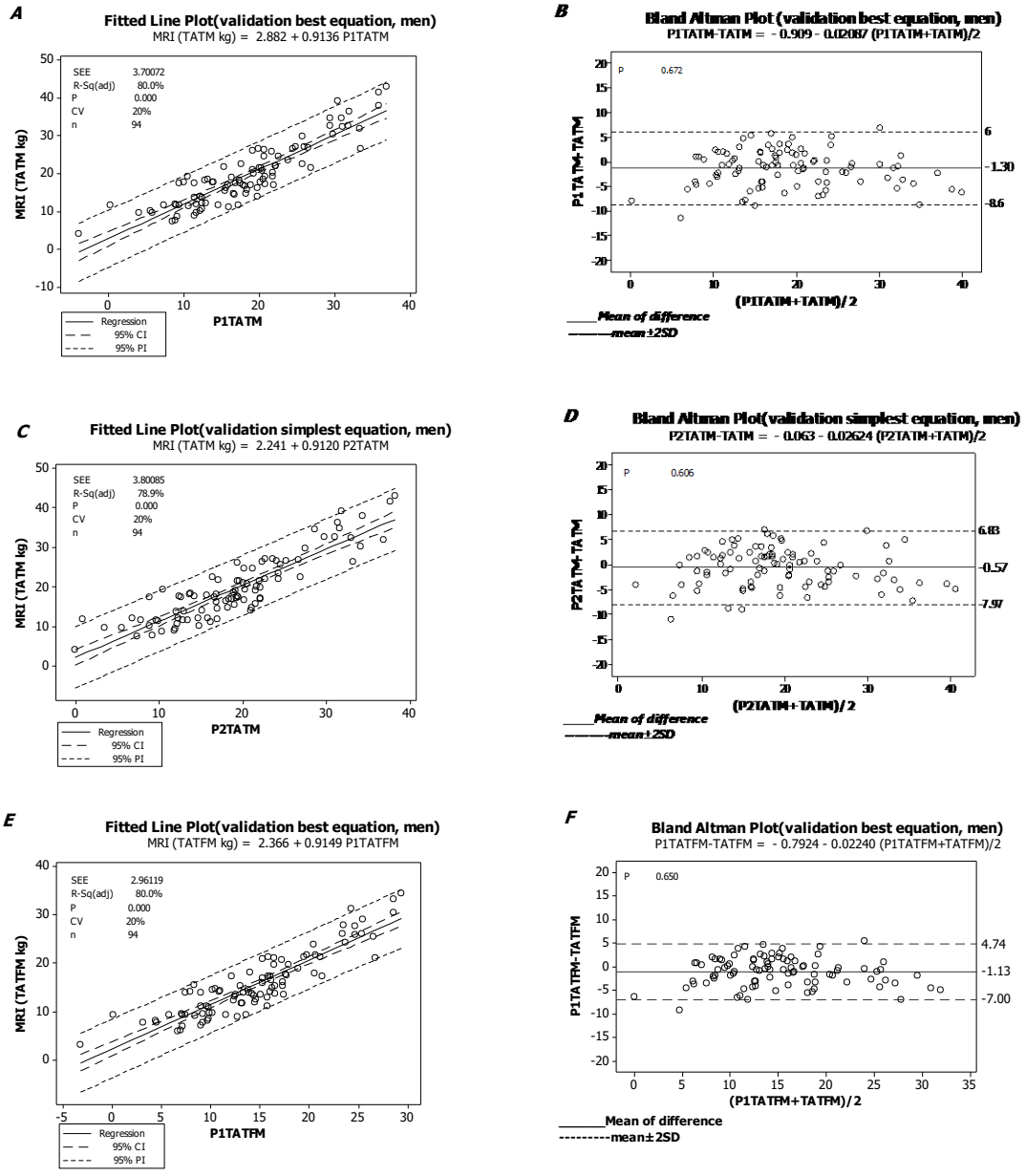
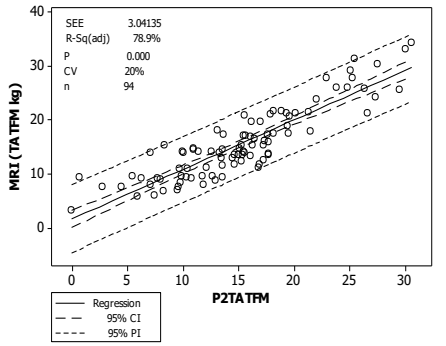


Figure 4-2 (derivation analysis women): Column 1 (A, C) are scatter-plots of MRI measured TATM (x-axis) against estimated TATM from prediction equations, while column 2 (B, D) are Bland Altman plots of difference between predicted and MRI-measured TATM (y axis) against their mean (x axis). Column 1 (E, G) are scatter-plots of MRI measured TATFM (x-axis) against estimated TATFM from prediction equations, while column 2 (F, H) are Bland Altman plots of difference between predicted and MRI-measured TATFM (y axis) against their mean (x axis). Lines represent mean difference of 0, regression and 95% confidence and prediction intervals.

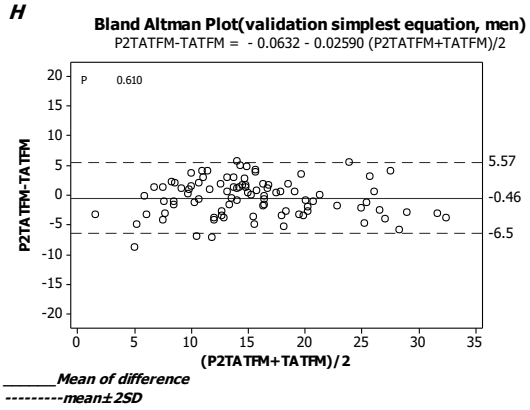
Figure 4-3: validation men



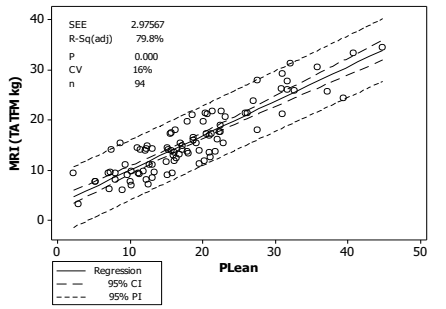
G


Fitted Line Plot(validation simplest equation, men)
MRI (TATFM kg) = 1.805 + 0.9116 P2TATFM
SEE 3.04135
R-Sq(adj) 78.9%
P 0.000
CV 20%
n 94
MRI (TATFM kg)
P2TATFM
Regression
95% CI
95% PI

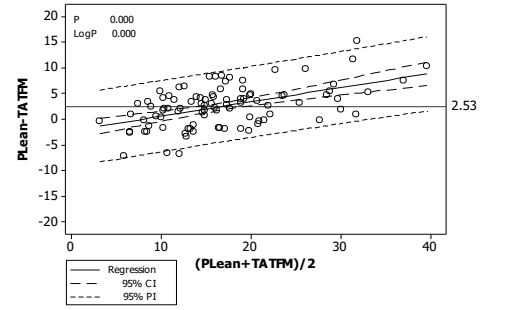
H


Bland Altman Plot(validation simplest equation, men)
P2TATFM-TATFM = - 0.0632 - 0.02590 (P2TATFM+TATFM)/2
P 0.610
5.57
-0.46
-6.5
P2TATFM-TATFM
(P2TATFM+TATFM)/2
Mean of difference
mean±2SD

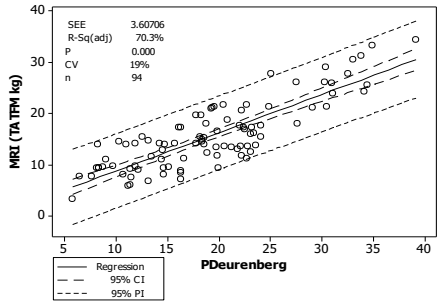
I


Fitted Line Plot(validation Lean et al, 1996 men)
MRI (TATFM kg) = 3.172 + 0.6862 PLean
SEE 2.97567
R-Sq(adj) 79.8%
P 0.000
CV 16%
n 94
MRI (TATFM kg)
PLean
Regression
95% CI
95% PI

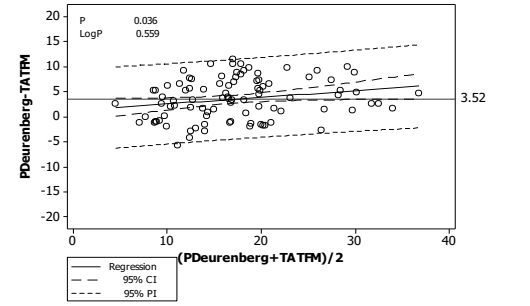
J


Bland Altman Plot(validation Lean et al, 1996 men)
PLean-TATFM = - 2.169 + 0.2781 (PLean+TATFM)/2
P 0.000
LogP 0.000
2.53
PLean-TATFM
(PLean+TATFM)/2
Regression
95% CI
95% PI

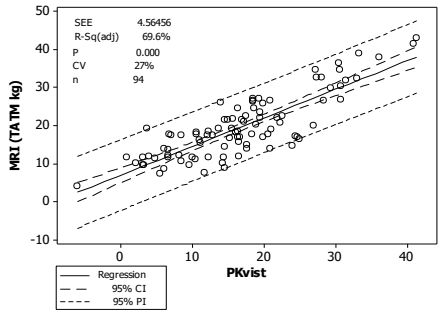
K


Fitted Line Plot(validation Deurenberg et al,1991 men)
MRI (TATFM kg) = 1.370 + 0.7449 PDeurenberg
SEE 3.60706
R-Sq(adj) 70.3%
P 0.000
CV 19%
n 94
MRI (TATFM kg)
PDeurenberg
Regression
95% CI
95% PI

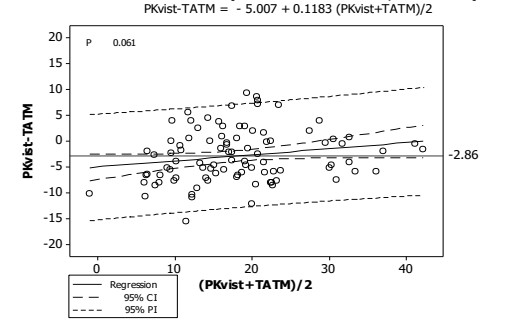
L


Bland Altman Plot(validation Deurenberg et al, 1991 men)
PDeurenberg-TATFM = 1.239 + 0.1311 (PDeurenberg+TATFM)/2
P 0.036
LogP 0.559
3.52
PDeurenberg-TATFM
(PDeurenberg+TATFM)/2
Regression
95% CI
95% PI

M


Fitted Line Plot(Validation Kvist et al, 1988 men)
MRI (TATM kg) = 7.037 + 0.7500 PKvist
SEE 4.56456
R-Sq(adj) 69.6%
P 0.000
CV 27%
n 94
MRI (TATM kg)
PKvist
Regression
95% CI
95% PI

N


Bland Altman Plot(validation Kvist et al, 1988 men)
PKvist-TATM = - 5.007 + 0.1183 (PKvist+TATM)/2
P 0.061
-2.86
PKvist-TATM
(PKvist+TATM)/2
Regression
95% CI
95% PI

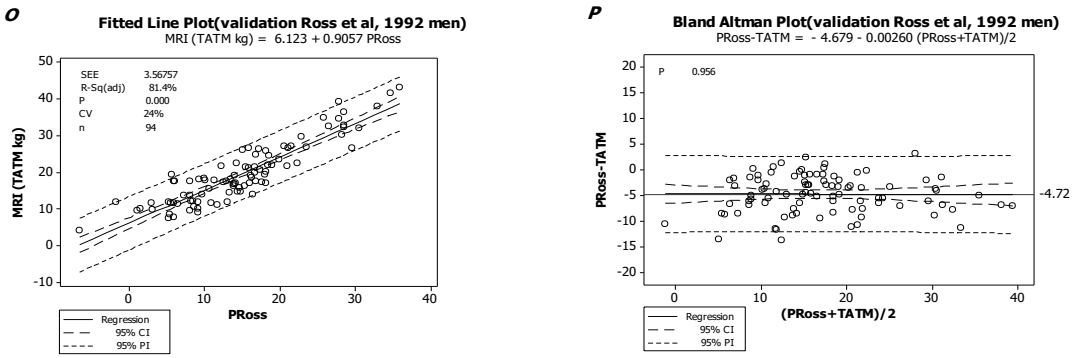
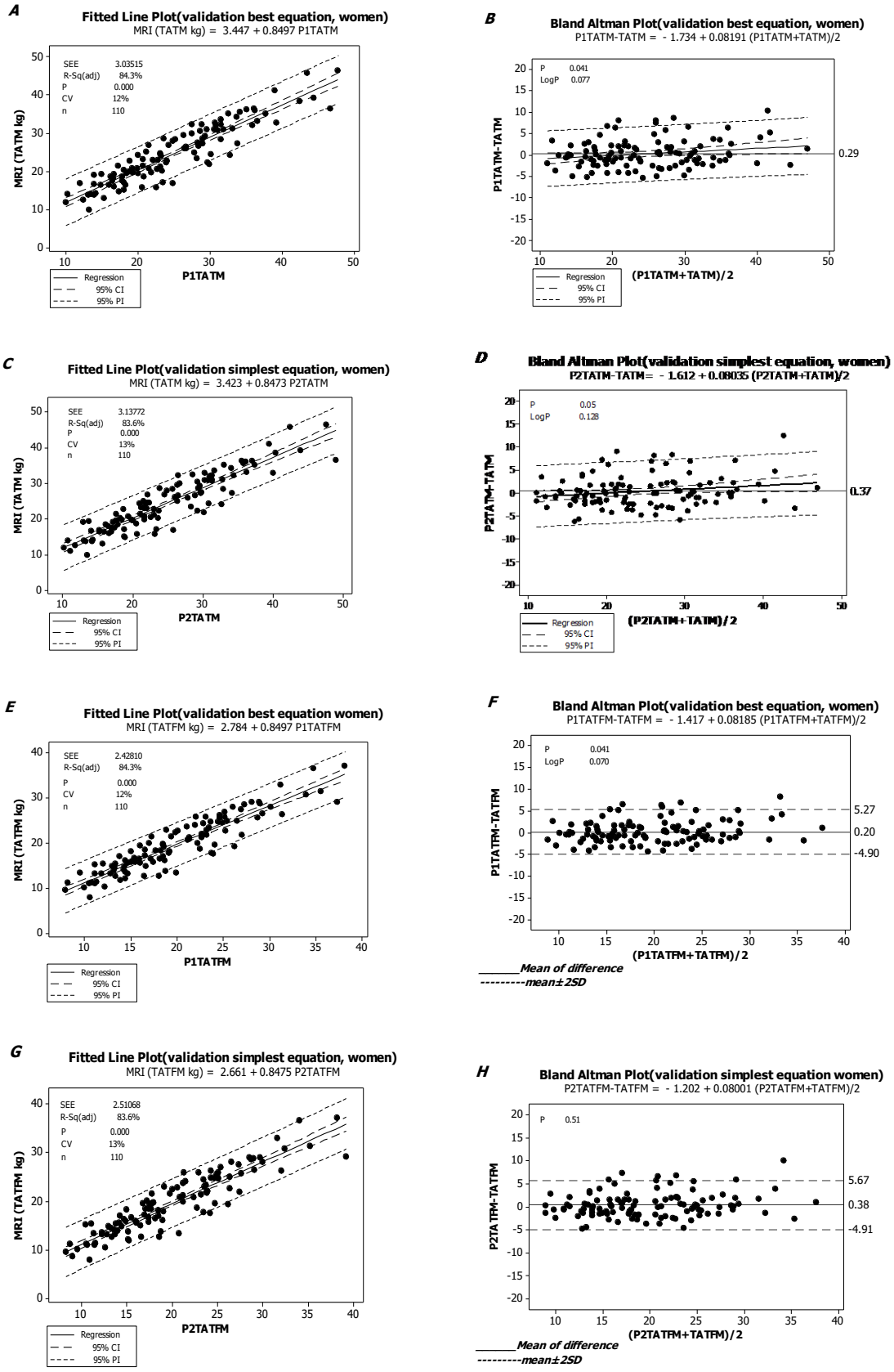


Figure 4-3: (validation men): Column 1 (A, C) TATM and (E, G) TATFM are scatter-plots of MRI measured (y-axis) against estimated TATM and TATFM from prediction equations, while column 2 (B, D) TATM and (F, H) TATFM are Bland Altman plots of difference between predicted and MRI-measured (y axis) against their mean (x axis). Plots (A, B) represent results from the validation of our best equation in men (P1TATM). (C, D) represent our validation of our simplest equation (P2TATM). Plots (E, F) are our validation of our best total adipose tissue fat mass equation (P1TATFM), and (G, H) our validation of our simplest total adipose tissue fat mass equation (P2TATFM). Plots (M, N) represent our validation of Kvist TATM equation (P-Kvist), Plots (O, P) represent our validation of Ross TATM equation (P-Ross). Plots (I, J, K, L) represent our comparison with Lean et al, 1996 and Deurenberg et al, 1991 total body fat equations. For the plots with no significant slope, Bland-Altman plots show the mean difference with limits of agreement around the mean difference a test for bias (mean difference significantly different from 0) using the one-sample t-test. For the plots with significant slope, Bland-Altman plots show the CI and PI around the regression line. P-values represent a test of significance of the slope.

Figure 4-4: validation women



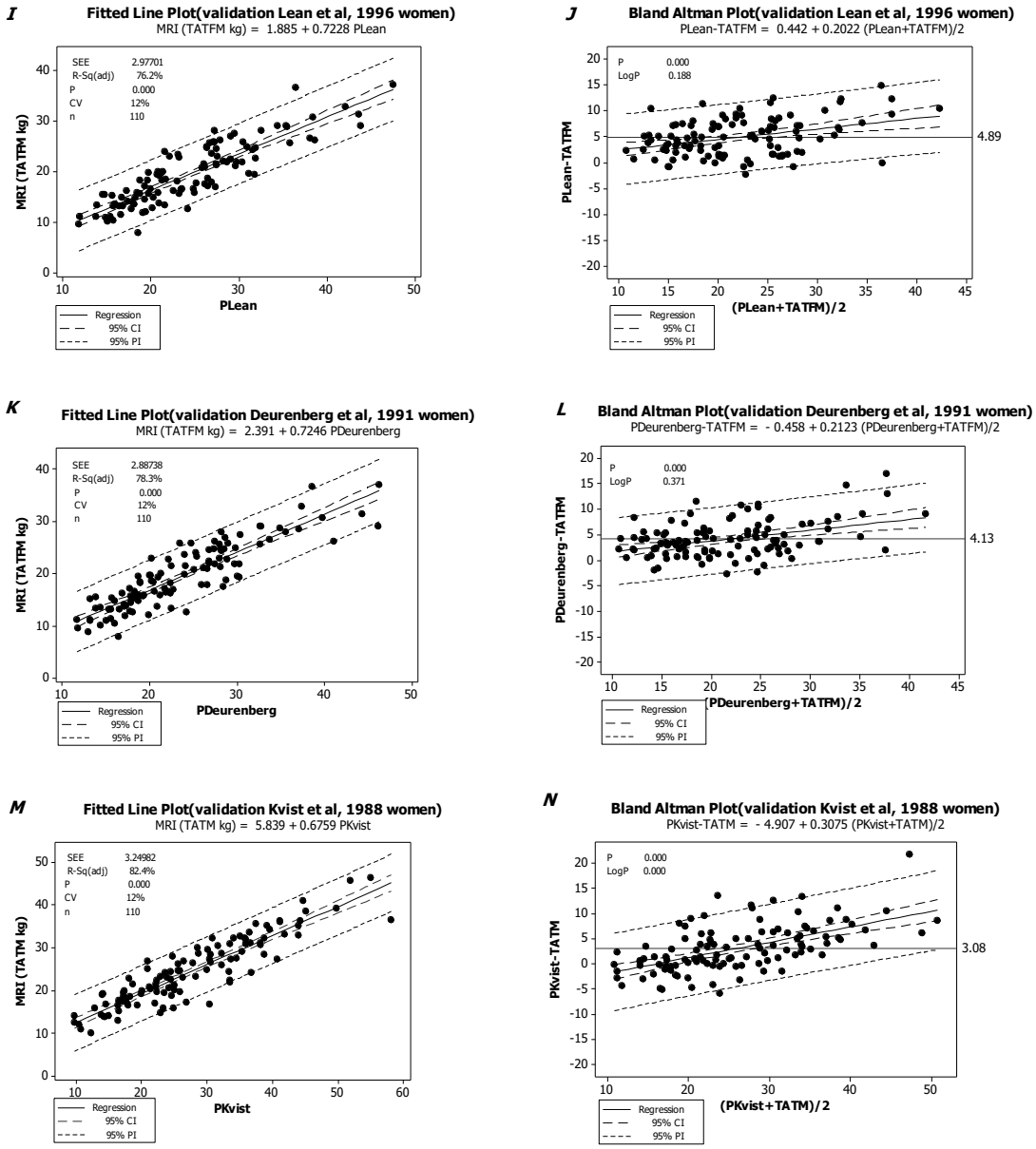
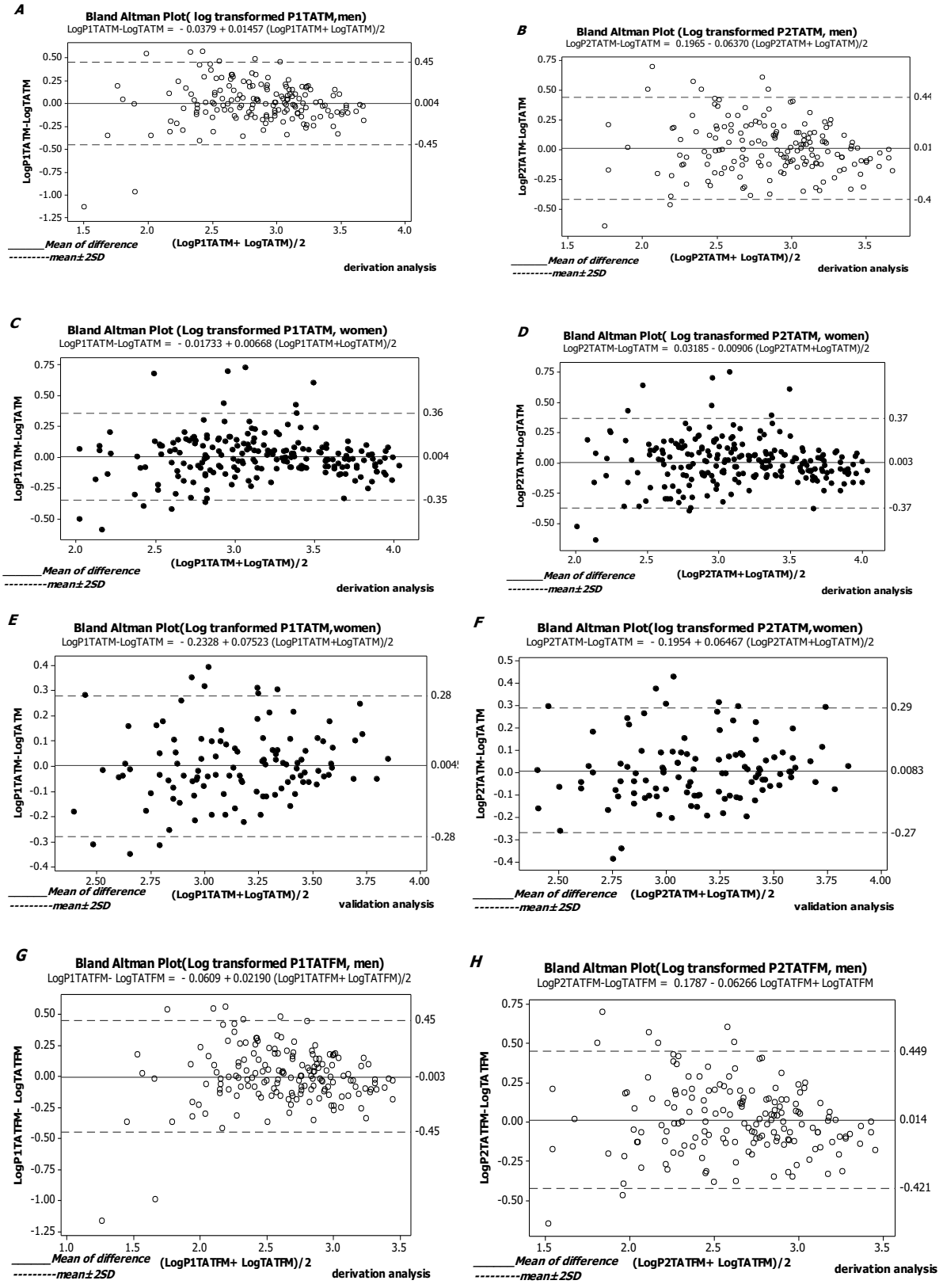
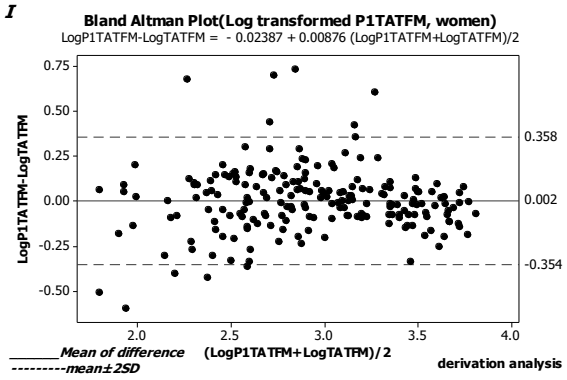


Figure 4-4 (validation women): Column 1 (A, C) TATM and (E, G) TATFM are scatter-plots of MRI measured (y-axis) against estimated TATM and TATFM from prediction equations, while column 2 (B, D) TATM and (F, H) TATFM are Bland Altman plots of difference between predicted and MRI-measured (y axis) against their mean (x axis). Plots (A, B) represent results from the validation of our best equation in women (P1TATM). (C, D) represent our validation of our simplest equation (P2TATM). Plots (E, F) is our validation of our best total adipose tissue fat mass equation (P1TATFM), and (G, H) our validation of our simplest total adipose tissue fat mass equation (P2TATFM). Plots (I, J) represent our comparison with (Lean et al, 1996) total body fat equation (P-Lean), Plots (K, L) represent our comparison with (Deurenberg et al 1991) total body fat equation (P-Deurenberg). Plots (M, N) represent our validation of (Kvist et al, 1998) equation (P-Kvist). For the plots with no significant slope, Bland-Altman plots show the mean difference with limits of agreement around the mean difference a test for bias (mean difference significantly different from 0) using the one-sample t-test. For the plots with significant slope, Bland-Altman plots show the CI and PI around the regression line. P-values represent a test of significance of the slope.

