Phenotyping the Dysregulation between BMI and Adiposity in Adult Subjects.

A thesis submitted for the degree of
Doctor of Philosophy

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Declaration of Originality

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ABSTRACT

AIMS
The purpose of this thesis was to fully quantify the dysregulation between body mass index (BMI) and body adiposity in 3 phenotypically different groups of adults for whom BMI may be particularly unreliable. The 3 groups were:


A secondary purpose of the thesis was to evaluate the accuracy of 2-C body composition devices and proxy measurements to accurately assess regional and whole body adiposity.

METHODS
In order to establish a cohort baseline, whole body and regional adiposity were quantified using MRI and MRS. Cohort = 500 healthy adults. Participant’s adiposity data obtained were: TAT, SAT, ASAT, NASAT, IAAT, IHCL, S-IMCL, S-IMCL. Anthropometric data included: height, weight, waist circumference, hip circumference (and skinfolds in some sub-groups).

Study 1: (A). In 21 healthy non-obese, males; 4 different 2-C body composition techniques (UWW, BIA, SKF, ADP) were compared to MRI adiposity data.
Study 1: (B). In 74 adult Caucasian (40 females and 34 males) abdominal adiposity was measured using an abdominal BIA device (Viscan) and compared to MRI adiposity.
Study 2: In 477 participants (343 male & 234 female) an in-depth comparison of BMI was conducted to identify TOFI individuals by developing a clinical index from the abdominal internal fat: subcutaneous abdominal fat (IAAT/ASAT) ratio for a normal range.
Study 3: 50 males, fitness tested using VO2 max and then categorized by their fitness (fit vs unfit), and fatness (fat vs slim) according to MRI adiposity data.
Study 4: 260 participants (68 Asian & 192 Caucasian) – age and BMI matched. Proxy measures WHR, WC etc compared. Apply study 3, TOFI cut-off to Asians adiposity data.

RESULTS
From the baseline adiposity data I confirmed that there is a wide range of regional body fat distributions (internal abdominal adipose tissue, IAAT; and abdominal subcutaneous adipose tissue, ASAT) by BMI, and that individuals with similar BMI values can show great variation in IAAT and ASAT.

Study 1, (A). When whole cohort data were compared to MRI adiposity data there was no significant difference between the measures derived. However when the cohort was divided by ethnicity (Asian vs Caucasian) differences were more apparent. Caucasian adiposity was overestimated by up to 3% and Asian adiposity was underestimated by up to 11%. BodPod would be best suited to measuring Asian adiposity and BIA devices would be best suited to measuring Caucasian adiposity.
Study 1. (B). The abdominal adiposity device (Viscan) using BIA method was not able to accurately measure IAAT in obese males and females. It appeared better at measuring subcutaneous adiposity (ASAT). It also appeared to be influenced by organ volumes in some cases – particularly the liver.

Study 2. The ‘Thin on the Outside – Fat on the Inside’ (TOFI) phenotype can be defined using the ratio of IAAT and ASAT (IAAT/ASAT). The resulting TOFI index provides a quantitative means of comparing intra-abdominal fat deposition and thereby identifying “at risk” individuals. In Caucasians, cut-off values of >1.0 in males and >0.45 in females are proposed for TOFI definition. Additionally, anthropometric measurements such as waist circumference (WC) and waist to height ratio (WHtR) are not appropriate for classifying the TOFI phenotype. This is because these surrogates generally correlated more with total and subcutaneous adipose tissue stores than internal or ectopic depots.

Study 3. IAAT and liver fat are lower in men who are fat, fit and active than in men who are fat, unfit and inactive. These ‘metabolically healthy’ individuals have the capacity to store excess fat in insulin-sensitive abdominal subcutaneous adipose tissue (ASAT) and this may help explain why the risk of chronic disease is lower in the ‘fat-fit’ than the ‘fat-unfit’. As a consequence, aerobic activity and the pursuit of physical fitness may be more appropriate goals in the battle against chronic disease than weight loss.

Study 4. Asian Indian males were found to be significantly ‘fatter’ with significantly higher subcutaneous fat depots compared to similar Caucasian males. Given the increased metabolic risks seen in the Asian population increased IAAT measures were not found to be significantly higher. Additionally, the TOFI classification was not useful in identifying ‘at risk’ individuals in the Asian group. Also, waist circumference measurements did not identify Asian males that had significantly elevated ASAT. However, elevated liver fat stores were seen in Asian males and females compared to Caucasians. Liver fat may therefore be a potential ‘at risk’ identifier in this ethnic group.