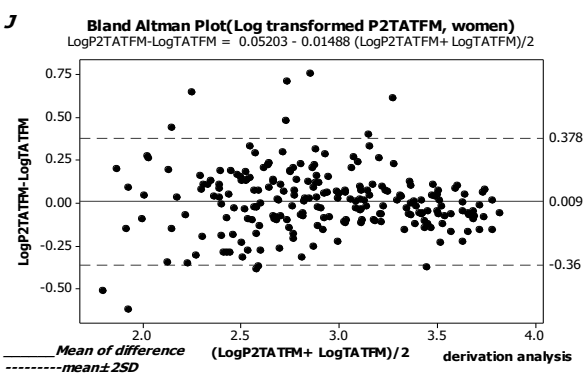
Figure 4-5: Log transformation



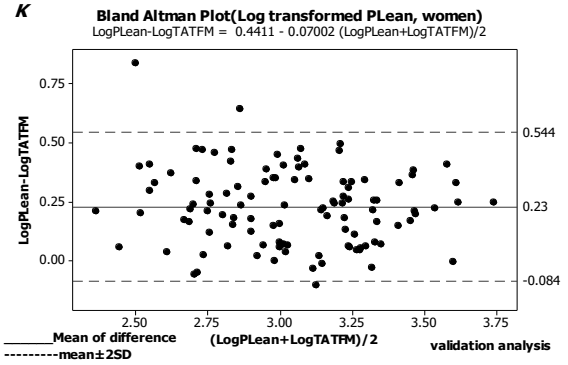
I


Bland Altman Plot(Log transformed P1TATFM, women)
LogP1TATFM-LogTATFM = - 0.02387 + 0.00876 (LogP1TATFM+LogTATFM)/2
LogP1TATFM-LogTATFM
0.358
0.002
-0.354
Mean of difference
mean±2SD
(LogP1TATFM+LogTATFM)/2
derivation analysis

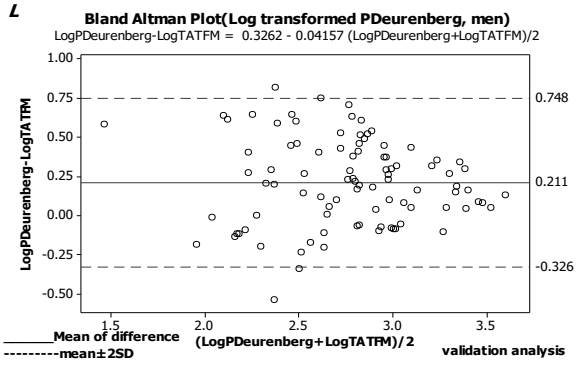
J


Bland Altman Plot(Log transformed P2TATFM, women)
LogP2TATFM-LogTATFM = 0.05203 - 0.01488 (LogP2TATFM+ LogTATFM)/2
LogP2TATFM-LogTATFM
0.378
0.009
-0.36
Mean of difference
mean±2SD
(LogP2TATFM+ LogTATFM)/2
derivation analysis

K


Bland Altman Plot(Log transformed PLean, women)
LogPLean-LogTATFM = 0.4411 - 0.07002 (LogPLean+LogTATFM)/2
LogPLean-LogTATFM
0.544
0.23
-0.084
Mean of difference
mean±2SD
(LogPLean+LogTATFM)/2
validation analysis

L


Bland Altman Plot(Log transformed PDeurenberg, men)
LogPDeurenberg-LogTATFM = 0.3262 - 0.04157 (LogPDeurenberg+LogTATFM)/2
LogPDeurenberg-LogTATFM
0.748
0.211
-0.326
Mean of difference
mean±2SD
(LogPDeurenberg+LogTATFM)/2
validation analysis

M

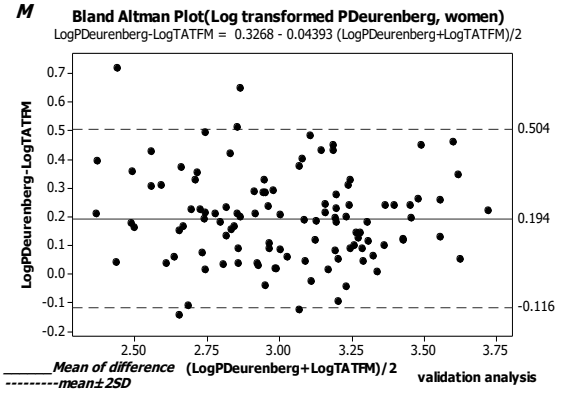

Bland Altman Plot(Log transformed PDeurenberg, women)
LogPDeurenberg-LogTATFM = 0.3268 - 0.04393 (LogPDeurenberg+LogTATFM)/2
LogPDeurenberg-LogTATFM
0.504
0.194
-0.116
Mean of difference
mean±2SD
(LogPDeurenberg+LogTATFM)/2
validation analysis

Table 4-1: Subject characteristics and variables

	Derivation Sample (n = 416)		Validation Sample (n = 204)	
	Men (n=194)	Women (n=222)	Men (n=94)	Women(n=110)
Age (yrs)	39.2 (13.9)	44.4 (16.2)	43.3 (15.9)	44.1 (16.4)
Body weight (kg)	79.8 (12.7)	67.1 (15.1)	77.4 (14.1)	66.3 (10.7)
Height (cm)	176.0 (6.8)	162.0 (7.2)	174.5 (7.3)	162.2 (5.9)
BMI (kg/m^2)	25.4 (3.7)	25.6 (5.5)	25.4 (4.0)	25.2 (4.1)
Hip circumference (cm)	99.7 (7.5)	101.2 (11.9)	97.5 (7.9)	99.6 (8.5)
Waist circumference (cm)	87.6 (10.7)	80.1 (13.4)	88.3 (12.1)	82.0 (11.1)
MRI SM (kg)	31.8 (5.5)	19.7 (3.4)	28.8 (5.8)	20.0 (3.4)
MRI TATM (kg)	18.4 (7.9)	25.6 (12.4)	18.4 (7.9)	25.8 (12.4)
Race:				
Caucasian (%)	41.4	42.9	36.6	33.6
African American (%)	29.0	31.9	26.9	36.4
Hispanic (%)	15	14.5	16.1	14.5
Asian (%)	14.5	11.1	20.4	15.5

Measurements reported as mean ± standard deviation, ---- BMI: body mass index (kg/m^2), MRI: magnetic resonance imaging, SM: skeletal muscle mass, TATM: total adipose tissue mass

Table 4-2: Explained variance (R2) from linear regressions

Variable		Women (R^2) %	Men (R^2) %	
	TATM mass	Muscle mass	TATM mass	Muscle mass
Age (yrs)	10.9	2.5	10.4	5.2
Body weight (kg)	82.8	38.4	58.6	54
Height (cm)	1.2	30.7	0.0	22.6
BMI (kg/m^2)	82.4	18.3	65.8	31.4
Waist circumference (cm)	77.5	16.5	76.8	11.6
Hip circumference (cm)	81.1	22.6	72.2	27.9
Waist/hip ratio	2.5	0.2	35.5	0.0
Mid-arm circumference (cm)	74.0	24.4	39.6	51.8
Mid-Thigh circumference (cm)	63.9	27.8	37.1	36.9
Mid-calf Circumference (cm)	21.0	12.4	29.9	44.5
Race*	9.4	16.36	6.02	15.89

Explained variance (R^2) in MRI total adipose tissue mass and whole body skeletal muscle mass, from simple linear regressions in the derivation study
*Race included four categories, Caucasians, African-American, Asian, and Hispanic. Race was analysed using general linear ANCOVA, TBAT: total body adipose tissue

Table 4-3: Prediction equations for total adipose tissue and total adipose tissue fat mass (men)

	Reference of PE	Reference method	Subjects	Prediction Equation	R^2 (%) derivation	R^2 (%) validation	SEE(kg)	CV (%)
Total adipose tissue mass	Present (P1TATM)	MRI	d (P): 194 v (P): 94	= - 0.128**BW** + 0.431**waist** + 0.607**hip**-69.0	0.82	0.80	d (P): 3.4 v (P): 3.7	18 20
	Present (P2TATM)	MRI	d (P): 194 v (P): 94	= 0.198**BW** + 0.478**waist** - 0.147**height**-12.8	0.79	0.79	d (P): 3.7 v (P): 3.8	20 20
	Ross[12]	MRI	D(R): 27 V(P): 94	= (1.003x**waist** – 56.475x **waist hip ratio** – 21.364) x 0.92	0.91	R:NR P:0.81	d (R):3.7 v (P):3.6	NR 24
	Kvist[13]	CT	d (K): 17 v (K): 7 v (P): 94	= (1.36**BW** / **height** [a] - 42.0) x 0.92	0.93	K: NR P: 0.70	d (K): 9.0% v (K): 11.4% v (P): 4.6	NR NR 27
Total adipose tissue fat mass	Present(P1TATFM)	MRI	d (P): 194 v (P): 94	=– 0.103**BW** + 0.345**waist** +0.485**hip**-55.2	0.82	0.80	d (P): 2.8 v (P): 3.0	18 20
	Present(P2TATFM)	MRI	d (P): 194 v (P): 94	= 0.158**BW** + 0.383**waist** -0.118**height**-10.2	0.79	0.79	d (P): 3.0 v (P): 3.0	20 20
	Lean[11]	UWW	d (L): 63 v (L): 146 v (P): 94	= (0.567**waist** + 0.101**age** – 31.8) x **BW**/100	0.78	L: 0.69 P: 0.80	d (L): 4.1 v (L): NR v (P): 3.0	NR NR 16
	Deurenberg[9]	UWW	d(D): 140 v(D): 148 v (P): 94	= (1.20**BMI** + 0.23**age** – 10.8 x 1 – 5.4) x **BW**/100	0.80	D:0.80 P:0.70	d (D): 4.2 v (D): 4.0 v (P): 3.6	17 16 19

Prediction equations for total adipose tissue mass and total adipose tissue fat (in kg) derived and validated in the present study, and comparison with existing equations published by Lean et al [11], Deurenberg et al [9] and, Kvist et al [13], Ross et al, [12]in men
PE: prediction equation, TATFM: total adipose tissue fat mass, TATM: total adipose tissue mass, UWW: under-water weighing, MRI: whole body magnetic resonance imaging, CT : whole body Ct-scan, P: present study, L: Lean et al, 1996 study, D: Deurenberg et al, 1991 study, R: Ross et al, 1992 study, BW: body weight, K: Kvist et al, study, NR: not reported, TATFM, TATM and BW in kg, , (waist, hip, height) in cm, BMI (kg/m^2) and age in years, a: height measurement in meter, d:derivation analysis, v: validation analysis.

Table 4-4: Prediction equations for total adipose tissue and total adipose tissue fat mass (women)

	Reference of PE	Reference method	Subjects	Prediction Equation	R^2 (%) derivation	R^2 (%) validation	SEE(kg)	CV (%)
Total adipose tissue mass	Present (P1TATM)	MRI	d (P): 222 v (P): 110	= 0.0726**age** + 0.634**BW** - 0.320**height** + 0.206**hip** +10.9	0.89	0.84	d (P): 4.2 v (P): 3.0	16 12
	Present (P2TATM)	MRI	d (P): 222 v (P): 110	= 0.789**BW** + 0.0786**age** - 0.342**height** + 24.5	0.88	0.84	d (P): 4.2 v (P): 3.0	16 13
	Kvist[13]	CT-scan	d (K): 10[b] v (K): 9[b] v (P): 110	= (1.61**BW** /**height** [a] – 38.3) x 0.92	0.96	K: NR P: 0.82	d (K): 6.8% v (K): 8.5% v (P): 3.2	NR NR 12
Total adipose tissue fat mass	Present (P1TOTAL BODY FAT)	MRI	d (P): 222 v (P): 110	= 0.0581**age** + 0.507**BW** – 0.256**height** + 0.165**hip** + 8.68	0.89	0.84	d (P): 3.4 v (P): 2.4	16 12
	Present (P2TOTAL BODY FAT)	MRI	d (P): 222 v (P): 110	= 0.631**BW** + 0.0629**age** – 0.273**height** + 19.6	0.88	0.84	d (P): 3.4 v (P): 2.5	16 13
	Lean[11]	UWW	d (L): 84 v (L): 238 v (P): 110	= (0.439**waist** + 0.221**age** -9.4) x **BW**/100	0.70	L: 0.75 P: 0.76	d (L): 4.7 v (L): NR v (P): 3.0	NR NR 12
	Deurenberg[9]	UWW	d(D): 216 v(D): 245 v (P): 110	= (1.20**BMI** + 0.23**age** – 10.8 x 0 – 5.4) x **BW**/100	0.80	D:0.80 P:0.78	d (D): 4.2 v (D): 4.0 v (P): 2.9	17 16 12

Prediction equations for total adipose tissue mass and total adipose tissue fat (in kg) derived and validated in the present study, and comparison with existing equations published by Lean et al [11], Deurenberg et al [9] and, Kvist et al [13], Ross et al, [12]in women; PE: prediction equation, TATFM: total adipose tissue fat, TATM: total adipose tissue mass, UWW: under-water weighing, MRI: whole body magnetic resonance imaging, CT : whole body Ct-scan, P: present study, L: Lean et al, 1996 study, D: Deurenberg et al, 1991 study, BW: body weight, K: Kvist et al, study. b: 9 women with ulcerative colitis were pooled with 10 healthy women, 19 women were ranked according to weight then allocated to derivation and cross-validation group, NR: not reported, TATFM, TATM and BW in kg, (waist, hip, height) in cm, BMI (kg/m ^{2}) and age in years, a: height measurement in meter, d:derivation analysis, v: validation analysis.

Chapter 5

5 Application in health surveys

Application of anthropometric prediction equations to data from Scottish and English National Health Surveys: associations of adipose tissue and skeletal muscle mass with diabetes/HbA1c and hypertension/blood pressure.

5.1 Abstract

Background: Associations between adipose tissue (AT) and skeletal muscle (SM) with metabolic illness and risk factors have only been poorly explored. The focus of the research literature has been on the relationships between metabolic risk factors and the anthropometric measurement of BMI, waist, WHR, waist height ratio (WHtR).

Aim: To explore the associations between the derived and validated AT and SM prediction equations with HbA1c, and blood pressure and with metabolic diseases. These prediction models will be compared to the older published prediction equations and anthropometric measurements.

Method: A cross-sectional analysis using data from the Scottish Health Survey (2003, 2008-2011) and Health Survey for England (2003-2006 – 2008-2013). A total of 32,657 men and 38,861 women were included in this analysis. ***Exclusion criteria***: age under 18 years, pregnant women and diabetic men and women on insulin. ***Anthropometrics and covariates:*** weight (kg), height (cm), BMI, waist and hip circumference (cm), age (years). ***Continuous outcomes:*** HbA1c (%), systolic and diastolic blood pressure. ***Categorical outcomes***: Type-2 diabetes mellitus, hypertension and both conditions are described as the combined 'metabolic illness'.

Prediction equations: the following equations were used to estimate AT and SM, expressed as % body weight (AT or SM / body weight x100): (Lee et al., 2000b) skeletal muscle prediction equation (SMLR), (Lean et al., 1996) body fat prediction equation (BFML), waist hip ratio (WHR), Waist/height ratio (WHtR) and body mass index (BMI).

Statistical analysis multiple regression was used to establish the best model fit for HbA1c and systolic and diastolic blood pressure measures. Binary logistic regression analysis was used to determine the associations between predicted AT, SM /anthropometry and categorical values diabetes, hypertension and metabolic illness.

Results: the new equations showed significant correlations with type-2 diabetes, hypertension and metabolic disease, HbA1c, systolic and diastolic blood pressure ($P<0.0001$). A high coefficient of determination with existing prediction equations and with anthropometric measurements was found: The correlations between BMI and %AT were; R^2 = men 87.4%, women 95.2%; and with %SM, R^2 = men 81.5%, women 81.1%.

In general, the coefficient of determination in this analyses were close, WHtR was the best predictor for HbA1c (R^2 = men 17.3%, women 21.2%). Whereas, %SM was the strongest for systolic blood pressure (R^2= men 9.8 and women 28.4). BMI had the highest coefficient of determination with diastolic blood pressure (R^2= men 6.3 and women 8.8). For the associations with 'metabolic disease', WHtR was again the best predictor (R^2 = men 16.8%, and women 24.4%).

When the population was divided by quintiles of %AT and of %SM, there were striking increases in Relative Risks (RR) of type-2 diabetes and of hypertension from Q2 to Q5 of %AT (men: T2DM RR=15, hypertension RR=3.9; women T2DM RR=23.6, hypertension RR=5.5). There were similar progressive increases in RR as %SM fell from Q1 to Q5 (men: T2DM RR=4.9, hypertension RR=3.2; women T2DM RR=9.9, hypertension RR=4.9). The prediction equations for %AT and %SM did not identify the same individuals with T2DM or hypertension in the quintile groups at highest risk.

Relative risk of BMI and WHtR increased with increase quintile. However, compared to the highest %AT quintile, BMI showed lower relative risk specially in diabetes, BMI RR=5.4, %AT RR=15 in men and BMI RR= 9.2 compared to %AT RR=23.6 in women. WHtR was close to %AT RR=22.4 and 23.6, yet, %AT was slightly higher than WHtR.

Conclusion: There are many predictors of metabolic ill-health in addition to BMI, which is still used almost exclusively in national health surveys. Both adipose tissue and skeletal muscle mass are significantly associated with metabolic disease, HbA1c, systolic and diastolic blood pressure and can be determined from

data routinely collected from health surveys. The inverse relationship between the prevalences of type-2 diabetes, hypertension and metabolic disease and the quintiles of %SM, and vice versa for %AT in both men and women suggest a protective role from skeletal muscle mass, independent of the risks from greater adipose tissue mass. However, %AT appears a more useful indicator for disease than %SM, BMI and to an extent WHtR.

5.2 Introduction

Epidemiological and health survey studies have advanced our understanding of risk factors for metabolic illnesses. Understanding the associations of body compartments and fat distribution with metabolic disease helps in determining causes of disease.

BMI has been used extensively to define the relationship between metabolic disease and body composition (Lim et al., 2011, Glogner et al., 2014, Logue et al., 2013, Sone et al., 2007). However, BMI has led to many uncertainties. In a systematic review (Romero-Corral et al., 2006), studied the association between mortality, obesity and cardiovascular disease events in patients with coronary artery disease. A total of 40 studies which included, 250,152 patients were used in the systematic review, with a mean follow-up 3.8 years. Overweight patients (BMI 25 – 29.9 kg/m^2) had the lowest risk of total mortality (RR = 0.87 [95%CI: 0.81-0.94]). Obese patients (30 – 35kg/m^2) and severe obese (>35kg/m^2) had no elevated risk for total mortality (RR=0.93 [95%CI: 0.85-1.03]) (RR=1.10 [95%CI: 0.87-1.41]) respectively. The highest risk for cardiovascular mortality was in the severely obese group (RR=1.88 [95%CI: 1.05-3.34]). The authors concluded that the inability of BMI to distinguish between lean and body fat could be a reason for these unexplained results. On the other hand, a large prospective collaborative study (Whitlock et al., 2009) investigated the association between BMI and overall and cause-specific mortality (vascular, diabetic, renal, hepatic, respiratory and neoplastic). A total of 894,567 men and women from 57 prospective studies who were followed for mean 8 ±6 years. For overweight men (BMI >25kg/m^2) there was a positive association, for each 5 kg/m^2 increase there was 30 % increase in total mortality (hazard ratio:1.29(1.27-1.32) and 60 – 120% for diabetic mortality (HR:2.16(1.89-2.46)). For BMI below 22.5kg/m^2 had inverse association with overall mortality. The study concluded that BMI on its own is inversely associated to and a strong predictor of overall mortality in adults with existing chronic disease.

Despite the common use of BMI, it is a poor measure of fatness because it is unable to differentiate between body compartments (Frankenfield et al., 2001,

Burkhauser and Cawley, 2008). As an alternative, waist circumference has been used to study associations with chronic health conditions (Han et al., 1995, Lean et al., 1995). Studies conclude that there is a significant relationship between waist circumference and metabolic illness, in general, the relationship is a little stronger than BMI. An Australian cohort study (to study the effect of waist circumference in predicting type-2 diabetes), included 803 diabetes-free adults (18 – 76 years), baseline data was collected between 1992 – 1998 and adults were followed up for 20 years. Waist circumference was strongly associated with the presence of type-2 diabetes ($P<0.001$), 1-unit increase in waist circumference had a type-2 diabetes hazard ratio of 1.7, (95%CI:1.3 – 2.2) in men and 2.1, (95%CI:1.7 – 2.6) in women (Adegbija et al., 2015). Using the same population, Wang et al found waist circumference to be a better predictor of cardiovascular disease than BMI, hip circumference and WHR using a Cox regression model. The rate ratio was 1.31 (95% CI: 1.11, 1.54), 1.29 (95% CI: 1.09, 1.53), 1.28 (95% CI: 1.08, 1.52) and 1.10 (95% CI: 0.93, 1.30) respectively, per one standard deviation increase in waist, BMI, hip and WHR (Wang and Hoy, 2004).

The following studies included a range of different (cross-sectional study) in Iranian population comprising 1000 adults aged between 20 – 80 years, BMI 21.3 – 47.5kg/m^2, included anthropometric measurements and fasting blood sugar concentrations. Overall, the prevalence of diabetes was 14%. Receiver operating characteristic curves (ROC) were used to estimate area under the ROC curve [AUC] to allow comparison of the strength of associations between parameters. For men the values were: Waist circumference =0.64, WHtR (AUC = 0.63), BMI (AUC = 0.62) and WHR (AUC = 0.60). Women: WHtR (AUC = 0.69) and WC (AUC = 0.68) were better than BMI (AUC = 0.67). In general, waist circumference was a better predictor of diabetes than BMI alone (Hajian-Tilaki and Heidari, 2015). Likewise, in a Cameroonian population (Mbanya et al., 2015) waist circumference was a better predictor for type-2 diabetes, (OR: 1.30 95%CI: 1.16-1.46) than BMI (OR: 1.05, 95%CI: 0.98 – 1.13).

Feng et al, analysed a north Chinese population (n = 8940) and found BMI to be a better predictor of hypertension (RR: 2.35, 95% CI: 2.18-2.50), and waist

circumference was a better predictor for risk of developing type-2 diabetes (RR: 2.05, 95%CI: 1.63-2.55) (Feng et al., 2012).

In the Framingham study (n = 4195, 53% women) logistic regression was used to evaluate the predictive power of waist circumference in the BMI categories of normal, overweight and obese to predict cardiovascular disease. Waist circumference was able to identify risk of first cardiovascular event in overweight women (OR: 1.86, 95%CI: 1.03-3.36) p = 0.04 only, not men (OR: 0.91, 95% CI 0.60-1.38), p = 0.66. Waist circumference did not appear to add to the strength of prediction of risk in all other BMI categories for men and women (Freiberg et al., 2008).

A few studies have included hip circumference as a predictor for metabolic illness. A Swedish cohort (Lissner et al., 2001b) studied the health risks associated with hip circumference, stressing the importance of hip circumference as an independent inverse risk estimator. In a systematic review (Cameron et al., 2013) examined evidence from studies which reported the associations between hip and/or waist circumference and the risk of developing diabetes, cardiovascular disease and mortality. The review indicated that hip circumference was as important as waist circumference in the assessment of obesity related health risk.

A random sample of 12,905 men and women between the ages 20-95 years, from the civil registries of Amsterdam were analysed to examine relationships between smaller hip circumference and health and lifestyle (Han et al., 1998). They found that smaller hip circumference was significantly associated with smoking, sedentary life style, increased age, high parity and lower education/achievement. They propose a smaller hip circumference may indicate gluteo-femoral muscle atrophy, thus acting as an additional predictor for the development of diabetes.

The use of BMI, waist, WHR and WHtR have all been as proxies for body composition. There have been other more specific prediction equations for body fat and for skeletal muscle mass. There has been no previous attempt to predict metabolic diseases, or risk factors, using more specific measurements of body

composition by estimating adipose tissue and muscle mass from simple anthropometric measures and determining their association(s) with metabolic disease.

The aim of this chapter was to explore in a number of representative health adult survey carried out in the UK database the relationships of the newly derived and validated prediction equations for SM and AT, and SM:AT, AT:SM ratio, with risk factors (HbA1c, systolic and diastolic blood pressure), and with diabetes and or hypertension. In addition, we aimed to compare coefficient of determination for the prediction equations devised as part of the thesis work (chapters 3,4) with those existing prediction equations and anthropometric indices. The strength of association between anthropometric measures and risk of disease is fair, however, it may be that using these anthropometric data to determine AT and SM may elicit stronger relationships, given the increasing evidence that estimates using prediction equations are valid. According to the published literature, this is the first analysis to determine these relationships.

5.3 Methods

5.3.1 Data collection

Study population*:* This cross-sectional analysis utilized data from two surveys of adults: 1) the Scottish Health Survey (SHS) (2003, 2008 till 2011) n=92,216, and 2) Health Survey for England (HSE) 2003-2006 and 2008-2013 n=140,627, total sample size was 232,841 adults. After data cleaning and the removal of data from subjects who failed to meet the inclusion criteria, final sample size of 125,256 (n= 55,878 men and 69,378 for women). As this study is a 'complete case analysis', any subjects with missing anthropometric measurements (weight, height, waist, and hip) were removed, and therefore the final sample size was 32,657 men and 38,861 women **(Table 5-1), (Figure 5-1).**

Inclusion criteria*:* non-pregnant female, age ≥18 years, those with type-2 diabetes not on insulin therapy, adults without type-2 diabetes, and hypertensive/non-hypertensive.

Outliers Excluded: Adults with these unlikely characters were excluded: weight < 45 kg in men and < 35 kg in women, height <146 cm in men and <135 cm in women, HbA1c <4% and >15%, diastolic blood pressure <40 mmHg and >120 mmHg, systolic blood pressure <85 mmHg and >199 mmHg, waist <64 cm and >155 cm, hip <65 cm, >180 cm, (**Table 5-1**).

Anthropometry and covariates: Participants were interviewed by a trained individual, either a nurse or trained interviewer, who measured weight (kg), height (cm), waist and hip circumference (cm) and recorded medication use. Age (years) was self-reported according to the participants last birthday.

Waist and hip circumference: Participants were asked to wear light clothing and stand straight and in a relaxed position, breathe normally and balance their weight evenly on both feet (25 – 30 cm apart). Arms were relaxed loosely by the subject's side. For waist measurements, the tape was positioned horizontally midway between the iliac crest and costal margin or lower rib. Measurement was recorded on exhalation. Nearest millimetre measurement was recorded, for accuracy measurements were taken in duplicate; a third measurement was taken only if the first two measurements were more than 3 cm apart. Hip circumference was defined as the widest circumference around the buttock and below the iliac crest, with tape positioned horizontally. Measurement to the nearest millimetre was recorded and repeated twice.

Continuous outcome variables: HbA1c (%) was measured using non-fasting blood samples taken by the nurse. The right arm blood pressure (systolic and diastolic blood pressure) was measured using Omron HEM 907 blood pressure monitor. Three readings were recorded at one-minute interval for the upper right arm, left arm only used when right arm not possible. Participants were seated leg uncrossed, their feet flat and they remained quiet for 5 min before the measurements were made.

Categorical outcome variables: Diabetes mellitus was recorded in three ways, doctor/nurse diagnosed, patient reported receiving treatment with oral anti-

diabetes medication and/or newly diagnosed on the basis of HbA1c>6.5%. Hypertension was reported by the subject as having been diagnosed by doctor/nurse, systolic blood pressure ≥140mmHg, Diastolic blood pressure ≥90mmHg Use of antihypertensive drugs was not included because these drugs are also prescribed for other conditions.

Treatments: If subjects responded yes for hypertension or diabetes; the nurse asked whether they were taking any medication for diabetes and/or hypertension. Treatments were recorded for diabetes as (Insulin injection, diabetes medicine, treatment for diabetes mellitus. Blood pressure treatment was recorded as medicine for blood pressure, other treatments for blood pressure) for diabetics and (medicine for blood pressure, other treatment for blood pressure) for hypertensive. Among insulin-treated adults, it was unclear who had type one and who had type-2 diabetes. Since most patients with type-2 diabetes do not take insulin injections, to avoid confusion in our analysis we removed participants taking insulin injections.

Metabolic disease: for the purpose of this chapter subjects that had diabetes and/or hypertension were combined as having 'metabolic disease'.

5.3.2 Data Cleaning and Statistical Analysis

Data were analysed using Minitab® 17.2.1. To start, sample was cleaned by removing extreme Outliers considered implausible (**Table 5-1**). Data was cleaned to exclude these implausible values (1 implausible combination of anthropometric indices which resulted in negative %SM was removed). The analysis was a complete case analysis, therefore all subjects with missing anthropometric data (weight, height, waist and hip) were removed **(Figure 5-1).**

Characteristics of included subjects are presented as mean (sd) **(Table 5-2).**

Binary logistic regression was used to calculate odds ratio (OR) and 95%CI in order to assess risk of diabetes and hypertension in relation to estimated muscle and adipose.

To compare the coefficient of determination (R^2) with outcome measures between prediction equations and anthropometric measurements, multiple regression analyses was used, adjusted for age and hypertension for the HbA1c analysis, and adjusted for age and diabetes for the blood pressure analyses.

Hosmer- Lemeshow is the statistical test used to assess goodness of fit for the logistic regression models. The test is used to assess how well the model fits the data across the full range, if the p value is less than 0.05, the null hypothesis is rejected, meaning the predicted value deviate from the expected value. Hosmer-Lemeshow groups the data by their estimated probabilities from lowest to highest, then it performs a Chi-square to conclude if the observed and expected values are significantly different. A large p-value indicates that there is consistency between the observed and expected values (Hosmer and Lemeshow, 2013).

Analyses were performed separately according to gender. Analysed data were naturally log transformed to sustain a normal distribution, with the exception for the binary logistic regression analysis, where data were not log transformed. A p-value <0.05 was considered significant.

5.3.3 Prediction Equations:

For the estimation of adipose tissue, muscle and body fat, the following prediction equations were used:

Adipose tissue prediction equation:

1. (Al-Gindan et al., 2014a); *(men***)** Total adipose tissue (kg) = 0.198 body weight (kg) +0.478 waist circumference (cm) - 0.147 height (cm) - 12.8, *women*: TATM(kg) = 0.789 body weight (kg) + 0.0786 age(y) - 0.342 height (cm) + 24.5.

To estimate percentage muscle mass and percentage adipose tissue the following equations were used: (muscle or adipose (Kg) /total body weight) x 100. The ratio %muscle mass/%adipose tissue was used to quantify SM:AT ratio.

Muscle mass prediction equations:

1. (Al-Gindan et al., 2014a); (**men**) SM (kg) = 39.5 + 0.665 body weight (kg) − 0.185 waist circumference (cm) − 0.418 hip circumference (cm) − 0.08 age (y). For (**women**): SM (kg) = 2.89 + 0.255 body weight (kg) − 0.175 hip circumference (cm) − 0.038 age (y) + 0.118 height (cm)

2. (Lee et al., 2000b) SM(kg) = 0.244Weight + 7.80 Height(m) + 6.6 sex (1 men, 0 women) - 0.098 age + (race) - 3.3

Percentage body fat prediction equation:

1. (Lean et al., 1996) Body fat (%) = 0.567 waist (cm) +0.101 age (years) – 31.8, for women: BF (%) = 0.439 waist (cm) +0.221 age (years)-9.4

BMI: body mass index = weight(kg)/(height(m))^2, WHtR: waist height ratio = waist (cm)/height (cm), WHR: waist hip ratio was computed as waist (cm)/hip(cm), to examine correlations and associations with HbA1C, systolic pressure, diastolic blood pressure, hypertension and diabetes.

5.4 Results

5.4.1 Subject Characteristics

The numbers of men and women with data for 'complete case analyses' are shown in **(Figure 5-1**). Subject characteristics are presented in (**Table 5-2**). Subject age ranged from 18-104 years, mean(sd): 51.1(17.2) and 50.5(17.2) for men and women respectively, weight ranged between 45.3-188kg in men, mean 84.3kg and in women weight ranged from 35.9-172kg, mean 71.3 kg **(Table 5-2), (Table 5-3).**

A total of 6.6% of men were recorded as diabetic and 38.7% as hypertensive, 4.5% women were recorded as diabetic and 32.9% hypertensive. Using the new %AT and %SM prediction equations, mean estimated muscle mass was 35.1% in men and in

women 28.2% body weight. Mean total adipose tissue was estimated as 29.4% in men and 40.2% in women.

5.4.2 Correlation and association of the new prediction equations with published prediction equations and anthropometric measurements

Al-gindan %AT and %SM prediction equations had a strong correlation and therefore association with already available prediction equations and anthropometric indices. SM prediction equations had high association (Lee et al,2000), R^2 = 81.5% and 81.1% for men and women respectively. Adipose tissue and % body fat had high correlations in both men and women R^2 = 86.9%, 71.4% respectively. BMI was highly correlated with the new equations R^2 = 87.4% and 95.2% for men and women (**Table 5-4**). The poorest association was between the %ATYY and the WHR, whereas the other R^2 values were close, in the 80-90% region.

5.4.3 Ranking model fit of the new prediction equations/published prediction equations/ anthropometric measurements with HbA1c, systolic and diastolic blood pressure

To test how well the new prediction equations, and other published prediction equations, fit the analysed data, multiple regression analyses were used, separately for men and women, and adjusted for age, presence of type-2 diabetes and/or hypertension, to avoid confounding. It was possible that those participants with diabetes might have hypertension and vice versa.

Adjustment for smoking was explored, but the analyses suggested a non-significant effect on HbA1c, systolic and diastolic blood pressure and metabolic disease (data not shown).

All the anthropometric measures and equations were predictive, generally explaining almost 10-29% of the variance in outcomes. Overall, there was little to

choose between the different measures and equations, and no clear ranking emerged. The exception was that hip was consistently poor.

For HbA1c, all relationships showed a significant R^2, accounting for up to 23% of the variance. WHtR had the highest R^2 = 17.3%, 23.0% for both men and women respectively.

For systolic blood pressure, there were general rather higher R^2 values for women than for men. SMLR (%) had the highest coefficient of determination for both men R^2= 9.8% and women R^2=28.4%, but others and notably BMI were close to this.

For diastolic blood pressure BMI had the highest correlation for both men and women: R^2= 6.3 and 8.8% (**Table 5-5**).

5.4.4 Association between the equations in adults with diabetes

For the categorical responses, diabetes and hypertension, binary logistic regression was used to assess association between outcome and prediction equations. Diabetes was defined in both surveys in three different ways; when doctor/nurse reported, receiving oral anti-diabetes medication and those participants found to have HbA1c measurements of at least 6.5% and therefore were included as undiagnosed cases.

Out of the 32,327 men, 2144 had diabetes. Highest coefficient of determination was seen with WHtR (R^2 = 15.9). Yet, coefficient of determination for all prediction equations and anthropometric measures were statistically not different to each other. For every 1-unit increase of predicted %SM, the OR of diabetes decreased by 26% using (Lee et al, 2000), on the other hand, the (Al-gindan et al, 2014) prediction equation 95%CI crossed 1(1.0, 1.02) and the coefficient did not show the inverse relation **(Table 5-6).** Adipose tissue in percentage (ATYY) gave high odds ratio OR:1.14, as adipose tissue increased, odds of diabetes increased by ≈ 14%. WHtR had the highest R^2 with diabetes in men (R^2 = 15.9%) **(Table 5-6).**

Unlike men, women BFML% showed higher R^2 = 18.4%, in addition to WHtR R^2 = 18.8%. Like BFML (%), waist had good associations (odds ratio 1.06, R^2= 18.4%). SM showed high R^2 values with SMLR (%) equation R^2= 15.5%, for every unit increase in SM (%) the odds of developing diabetes decreased by 44% **(Table 5-7).**

5.4.5 Association between the new and published prediction equations with hypertension

Hypertension was defined by being reported by the subject to have been diagnosed by a doctor or nurse, systolic blood pressure ≥140 and diastolic blood pressure ≥90 mmHg. A total of 12,624 men and 12,804 women were included as hypertensive. For men, WHtR had the highest R^2 = 15.4% OR: 1.07. BMI and ATYY (%) were higher in women R^2 = 23.2% and odds ratio = 1.08 for ATYY (%) and 1.10 for BMI.

The inverse relationship between muscle and hypertension was evident in all SM equations (coefficient –ve). In general, SMLR (%) had slightly higher correlation and odds ratio values than SMYY (%), for every 1-unit increase in muscle mass percentage there was an 18% odds of decreased hypertension in men and 33% lower odds of developing hypertension in women **(Table 5-8), (Table 5-9).**

5.4.6 Prediction of metabolic disease: diabetes and/or hypertension.

The anthropometric variables used within all four prediction equations were all able to predict metabolic disease. All equations and anthropometric indices provided were significant $p<0.001$. Again WHtR provided the highest R^2 = 16.8% for men and 24.4% for women, minimally better than waist circumference alone or the anthropometric equations **(Table 5-10, Table 5-11).**

In men SMYY (%) had lower correlations compared to SMLR (%). The highest odds ratio for SM was that derived by the Lee et al equation (2000). The SM equation showed a 20% greater likelihood of developing metabolic disease for every unit decrease in %SM for men.

For women, every unit increase in %SM, odds of metabolic disease decrease by 36% using (Lee et al, 2000) prediction equation. BMI had slightly higher odds ratio and R^2 than ATYY (%) (OR = 1.12, 1.10) and (R^2= 16.7%, 16.5%) for men, coefficient of determination was the same as BMI in women R^2= 23.8%.

5.4.7 Prevalence of diabetes, hypertension and metabolic disease and mean HbA1c and blood pressure by quintiles of %AT and %SM

There were consistent gradients of risk for both diabetes and hypertension across the quintiles of %AT and %SM. Prevalence of diabetes was maximal in the fifth quintile of %AT and first quintile of %SM (16.5%, 13.4% in men and 11.8%, 10.9% in women, respectively). Hypertension was at its peak in the fifth quintile of %AT and first quintile of %SM (60.9% and 60.6 in men and 56.8%, 58.1% in women, respectively).

The highest mean HbA1c was seen in the fifth quintile of %AT (6.0% and 5.9% for men and women respectively). The lowest mean HbA1c for %SM was seen in the fifth quintile (5.3% for both men and women).

Likewise, mean systolic and diastolic blood pressure were lower with higher %SM and higher with lower %AT. The lowest %AT quintile had the lowest mean systolic and diastolic blood pressure compared to Q2, Q3, Q4 and Q5. The highest %SM quintile had the lowest mean systolic and diastolic blood pressure **(Table 5-12), (Table 5-13).** In general, results indicate that relationships are reversed, so %AT and %SM have opposite influences on metabolic risks and diseases.

Relative Risk of diabetes, hypertension and metabolic illness in men and women are shown in **(Table 5-14, Table 5-15).** Compared to Q1, relative risk of diabetes increased with increase %AT quintiles: Q2 = 2.5%, Q3 = 3.9%, Q4 = 7.9% and Q5 = 15% for diabetes in men. The same pattern was seen for hypertension and metabolic illness, however with the higher prevalence in Q1, RR in Q4 and Q5 were much lower than seen with type 2 diabetes (3.2% and 4.1% respectively).

In women RR for diabetes increased with %AT (2.8%, 6%, 11.8%, 23.6% for Q2-Q5, using Q1 as the baseline). RR of diabetes rose with reduced %SM quintile (9.9%, 5%, 2.9% and 1.7% in Q1, Q2, Q3, and Q4 of %SM, using Q5 as baseline) **(Table 5-14, Table 5-15).** The same pattern was seen for hypertension and metabolic illness with Q1 of diabetes much higher than Q1 of hypertension and metabolic disease 9.9% compared to 4.9% and 4.8% and in %AT 23.6% compared to 5.5% and 5.6%.

Across the five BMI quintile groups, prevalence of diabetes, hypertension and metabolic illness all increased with increasing BMI. Relative Risks were maximal the fifth quintile for type 2 diabetes (RR= 6.6% in men and 7.9% in women) and for both hypertension and metabolic disease (RR=2.5% in men and 2.6% in women) **(Table 5-16, Table 5-17).** Compared to relative risk of Q5%AT, BMI showed lower relative risk for diabetes: 5.4% compared to 15% in men and 9.2% compared to 23.6% in women. BMI relative risk for hypertension and metabolic disease were lower than %AT, however not as drastic as diabetes: RR (hypertension)= 2.5% for Q5 BMI and 3.9% for Q5 %AT, RR (metabolic disease) = 2.5% for Q5 BMI and 4.1 for Q5 %AT in men. Same pattern was seen in women. WHtR was close to %AT, both indicate higher RR for their fifth quintile compared to hypertension and metabolic disease **(Table 5-14, Table 5-15).**

Tables 5.18 and 5.19 show that, among the people with type 2 diabetes, using Q5 of %AT or Q1 of %SM will identify those with diabetes who have BMI below 30kg/m^2. To highlight the importance of the assessment of %SM, tables 5.20 and 5.21 report the number of subjects in the quintiles of %SM who are in different quintiles of BMI and waist. In men the first %SM quintile have 21.4% people with normal BMI from them 7.9 are diabetic. On the other hand, in the same %SM quintile 13.8% have waist circumference (Q2:88.29-94.9cm) 7.9% of them are diabetic. In women the numbers were lower, 0.78% of Normal BMI were present in the first %SM quintile, 10% of them are diabetic. There were 2.2% of women with waist circumference (Q2:76.51-83.3cm) in the first %SM quintile 5.3% of them were diabetic.

5.5 Discussion

The prediction equations for adipose tissue and muscle mass, developed and validated in chapters 3 and 4 were applied to a large UK population of adults, to determine their associations with indicators of 'obesity-related' metabolic diseases. Many significant relationships were found, but differences between the strength of associations for different equations were small. Because the overall prevalence of hypertension was much greater than type 2 diabetes, the associations with 'metabolic illnesses' with the combination of the two conditions, were essentially the same as for hypertension alone.

In this study, the significant inverse associations between SM (%) and SM:AT with diabetes, hypertension, HbA1c and blood pressure were dominant in all analyses. An inverse relationship between SM and diabetes has been reported previously in the literature. A cross-sectional study, carried out in Korean Asian adults, showed muscle mass was 2- to 4-fold lower in participants with type-2 diabetes (Kim et al., 2014) than those without. Another cross-sectional study (Srikanthan and Karlamangla, 2011), studied whether muscle mass was protective in improving glucose regulation among 13,644 subjects in the US National Health and Nutrition Examination Survey. An inverse association was evident from the lowest quartile to the highest quartile of 'skeletal muscle index' as measured by bioelectrical impedance. After adjusting for sex, age, race and obesity, a 10% increase in skeletal muscle index was associated with 11% reduction in insulin resistance HOMA-IR (95%CI: 6–15%) and a 12% decrease in prediabetes as defined by glycated haemoglobin (95% CI:1–21). These present analyses concur with existing relationships, suggesting that increasing muscle mass could be protective against the development of insulin resistance.

Secondly, SM in kg showed little association, with poor correlations and an absence of inverse relationships, however SM as percentage body weight showed strong associations with disease outcomes. This may reflect the fact that skeletal muscle mass can be elevated in obesity. Thus analyses in this chapter focused on SM and AT calculated as percentages of body weight. As in our analysis, (Srikanthan and

Karlamangla, 2011) found that calculated skeletal muscle mass, relative to body weight, (by dividing muscle mass and body weight and converting it to percentage) showed higher inverse correlation with insulin resistance and prediabetes.

Association of the outcomes of prediction equations with HbA1c and blood pressure

In general, women had stronger associations than men. Their coefficient of determination with systolic blood pressure and HbA1c were higher than diastolic blood pressure, while men had higher associations with HbA1c compared to systolic and diastolic blood pressure, diastolic blood pressure had the weakest associations.

In testing how good the prediction equations were in fitting with the data, WHtR showed highest coefficient of determination for HbA1c R^2= 17.3% in men and 23.0% for women, and SMLR was best for systolic blood pressure**.** BMI had the strongest associations with diastolic blood pressure in men and women although all were considerably weak R^2= 6.3% and 8.8 % respectively. Unexpectedly, as it may indicate muscle mass, hip circumference (alone) showed the lowest correlations in all the analysis. BMI, ATYY and BFML equations had almost the same correlations.

Association of the prediction equations with diabetes, hypertension and metabolic illness

WHtR has been explored lately in the literature showing promising associations. In a prospective cohort study, data from 10,258 men and women were pooled to assess associations between weight, BMI, waist circumference, WHR, WHtR and type-2 diabetes. They found stronger associations of diabetes with waist circumference and WHtR compared to BMI (Hartwig et al., 2016).

A meta-analysis and systematic review aimed to study the WHtR potential by comparing it to waist circumference and BMI in terms of strength of association to cardio-metabolic risk factors. The analysis was considerable, comprising data for 300,000 adults reported in the 31 included papers. WHtR to be superior over BMI, WHtR enhanced discrimination by 4-5% over BMI (Ashwell et al., 2012). Ashwell

warned that continuing to use BMI as the only indicator of health risk would mean that 10% of the UK population and more than 25% who are misclassified to be normal by BMI may not be alerted of health risks (Ashwell and Gibson, 2014)

Likewise, our analysis showed the superiority of WHtR in its relation with diabetes, hypertension and metabolic illness. In addition, WHtR has shown better associations than BMI in recognizing cardio-metabolic disorders $p<0.01$ (Kahn and Bullard, 2016) in 3071 non-diabetic men and women.

By investigating the prevalence of diabetes, hypertension and metabolic illness across the quintiles of %AT and %SM, we can see that increased %AT will increase prevalence of diabetes, hypertension and metabolic disease in men and women. On the other hand, the protective role of %SM is evident across the full range of quintiles for men and women.

The top quintiles of BMI, WHtR and of %AT all have the same number of people, that means that (by quintiles) the greatest number of people with diabetes are in the top quintile of %AT, indicating that %AT is a more useful predictor of disease. While the top quintile of BMI does identify a lot of people with diabetes, the top quintile of %AT identifies more. So there are people who have diabetes without a very high BMI who can be identified by their high %AT.

Whole body skeletal muscle and adipose tissue ratios

In this analysis we explored SM:AT and AT:SM ratio, computed using the new SM and AT prediction equations. The findings suggest that the higher SM:AT ratio is more protective against the development of diabetes and hypertension, and the lower AT:SM ratio is the more protective against diabetes and hypertension. SM:AT and AT:SM ratio may have value in determining risk of metabolic disease.

To date the limited literature on this element has been poorly investigated. One related attempt perhaps related attempt was to explore muscle mass visceral fat ratio (Kim et al., 2011). In the Korean Sarcopenic Obesity Study, 526 healthy adults

20 – 80 years old were enrolled, 68% women, using dual energy x-ray absorptiometry and computed tomography they measured muscle mass and visceral fat respectively then computed muscle-fat ratio. Their results show an independent negative association between arterial stiffness and metabolic syndrome. The same research group computed the opposite, visceral fat to muscle ratio and found it positively associated with insulin resistance, (Kim et al., 2004) in 114, nondiabetic, overweight and obese middle aged women. Higher visceral fat muscle ratio, was evident in older women, with higher triglyceride and insulin-AUC, and lower glucose insulin ratio, than women with low visceral fat muscle ratio. This chapter also showed an inverse relationship between SM:AT ratio and HbA1c/ blood pressure.

Study Limitations.

There are some important limitations to this present analysis. The new prediction equations were derived and validated in relatively small numbers of mixed-race North American adults. They have not yet been validated in other populations. The HSE and SHS databases include very large numbers of subjects who were mostly Caucasian, so they may have been more uniform in terms of body composition and anthropometry, which would weaken the estimations from the new equations. A database with a wider range of variables might tend to show stronger correlations, and the inter-relationships between prediction equations would probably vary. It would be unwise to declare from the present analysis that the ranking of predictions should lead to adopting one or other of those with the best predictions. This type of analysis needs to be conducted on other datasets with different racial and ethnic profiles. It is possible that larger studies in the future might define better prediction equations.

The Health survey of England and Scottish Health Survey were typical nationally representative health surveys, but the design of these rolling cross-sectional surveys changed, such that not all the measures were made for every subject in every year. Thus, there were more missing data than expected, this was especially true for hip and waist circumferences. In addition, it was not possible

to distinguish between type-1 and type-2 diabetes, and use of reported medication to identify hypertension could be misleading, given many drugs have multiple medical indications. An analyses of data after excluding patients on insulin treatment, explored who may have had type-1 diabetes, correlations and odds ratio increased. It can be speculated that this reduction in numbers may have weakened the relationships.

The present analyses examined the data only for linear relationships. It is possible that stronger non-linear relationships exist.

The present study is on a cross-sectional database. It has been shown previously that 'prediction' of current disease status is likely to be different to prediction of future disease incidence. For example, WHR was found to relate more strongly than waist circumference alone to type-2 diabetes in cross-sectional surveys, but not in longitudinal studies where waist had the stronger relationship. The explanation could be the recent fall in hips circumference (through loss of gluteal muscle mass) that often occurs with the onset of type-2 diabetes with the impact to elevate the WHR. However, a greater body fat content, indicated by high waist circumference but not by WHR, remains an important predictor of type-2 diabetes incidence (Lissner et al., 2001b, Han et al., 1995). Surprisingly, in this chapter hip circumference gave the lowest associations and odds ratios with metabolic disease. The SM equation (Algindan et al, 2014) includes hip circumference but had lower correlations of SM than the (Lee et al, 2000) SM equation, which is based mainly on demographic variables.

After reviewing the literature relative risk and R^2 were the choice for the statistical analyses in this section. Whilst there are other effective techniques to compare methods such as ROC curves, which look at cut offs for specific measurements, they measure sensitivity and specificity of predicting an outcome and plot them together. In these analyses we do not have specific cut-offs to determine, but considered the opportunities offered by relative risk to adjust for other key variables. ROC analysis might be useful for future investigations. Other

methods that have been used in the literature include hazard ratio and Cox which need a measure of time and odds ratio that has been used in our analyses.

Variables such as BMI, waist and WHtR are useful in public health surveys (to predict population levels of disease or risks, not to identify individuals), but not because those measures are simple predictors of adipose tissue or fat mass. BMI and WHtR contain other information and health-predictive components (height-related).

To gain information about disease development it is valuable to determine the influences of different compartments of body composition. These equations are better estimates of adipose tissue or fat mass, and of skeletal muscle mass, both of which may prove additional information for public health. It is clear from the results in this chapter that high %AT and low %SM are predictive of ill-health, for both men and women. In addition, mean HbA1c, systolic and diastolic blood pressure were higher in the highest %AT quintile and lower in highest %SM quintile for both men and women. Importantly, the highest risk quintiles of %AT and of %SM identified different people with diabetes and with hypertension, so both estimates are valuable, and they may in practice reflect different aspects of disease development. The highest risk quintiles of prediction equations also identified people with diabetes and/or hypertension who were not in the top quintile of BMI.

5.5 Conclusion

Prediction equations for both skeletal muscle mass and body fat are significantly associated with metabolic disease (diabetes and/or hypertension), HbA1c, systolic and diastolic blood pressure, identifying different people at risk. The prediction equations for %AT and %SM identify some people with diabetes and hypertension who would not be identified if only BMI>30 is used. The prediction equations show more people with metabolic illness are in the top quintile of %AT and in the lowest quintile of %SM, with steep gradients. This analysis highlights the importance of using %SM as an indicator for diabetes when some categories such as people with

low %SM but don't have large waist and high BMI, this group cannot be identified by using BMI and waist circumference.

The prediction equations offer potential for use in the analysis of future health surveys. The established body-composition indicators, BMI, waist, performed similarly, and waist/height ratio showed particularly strong correlations, yet %SM and %AT offer different information.

Further exploration would be valuable, especially in longitudinal follow-up studies.

Figure 5-1: process of data cleaning for the Scottish Health Survey and Health Survey of England.

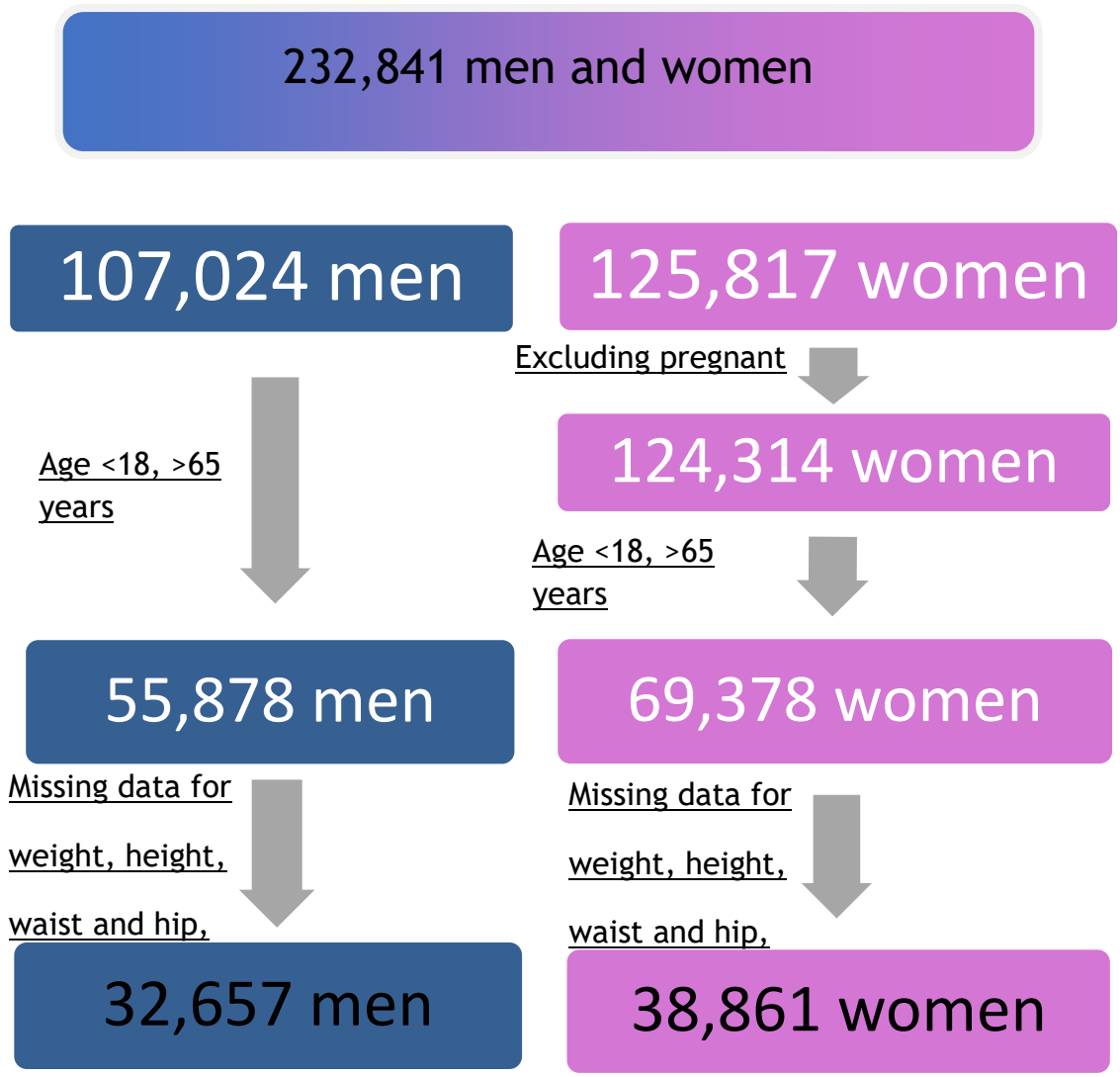


Table 5-1: Process of cleaning data from the Scottish Health Survey and Health Survey of England. Extreme outliers' removal from analysed data.

variable	Outliers men	n	Outliers women	n
Weight	<45 kg	46	<35 kg	61
Height	<146 cm	14	<135 cm	15
Waist	<64, >155 (cm)	24	<64, >155 (cm)	28
Hip	<65, >180 (cm)	430	<65, >180 (cm)	424
HbA1c	<4, >15%	31	<4, >15%	34
Systolic BP	<85, >199 mmHg	62	<85, >199 mmHg	61
Diastolic BP	<40, >120 mmHg	35	<40, >120 mmHg	37

Sample cleaning, n: number of removed extreme Outliers which are considered implausible. Anthropometric outliers were chosen by using CDC reference data as a general guide (Fryar CD, 2012), Blood pressure cut-offs were arbitrary, based on clinical experience.

Table 5-2: Subject characteristics

	Analysed data	
	Men	Women
Sample size (n)	32,657	38,861
Age (years)	51.1(17.2)	50.5(17.2)
Body height (cm)	174.6(7.2)	161.2 (6.8)
Body weight (kg)	84.3(14.7)	71.3 (14.8)
Hip circumference (cm)	104.5(9.2)	105.3 (11.8)
Waist circumference (cm)	98.5(12.1)	88.3 (13.1)
BMI* (kg/m^2)	27.7(4.4)	27.4 (5.5)
WHR	0.94(0.1)	0.84(0.1)
WHtR	0.56(0.1)	0.55(0.1)
Adipose tissue (%)	29.4(5.9)	40.2 (7.8)
Muscle mass (%)	35.1(4.7)	28.2(3.5)
Body fat (%)	29.2(7.6)	40.5(7.6)
HbA1c (%)*	5.6 (0.8)	5.6 (0.7)
SBP (mmHg)	131.5 (15.6)	125.4 (18.5)
DBP (mmHg)	74.7 (11)	73.3 (10.7)
Diabetes %*	2144 (6.6%)	1747 (4.5%)
Hypertension %	12,624 (38.7%)	12,804 (32.9)
Insulin	330 (1.01%)	315 (0.81%)

Value is presented as mean(sd); BMI: body mass index; adipose tissue (%): predicted by (Al-Gindan et al, 2015) then converted to (%) AT/body weight x100); Muscle mass (%): predicted by (Al-Gindan et al 2014) then converted to (%) SM/body weight x100; HbA1c: Glycated haemoglobin; DBP: diastolic blood pressure; SBP: systolic blood pressure. Diabetes mellitus was recorded when doctor/nurse reported, patient reported receiving treatment with oral medicine and HbA1c ≥6.5. Hypertension was recorded when doctor/nurse reported, systolic blood pressure ≥140 and diastolic blood pressure ≥90 mmHg. *: calculated after removing participants taking insulin.

Table 5-3: range of subject characteristics

	Men n = 32,657		Women n = 38,861	
	Minimum	Maximum	Minimum	Maximum
Age (years)	18	104	18	100
Height (cm)	146	210.3	135.6	188.4
Weight (kg)	45.3	188	35.9	172
Hip (cm)	65	166.5	65	179.9
Waist (cm)	64.5	154.3	64	152.1
BMI (kg/m^2)	13.3	56	14.3	63.1
WHR	0.6	1.7	0.8	1.6
WHtR	0.4	0.9	0.4	0.97
ATYY (%)	3.7	58.1	5.4	63
SMYY (%)	4.0	61.5	13.1	42.8
BFML(%)	6.9	62.3	22.7	73.4
HbA1c (%)	4	14.5	4	14
SBP (mmHg)	86	199	85	199
DBP (mmHg)	40	120	40	120

BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio, AT (%): predicted by (Al-Gindan et al, 2015) then converted to (%) AT/body weight x100); SM (%): predicted by (Al-Gindan et al 2014) then converted to (%) SM/body weight x100; BF (%): predicted by (Lean et al, 1996); HbA1c: Glycated haemoglobin; DBP: diastolic blood pressure; SBP: systolic blood pressure.

Table 5-4: Association (R^2) of adipose tissue mass and muscle mass with already published prediction equations and anthropometric indices

Equations	R^2	
	men	women
SMYY (kg) & SMLR (kg)	81.5	81.1
ATYY (%) & BFML (%)	86.9	71.4
ATYY (kg) & BMI	87.4	95.2
ATYY (kg) & WHR	34.8	11.9
ATYY (kg) & WHtR	87.2	69.3

BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio, ATYY: predicted by (Al-Gindan et al, 2015); SMYY: predicted by (Al-Gindan et al 2014); SMLR: predicted by (Lee et al, 2000); BFML: predicted by (Lean et al, 1996);

Table 5-5: Multiple regression analysis (R^2) of HbA1c, systolic and diastolic blood pressure with published prediction equations and anthropometric measurements adjusted for age and hypertension (for HbA1c), age and type-2 diabetes (for systolic and diastolic blood pressure)

	HbA1c		Systolic		Diastolic	
Variable	**men**	**women**	**men**	**women**	**men**	**women**
Sample size	**32,327**	**38,546**	**32,657**	**38,861**	**32,657**	**38,861**
SMYY(%)	14.3	20.3	7.3	26.7	1.4	5.2
SMLR(%)	16.2	21.2	9.8	28.4	3.8	2.8
ATYY(%)	16.0	20.4	9.0	27.2	5.3	7.7
BF(%)	16.1	22.6	9.1	27.4	5.7	7.2
SM:AT(%)	15.6	20.5	8.6	27.1	2.7	7.2
AT:SM(%)	15.6	20.5	8.6	27.1	2.7	7.2
BMI (kg/m^2)	16.4	21.3	9.3	27.0	6.3	8.8
WHR	15.9	20.9	7.9	25.4	3.9	3.4
WHtR	17.3	23.0	9.1	27.0	5.5	8.0
Waist (cm)	16.6	22.5	9.0	26.8	5.6	7.8
Hip (cm)	14.7	19.5	8.0	25.6	2.3	4.8

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. All data log transformed to insure normal distribution. All P-value significant (≤0.05). Subjects taking insulin were removed from HbA1c analysis only.

Table 5-6: Association between categorical variable diabetes and continuous variables muscle, fat, adipose and anthropometric measurements in men, adjusted for age and hypertension using binary logistic regression

	Odds Ratio	R2	Coefficient of variation
Sample size	**32,327**		
SMYY(%)	1.01 (1.0,1.02)	11.16	0.01
SMLR(%)	0.74(0.72, 0.76)	14.7	-0.3
ATYY(%)	1.14(1.13, 1.15)	15.5	0.1
BFML(%)	1.09(1.08, 1.10)	15.2	0.09
SM:AT*	0.98(0.98, 0.98)	13.1	-0.02
AT:SM*	1.02(1.01, 1.02)	12.7	0.02
BMI (kg/m^2)	1.14(1.12, 1.15)	15.1	0.13
WHR*	1.06(1.06,1.07)	14.6	0.06
WHtR*	1.10(1.09, 1.11)	15.9	0.10
Waist (cm)	1.05(1.05, 1.06)	15.2	0.05
Hip (cm)	1.03(1.02, 1.03)	11.8	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis. For this analysis data is not log transformed. All P-value significant (≤0.05). Subjects taking insulin were removed (n= 330 men). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5), out of the 32,327 subjects, 2144 were diabetic.

Table 5-7: Association between categorical variable diabetes and continuous variables muscle, fat, adipose and anthropometric measurements in women, adjusted for age and hypertension using binary logistic regression.

	Odds Ratio	R2	Coefficient of variation
Sample size	**38,546**		
SMYY(%)	1.03(1.03, 1.03)	13.6	-0.16
SMLR(%)	0.56(0.53, 0.59)	15.5	-0.58
ATYY(%)	1.12(1.11,1.13)	16.6	0.11
BFML(%)	1.15(1.14, 1.16)	18.4	0.13
SM:AT*	0.96(0.96,0.96)	15.3	-0.04
AT:SM*	1.01(1.01,1.01)	15.2	0.03
BMI (kg/m^2)	1.12(1.11, 1.13)	16.3	0.11
WHR*	1.05(1.05, 1.06)	14.1	0.05
WHtR*	1.10(1.09, 1.11)	18.8	0.10
Waist (cm)	1.06(1.06, 1.07)	18.4	0.06
Hip (cm)	1.03(1.04, 1.04)	13.1	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis. For this analysis data is not log transformed. All P-value significant (≤0.05). Subjects taking insulin were removed (n=315 women). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5) out of the 38,546 subjects, 1747 are diabetic.

Table 5-8: Associations between categorical variable hypertension and continuous variables muscle, fat, adipose and anthropometric measurements in men, adjusted for age and diabetes using binary logistic regression.

	Odds Ratio	R2	Coefficient of variation
Sample size	**32,657**		
SMYY(%)	0.98(0.98, 0.99)	12.8	-0.02
SMLR(%)	0.82(0.81,0.83)	15.2	-0.2
ATYY(%)	1.09(1.08, 1.09)	15.1	0.08
BFML(%)	1.07(1.06, 1.07)	15.2	0.06
SM:AT*	1.0(1.0, 1.0)	14.2	-0.01
AT:SM*	1.01(1.01, 1.02)	14.0	0.01
BMI (kg/m^2)	1.11(1.10, 1.11)	15.4	0.10
WHR*	1.04(1.03, 1.04)	13.9	0.03
WHtR*	1.07(1.06, 1.07)	15.4	0.07
Waist (cm)	1.04(1.04, 1.04)	15.2	0.04
Hip (cm)	1.03(1.03, 1.03)	13.8	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis. For this analysis data is not log transformed. All P-value significant (≤0.05). Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90mmHg. Out of the 32,657 subjects, 12,624 are hypertensive.

Table 5-9: Associations between categorical variable hypertension and continuous variables muscle, fat, adipose and anthropometric measurements in men, adjusted for age and diabetes using binary logistic regression.

	Odds Ratio	R2	Coefficient of variation
Sample size	**38,861**		
SMYY(%)	0.87(0.86, 0.88)	21.9	-0.13
SMLR(%)	0.67(0.66, 0.69)	22.5	-0.40
ATYY(%)	1.08(1.08, 1.09)	23.2	0.08
BFML(%)	1.09(1.09, 1.10)	22.9	0.09
SM:AT*	0.98(0.97, 0.98)	22.3	-0.02
AT:SM*	1.01(1.01, 1.01)	22.6	0.01
BMI (kg/m^2)	1.10(1.10, 1.10)	23.2	0.10
WHR*	1.03(1.03, 1.04)	20.8	0.03
WHtR*	1.07(1.06, 1.07)	23.1	0.06
Waist (cm)	1.04(1.04, 1.04)	22.9	0.04
Hip (cm)	1.03(1.03, 1.03)	21.4	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis. For this analysis data is not log transformed. All P-value significant (≤0.05). Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90mmHg. Out of the 38,861 subjects, 12,804 are hypertensive.

Table 5-10: Associations of outcomes of SM and AT mass derived from the prediction equations and anthropometric measurements with metabolic disease' in men, using binary logistic regression adjusted for age.

	Odds Ratio	R2	Coefficient of variation
Sample size	**32,327**		
SMYY(%)	0.99 (0.98,0.99)	13.4	-0.01
SMLR(%)	0.80 (0.79, 0.81)	16.4	-0.2
ATYY(%)	1.10 (1.09, 1.11)	16.5	0.1
BFML(%)	1.08 (1.07, 1.08)	16.6	0.1
SM:AT*	0.99(0.99, 0.99)	15.1	-0.01
AT:SM*	1.02(1.02, 1.02)	15.1	0.02
BMI (kg/m^2)	1.12 (1.11, 1.12)	16.7	0.1
WHR*	1.05(1.04, 1.05)	15.2	0.04
WHtR*	1.08(1.07, 1.08)	16.8	0.08
Waist (cm)	1.04(1.04,1.04)	16.6	0.04
Hip (cm)	1.03(1.03, 1.03)	14.5	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis Data not log transformed. All P-value significant (≤0.05). Subjects taking insulin were removed from analysis (n=330). Subjects with hypertension and or diabetes = 13055.

Table 5-11: Association of prediction equations and anthropometric measurements with 'metabolic disease' in women, using binary logistic regression adjusted for age.

	Odds Ratio	R2	Coefficient of variation
Sample size	**38,546**		
SMYY(%)	0.86 (0.85, 0.87)	22.0	-0.2
SMLR(%)	0.64 (0.62,0.65)	23.0	-0.4
ATYY(%)	1.09(1.09, 1.10)	23.8	0.1
BFML(%)	1.11(1.10, 1.11)	23.7	0.1
SM:AT*	0.97(0.97, 0.97)	22.8	-0.03
AT:SM*	1.01(1.01, 1.01)	23.3	0.01
BMI (kg/m^2)	1.11(1.11, 1.12)	23.8	0.1
WHR*	1.04(1.04, 1.05)	21.2	0.04
WHtR*	1.08(1.07, 1.08)	24.4	0.07
Waist (cm)	1.05 (1.04, 1.05)	23.7	0.05
Hip (cm)	1.03(1.03, 1.04)	21.4	0.03

SMYY: skeletal muscle predicted by (Al-Gindan et al, 2014); ATYY: adipose tissue predicted by (AL-Gindan et al, 2015): SMLR: skeletal muscle predicted by (Lee et al, 2000); BFML: body fat predicted by (Lean et al, 1996); SM:AT: SMYY/AYYY: AT:SM: ATYY/SMYY; BMI: body mass index; WHR: waist hip ratio, WHtR: waist height ratio. *: scale of the original observation changed by multiplying by 100 before entering it into the analysis. Data not log transformed. All P-value significant (≤0.05). Subjects taking insulin were removed from analysis (n=315). Subjects with hypertension and or diabetes = 13135.

Table 5-12: Prevalence of diabetes, hypertension and metabolic disease and mean of HbA1c and blood pressure in 20%, 40%, 60% 80% quintile of %AT and %SM estimated by (Al-gindan et al, 2014,2015) prediction equations in men

Adipose tissue(AT%)					
	Q1 3.7-24.7	Q2 24.8-28.3	Q3 28.4-31.2	Q4 31.3-34.3	Q5 34.4-58.1
N	6465	6466	6465	6465	6464
Diabetes(%)	70(1.1%)	172(2.7%)	275(4.3%)	560(8.7%)	1066(16.5%)
Hypertension(%)	1004(15.5%)	1794(27.7%)	2487(38.5%)	3169(49.0%)	3936(60.9%)
Metabolic illness(%)	1037(16.0%)	1877(29.0%)	2577(39.9%)	3352(51.8)	4210(65.1%)
HbA1c (mean(sd))	5.3(0.4)	5.4(0.5)	5.5(0.6)	5.7(0.8)	6.0(0.9)
Systolic BP (mean(sd))	125.2(13.1)	129.4(14.2)	132.2(15.1)	134.2(15.8)	136.2(17.1)
Diastolic BP (mean(sd))	70.2(9.9)	74.1(10.1)	75.9(10.7)	76.7(11.0)	76.4(11.9)

Muscle mass (SM%)					
	Q1 4.0-31.7	Q2 31.8-34.1	Q3 34.2-36.0	Q4 36.1-38.3	Q5 38.4-61.5
N	6465	6466	6466	6465	6465
Diabetes (%)	869(13.4%)	557(8.6%)	347(5.4%)	195(3.0%)	176(2.7%)
Hypertension (%)	3917(60.6%)	3148(48.7%)	2360(36.5%)	1748(27.0%)	1219(18.9%)
Metabolic illness (%)	4143(64.1%)	3313(51.2%)	2501(38.7%)	1814(28.1%)	1284(19.9%)
HbA1c (mean(sd))	5.9(0.8)	5.7(0.7)	5.6(0.7)	5.5(0.7)	5.3(0.6)
Systolic BP (mean(sd))	136.5(18.0)	133.9(16.5)	130.9(15.0)	129.1(13.0)	126.8(12.8)
Diastolic BP (mean(sd))	73.0(11.5)	76.3(10.8)	76.9(10.8)	75.5(10.5)	71.9(10.6)

Results reported as percentage of all people with type-two diabetes, hypertension and metabolic disease for each quintile; mean(sd) of HbA1c, systolic and diastolic blood pressure for each quintile; N: number of subjects; Q1:first quintile (minimum – 20%), Q2:second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (80% - maximum); Prediction equation for %AT developed by (Algindan et al, 2015); Prediction equation for %SM developed by (Algindan et al, 2014); Subjects taking insulin were removed (n= 330 men, n=315 women) . Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 32,325 men, 2144 are diabetic; Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90mmHg, out of the 32,325 men, 12,391 are hypertensive; metabolic illness: Subjects with hypertension and or diabetes = 13054 men.

Table 5-13: Prevalence of diabetes, hypertension and metabolic disease and mean of HbA1c and blood pressure in 20%, 40%, 60% 80% quintile of %AT and %SM estimated by (Al-gindan et al, 2014,2015) prediction equations in women.

Adipose tissue (ATYY%)					
	Q1 5.4-33.5	Q2 33.6-38.4	Q3 38.5-42.5	Q4 42.6-47.0	Q5 47.1-63.0
N	7708	7708	7710	7709	7709
Diabetes (%)	36(0.5%)	112(1.4%)	229(3.0%)	456(5.9%)	914(11.8%)
Hypertension (%)	786(10.2%)	1561(20.3%)	2463(31.9%)	3424(44.4%)	4377(56.8%)
Metabolic illness (%)	809(10.5%)	1612(20.9%)	2554(33.1%)	3565(46.2%)	4595(59.6%)
HbA1c (mean(sd))	5.3(0.4)	5.4(0.5)	5.5(0.5)	5.7(0.7)	5.9(0.8)
Systolic BP (mean(sd)	114.9(14.3)	121.1(16.3)	126.2(17.9)	130.7(18.7)	133.4(18.7)
Diastolic BP (mean(sd))	68.9(9.5)	71.3(9.7)	73.5(10.1)	75.4(10.7)	77.2(11.1)
Muscle mass (SMYY%)					
	Q1 13.1-25.2	Q2 25.3-27.2	Q3 27.3-28.9	Q4 29.0-31.1	Q5 31.2-42.8
N	7708	7710	7709	7709	7709
Diabetes (%)	846(10.9%)	427(5.5%)	244(3.2%)	145(1.9%)	85(1.1%)
Hypertension (%)	4477(58.1%)	3350(43.5%)	2380(30.9%)	1489(19.3%)	914(11.8%)
Metabolic illness (%)	4674(60.6%)	3488(45.2%)	2474(32.1%)	1542(20.0%)	956(12.4%)
HbA1c (mean(sd))	5.8(0.8)	5.7(0.7)	5.5(0.5)	5.4(0.5)	5.3(0.5)
Systolic BP (mean(sd))	134.3(18.9)	130.4(18.8)	125.4(17.4)	120.7(16.2)	115.6(14.5)
Diastolic BP (mean(sd))	76.5(11.2)	75.3(10.7)	73.5(10.2)	71.6(9.9)	69.5(9.7)

Results reported as percentage of all people with type-two diabetes, hypertension and metabolic disease for each quintile; mean(sd) of HbA1c, systolic and diastolic blood pressure for each quintile; N:number of subjects; Q1: first quintile (minimum), Q2: second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (maximum); Prediction equation for %AT developed by (Algindan et al, 2015); Prediction equation for %SM developed by (Algindan et al, 2014);Subjects taking insulin were removed (n= 330 men, n=315 women). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 38,546 women, 1747 are diabetic; Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90, out of the 38,546 women, 12,610 are hypertensive; metabolic illness: Subjects with hypertension and or diabetes = 13134women.

Table 5-14: Relative risk of having diabetes, hypertension and metabolic disease in %AT, %SM, BMI and WHtR quintiles relative to reference population (Q1 for %AT, BMI, WHtR) and Q5 for %SM) in men.

Adipose tissue (AT%)					
	Q1 3.7-24.7	Q2 24.8-28.3	Q3 28.4-31.2	Q4 31.3-34.3	Q5 34.4-58.1
RR diabetes (%)	----	2.5	3.9	7.9	15
RR hypertension (%)	----	1.9	2.5	3.2	3.9
RR metabolic illness (%)	----	1.8	2.4	3.2	4.1

Muscle mass (SM %)					
	Q1 3.7-24.7	Q2 24.8-28.3	Q3 28.4-31.2	Q4 31.3-34.3	Q5 34.4-58.1
RR diabetes (%)	4.9	3.2	2	1.1	----
RR hypertension (%)	3.2	2.6	1.9	1.4	----
RR metabolic illness (%)	3.2	2.6	1.9	1.4	----

BMI kg/m^2					
	Q1 13.3-24.0	Q2 24.1-26.3	Q3 26.4-28.2	Q4 28.3-30.9	Q5 31.0-56.0
RR diabetes (%)	----	1.6	2.3	3.0	5.4
RR hypertension (%)	----	1.5	1.8	2.1	2.5
RR metabolic illness (%)	----	1.5	1.8	2.1	2.5

WHtR					
	Q1 0.35-0.50	Q2 0.51-0.54	Q3 0.55-0.57	Q4 0.58-0.62	Q5 0.630.94
RR diabetes (%)	----	2.5	4.3	7.9	14.5
RR hypertension (%)	----	1.8	2.5	3.2	3.8
RR metabolic illness (%)	----	1.8	2.5	3.2	4.0

Results reported as relative risk of having type-two diabetes, hypertension and metabolic disease in each quintile using Q1 as baseline for %AT, Q5 as baseline for %SM, Q1 as baseline for BMI and WHtR; Q1:first quintile (minimum – 20%), Q2:second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (80% - maximum); Prediction equation for %AT developed by (Algindan et al, 2015); Prediction equation for %SM developed by (Algindan et al, 2014); Subjects taking insulin were removed (n= 330 men, n=315 women) . Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 32,325 men, 2144 are diabetic; Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90, Out of the 32,325 men, 12,391 are hypertensive; metabolic illness: Subjects with hypertension and or diabetes = 13054 men.

Table 5-15: Relative risk of diabetes, hypertension and metabolic disease in %AT,%SM, BMI and WHtR quintiles in women.

Adipose tissue (AT%)					
	Q1 5.4-33.5	Q2 33.6-38.4	Q3 38.5-42.5	Q4 42.6-47.0	Q5 47.1-63.0
RR diabetes (%)	---	2.8	6	11.8	23.6
RR hypertension (%)	---	2.0	3.1	4.3	5.5
RR metabolic illness (%)	---	2.0	3.1	4.4	5.6

Muscle mass (SM %)					
	Q1 13.1-25.2	Q2 25.3-27.2	Q3 27.3-28.9	Q4 29.0-31.1	Q5 31.2-42.8
RR diabetes (%)	9.9	5	2.9	1.7	---
RR hypertension (%)	4.9	3.7	2.6	1.6	---
RR metabolic illness (%)	4.8	3.6	2.6	1.6	---

BMI kg/m^2					
	Q1 13.3-24.0	Q2 24.1-26.3	Q3 26.4-28.2	Q4 28.3-30.9	Q5 31.0-56.0
RR diabetes (%)	----	1.8	3.2	5.2	9.2
RR hypertension (%)	----	1.5	2.0	2.5	3.0
RR metabolic illness (%)	----	1.5	2.0	2.5	3.1

WHtR					
	Q1 0.35-0.50	Q2 0.51-0.54	Q3 0.55-0.57	Q4 0.58-0.62	Q5 0.630.94
RR diabetes (%)	----	1.9	4.6	9.8	22.4
RR hypertension (%)	----	1.9	2.8	3.9	4.9
RR metabolic illness (%)	----	1.9	2.8	3.9	5.0

Results reported as relative risk of having type-two diabetes, hypertension and metabolic disease in each quintile using Q1 as baseline for %AT and Q5 as baseline for %SM; Q1 as baseline for BMI and WHtR; Q1: first quintile (minimum), Q2: second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (maximum); Prediction equation for %AT developed by (Algindan et al, 2015); Prediction equation for %SM developed by (Algindan et al, 2014);Subjects taking insulin were removed (n= 330 men, n=315 women). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 38,546 women, 1747 are diabetic; Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90, out of the 38,546 women, 12,610 are hypertensive; metabolic illness: Subjects with hypertension and or diabetes = 13134 women.

Table 5-16: Prevalence of diabetes, hypertension and metabolic illness in different BMI categories and distribution of %AT and %SM across the BMI categories in men.

BMI (n)	16 – 18.4 kg/m² (185)					18.5-24.9 kg/m² (14,022)					25.0-29.5 kg/m² (12,406)					30.0-34.9 kg/m² (6,556)					≥35.0 kg/m² (3,736)				
Diabetes (%)	0.5					2.8					5.6					10.6					18.5				
RR diabetes	0.2					-----					2					3.7					6.6				
Hypertension (%)	14.6					23.8					38.0					51.8					58.8				
RR hypertension	0.6					-----					1.6					2.2					2.5				
Metabolic illness (%)	15.1					25.0					39.9					54.8					62.6				
RR metabolic illness	0.6					-----					1.6					2.2					2.5				
AT quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	98.4	1.6	0	0	0	58.7	26.5	10.3	4.0	1.3	8.7	27.3	30.5	22.4	11.1	0.3	3.1	15.4	35.8	45.4	0.1	0.2	1.6	14.0	84.2
SM quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	8.6	4.3	8.6	8.6	69.7	15.6	14.1	15.2	19.5	35.6	20.6	21.0	21.0	20.9	16.6	22.3	24.8	23.2	19.7	10.0	28.9	23.7	24.0	17.6	5.7

n: number of subjects; Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90; metabolic illness: Subjects with hypertension and/or diabetes; Q: quintile; %AT(whole body adipose tissue predicted by (Algindan et al, 2015)) Q1 = 3.8-24.7, Q2 = 24.8 – 28.3, Q3 = 28.4 – 31.2, Q4 = 31.3 – 34.3, Q5 = 34.4 – 58.1; %SM (whole body skeletal muscle predicted by (Algindan et al, 2014) Q1 = 4.0 – 31.7, Q2 = 31.8 – 34.1, Q3 = 34.2 – 36.0, Q4 = 36.1 – 38.3, Q5 = 38.4 – 61.5.

Table 5-17: Prevalence of diabetes, hypertension and metabolic illness in different BMI categories and distribution of %AT and %SM across the BMI categories in women.

BMI (n)	16 – 18.4 kg/m² (328)					18.5-24.9 kg/m² (14,022)					25.0-29.5 kg/m² (12,406)					30.0-34.9 kg/m² (6,556)					≥35.0 kg/m² (3,736)				
Diabetes (%)	0.9					1.5					4.0					7.7					11.9				
RR diabetes	0.6										2.7					5.1					7.9				
Hypertension (%)	17.1					20.1					33.5					47.0					51.4				
RR hypertension	0.8										1.7					2.3					2.6				
Metabolic illness (%)	17.4					20.7					34.8					48.9					54.6				
RR metabolic illness	0.8										1.7					2.4					2.6				
AT quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	100	0	0	0	0	52.2	39.2	8.3	2.9	0	0	16.4	50.5	31.9	1.3	0	0	0.8	44.6	54.6	0	0	0	0.05	99.9
SM quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	0	0	0.30	2.4	97.3	0.4	3.5	13.2	35.9	47.0	8.8	29.0	39.2	19.2	3.7	45.5	41.6	8.6	1.2	3.0	86.4	10.3	1.7	1.1	0.4

n: number of subjects; Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); Hypertension variable included doctor/nurse reported, systolic blood pressure≥140 mmHg, diastolic blood pressure ≥90; metabolic illness: Subjects with hypertension and/or diabetes; Q: quintile; %AT (whole body adipose tissue predicted by (Algindan et al, 2015)) Q1: 5.4 – 33.5, Q2:33.6 – 38.4, Q3: 38.5 – 42.5, Q4:42.6 – 47.0, Q5:47.1 – 63.0; %SM (whole body skeletal muscle predicted by (Algindan et al, 2014) Q1: 13.1 – 25.2, Q2:25.3 – 27.2, Q3:27.3 – 28.9, Q4: 29.0 – 31.1, Q5:31.2 – 42.8

Table 5-18: Distribution of %AT and %SM for subjects with diabetes in different BMI categories in men.

BMI (n)	16 - 18.4 kg/m² (1)					18.5-24.9 kg/m² (240)					25.0-29.5 kg/m² (764)					30.0-34.9 kg/m² (678)					≥35.0 kg/m² (354)				
AT quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	100	0	0	0	0	20.4	30.8	23.7	17.9	7.1	2.2	12.0	22.0	36.2	27.3	0.1	0.4	5.5	27.9	66.1	0.3	0	0.3	3.7	95.8
SM quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	0	0	0	0	100	43.3	22.5	14.2	7.1	12.9	41.9	25.7	15.2	8.1	9.2	36.0	29.1	17.3	10.8	6.9	43.8	24.3	18.9	9.3	0.8

n: number of subjects; total number of men with diabetes 2144; Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5; Q: quintile; %AT (whole body adipose tissue predicted by (Algindan et al, 2015)) Q1 = 3.8-24.7, Q2 = 24.8 – 28.3, Q3 = 28.4 – 31.2, Q4 = 31.3 – 34.3, Q5 = 34.4 – 58.1; %SM (whole body skeletal muscle predicted by (Algindan et al, 2014) Q1 = 4.0 – 31.7, Q2 = 31.8 – 34.1, Q3 = 34.2 – 36.0, Q4 = 36.1 – 38.3, Q5 = 38.4 – 61.5.

Table 5-19: Distribution of %AT and %SM for subjects with diabetes in different BMI categories in women.

BMI (n)	16 - 18.4 kg/m² (3)					18.5-24.9 kg/m² (213)					25.0-29.5 kg/m² (501)					30.0-34.9 kg/m² (504)					≥35.0 kg/m² (443)				
AT quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	100	0	0	0	0	15.5	46.0	36.6	1.9	0	0	2.8	28.9	64.5	3.8	0	0	0	15.9	84.1	0	0	0	0	100
SM quintile	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
%	0	0	0	0	100	2.8	13.1	30.5	38.5	15.0	19.5	42.7	25.1	8.2	4.4	62.3	24.6	6.5	2.0	4.6	89.2	6.3	2.7	2.3	0

n: number of subjects; total number of women with diabetes 1747; Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5; Q: quintile; %AT (whole body adipose tissue predicted by (Algindan et al, 2015)) Q1: 5.4 – 33.5, Q2:33.6 – 38.4, Q3: 38.5 – 42.5, Q4:42.6 – 47.0, Q5:47.1 – 63.0; %SM (whole body skeletal muscle predicted by (Algindan et al, 2014) Q1: 13.1 – 25.2, Q2:25.3 – 27.2, Q3:27.3 – 28.9, Q4: 29.0 – 31.1, Q5:31.2 – 42.8

Table 5-20: numbers and percentage of people in quintiles of %SM who are in different quintiles of Waist and of BMI, and the number and percentage of type-2 diabetes in each BMI and waist quintile in men.

SM%	Q1(4.0-31.7)					Q2(31.8-34.1)					Q3(34.2-36.0)					Q4(36.1-38.3)					Q5(38.4-61.5)				
N	6465					6466					6466					6465					6465				
T2DM	869					557					347					195					176				
%*	13.4					8.1					5.4					3.01					2.7				
BMI	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
N*	20	1386	3070	1436	552	14	1249	3150	1600	453	18	1356	2879	1754	459	17	1672	2929	1497	350	132	3066	2337	817	113
%**	0.31	21.4	47.4	22.2	8.5	0.22	19.3	48.7	24.7	7.0	0.28	21	44.5	27.1	7.1	0.26	25.9	45.3	23.2	5.4	2.04	47.4	36.1	12.6	1.7
T2DM (n)	0	110	356	248	155	0	57	214	200	86	0	35	116	129	67	0	17	63	82	33	1	31	73	58	13
%***	0	7.9	11.6	17.3	28.1	0	4.6	6.8	12.5	19	0	2.5	4.03	7.4	14.6	0	1.1	2.1	5.5	9.4	0.8	1.01	3.1	7.1	11.5
Waist	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
N*	382	894	1293	1730	2166	509	1087	1514	1628	1728	760	1369	1490	1482	1365	1354	1706	1413	1111	881	3460	1418	776	499	312
%**	5.9	13.8	20	26.8	33.5	7.9	16.8	23.4	25.2	26.7	11.7	21.2	23	22.9	21.1	20.9	26.4	21.9	17.2	13.6	53.5	21.9	12.0	7.7	4.8
T2DM (n)	17	71	137	211	433	24	52	93	127	261	16	44	61	72	154	16	31	37	30	81	30	25	42	41	38
%***	4.4	7.9	10.6	12.2	20	4.7	4.8	6.1	7.8	15.1	2.1	3.2	4.1	4.9	11.2	1.2	1.9	2.6	2.7	9.2	0.9	1.8	5.4	8.2	12.2

Results reported as "number of subjects (N) and percentage (%)"; T2DM: type-2 diabetes; Q1: first quintile (minimum), Q2: second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (maximum); Prediction equation for %SM developed by (Algindan et al, 2014); Subjects taking insulin were removed (n= 330 men, n=315 women). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 38,546 women, 1747 are diabetic; BMI quintiles: Q1 (13 – 18.4 kg/m^2), Q2 (18.5-24.9 kg/m^2), Q3 (25-29.9 kg/m^2), Q4 (30 – 34.9 kg/m^2), Q5 (35 – 64 kg/m^2); waist quintiles: Q1(64.5-88.28cm), Q2(88.29-94.9cm), Q3(94.91-100.7cm), Q4(100.71-108.05cm), Q5(108.06-154.35cm); N*: numbers of people in quintiles of %SM who are in different quintiles of Waist and of BMI; %**: percentage of people in quintiles of %SM who are in different quintiles of Waist and of BMI; %***: percentage of diabetics in each BMI and waist quintiles.

Table 5-21: numbers and percentage of people in quintiles of %SM who are in different quintiles of Waist and of BMI, and the number and percentage of type-2 diabetes in each BMI and waist quintile in women.

SM%	Q1(13.1-25.2)					Q2(25.3-27.2)					Q3(27.3-28.9)					Q4(29—31.1)					Q5(31.2-42.8)				
N	7708					7710					7705					7709					7709				
T2DM	846					427					244					145					85				
%*	11					5.5					3.1					1.9					1.1				
BMI	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
N*	0	60	1101	3258	3289	0	487	3636	3183	404	1	1856	4967	813	68	8	5031	2528	98	44	339	6628	485	241	16
%**	0	0.78	14.3	42.3	42.7	0	6.3	47.2	41.3	5.2	0.01	24.1	64.5	10.6	0.9	0.1	65.3	32.8	1.3	0.6	4.4	86	6.3	3.1	0.2
T2DM (n)	0	6	99	337	404	0	28	216	153	30	0	65	128	39	12	0	82	145	14	8	3	32	22	27	1
%***	0	10	9	10.3	12.3	0	5.7	5.9	4.8	7.4	0	3.5	2.6	4.8	17.6	0	1.6	5.7	14.3	18.2	0.9	0.5	4.5	11.2	6.2
Waist	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
N*	25	171	643	1894	4975	154	811	1923	2913	1909	626	1946	2640	1951	546	1909	3083	1943	633	141	4999	1729	569	283	129
%**	0.32	2.2	8.3	24.6	64.5	2	10.5	24.9	37.8	24.8	8.1	25.3	34.3	25.3	7.1	24.8	40	25.2	8.2	1.8	64.8	22.4	7.4	3.7	1.7
T2DM (n)	0	9	35	140	662	3	25	97	135	167	14	28	60	86	56	21	35	40	27	22	19	14	13	15	24
%***	0	5.3	5.4	7.4	13.3	1.9	3.1	5.0	4.6	8.7	2.2	1.4	2.3	4.4	10.3	1.1	1.1	2.1	4.3	1.6	0.4	0.1	2.3	5.3	18.6

Results reported as "number of subjects (N) and percentage (%)"; T2DM: type-2 diabetes; Q1: first quintile (minimum), Q2: second quintile (20%), Q3: third quintile (40%), Q4: fourth quintile (60%), Q5: fifth quintile (maximum); Prediction equation for %SM developed by (Algindan et al, 2014); Subjects taking insulin were removed (n= 330 men, n=315 women). Diabetes variable included (doctor/nurse report, taking medication for diabetes and HbA1c>6.5); out of the 38,546 women, 1747 are diabetic; BMI quintiles: Q1 (13 – 18.4 kg/m^2), Q2 (18.5-24.9 kg/m^2), Q3 (25-29.9 kg/m^2), Q4 (30 – 34.9 kg/m^2), Q5 (35 – 64 kg/m^2); waist quintiles: Q1(64.0-76.5cm), Q2(76.51-83.3cm), Q3(83.31-90.1cm), Q4(90.11-98.95cm), Q5(98.96-116.35cm); N*: numbers of people in quintiles of %SM who are in different quintiles of Waist and of BMI; %**: percentage of people in quintiles of %SM who are in different quintiles of Waist and of BMI; %***: percentage of diabetics in each BMI and waist quintiles.

Chapter 6

6 Application of prediction equations to obese women before and after weight loss

(A pilot study)

Anthropometric prediction of total adipose tissue/fat mass and muscle mass during weight loss, using MRI as a reference method

6.1 **Abstract**

Background: Prediction equations for adipose and muscle mass, based on simple anthropometric measurements, may have particular value in weight management interventions, provided they remain predictive after weight loss.

Aims: 1) To examine changes in body composition using magnetic resonance imaging (MRI) and 2) to assess the validity of the previously developed adipose and muscle mass prediction equations, before and after substantial (target >15kg) non-surgical weight loss.

Subjects and Methods: 10 obese, non-diabetic women (BMI>30, 18-65 years), recruited from GP practices and through distribution of posters around the University of Glasgow campus. At baseline, all participants underwent whole-body MRI, anthropometric measurements (weight; height; waist and hip circumference) and muscle strength measurements. These measurements were repeated after weight loss from total diet replacement (820kcal/day, Counterweight-Plus) for 12-20 weeks.

Results: Subjects showed a mean weight loss of 15.3(standard deviation:8.6) kg, from 99.6(14.6) kg to 84.3(12.3) kg, and a reduction in waist circumference of 16.5(7.2) cm, from 112.3(12.4) cm to 95.4(13.0) cm. High correlation and good agreement was seen between MRI-measured and predicted adipose tissue mass, both before (R^2=86.2; p=0.001) and after (R^2=91.6; p=0.001) weight loss. Muscle mass prediction equations revealed a weaker correlation with MRI measurements before (R^2=78.0; p=0.001) and, in particular, after (R^2=51.7; p=0.017) weight loss. There were no significant associations between MRI-measured muscle mass and muscle strength, either before or after weight loss.

Conclusion: Estimated adipose tissue mass, calculated using an anthropometric equation, agrees well with MRI measurement, both before and after substantial weight loss. Muscle mass was estimated less reliably, particularly after weight loss.

6.2 Introduction

One of the major reasons for body composition assessment is to track weight change over time. There are many factors that contribute to changes in body composition, such as pregnancy, growth, ageing and disease, in addition to changing eating habits and physical activity. Altered body composition is routinely tracked in clinical practice using the body mass index (BMI) and weight; however, these variables do not indicate muscle, fat and adipose tissue masses, which are more related to body changes.

Assessment of body composition changes arising from weight loss is important. High fat mass and low muscle mass are both associated with numerous co-morbidities (Atlantis et al., 2009, Sayer et al., 2013) and, when presenting together, are known as sarcopenic obesity (SO) (Kohara, 2014). The prevalence of SO is unclear, primarily due to weakness in the methodologies available to determine fat and muscle mass, and a lack of any recognised definitions (Batsis et al., 2013). There are additional negative health impacts in sarcopenia with obesity (Heber et al., 1996). Clinically useful approaches to determine muscle and fat mass, and in turn SO, are therefore justified, and may have particular value in weight management interventions.

Typically, outcomes of weight management programmes are reported in terms of kilograms (kg) or as percentage (%) change in body weight. However, whilst muscle mass usually decreases during intentional weight loss, these changes are rarely measured directly (Benton et al., 2011).

A systematic review (Weinheimer et al., 2010) assessed the effect of exercise and energy restriction (ER) – separately or combined – on fat-free mass (FFM) from 52 studies. The findings indicated that energy restriction alone leads to moderate weight loss <10 and ≥ 5 kg in 61 % of energy restricted group, fat free mass (FFM) loss <3 and ≥1.5 in 56% of the ER group. However, half the ER groups lost approximately a quarter of their body weight as FFM, using a number of different assessment approaches. Adding exercise to ER reduced FFM loss by 11%. In the

studies included in this review, FFM was measured using four different techniques and limited to an experimental setting; dual energy X-ray absorptiometry (DEXA), hydrostatic weighing, air displacement plethymography, body potassium. Studies using skin-fold and bioelectrical impedance were excluded.

Prediction equations for fat mass from simple anthropometric measurements (age; body weight; height; waist circumference, hip circumference) are reasonably well established (Lean et al., 1996). Our recent study (Al-Gindan et al., 2014a) validated prediction equations for muscle mass (separately for men and women), using simple anthropometric measurements (age, body weight, height, waist circumference, and hip circumference). In this study, prediction equations showed a stronger correlation with measurements in men than in women (R^2=0.79 and 0.60, respectively), Our validation study findings are in agreement with others based on different measures, with women showing weaker correlations than men (Chen et al., 2011a, Lee et al., 2000a, Ross et al., 1994a).

As part of this thesis, anthropometric equations were also developed and validated, for both men and women, to estimate adipose tissue, using whole-body MRI measurements as reference. In contrast to our muscle mass prediction equations, the adipose tissue mass prediction equations explained more variance in women than in men (Al-Gindan et al., 2015).

The relationships between muscle mass estimated from these equations and functional outcomes, such as muscle strength, have yet to be investigated and evaluated in severe obesity or after weight loss.

The present study compares estimated adipose tissue and muscle mass, using these prediction equations, with measured values from whole-body MRI scans in obese women. We will then test their ability to quantify MRI-measured changes in body composition following a well-established weight loss programme aimed at causing major weight loss (>15kg). Also, we will investigate the relationships between estimated muscle mass and functional muscle strength.

6.3 **Methods**

13 obese, non-diabetic women (BMI >30, 18-65 years), were recruited directly from General Practice and through poster advertisement around Glasgow Royal Infirmary. Ethical approval (appendix 3)

At baseline, all participants underwent whole-body MRI scans, anthropometric measurements (weight; height; waist and hip circumferences) and muscle strength measurements (hand grip; quadriceps and hamstring strength).

Measurements were repeated after 12-20 weeks of subjects following the weight management programme Counterweight-Plus, which is based on a 820kcal/day nutritionally complete formula diet, aiming to achieve a weight loss of >15kg, as previously described (Lean et al., 2013a).

Inclusion criteria: Written informed consent was sought from females aged 18-65, with BMI≥30kg/m2 (<45kg/m^2), weight <200kg and waist circumference <60 cm (upper limits for MRI scanning). These subjects were non-diabetic, without other known illnesses or disabilities which would affect body weight or prevent completion of the program.

Exclusion criteria: Adults with known illnesses, such as cancer, myocardial infarction in the previous six months, heart failure, diabetes (known to alter body composition), as well as disability that would affect weight loss, MRI-incompatible implanted electronic devices or ferromagnetic foreign bodies, learning difficulties, poor understanding of English, pregnant/ considering pregnancy, substance abuse, history of hospitalisation for depression, use of antipsychotic drugs, current participation in another clinical research trial, weight loss >5kg within the last six months, parallel anti-obesity therapy or a diagnosed eating disorder.

Dietary plan: The Counterweight-Plus Programme is a structured weight management programme, comprising three phases. First, a nutritionally replete Total Diet Replacement Plan (TDR) of 820 calories/day is supplied to the subjects

as formula soups and shakes to be prepared with water. The TDR, if completed they could generate a weight loss of 15-20kg over 12-16 weeks. Second, in a structured Food Reintroduction phase, meals made from normal foods are reintroduced one at a time. Some further weight loss is expected during this phase. Third is a structured, long-term Weight Loss Maintenance (WLM) phase, during which the formula soups and shakes are used, usually once a day as meal replacement, in conjunction with planned food-based meals. Throughout the Counterweight-Plus programme, elements of theory-based behavioural change methods (e.g. Cognitive Behavioural Therapy; CBT) are employed, and physical activity is encouraged, and supported using step-counters. A formal exercise programme is, however, not routinely incorporated, recognising that severely obese people find exercise difficult and many are already exercising maximally because of their weight.

This chapter covers the Total Diet Replacement (TDR) phase only.

6.3.1 Anthropometric measurements

Body weight was measured to the nearest 0.1kg using a Tanita scale. To allow for clothing, 1.0 kg was added to all participants' weights. A wall-mounted stadiometer was used to measure standing height to the nearest 0.1cm. Anthropometric circumferences were obtained using a heavy-duty inelastic plastic fibre tape measure: waist was measured as the midpoint between the lowest rib and the hip bone; hips at the level of the pubic symphysis and the greatest gluteal protuberance (WHO, 2011). Yasmin Algindan and Naomi Brosnahan took the anthropometric measurements.

6.3.2 MRI measurements

MRI data was collected in the same MRI department, with a single MRI analyst performing all measurements (Rosario Lopez Gonzalez). MR imaging was carried out using a Philips Ingenia 1.5T scanner. Standard T1-weighted imaging technique was used: transversal T1-w (TR/TE = 416/6ms, matrix size = 424x397, FoV = 558x558mm^2) with a slice thickness of 5 mm. For segmentation, image J (NIH, open access) and the extended MR workspace Philips were tested. A threshold method

was used to segment fat, muscle was measured using both threshold and manual segmentation. After evaluation of packages; image J (NIH, open access), and the segmentation tool available in the MRI Philips workstation, other non-free software, the radiology team found that MRI Philips was the best free software available at the time of testing. Adipose tissue and muscle content were quantified using a combination of threshold and manual segmentation techniques. Scanning time was 45–60 min. In order to acquire a whole-body scan, participants were first scanned with one arm up, then the second arm was scanned by itself. To quantify whole-body adipose tissue and muscle mass, the whole-body excluding the arms and the arm scanned individually multiply by 2 were added together, measurements were obtained in cm^3. MRI measured waist and hip circumferences (in mm) were taken: the waist was measured at the midpoint between the lowest rib and the hip bone; hips were measured at the level of the pubic symphysis and the greatest gluteal protuberance. Waist and hip measurements were converted from millimetres to centimetres. MRI-measured muscle and adipose volumes were converted from cm^3 to litre. Whole-body muscle and adipose tissue volume estimates were converted to mass using the assumed densities of 1.04kg/l and 0.92kg/l, to convert total adipose tissue volume to mass (Garrow, 1975). To account for inter-observer variability, Rosario Lopez Gonzalez and Yasmin Algindan measured fat and muscle volume three times for a participant's abdominal section (by varying threshold values and manual segmentation).

6.3.3 Functional assessment

Subjects' muscle strength was assessed before and after the weight loss intervention. Muscle strength was measured for both the upper (grip strength) and lower (quadriceps and hamstrings) limbs.

grip strength was measured by a standard Jamar® Hand Dynamometer (Lafayette Instruments, Lafayette, IN), each subject completed three isometric maximal strength tests with both hands. Subjects were asked to sit with their shoulder adducted and neutrally rotated, forearm in neutral position, elbow flexed at 90° and wrist between 0° and 30° extensions and between 0° and 15° ulnar deviations (Mathiowetz et al., 1985). Results were documented in Newton.

Magnitude of isometric contractions of the quadriceps and hamstring muscle was measured using digital myometer from MIE Medical Research Limited. Participant sat on an examination bed and the strap for the myometer was wrapped around the participant's ankle, lower limb was set to 90°. The perpendicular distance from the knee to the ankle was logged to calculate the moment of force. For quadriceps, participant pulled against the myometer strap to extend knee joint. For the hamstring test the participant pulled against the myometer strap to flex the knee joint more than the 90 degrees.

6.3.4 Statistical analysis

In a single sample weight-loss intervention study, there is no value in studying control subjects who do not lose weight; furthermore, the intervention sample was expected to include a relatively wide range of weight lost, reflecting variable compliance with the diet plan. Paired t tests were used to statistically assess weight differences between two groups (Figure 6-1).

To assess the relationship between MRI-measured adipose tissue and muscle mass and predicted adipose tissue/muscle mass and muscle strength, Pearson correlation coefficient and linear regression analysis were used. Mean differences and 95% limit of agreement between methods were estimated using Bland Altman analysis.

6.4 Results

6.4.1 Subject characteristics

Of the 13 women who agreed to participate and satisfied the inclusion criteria, one subject did not start the diet (after taking all baseline measurements) due to family circumstances. Two subjects dropped out because they could not stick to the diet plan. As a result, data was collected from 10 women who successfully completed the TDR phase of the Counterweight-Plus programme.

Characteristics of the 10 participants who completed the diet programme are shown in Table 6.2. The mean age was 51.1(6.8) years; mean weight loss was 15.3(8.6) kg, from baseline 99.6(14.6) kg to final weight 84.3(12.3) kg. The greatest individual weight loss was 32.1kg, while the lowest was 8.4kg.

Mean waist circumference decreased by 16.5(7.2) cm, from 112.3(12.4) cm to 95.4(13.0) cm, significant by paired t-test (p=0.001), while mean hip circumference also significantly decreased by 10.8(7.0) cm, from 123.7(11.6) cm to 112.6(9.4) cm (p=0.002).

Comparing means of MRI-measured and tape-measured waist and hip circumferences, a considerable difference was seen between tape measured 112.3(12.4) cm, and MRI-measured 121.8(11.0) cm waist circumferences, before weight loss. Before weight loss, there was less difference between tape-measured hip circumference, 123.7(11.6) cm, and MRI-measured hip circumference, 125.6(7.6) cm. After weight loss, tape-measured and MRI-measured hip circumference were 112.6(9.4) cm and 116.9(6.8) cm, respectively.

MRI-measured whole-body skeletal muscle mass ranged from 24.6-37.3 kg, while whole-body adipose tissue ranged from 32.6-68.5 kg. After 12–20 weeks of total diet replacement, there was a small decrease, Mean difference 0.71(1.3) kg in whole-body skeletal muscle mass; mean 29.5(4.4) kg, 28.8 (4.1) kg before and after weight loss. Adipose tissue mean difference was 10.6(5.7), mean 50.3(12.7) kg, before and 39.7(13.0) kg after weight loss **(Table 6-2).**

Due to time constraints, muscle strength measurements were completed for only seven participants; one participant left the study before providing her final strength measurements, while four participants are waiting to be booked for their second strength measurements.

6.4.2 Associations between MRI- and tape-measured waist and hip circumferences

Significant correlations were seen between MRI- and tape-measured waist and hip circumferences, waist: R^2= 89% and 54.2%, and hip: R^2 = 47.7% and 55.5% both before and after weight loss P<0.05 **(Table 6-3).** Correlations between tape and MRI measured waist circumferences was highest and SEE was lowest for the waist circumference before weight loss: R^2=89%, SEE=2.8cm. Mean waist and hip circumferences as measured by tape-measurement were lower than the MRI-generated figure, both before and after weight loss.

Bland Altman analysis of the above data **(Figure 6-2)** shows a positive relationship between MRI-measured and tape-measured waist circumferences before weight loss: lower values of tape-measured waist were underestimated and higher values were overestimated. No relationship was seen between tape- and MRI-measured waist after weight loss.

6.4.3 The relationship between MRI-measurements of muscle mass and adipose tissue, prediction equations and anthropometric measurements

Prior to weight loss, estimated muscle mass, using the previously described prediction equations (Algindan et al., 2014), significantly correlated with MRI-measured muscle mass (R^2=78%, SEE=2kg; see Table 6.4). After weight loss, a lower but still statistically significant correlation was seen (R^2=51.7, SEE=3.7kg). These predicted equations had better correlations than the muscle equation of Lee et al. (2000), who reported a correlation of R^2=66.1% (SEE=2.5kg) before weight loss and R^2=23.8% (SEE= 3.5kg) after weight loss. Adipose tissue mass (AT-PR; **Table 6-4**) showed significantly high correlations before and after weight loss R^2=86.2%, 91.6%, SEE =4.7kg, 3.7kg respectively. Compared to measures of fatness (BF%, waist hip ratio (WHR), waist height ratio (WHtR), waist and BMI) the validated prediction equations had better correlations, except for BMI, and showed the highest correlations with MRI-measured body fat (R^2=97.6%; **Table 6-4**).

6.4.4 Agreement between MRI-measured and predicted muscle and adipose tissue mass

All data, except for adipose tissue before weight, loss show a negative relationship; lower values are overestimated and higher values are underestimated. Bland Altman analysis of data (**Figure 6-3**) revealed that there was no bias between measured and predicted adipose tissue before weight loss. However, a slight overestimation of adipose tissue mass was evident before weight loss (mean difference = 1.73; **Figure 6-3**).

6.4.5 Association of MRI-measured muscle mass and adipose tissue with muscle strength

Muscle strength had no correlations with MRI-measured muscle mass, nor with adipose tissue mass: all p values were not significant ($p>0.05$; Table 6.5). Although individual subject characteristics (**Table 6-5**) indicated that hand grip, hamstring and quadriceps strength all decreased after weight loss, p-values using paired-t test for strengths before and after weight loss were not significant. Difference between hand grip strengths before and after weight loss was 1.33(3.8) Nm, p value from paired-t test = 0.433, difference between quadriceps strengths (knee extension) before and after weight loss was 13.2(36) Nm, p-value from paired-t test = 0.41. Difference between hamstring strengths (Knee flexion) before and after weight loss was 9.7(41.9) Nm, paired-t test p-value = 0.597.

6.5 Discussion

The Counterweight-Plus programme was effective in achieving substantial weight loss in all 10 participants, with a mean 15.3(8.6) kg loss in weight after 12-20 weeks of TDR. The maximum recorded individual weight lost was 32.1kg, while the minimum weight lost was 8.4kg.

There were wide variations in the changes in muscle mass during weight loss, and hence compliance with the regime. Six participants lost between 0.6-3.1kg of MRI-measured muscle mass, while four participants gained 0.09–1.1 kg. Using the anthropometry-based equations of (Al-Gindan et al., 2015), nine participants were

found to lose 0.9-4.9kg muscle mass, while one participant gained 0.02kg. There is sparse comparable data currently in the literature, as muscle mass is not routinely measured in weight loss studies. Many studies have estimated FFM using DEXA and/or BIA (Minderico et al., 2008, Thomson et al., 2007, Grossman and Payne, 2016), where FFM includes mass of bone, internal organs and muscle. A measurement of solely muscle mass, strength and function is crucial to understanding the physical and metabolic consequences of weight loss, as muscle plays key roles in whole-body protein metabolism, and in insulin sensitivity and carbohydrate tolerance (Wolfe, 2006). Muscle mass contributes to approximately 50% of total body weight which decrease with age. This decrease in muscle mass is parallel to mobility and muscle function (Goodpaster et al., 2006). Many factors relate to loss in muscle and strength most importantly physical inactivity, almost inevitable with aging. On the other hand, basal metabolic rate decreases as a result of skeletal muscle loss, and leads to a lower energy expenditure. The decreased metabolic rate, physical inactivity and lower postprandial energy expenditure due to lower fat oxidation is positively associated with increased aging. Muscle quality is also affected on aging due to fat infiltration of the muscle, which compounds poor physical performance (Kalyani et al., 2014)

The present study found no association between muscle mass and muscle strength in three different muscle groups. This could be due to many factors, but is likely here to be a result of the small sample size. Some participants complained of discomfort during strength measurements, so may not have truly tried their maximum. It is possible, or even likely, that there is no simple relationship between muscle bulk and its functional capacity, as many other factors affect muscle quality. A meta-analysis was conducted by Schaap et al. (2013) to determine the relationship between different body composition measures (BMI; waist and mid-arm circumference; WHR; fat mass; muscle mass; muscle fat infiltration) and muscle strength in men and women, with measures of functional decline as the outcome. Fifty papers met the inclusion criteria (prospective, longitudinal design, in English, age ≥65 years). The findings showed that low muscle strength and BMI≥30 were associated with functional decline (pooled odds ratio: 1.86, 95%CI: 1.32, 2.64, and OR: 1.60, 95%CI: 1.43, 1.80) for muscle strength

and BMI respectively. Muscle mass was not associated with functional decline pooled odds ratio: 1.19, 95% CI: 0.98, 1.45) (Schaap et al., 2013).

An important finding in this study is the agreement between MRI-measured adipose tissue and estimates made using the simple anthropometric equation introduced by (Al-Gindan et al., 2015).

Although measured and estimated muscle mass showed a high correlation, Bland Atman analysis of the data suggested bias. The sample size was very small. A Bland Altman plot will be more usefully done on a bigger number when more participants are recruited in this study.

This study examined the correlations with MRI-measured adipose tissue of various commonly measured anthropometric variables, as well as with estimates from the equation of (Al-Gindan et al., 2015) (Table 6-4). Again, the sample size here is too small to judge the true value of these methods, or to differentiate between them with any confidence, but nevertheless, BMI had the highest correlations and lowest SEE. Waist and hip measurements had moderate correlations, while WHtR showed somewhat weaker correlations. WHtR had no significant association with MRI-measured body fat. This analysis is shown only to illustrate the method that will be used for a larger number of subjects.

There is a debate in the literature about the different value and appropriate use of anthropometric measurements for body composition analysis and to predict health or disease, between the value of BMI, WHR, WHtR, waist and hip circumferences. In general, waist circumference measurement appears to have at least as much value as more complex variables. Using these measurements in an obese population is a different matter, especially when it comes to waist circumference. It is difficult to measure waist circumference in obese subjects, as finding the lowest rib and hip bone is sometimes a challenge. In addition, a large pendulous belly when standing affects, the measurement. There was a big difference, in the obese women studied here, between mean waist circumference measured by MRI and tape, while there was much less difference for hip circumference. This is

probably because waist circumference was measured with participants standing, while MRI scanning was carried out while participants were lying down.

There were two main limitations in this study: firstly, the small sample size available for analysis in this thesis. Nevertheless, this chapter is a pilot study, the group that has been investigated is definitely not intended for classification or diagnosis, this work simply suggest a correlation analysis using the results within a group. Secondly the difficulty in arranging appointments to allow the MRI, strength and anthropometric measurements to be simultaneous. The interval between these measurements varied up to 2 weeks.

Measuring whole-body MRI scans of obese subjects is not straightforward. There are absolute limits of BMI, weight and waist circumference, imposed by the apparatus, which are reflected in the exclusion criteria for this study. Several subjects were close to those limits, which made it impossible to include both arms beside the trunk. This problem was solved by making the measurements with one arm held above the head (thereby excluding it from the image) and then measuring that arm separately, and adding it to the total. It appears that this is the first time this pragmatic technique has been applied for the measurement of whole-body MRI in obese subjects.

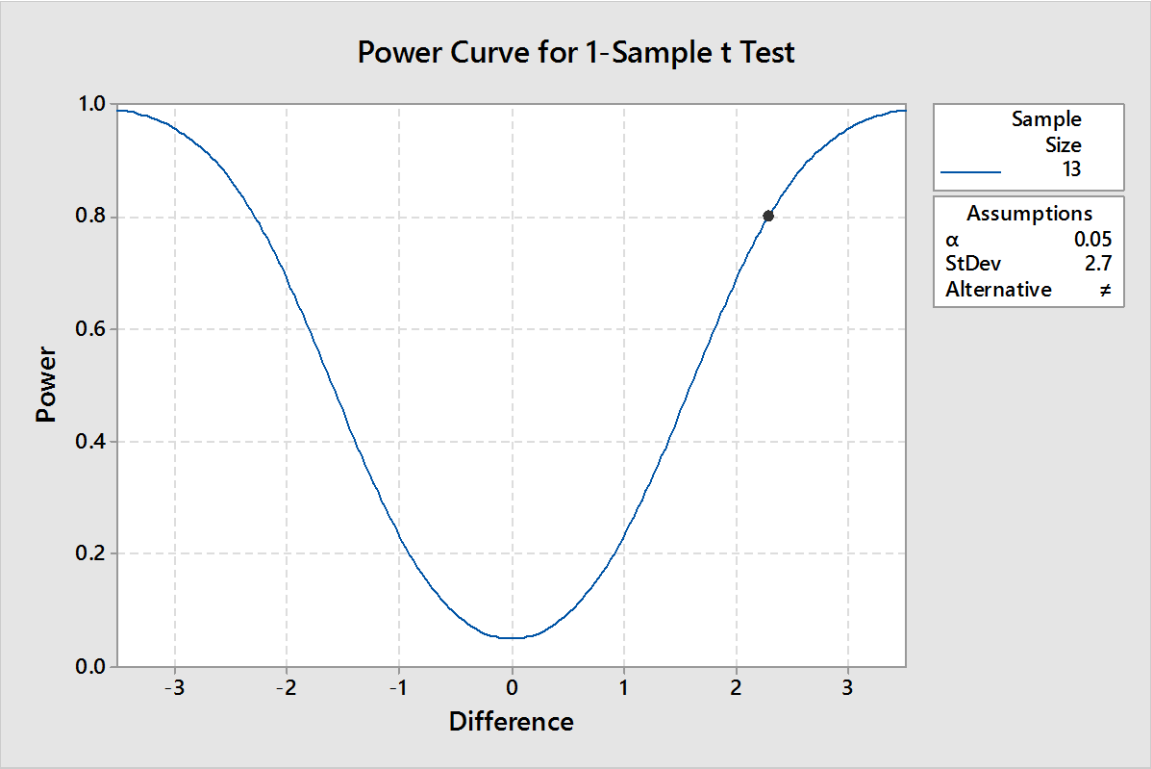
6.6 **Conclusions**

Total adipose tissue, estimated using anthropometric equations, showed high correlations before and after weight loss, and good agreement with MRI measured adipose tissue before weight loss. More studies are needed to establish a ranking of anthropometric estimates; however, in obese women, waist circumference is probably not the best anthropometric predictor of adipose tissue mass, and BMI may be the best simple measure.

Muscle mass estimates from anthropometric equations also had reasonably high correlations; however, limits of agreement were less good than for adipose tissue,

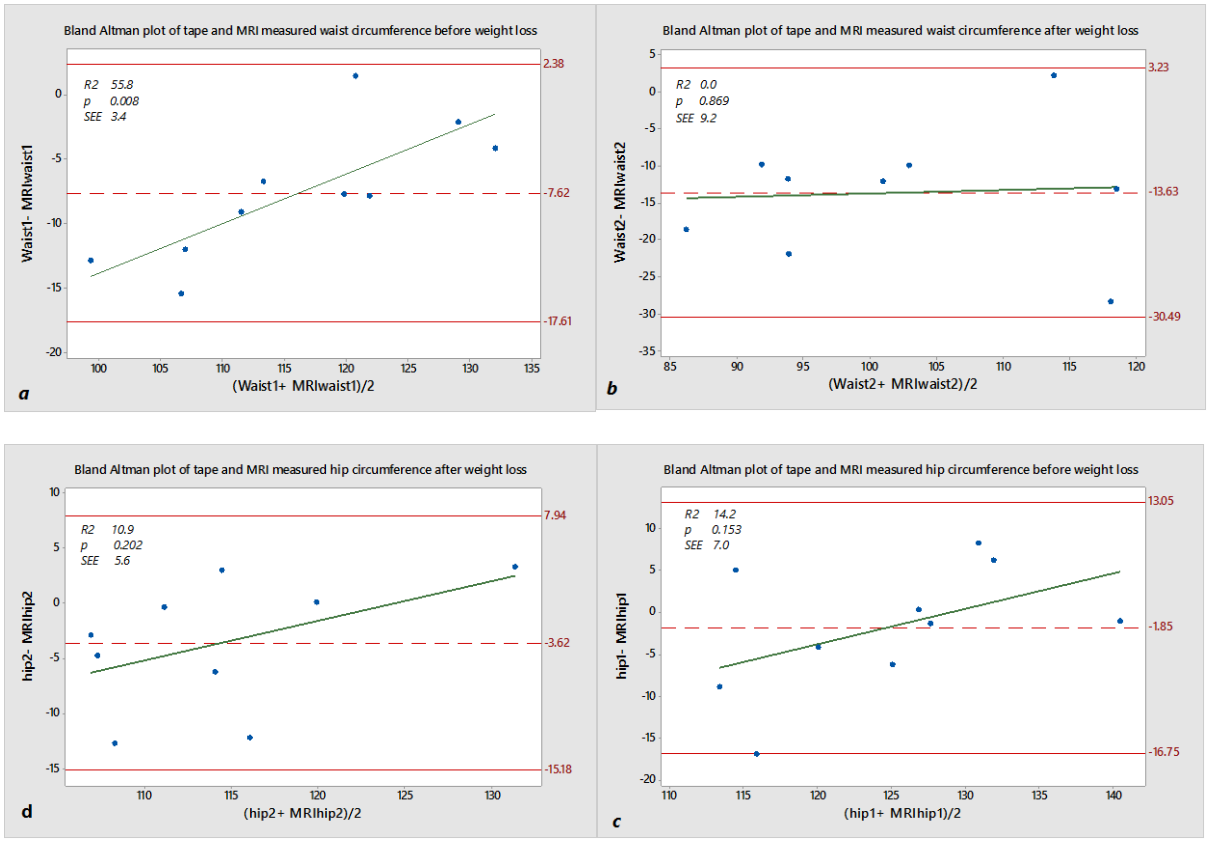
and fell further after weight loss. Further work is needed to confirm this finding and to establish a relationship between changes in muscle mass and strength during weight loss.

Figure 6-1: Power calculation using 1-sample t-test in Minitab



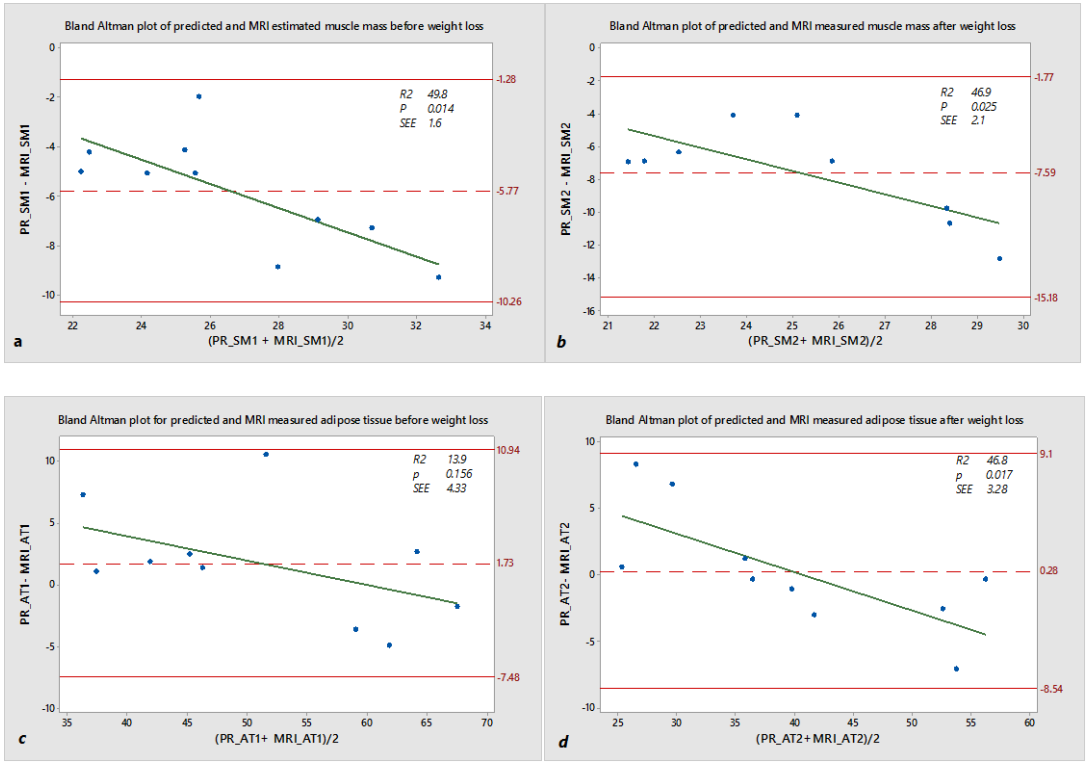
1-Sample t Test, α = 0.05, Assumed standard deviation = 2.7, power 0.8, difference 2.28

Figure 6-2: Bland Altman plots of MRI-measured waist and hip circumferences and tape-measured waist and hip circumferences, before and after weight loss.



Waist1(a):tape measured waist circumference in (cm) before weight loss, MRIwaist1(a): MRI measured waist circumference in (cm) before weight loss, Waist2(b):tape measured waist circumference in (cm) after weight loss, MRIwaist2(b): MRI measured waist circumference in (cm) after weight loss, hip1(c):tape measured hip circumference in (cm) before weight loss, MRIhip1(c): MRI measured hip circumference in (cm) before weight loss hip2(a):tape measured hip circumference in (cm) after weight loss, MRIhip2(d): MRI measured hip circumference in (cm) after weight loss, red dotted line: mean difference, red line: mean±2SD, green line: regression line, SEE standard error of the estimate, R2: linear regression of mean and average, $p<0.05$ significant

Figure 6-3: Bland Altman plot of MRI- measured muscle mass and adipose tissue and predicted muscle mass and adipose tissue estimates



Dotted line (---): mean of difference; upper and lower red lines: mean±1.96SD; green line: line of regression, SMPR.1, SPMR.2: muscle mass predicted by (Algindan et al 2014) before and after weight loss; ATPR.1, ATPR.2: adipose tissue predicted by (Algindan et al, 2015) before and after weight loss; SMMRI.1, SMMRI.2: MRI measured skeletal muscle before and after weight loss; ATMRI.1, ATMRI.2: MRI measured adipose tissue before and after weight loss; BMI: body mass index. Data shows a negative correlation between the MRI-measured values and predicted estimates.

Table 6-1: MRI inter-observer variability for two observers making three measurements each on one participant's abdominal section.

	Observer 1	Observer 2	Observer1	ObserveR2
		Muscle mass		Adipose tissue volume
volume				
Mean (cm^3)	15977.7	16927.5	6715.7	6391.3
StDev (cm^3)	223.9	214.1	43.0	113.8
CoV(%)	1.4	1.2	0.64	1.78

StDev: standard deviation, CoV: Coefficient of variation,
% difference between observers for mean muscle area was 4.95%,
% difference between observers for mean fat area was 5.77%.

Table 6-2: Characteristics of participants who completed the total diet replacement phase:

	Before weight loss		After weight loss	
	Mean(sd)	Min/max	Mean(sd)	Min/max
Age (years)	51.1(6.8)	38-57	-----	-----
Height(cm)	161.1(6.2)	154- 175	-----	-----
Weight (kg)	99.6(14.6)	81.3- 118.6	84.3(12.3)	65.7- 105.1
Weight change	-----	-----	-15.3(8.6)	-8.4 - -32.1
BMI(kg/m^2)	38.3(4.6)	32.5- 44.6	32.5(4.9)	26.6- 39.5
Waist circumference(cm)	112.3(12.4)	93.0- 130.0	95.4(13.0)*	77.0- 115.0
Waist-MRI (cm)	121.8(11.0)	105.8- 141.9	109.7(12.0)	95.5- 132.2
Hip circumference (cm)	123.7(11.6)	107.5- 140.0	112.6(9.4)*	102.0- 133.0
Hip-MRI(cm)	125.6(7.6)	112.0- 141	116.9(6.8)	108.4- 129.7
SM-MRI (kg)	29.5(4.4)	24.6- 37.3	28.8(4.1)	24.9- 35.9
AT-MRI (kg)	50.3(12.7)	32.6- 68.5	39.7(13.0)	22.4- 57.3
SM-PR (kg)	23.7(2.7)	19.8- 28.0	21.4(2.7)	17.9- 23.5
AT-PR (kg)	52.0(10.5)	37.9- 66.7	39.9(9.8)	25.6- 56.2
Hand grip strength (Nm)**	29(3.4)	24 - 33	27.2(3.1)	23 - 30
Knee extension (Nm)**	352.3(87.1)	192 - 473	336(86.3)	194 - 462
Knee flexion (Nm)**	155(39.5)	90 - 205	145.8(37.6)	90 - 205

Results reported as mean (SD); BMI: body mass index; Waist-MRI: waist measured by MRI in centimetres the area between lower rib and upper hip bone; hip-MRI: hip measured by MRI in centimetres, the largest area of hip. SM-MRI: Skeletal muscle measured by MRI in kg; AT-MRI: adipose tissue measured by MRI in kg: SM-PR: skeletal muscle mass predicted by (Algindan et al, 2014) Skeletal muscle mass(kg) = 2.89 + 0.255 body weight (kg) − 0.175 hip circumference (cm) − 0.038 age (y) + 0.118 height (cm); AT-PR: adipose tissue predicted by (Algindan et al, 2015) Total adipose tissue(kg) **=** 0.789 body weight (kg) + 0.0786 age(y) - 0.342 height (cm) + 24.5. Nm: Newton meters. The highest reading of three for each hand and leg was used in the analysis; *9 participants; **7 participants.

Table 6-3: Linear regression between MRI-measured waist and tape-measured waist and hip circumferences

	Before weight loss			After weight loss		
	R^2	P	SEE	R^2	P	SEE
MRI-waist, waist circumference	*89.0*	*0.001*	*2.8*	*54.2*	*0.014*	*8.4*
MRI-hip, hip circumference	*47.7*	*0.016*	*5.5*	*55.5*	*0.013*	*4.5*

R2: linear regression, SEE: standard error of the estimate; MRI-waist: MRI measured waist circumference in (cm), MRI-hip: MRI measured hip circumference in (cm), waist circumference and hip circumference both were measured using an inelastic plastic fiber tape. Measurements: waist measured as the midpoint between the lowest rib and the hip bone; hips at the level of the pubic symphysis and the greatest gluteal protuberance for both MRI and tape measures.

Table 6-4:Linear regression of MRI-measured muscle mass, adipose tissue and body fat with prediction equations and anthropometric measurements.

	Before weight loss			After weight loss		
	R^2	P	SEE	R^2	P	SEE
SM-MRI (kg) and SM-PR(kg)	*78.0*	*0.001*	*2.0*	*51.7*	*0.017*	*2.9*
AT-MRI (kg) and AT-PR(kg)	*86.2*	*0.001*	*4.7*	*91.6*	*0.001*	*3.7*
SM-MRI(kg) and SM-LR(kg)	*66.1*	*0.003*	*2.5*	*23.8*	*0.087*	*3.5*
BF-MRI (%) and BF-ML (%)	*36.5*	*0.038*	*4.5*	*52.6*	*0.016*	*4.9*
BF-MRI (kg) and BMI	*97.6*	*0.001*	*1.6*	*93.9*	*0.001*	*2.5*
BF-MRI(kg) and WHR	*0.2*	*0.688*	*10.7*	*2.9*	*0.302*	*9.6*
BF-MRI(kg) and WHtR	*48.6*	*0.051*	*7.3*	*55.4*	*0.013*	*6.5*
BF-MRI(kg) and waist	*62.6*	*0.004*	*6.2*	*63.2*	*0.006*	*5.9*
BF-MRI(kg) and hip	*68.6*	*0.002*	*5.6*	*50.3*	*0.019*	*6.9*

R2: regression analysis; $p<0.05$ significant; SEE: standard error of the estimate; SM-MRI: skeletal muscle measured by MRI in kg; AT-MRI: adipose tissue measured by MRI in kg: SM-PR: skeletal muscle mass predicted by (Algindan et al, 2014) Skeletal muscle mass(kg) = 2.89 + 0.255 body weight (kg) − 0.175 hip circumference (cm) − 0.038 age (y) + 0.118 height (cm); AT-PR: adipose tissue predicted by (Algindan et al, 2015) Total adipose tissue(kg) **=** 0.789 body weight (kg) + 0.0786 age(y) - 0.342 height (cm) + 24.5; SM-LR: whole-body skeletal muscle calculated using (Lee et al, 2000) equation =0.244Weight + 7.80 Height(m) + 6.6 sex(1 men, 0 women - 0.098 age + (0 for Caucasians) - 3.3; BF-MRI (kg): calculated using assumption (AT-MRI (kg) x 0.80); BF-ML: % body fat predicted using the Lean et al. (1996) equation = 0.439 waist (cm) +0.221 age (years)-9.4;

Table 6-5: Correlations of MRI-measured muscle and adipose tissue mass with muscle strengths.

	Before weight loss			*After weight loss*		
	R^2	*p*	*SEE*	*R^2*	*p*	*SEE*
SM-MRI(kg), Grip strength (Nm)	*0.001*	*0.616*	*5.4*	*9.2*	*0.287*	*4.7*
AT-MRI(kg), Grip strength (Nm)	*0.4*	*0.359*	*12.6*	*0.001*	*0.947*	*14.2*
SM-MRI(kg), Knee extension (Nm)	*0.001*	*0.847*	*5.5*	*0.001*	*0.968*	*5.6*
AT-MRI(kg), Knee extension (Nm)	*27.7*	*0.129*	*10.7*	*0.001*	*0.393*	*12.8*
SM-MRI(kg), Knee flexion (Nm	*35*	*0.095*	*4.1*	*0.001*	*0.895*	*5.5*
AT-MRI(kg), Knee flexion (Nm)	*0.001*	*0.977*	*13.8*	*0.001*	*0.728*	*14.0*

R2: regression analysis; $p<0.05$ significant; SEE: standard error of the estimate; SM-MRI: Skeletal muscle measured by MRI in kg; AT-MRI: adipose tissue measured by MRI in kg: Nm: right hand grip, knee extensions measured in Newton meters (Nm). Knee extension measured quadriceps strength; knee flexion measured hamstring strength. The highest of three readings of for both hands and legs was used in the analysis

Chapter 7

7 General conclusion

The purpose of all research is to establish important questions which are unanswered ('Research Questions'), and then to design appropriate, valid, scientific methods to try to answer them. Finally, conclusions must be drawn, to declare to what extent, or with what confidence, the Research Questions have been answered. Almost inevitably, in the course of research, limitations are recognized, and new research questions emerge for the future.

This thesis set out to investigate the use of simple anthropometric measurements to estimate muscle mass and adipose tissue mass in health and during weight loss, using MRI as the reference method. It also explored the use of the equations for identifying individuals with metabolic diseases in population surveys, to assess whether they might be more informative that the current usual use of BMI.

This final Conclusions chapter brings together the answers to the Research Questions posed in each chapter, summaries general findings and identifies some new Research Questions for future research. Detailed findings and conclusions are provided in detail in each chapter.

To answer the research questions, this study went through four parts; 1. Literature review, 2. Derivation and validation analyses, 3. Application in metabolic disease, 4. Application during weight loss.

7.1 **Literature review**

The literature for body fat and adipose tissue estimation was found to be confused, while the literature for muscle mass was scarce. It is critical that studies are using incorrect reference methods and assumptions based on small sample size and bad choice of reference methods. Saying that, human body composition literature is already based on theories that were established from studies with sample size and method limitations. The most accurate reference method in human body composition is the direct chemical analysis of cadaver (Heymsfield et al., 2015). Nevertheless, the famous Brussels cadaver study was based on a small sample size; 25 cadavers which were selected from 75 subjects on the basis of least emaciation and most normal appearance (Clarys et al., 2005). Another highly

cited cadaver study that was based on a 35 year old man that died from a myocardial infarction (Mitchell et al., 1945, Heymsfield et al., 2015). To date there is no gold standard reference method without limitations. Although MRI is considered the best available reference method. Due to the high cost of MRI, not many studies used it as reference method, others mistakenly used other less accurate methods such as DEXA. To account for MRI high cost, some studies used regional MRI as marker of whole body MRI and others coped with small sample size. There is a definite need for higher standard reference values and normative ranges to base our new body composition research on.

A consensus in applied reference methods will simplify the search for a simple field method that can be accurately used both clinically and epidemiologically. Currently the two studied field methods are anthropometry and bioelectrical impedance with none of them showing superiority over the other. With the use of an accurate reference method, the development of prediction equations to estimate body composition will be simplified. In the literature review, many prediction equations were retrieved, however Giving the numerous published prediction equations, it is difficult to choose the best prediction equation that accurately estimates the body compartment of interest, as all incorporate various limitations which were: 1. Most studies had small sample sizes, mainly because of the high cost of MRI, 2. Some studies did not validate their prediction equation in a separate population, 3. Agreement between methods was not assessed beyond simple regression analysis, 4. Studies in the literature that investigated regional muscle, adipose and fat mass/volume, rather than whole-body muscle, fat or adipose tissue mass/volume, were sometimes assumed to be representative of the whole-body data.

If prediction equations are the choice for body composition assessment, it is crucial to select the best prediction equation, there are certain criteria to look for:

1. Use of best or gold standard reference method, prediction error could come from reference method error,

2. Large sample size (i.e. n>100),

3. Small standard error of the estimate

4. High correlation (r>80%) between reference measurement and prediction

5. Validation of the prediction equation in a separate population.

In addition, it is important that the prediction equations were tested for agreement between methods using Bland Altman Plots.

In the first two chapters We managed to retrieve four body fat and adipose tissue prediction equations; (Deurenberg et al, 1991), (Lean et al, 1996), (Ross et al, 1992) and (Kvist et al, 1998). And for muscle mass, a systematic review was conducted. Out of 12 papers that used MRI as reference method for the derivation of muscle equations, only one published paper based on the above criteria was satisfactory (Lee et al, 2000). Thus, only the above five prediction equation were used as a comparator to the adipose/fat and muscle mass equations developed in the present thesis.

7.2 Derivation and validation of prediction equations to estimate muscle and adipose tissue mass

Sample size is a major limitation in the literature. The derivation and validation analysis in this thesis were based on existing databases, made available through a collaborative link within studies, sample sizes, = 423 for the derivation study and 197 for the validation analyses, the adults studied represented a range of different races, age and body masses. The reference method of choice was whole body MRI and the field method was simply anthropometric measurements.

Waist circumference was the best predictor for adipose tissue in men and body weight in women. Moreover, muscle mass was best predicted by body weight in both men and women.

Starting with stepwise regression analysis, then choosing the highest R^2 from multiple regression analysis for further investigation, prediction equations to estimate adipose tissue mass and muscle mass were developed for men and women. The combination of body weight, waist and hip was the best predictor for adipose tissue in men. In women, age, body weight, height and hip circumference best predicted adipose tissue. Combination of body weight, waist, hip and age were the best predictors in men muscle mass and body weight, hip, age and height were the best predictors for women's muscle mass.

Few published studies have explored hip circumference as a simple predictor of muscle mass. In this thesis adding hip circumference into multiple regression was identified as a consistently powerful indicator of muscle mass and also of adipose tissue. It is likely that muscle mass is importantly affected by gluteal muscle bulk, and in obese people also by fat.

Adding 'race' as a variable did not advantage the prediction equations. Instead, adding anthropometric measurements gave better outcomes. Thus the anthropometric measures were able to capture any differences in body composition from race.

The validation analysis of the prediction equations was satisfactory. Comparing them with prediction equations in the literature, they were similar or even better. The validated prediction equations gave higher correlations with MRI adipose tissue mass compared to BMI. Correlations against whole body adipose tissue mass R^2 = 79%, 84% for men and women adipose tissue prediction equations, 65.8 and 82.4 for men and women BMI. BMI when proposed by the Belgian mathematician Adolphe Quetelet, was not intended to assess obesity. It was mainly to measure growth of normal man. It wasn't until 1972 that Ancel Keys confirmed the validity of Quetelet index and named it body mass index. Since then BMI has been used to link access weight to mortality and morbidity (Eknoyan, 2008). It is well understood and appreciated the importance of BMI in the field of human body composition, in addition to its importance is in many other fields, such as medicine, economics and day to day practices. It is used in every hospital, clinic and even when applying for some jobs. It is a simple important tool.

However, if we are looking to investigate body composition in more detail for medical purposes, we need to identify body compartments where BMI is not the best tool for that. Muscle mass and adipose tissue have been linked to Disease and effective treatments. Thus, we need to find other measures to link body composition with disease.

7.3 Application: in metabolic illness

Testing the prediction equations in large health surveys (chapter 5), presented an understanding on how good the prediction equations predict chronic illness; diabetes and/or hypertension. Using the prediction equations, both muscle mass and adipose tissue mass were significantly associated with diabetes, hypertension, HbA1c and blood pressure.

After adjusting for age, the adipose tissue prediction equations correlations with diabetes were higher in men compared to women R^2= 17.6% and 10.8%. On the other hand, the muscle mass prediction equation associations with diabetes were the same for men and women. Adipose tissue prediction equations association with hypertension were higher in men compared to women R^2= 13.9% and 10.9% respectively, and vice versa for the muscle mass prediction equations R^2= 11.2% for men and 13.8% for women.

Muscle mass and adipose tissue mass prediction equations were significantly associated with HbA1c, systolic and diastolic blood pressure. Although correlations were not the highest, they were comparable with existing prediction equations and anthropometric indices. In general, women skeletal muscle prediction equations showed higher correlations than men, for those with type-2 diabetes and elevated blood pressure. The adipose tissue prediction equations presented higher association in men compared to women when analysed against HbA1c and systolic blood pressure. Diastolic blood pressure and adipose tissue mass were slightly higher in men. All the analysis was carried out adjusting for age, associations were much lower when not adjusted for age.

Prediction equations did not show superiority in associations, WHtR presented the highest correlation with HbA1c, diabetes and hypertension. Muscle mass predicted by (Lee et al, 2000), showed strongest association with systolic blood pressure. Diastolic blood pressure was best predicted by the new adipose tissue mass prediction equation in men and by BMI in women.

Although the prediction equations did not show the highest correlations compared to WHtR and muscle mass predicted by (Lee et al,200) equation, they were not far from WHtR strength of association; diabetes: R^2 = 18.6 and 17.6, hypertension: R^2 = 14.3 and 13.9 for WHtR and adipose tissue mass respectively in men. Differences were larger in women; diabetes: R^2 = 20.8 and 10.8, hypertension: R^2 = 15.3 and 10.9 for WHtR and adipose tissue mass respectively.

The results of chapter 5 indicate that the prediction equations are not superior to WHtR, (Lee et al,2000) the Lee equation and BMI, this only confirms that there is further investigation needed to reach to a consensus when it comes to the relation between body compartments (adipose tissue and muscle mass) and chronic illness. WHtR and BMI alone will not explain the complex association of muscle and adipose tissue. The (Lee et al, 2000) whole body skeletal muscle equation is definitely a well-developed and evaluated equation. It has shown impressive associations throughout the analyses in this thesis (chapters 2, 4, 5), however the equation uses race as a variable which is set for an American population, this could show bias if used in other populations for which there are little racial differences between a population.

This chapter adds to our understanding of the patho-physiology of type-2 diabetes in body composition. Although we can identify people with type-2 diabetes in health survey using HbA1c, it is less expensive and more practical using prediction equations. On the other hand, people at “risk” of future type-2 diabetes in health surveys are currently identified by high waist and BMI. The data in chapter 5 suggest that it may be possible to identify another category, ie people with low muscle mass (sarcopenic) but without a large waist or high body fat. This suggestion will need confirmation in longitudinal studies, to illustrate the possible value of estimating %SM.

This work is not trying to compete with age, height and family history as the strongest predictors of type 2 diabetes or hypertension, in risk prediction models such as Framingham. Body composition does add some predictive power, but BMI is a poor indicator of body composition, and this thesis has generated some evidence that with better characterisation of body composition, the prediction of these metabolic diseases might be improved in epidemiology. Body composition is also a potentially alterable element in the aetiology of these diseases, another reason to seek better estimates than BMI.

7.4 Application: during weight loss

The final study in this thesis exploring the equations during weight loss. Adipose tissue mass and muscle mass and prediction equations gave moderate to high correlations with MRI measured adipose tissue mass and muscle mass. Adipose tissue mass was superior in correlation and agreement with MRI measured adipose tissue mass. The relation between muscle strength and MRI measured muscle mass was explored, no significant correlation was detected.

Compared to all measures of fatness, adipose tissue mass prediction equation presented superiority in the level of association and agreement with MRI measured whole body adipose tissue, the values were; R^2= 86.2% AND 91.6% before and after weight loss.

The muscle mass prediction equations showed significantly high correlations with whole body MRI measured muscle mass before weight loss R^2= 78%. After weight loss significantly moderate associations were observed R^2= 51.7%. The new muscle prediction equations showed higher correlations than the existing muscle mass prediction equation.

The prediction equations were able to estimate change across wide range of weight change. Associations between adipose tissue mass change was significant and highly correlated with MRI-adipose tissue change R2= 85.4%. However, there is no association between change in predicted muscle mass and change in MRI measured muscle mass $p>0.05$.

Assessing inter-observer variability in whole body muscle volume measurements by different researchers indicated that although MRI is considered a 'gold standard' reference method, it is still dependent on individual observation of muscle and adipose tissue areas on scans. Thus, human error is expected. This could be automated, but still the allocation of MRI characteristics to identify tissues remains operator-dependent and therefore can also introduce errors. As highlighted in the previous chapters, the prediction equations are intended to use for epidemiological research only. In chapter 6, the pilot study has only 10 subjects. Using a larger sample size could give better associations. It is important to highlight that the analysis in chapter 6 is a start for a larger sample size analysis, thus this chapter was not intended to categorize individuals, instead we suggest a correlation analysis using the results within a group.

A final conclusion, we can now estimate adipose tissue mass adequately for many purposes, as population or group means, and those measurements remain after drastic weight loss. However, we must be very careful not to assume greater accuracy than the data show, and use these methods for assessing fatness and categorising individuals. The R^2 value for estimates is about 86.2 – 91.6%, which leaves unexplained variability for individuals. This caveat applies equally or more so to BMI, but BMI has unfortunately been used inappropriately very widely to make judgements about the fatness of individuals.

For estimating muscle mass, with R^2= 78 – 51.7%, the equations developed are weaker than for adipose tissue so the warning should be even stronger, not to use them to categorise individuals.

This thesis has not yet established any criteria from which to identify 'sarcopenia', because the quality of muscle varies widely. Loss of muscle mass (as estimated by the equations) within individuals observed over time might now be more easily detectable. This deserves future research. However, estimating muscle mass alone is insufficient. Measurement of muscle quality, as reflected by strength, is one aspect. That did not form a major part of the present thesis, but there was some evidence for a small decline in strength during weight loss, among obese people who were not able to increase physical activity greatly.

7.5 Future directions:

Human body composition is a rich area of research that is still thirsty for more research. From this thesis some future directions are required:

1. Validation of the adipose tissue mass and muscle mass prediction equations in a large dataset from different populations, as predictors of health and disease conditions.

Research Question: How well do the adipose tissue mass and muscle mass prediction equations perform in healthy children, populations of different ethnic groups (other than the American identified race: Caucasian, African American, Asian and Hispanic)?

2. Investigating the value of prediction equations over time in progressive diseases.

Research Question: how well do the adipose tissue mass and muscle mass prediction equations perform during chronic illness such as cancer and heart disease?

3. Exploring the quality of the prediction equations (against MRI measurements) before and after major weight loss with bariatric surgery.

Research Question: How well do the prediction equations detect adipose tissue mass and muscle mass before and after major weight loss with bariatric surgery?

4. Testing the ability of the prediction equations to accurately detect adipose tissue mass and muscle mass change during non-surgical weight loss in a larger sample size. The study described in Chapter 6 is ongoing (n = 20) with expected results after one year.

Research Question: How well do the prediction equations detect substantial non-surgical weight loss?

5. Bioelectrical impedance is widely used in clinics and research settings, the weight loss study (Chapter 6) has data from bioelectrical impedance. It will be enlightening to compare estimates from bioelectrical impedance and the prediction equations against MRI-measured adipose tissue and muscle mass.

Research Question: Can the prediction equations replace bioelectrical impedance?

6. Muscle strength analysis with a larger sample size is needed to establish whether there are associations, and to search for criteria for sarcopaenia.

Research Question: How well do the prediction equations correlate with muscle strength? And can muscle strength be added as part of a prediction equation to diagnose sarcopenia?

7. Measuring the association between quality of life and the adipose tissue mass and muscle mass prediction equations

Research Question: How well do the prediction equations associate with measures of quality of life?

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9 Appendices

Appendix 1: Predicting muscle mass from anthropometry, using magnetic resonance imaging (MRI) as reference: a systematic review

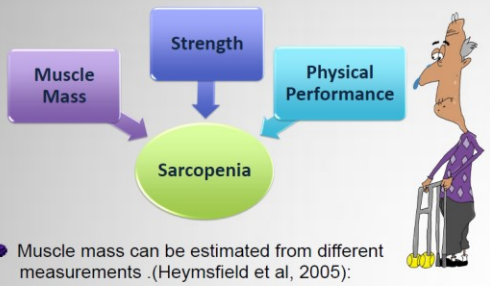
University of Glasgow | College of Medical, Veterinary & Life Sciences

Predicting muscle mass of adults from anthropometry, using magnetic resonance imaging as reference: a systematic review

Y.Y.Al-Gindan, C.R.Hankey, W.S.Leslie, L.Govan and M.E.J.Lean.
School of Medicine, Glasgow Royal Infirmary, Glasgow G4 0SF, UK

Background:

- The term **sarcopenia** has been used for over 20 years to indicate lack of muscle (Morley et al, 2010).
- There is no agreed definition, nor diagnostic criteria (Cooper C et al, 2011)



- Muscle mass can be estimated from different measurements .(Heymsfield et al, 2005):

Objective:

To evaluate published studies using anthropometric measures to predict the muscle mass/volume of adults, measured by MRI (as reference method) .

Methods:

Two researchers searched literature and assessed studies independently (YA and WL), following guidelines of the Cochrane Library hand book for systematic reviews. Searches comprised (Medline, Embase, Web of Science, Pubmed, Cochrane Library) .

Results:

Reference	N (M/F)	Predictive Variables	R^2
Whole Body Muscle Mass			
Lee, 2000	150F/	1.BW, HT, AGE, SEX, RACE	D: 0.86,V: 0.79
	174M	2.HT, CAG, CTG, CCG, SEX AGE,RACE	D: 0.91,V: 0.83
Ross, 1994	40F	1.BW,HC	D: 0.62
	17M	2.BW,WC,TC	D: 0.89
Limb Region Muscle Mass			
Chen, 2011 (Thigh)	36 F	1.age, BW, TC	D: 0.62
	33M	2.age, BW, TC, WC	D: 0.68
Knapik, 1996 (Thigh)	9F /9M	CTG + TL	D: 0.92
Housh, 1994 (Thigh)	43M	1. CTG	D: 0.72,V: 0.64
		2. CTG	D: 0.52,V: 0.29
		3. CTG	D:0.74,V: 0.77
Fuller, 1999 (Thigh, calf)	8M/8F	CTG	D: 0.35
		CCG	D: 0.69
Tothill, 2002 (Thigh)	9M/10F	CTG	D: 0.80
Mathur, 2008 (Thigh)	22F/	1. CTG	V: 0.06
	18M	2. CTG	V:0.08
Tonson, 2008 (Arm)	46M	CAG	V:0.90
Nakamura, 2006 (Thigh)	16F	TC	V:0.12
Bamman, 2000 (Calf)	39F	CCG	V:0.45
Baumgartner, 1992	17F /8M	CAG	V:0.69
(arm, thigh)		CTG	V:0.43

D: derivation study, V: validation study

BW: body weight, TC: thigh circumference, WC: waist circumference, HC: hip circumference, HT: height, TL: thigh length, SF: skin -fold, CAG: SF corrected arm girth, CTG: SF corrected thigh girth, CCG:SF corrected calf girth, CC: calf circumference, UAC: upper arm circumference, MTC mid thigh circumference

Evaluation and conclusions:

- **Most studies assessed only regional MRI muscle mass/volume.**
- **Most used limb circumference adjusted for skin-fold thickness which limits practical application.**
- **The link between limb muscle volume and whole body muscle volume is not well established.**
- **None of these studies has assessed change over time.**
- **The simplest method for whole body muscle prediction is Ross et al 1994, but this has not been validated in a separate population.**

References:

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Cooper C, Dere W, Evans W.(2012). Osteoporos Int, 23:1839-1848
Heymsfield S, Lohman T, Wong Z.(2005)Human Kinetics Publisher: 209- 212

University of Glasgow, charity number SC004401

Appendix 2: Derivation and validation of simple anthropometric equations to predict adipose tissue fat mass, using MRI as reference method.

University of Glasgow | College of Medical, Veterinary & Life Sciences

Derivation and validation of simple anthropometric equations to predict adipose tissue fat mass, using MRI as reference method

Yasmin Y Al-Gindan, Catherine Hankey, Lindsay Govan, Dympna Gallagher, Steven B Heymsfield, Michael EJ Lean

Background

MRI is the reference method for quantification of fat mass from adipose tissue measurements. It has been validated in human cadavers (1,2).

Practical estimation of total adipose tissue and total fat mass, is valuable for epidemiology and clinical use, but current prediction equations are not based on MRI.

Objective

Derivation & validation of new anthropometric equations to estimate total adipose tissue fat mass (TATFM) derived from MRI measurements, & to validate existing prediction equations.

Methods

Derivation-sample: n = 416 (222 women), aged 18–88 years; BMI I5.9-40.8 kg/m^2.

Validation-sample: n = 204 (110 women), aged 18–86 years; BMI 15.7-36.4 kg/m^2.

Both samples included mixed ethnic/racial groups.

All underwent whole-body MRI to quantify total adipose tissue volume, which was converted to adipose tissue weight by multiplying by 0.92, & this was converted to TATFM by multiplying by 0.8 (1).

Prediction equations for TATFM were developed from anthropometric measures, & investigated for agreement & bias, before validation in separate datasets.

Results

The best equations, with high R^2 & good agreement without bias in Bland-Altman plots, were:

Men TATFM (kg) =
-5.95 + 0.901**waist**(cm) - 0.103 **BW(kg)** - 56.5 **waist/hip**
R^2 = 0.81, SEE = 2.9 (derivation analysis) (Figures 1A & 1B)

Women TATFM (kg) =
8.68 + 0.0581 **age(y)** + 0.507 **BW(kg)** - 0.256 **height(cm)** + 0.165 **hip(cm)**
R^2= 0.84, SEE = 2.4 (derivation analysis) (Figures 1C & 1D)

Adding a 'race' variable did not add predictive power.

Validation of Lean et al, (3) & Deurenberg et al, (4) equations was good, but below that of the new equations (Figures 2 & 3)

Conclusions

New validated equations, using simple anthropometric measurements, estimate MRI-measured TATFM with higher correlations & better agreement than existing equations

Figure 1: validation of new MRI-derived equations

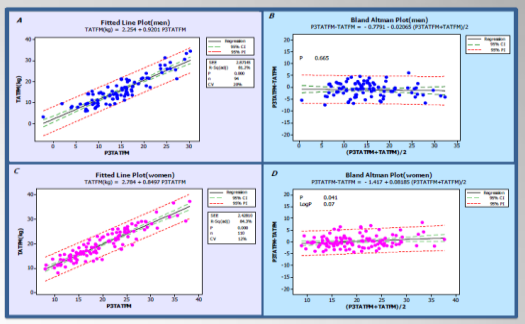


Figure 2: validation of under-water weighing-derived equations (Lean et al, 1996) (3)

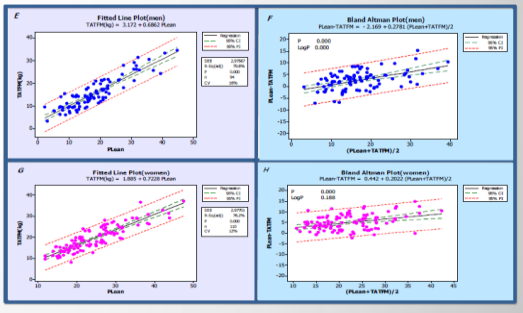
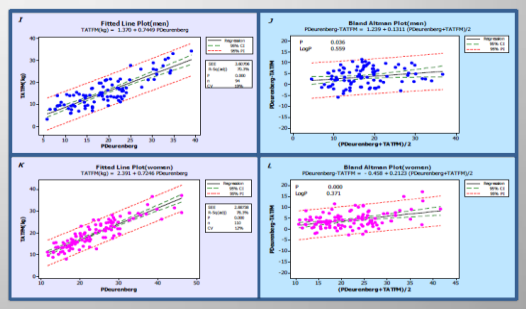


Figure 3: validation of underwater-weighing-derived equations (Deurenberg et al, 1991) (4)



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4. Deurenberg et al, 1991. Br J Nutr, 1991(65).

Acknowledgement

This work forms part of a PhD program at the University of Glasgow, supported by a grant from University of Dammam

Appendix 3: Ethical approval for the BEYOND weight loss study

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 5
Ground Floor - Tennent Building
Western Infirmary
38 Church Street
Glasgow G11
6NT

Date 20 January 2015

Direct line 0141 211 2102
E-mail WoSREC5@ggc.scot.nhs.uk

Professor Michael Lean
New Lister Building, Room 2.19
Glasgow Royal Infirmary
10-16 Alexander Parade
G31 2ER

Dear Prof Lean

Study title: **Body composition and Energy Expenditure with Total Diet Replacement during weight loss and maintenance**

REC reference: **14/WS/1164**

10 **IRAS project ID: 152818**

Thank you for your letter received on 13 January 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a Sub-Committee of the REC. A list of the Sub-Committee members is attached.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish

to make a request to defer, or require further information, please contact the REC Manager, Mrs Sharon Macgregor, WoSREC5@ggc.scot.nhs.uk. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

11. Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

12. Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

13. Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

14. Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [BEYOND poster]	4	29 November 2014
Covering letter on headed paper [Cover letter]	1	05 December 2014

Evidence of Sponsor insurance or indemnity (non NHS Sponsors		30 July 2014
only)		
GP/consultant information sheets or letters [GP notification letter]	2	03 December 2014
GP/consultant information sheets or letters [GP referral letter]	4	12 January 2015
Interview schedules or topic guides for participants [Assessment Schedule]	3	29 November 2014
Other [Dr. Lindsay supervisor]		01 September 2014
Other [Cover letter (response letter)]	2	08 January 2015
Participant consent form [Consent form]	5	08 January 2015
Participant information sheet (PIS) [Participant Information sheet]	6	08 January 2015
Participant information sheet (PIS) [MRI information sheet]	2	08 January 2015
REC Application Form [REC_Form_08122014]		08 December 2014
Research protocol or project proposal [Beyond weight loss protocol]	V6	08 January 2015
Summary CV for Chief Investigator (CI) [Prof. Micheal Lean]	1	03 November 2014
Summary CV for student [Yasmin Algindan CV]		01 November 2014
Summary CV for supervisor (student research) [Dr. Catherine Hankey CV]		04 September 2014
Validated questionnaire [Counterweight Plus Protocol for Assessing Binge Eating Disorder]		
Validated questionnaire [EQ-5D questionnaire]		
Validated questionnaire [WOMAC questionnaire]		

15. Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

16. After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports

- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

17. User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/qualityassurance/

18. HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

14/WS/1164	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Gregory Ofili

19. Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

"After ethical review – guidance for researchers"

Copy to: Ms Emma-Jane Gault, Glasgow University

Mrs Maureen Travers, NHS Greater Glasgow & Clyde

20. West of Scotland REC 5

Attendance at Sub-Committee of the REC meeting

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Ahmed Khan	Consultant Psychiatrist	Yes	
Mrs Liz Tregonning (Alternate ViceChair)	Retired (Special Needs Teacher)	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Mrs Sharon Macgregor	Co-ordinator

Appendix 4: Quantification of whole body fat and muscle using Magnetic Resonance Imaging

University of Glasgow | College of Medical, Veterinary & Life Sciences

NHS Greater Glasgow and Clyde

Quantification of whole body fat and muscle using Magnetic Resonance Imaging

MR Lopez Gonzalez[1], Y Algindan[2], N Brosnahan[2,*], G Thom[2], L Govan[2], C Hankey[2], G. Roditi[3] and M EJ Lean[2]
[1]Department of Clinical Physics and Bioengineering, NHS Greater Glasgow and Clyde, Glasgow, UK
[2]Department of Human Nutrition, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK.
[3]Department of Radiology, NHS Greater Glasgow and Clyde, Glasgow, UK. *Counterweight Ltd.

Purpose/Introduction

Clinical methodologies are necessary to assess body composition (i.e. fat and muscle), indirect methods have lower accuracy compared with current imaging methods such as CT and MRI [1-2].

The aim of the BEYOND Weight Loss study is to develop a method to measure fat content and distribution and muscle mass accurately. In this study we optimised an MRI acquisition protocol for the quantification of whole body adipose tissue and muscle in volunteers.

MRI-based methods allow for very high resolution assessment of regional variations of fat and muscle composition. MRI-based techniques to assess body composition are: standard T1-weighted imaging and based on the so-called "DIXON" sequences, which are sequences designed to generate images containing only fat or water [3].

Subjects/Methods

This study was undertaken following ethical approval and with informed written consent. The data presented here is from females and a male volunteer, 18-65 years, BMI 30-45kg/m^2. Acquisitions were performed at 1.5T (Philips Ingenia).

(i) Dixon technique: Breath hold mDixon (TR/TE=5.6/4.6ms, Matrix size=392x339, FoV=450mm).
(ii) Standard T1-w imaging: Transversal T1-w (TR/TE=412/6ms, Matrix size=424x397, FoV=558mm) with slice thickness of 5 mm.

The segmentation tools used were ImageJ and the Extended MR workspace Philips. Fat and muscle tissue content was quantified by using threshold and manual segmentation techniques.

Results: mDIXON

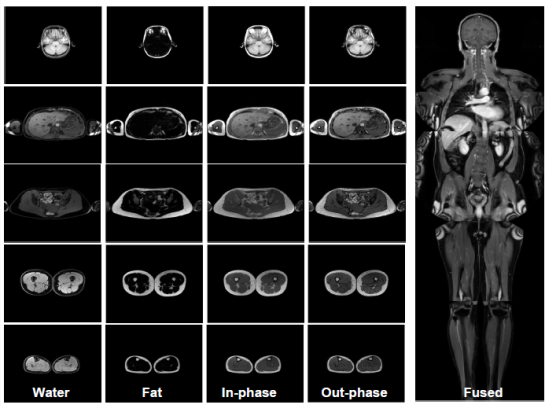

Figure 1: mDixon: Transversal slices of the whole body of volunteer 1.

ImageJ Analysis

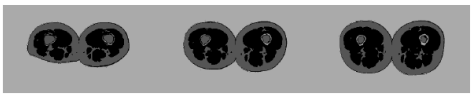

Figure 2: Semi-automatic segmentation adjusting threshold values using ImageJ.

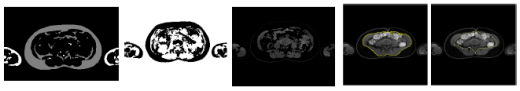

Figure 3: ImageJ data analysis. Left: Semi-automated segmentation masks and data with masks applied. Right: Manual segmentation in abdomen and thorax.

MR workspace analysis

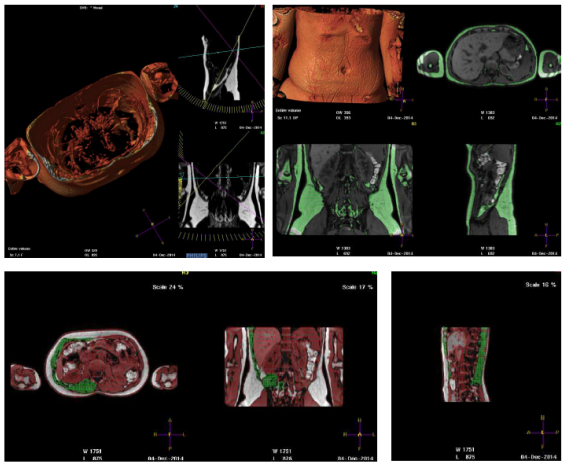

Figure 4: Fat tissue segmentation (Top). Skeletal muscle segmentation (Bottom).

Whole Body MR mDixon Advantages/Disadvantages

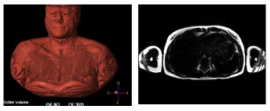

Figure 5: The mDixon images present susceptibility artefacts when scanning our volunteers, though the total scanning time was less than 20 minutes compared with under an hour in the case of the SE T1-w approach.

Results: T1-w Imaging

The inter-observer variability

FAT		
Mean (cm^3)	15977.7	16927.1
StDev	223.8	214.1
CoV (%)	1.4	1.2
MUSCLE		
Mean (cm^3)	6715.7	6391.3
StDev	43.0	113.8
CoV (%)	0.6	1.7

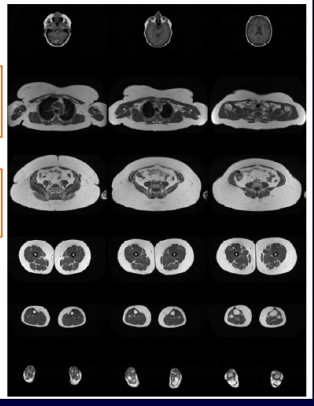

Figure 6: Transversal T1-w whole body scan in a female volunteer.

Conclusions and Future work

- Using the MRI mDixon and SE T1-w protocols we were able to measure adipose tissue and skeletal muscle with accuracy.
- The T1-w imaging protocol will be used in BEYOND participants in order to reduce susceptibility artefacts.
- BEYOND participants will follow the Counterweight Plus for 12-20 weeks.
- Participants will have a baseline and a follow up scans to assess fat and muscle changes before and after Counterweight Plus.

References

[1] Baumgartner R N, et al. Am J Clin Nutr 1988;48:936-945. [2] Foster MA et al Magn. Reson.Imag 1984;2:187-192. [3] Dixon WT, Radiology 1984;153:189–194.

Appendix 5: Body Composition and Energy Expenditure with Total Diet Replacement during weight loss and maintenance (BEYOND): study protocol

Body Composition and Energy Expenditure with Total Diet Replacement during weight loss and maintenance (BEYOND): study protocol

Algindan Y[1+], Brosnahan N [1,2], Thom G[1], McCombie L[2], Hankey C[1], Lean M (Chief Investigator), Govan L[1], Gerasimidis K[1], Rizou E[1], Dombrowski S[3], Banger M[4], Roditi G[5], Lopez-Gonzalez R[5]. *[1]University of Glasgow, [2] Counterweight Ltd., [3] University of Stirling, [4] University of Strathclyde*
+ Lead Author

Introduction: Weight loss >15 kg is the national target recommended by the SIGN obesity guideline. Long-term maintenance of non-surgical weight loss remains the most significant problem in obesity treatment.

Aims: This study seeks to integrate physiological and behavioural perspectives to advance the field of weight loss and maintenance science by investigating:

1. Changes in body composition with substantial weight loss using published anthropometric prediction equations and MRI as reference
2. Metabolic adaptations (including resting and non resting energy expenditure, gut microbiota and hormonal factors)
3. Participant views and experiences of weight loss and weight loss maintenance and how they change during intervention
4. Assess binge eating disorder, quality of life and strategies to prevent weight regain

Methods:

- 20 patients will be recruited via posters
- Main inclusion criteria: women aged 18-65 years, all ethnicities, body mass index >30kg/m^2 - <45kg/m^2
- 'Counterweight Plus' weight management programme, which includes total diet replacement, food reintroduction and weight loss maintenance
- Study measurements including bloods, resting energy expenditure and stool samples will be taken during each phase of the programme, and once body weight is stable

Results:

Primary outcomes:

- Muscle and adipose tissue mass will be estimated using published prediction equations and compared with measured values from whole body MRI

Secondary outcomes:

- Functional muscle strength and QOL
- Changes in key psychological process measures
- Changes in resting and non resting energy expenditure
- Hormonal changes; to include leptin, ghrelin, thyroid profile
- Changes in gut microbiota and colonic inflammation
- Weight change, WC, HC, adherence, and compliance
- Repeated use of Total Diet Replacement and Food Re-introduction plans as a weight loss maintenance strategy

Conclusion:

This study will:

- Establish validation for predictive equations for muscle and adipose tissue mass and their relation to muscle strength
- Improve our understanding of the physiological and psychological changes that occur prospectively during weight loss and maintenance
- Inform our knowledge of effective approaches towards
- relapse prevention and long term weight loss maintenance

Initial results available from 2016.

'Counterweight Plus' weight management programme

SCREENING VISIT	Check entry criteria/readiness to change Secure informed consent
TOTAL DIET REPLACEMENT (TDR) (0-12* weeks)	800+ Calories per day (Cambridge Weight Plan Pro800; shakes/soups)
FOOD REINTRODUCTION (12-18 weeks)	Gradual introduction of portion-controlled balanced meals TDR reduced as meals introduced Physical activity gradually increased
WEIGHT LOSS MAINTENANCE (19-104 weeks)	All nutrition from normal food and drink Focus on maintaining lifestyle Stepped approach to weight regain prevention Relapse management & 'Rescue Package' for weight regain or return of T2DM Sustainable increase in physical activity maintained

** TDR can be extended for up to a maximum of 20 weeks*

Trial Registration: ISRCTN03267836

Sponsor: NHS Greater Glasgow and Clyde

Funding: Department of Clinical Nutrition University of Dammam, Department of Human Nutrition University of Glasgow

Investigators: Prof. M Lean, Dr C Hankey, Dr L Govan, Y Algindan, G Thom, N Brosnahan, Dr G Roditi, Dr Lopez-Gonzalez, M Badger, Louise McCombie, Hazel Ross

Conflicts of Interest:

- ML and NB have received funding from Cambridge Weight Plan for conference attendance and for related departmental research
- NB and LM are shareholders and employees of Counterweight Ltd.

Appendix 6: BEYOND participant information sheet, consent form and schedule of assessment:

School of Medicine

Body composition and Energy Expenditure with Total diet replacement during weight loss and maintenance

BEYOND Weight Loss Study – Participant Information Sheet

You are being invited to take part in a research study. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. This study will be conducted at the Royal Infirmary hospital, supervised by Professor Michael EJ Lean and will contribute to a PhD qualification.

What is the purpose of the study?

The purpose of this study is to understand more about the changes to our bodies during and after weight loss. We aim to do this in a number of ways.

We will measure the total amount of muscle and fat in your body before a weight loss programme using magnetic resonance scan (MRI), and then again after you have completed the weight loss programme. This will provide important information on how much of your weight loss is from fat, and how much is from muscle. Scan results will be compared with measurements of weight and height, and hip and waist circumferences.

Along with this your handgrip and lower limb strength will be measured through special equipment similar to what you might find in a gym. An assessment of your range of motion and muscle force will also be recorded during repeated flat walking for about 5m. You will be required to have reflective markers stuck to your skin or tight clothing that will be tracked by a camera system in a specially designed laboratory. We will look at whether there are any relationships between all these measurements, estimates of muscle strength and your walking assessment.

You will also have your metabolic rate measured at every appointment whilst you are losing weight. This will provide important information about how many calories your body is using. This will reduce with weight loss, but we do not know by how much, and will differ between individuals. The results of this will help us to advise you on how best to maintain your weight loss. A fasting blood sample will also be taken at each visit during the weight loss phase of the programme; this will give us information on important hormonal changes that occur whilst you are losing weight.

We are also keen to understand how your views and experiences change whilst you are losing and maintaining weight loss. We will ask you to complete a short questionnaire at each visit, and will also ask you to attend interviews about key topics such as motivation, self-belief, overall satisfaction and the support you have at key intervals during your weight loss journey.

These will take no longer than 30 minutes, and will be at your study appointments, about seven times over a two-year period. Interviews will be audio recorded with a voice-recorder, transcribed verbatim and analysed. Following transcription audio files will be destroyed. All files will be kept on password protected computers at all times and will only be accessible to members of the research team.

How will I lose weight?

We will provide you with a structured weight management programme (Counterweight Plus). The programme aims to achieve at least 15-20 kg (2 ½ - 3 stones) weight loss. During the programme you will be given full support/advice from the research dietitian/nutritionist for two years.

This will include following a Total Diet Replacement (TDR) for 12 weeks. If you are doing well and wish to continue losing weight, you can stay on the TDR for up to 20 weeks. You will stop all of your usual food intake on the TDR. The diet is a combination of nutritionally balanced soups and shakes. You will be able to have four soups/shakes per day, totalling 825–853 calories. This will be provided to you free of charge. The TDR is followed by food re-introduction over a period of 6 – 8 weeks, which means reducing the soups/shakes and adding back in normal food and increasing your calorie intake gradually to minimise the risk of you regaining weight. This is followed by a weight loss maintenance programme to enable you to manage your weight in the long term. There will be a total of 34 visits during the two-year study period.

Why have I been chosen?

You volunteered as you could benefit from substantial weight loss.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. You can opt out of some of the assessments, if you choose.

What will happen to me if I take part?

After confirming with your GP your suitability to participate in our study (your GP will be asked to provide details of your medical history and medications (if any)), you will be asked to follow an intensive weight loss programme and have some additional measurements of your body taken every two weeks. There are MRI scans, weight, height and waist and hip circumferences and muscle strength measurements (hand grip lower and upper limb strength), resting and non-resting metabolic rate, blood, urine and faecal samples; in addition, you will be given short questionnaires covering eating behaviours, quality of life, activity performance and key psychological measures. In total appointments will take 1 hour per week, except for the baseline appointment and the end of the total diet replacement phase. During the maintenance phase appointments are 23 minutes per week (for more details please refer to the schedule of assessment)

What do I have to do?

Before you start the Counterweight Plus weight management programme you will be invited to attend the Royal Infirmary hospital for an assessment appointment. You will be met by the researcher and taken to radiology to have your MRI scan. You will be asked to lie down and be put into a scanning tunnel, and asked to remain still for 45 minutes. If you are uncomfortable you can push a buzzer and ask staff to release you from the scanner. Your muscle strength will be estimated using hand grip strength lower limb strength and muscle performance from flat walking; this will take around 30-45 minutes. These measures will be repeated after 12 weeks.

You will have your resting metabolic rate (RMR) measured and a 20 ml fasting blood sample taken every two weeks at the clinical research facility (CRF) in the Royal Infirmary until your weight stabilises and then at 6, 9, 12, 18 and 24 months. RMR is the amount of calories your body uses at rest to maintain vital bodily functions such as breathing, keeping your heart beating and pumping blood around the body. This will take about 30 minutes, using a special machine called an indirect calorimeter and involves lying flat on a bed breathing into a ventilated hood (see picture). We ask you just to breathe normally inside the hood. At the end of the test we will be able to tell you how many calories your body is burning. We will also ask you to complete an almost identical test whilst you are doing some low intensity exercise (walking on a treadmill). You will breathe through a specially designed mask which will measure how much oxygen you are consuming. This will tell us how many calories your body is using whilst you are doing usual lifestyle-related physical activity, and whether this changes much after you have lost weight. **In addition to this, we are** interested to monitor whether there are any changes in your physical activity level as you lose weight. To this end, we will ask you to wear an accelerometer on up to 4 occasions during the 2-year study to track your physical activity level over a 7-day period. An accelerometer is a lightweight wrist worn device (like a watch) which objectively measures all of your movement, resting and sleeping patterns. It is water-proof and doesn't need to be removed during the 7-day period.

What are the possible disadvantages and risks of taking part?

The effects of magnetic fields in an MRI scanner have been widely investigated; there are no known significant risks. You will lie down and may be bothered by the noise made by the

magnet during the procedure, for that you will be asked to wear earplugs while in the magnet. You may not participate in this study if you have implanted electronic or metallic devices.

There are very few health risks from following this weight management programme. Some people may experience symptoms during weight loss. These are usually temporary and go away once body weight is stable at a lower level.

- Constipation, we advise taking Fybogel (a fibre supplement) to overcome this.
- Dizziness is possible when standing up suddenly. This is due to the body adjusting to a healthier lower blood pressure and happens mainly in those who were taking medication to control their blood pressure. If this occurs, take more time when standing up, and don't become dehydrated, drink plenty of water.
- Gall-stones, may cause abdominal pain, most often a consequence of existing gall-stones. The diet we are using contains some fat, which further minimises the risk of gall-stone problems

Taking part will involve change in lifestyle and substantial time commitment. The weight management programme is challenging, but you will be given full support throughout the study.

What are the possible benefits of taking part?

If you follow the protocol, you will lose a substantial amount of weight. The information that is collected during this study will give us a better understanding of the effect of weight loss on the quantities and quality of fat and muscle mass in your body and how this is affected with weight loss.

Will my taking part in this study be kept confidential?

Your GP will be notified of your participation. All information which is collected about you during the course of the research will be kept strictly confidential, identified by an ID number. Any information about you will have your name and address removed so that you cannot be recognised from it. Information will be stored in University of Glasgow computers at the Royal Infirmary with password that only the research team has access to. Any data shared with other researchers will be completely anonymised. Information will be stored for at least 10 years Information may be accessed by representatives of the study sponsor (NHS GG&C) to make sure the study is conducted correctly.

What will happen to the results of the research study?

After the project is finished, we will post out information about the findings of this research project to everyone who takes part. The research findings will be written into reports which will be published. It will not be possible to identify any of the individuals who take part in the project from the reports, as all the information will be anonymised. The information will also be used as part of a PhD student project.

Who is organising and funding the research?

This project has been planned by a PhD student and two research associates at the University of Glasgow, department of human nutrition. Funding's are from student PhD fees and department of Human Nutrition research account.

One of the research associates (Naomi Brosnahan) who plans to register for a PhD at the University of Glasgow is currently an employee and shareholder in Counterweight Ltd (the company which is supplying the formula diet for the present study).

Who has reviewed the study?

This project has been reviewed and approved by West of Scotland Research Ethics Committee 5 and NHS Greater Glasgow & Clyde Research and Development Department.

How can I find out more about this project?

If you would like to find out more about this project, please contact Yasmin Algindan, Naomi Brosnahan or George Thom the research dietitian/nutritionist.

Thank you for taking the time to read this information sheet.

Researcher (PhD student):

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University of Glasgow

School of Medicine

NHS Greater Glasgow and Clyde

BEYOND WEIGHT LOSS STUDY

Body composition and Energy Expenditure with Total diet replacement during weight loss and maintenance

Consent form

Please initial each box

1) I agree that my GP can be notified of my participation in the study ☐

2) I agree that my GP will be contacted to provide research team with details of my medical history and medications (if any) ☐

3) I understand that my information may be looked at by representatives of the study sponsor (NHS GG&C) for audit purposes ☐

4) I agree that my anonymised data may be shared with other researchers ☐

5) I confirm that I have read and understood the information sheet V8.3 for the study dated 07/07/2015 and have had the opportunity to ask questions ☐

6) I understand that taking part is voluntary and that I am free to withdraw at any time, without giving reasons, without my medical care or legal rights being affected ☐

7) I agree to undertake whole body MRI scans twice; at the beginning and end of the total diet replacement stage. ☐

☐

8) I agree to undertake muscle strength and performance measurements at the beginning and end of the study ☐

9) I agree to undertake weight, height, waist, hip measurements and questionnaires during the study ☐

10) I agree to undertake indirect calorimetry testing for measurement of resting and non-resting metabolic rate at the start and end of the study, and periodically throughout the intervention period ☐

11) I agree to provide blood, stool and urine samples ☐

12) I agree to measurements of my physical activity level at key study intervals ☐

13) I agree that my samples blood and stool samples could be retained anonymised for use in future studies ☐

14) I agree to take part in interviews to discuss my views and experiences whilst I am losing and maintaining weight loss and grant permission for the interview session to be recorded and saved for purpose of review by the researcher ☐

15) I agree to take part in this study

One copy of the consent form will go to the participant and the second copy will go to the researcher.

Name of participant (please print name).....

Date..... Signature.....

Researcher (please print name).....

Date..... Signature.....

BEYOND WEIGHT LOSS STUDY SCHEDULE OF ASSESSMENTS

		Total Diet Replacement Phase							Food Reintroduction Phase			
Appointment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Week	**Time (min)**	**0 Baseline**	**0+1**	**0+2**	**0+4**	**0+6**	**0+8**	**0+10**	**0+12**	**0+13**	**0+15**	**0+17**	**0+19**
Study Procedure													
Review Inclusion/Exclusion Criteria	5	√											
Review & discuss study participation (readiness to change)	10	√											
Obtain Informed Consent	10	√											
Height	1	√											
Weight	1	√	√	√	√	√	√	√	√	√	√	√	√
Waist Circumference	2	√							√				√
Hip Circumference	2	√							√				√
Blood Pressure	2	√	√	√	√	√	√	√	√	√	√	√	√
Blood samples	5		√	√	√	√	√	√	√	√	√	√	√
EQ-5D questionnaire	2	√								√			
Binge Eating Questionnaire	2	√								√			√
Psychology quantitative questionnaire	2			√	√	√	√	√	√	√	√	√	√
Psychology qualitative interview	20		√*					√*					√*	
Indirect Calorimetry	30	√	√	√	√	√	√	√	√	√	√	√	√	√
Gut Microflora	0		√		√					√		√	
*MRI Scanning	45	√							√				
WOMAX Index Questionnaire	2	√							√				
Gait and Muscle Strength	30	√							√				
Counterweight Plus (CWP)	60-20		60*	20	20	20	20	20*	20	20	20	20	20*
Total time (min)	NA	114	98	60	60	60	60	60	141	64	60	60	66

*=incorporated within CWP appointment