CONCLUSION

The results of this thesis confirm BMI may be an inexpensive, non-invasive measure of obesity for predicting the risk of related complications, but its accuracy is limited by its dysregulation with adiposity. While obesity means excess body fat, the current definition of obesity using BMI is based on body weight regardless of its composition. The studies in this thesis have highlighted that fact that there are several different sub-populations of individuals for whom BMI does not tell the whole story. The Fat-Fit, the TOFI and the Asian Indian are specific phenotypic examples of these sub-populations. This is evidence of the fact that BMI should not be considered as the only measure of obesity.

The results of this thesis also confirm that some techniques to measure adiposity are suboptimal for measuring percent body fat. For this reason MRI and other high quality (and high cost) imaging methods are still the best method for health risk based research.
Acknowledgements

I would like to thank my supervisors Prof. Gary Frost and Prof. Jimmy Bell at Imperial College and the United Kingdom Medical Research Council (MRC) respectively for their guidance, support and patience throughout this PhD process. I am also grateful to the MRC for financial support with scanning costs.

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I would like to thank my friends and family for their support too and for listening to my ‘fat’ related musings.

Finally I would like to thank my wife Rachel not just for supporting me with unwavering optimism throughout the process, but for enduring the hardships that come with PhD work and for sacrificing so much so that I could get to this stage. Thank you for being there and for being my rock.

This thesis is dedicated to Rachel, my wife and my best friend, as well as to Amber who at the age of 2 yrs has endured “daddy’s working” far too often.

Thank you everyone.
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<td>%BF</td>
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<td>Nuclear magnetic resonance</td>
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<tr>
<td>PEM</td>
<td>Protein energy deficiency</td>
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<tr>
<td>PRESS</td>
<td>Point-resolved spectroscopy</td>
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<tr>
<td>RER</td>
<td>Respiratory exchange ratio</td>
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<tr>
<td>S-IMCL</td>
<td>Soleus Intramyocellular lipids</td>
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<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<tr>
<td>SKF</td>
<td>Skinfold</td>
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<tr>
<td>SSA</td>
<td>Site-specific assessment</td>
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<tr>
<td>STPD</td>
<td>Standard temperature and pressure, dry</td>
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<tr>
<td>T-IMCL</td>
<td>Tibialis Intramyocellular lipids</td>
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<td>T2D</td>
<td>Type 2 Diabetes</td>
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<td>TAG</td>
<td>Triacylglyceride</td>
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<td>TAT</td>
<td>Total adipose tissue</td>
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<td>TBD</td>
<td>Total body density</td>
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<td>TBW</td>
<td>Total body water</td>
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<td>Thoracic gas volume</td>
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<td>TNF</td>
<td>Tumour necrosis factor</td>
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<tr>
<td>TOFI</td>
<td>Thin Outside, Fat Inside</td>
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<tr>
<td>UWW</td>
<td>Underwater weighing</td>
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<tr>
<td>VCO₂</td>
<td>Volume of carbon dioxide</td>
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<td>VE</td>
<td>Ventilation</td>
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<td>VLDL</td>
<td>Very low-density lipoprotein</td>
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<tr>
<td>VO₂</td>
<td>Volume of oxygen</td>
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<td>WC</td>
<td>Waist circumference</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>Waist-to-hip-ratio</td>
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<td>Waist-to-height ratio</td>
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<td>WSR</td>
<td>Waist to stature ratio</td>
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Chapter 1. Introduction

1.1. General Introduction

Obesity is classified as a disease of multifaceted aetiology, with its own disabling capacities, pathophysologies and comorbidities. It meets the medical definition of disease in that it is a physiological dysfunction with environmental, genetic and endocrine aetiologies. Obesity is a response to environmental stimuli, genetic predisposition and metabolic abnormalities, and has a characteristic set of signs and symptoms with consistent anatomical alterations. It is a chronic condition characterized by excess fat deposition in adipose tissue and other organs, for example, liver, skeletal muscle, heart, and pancreatic islets and is associated with a large number of debilitating and life-threatening disorders. The excess adipose tissue adversely effects health by increasing the work of the heart, altering pulmonary, endocrine and immunological functions. This has enormous public health consequences as obesity is a strong risk factor (relative risk >3) for type-2 diabetes and dyslipidemia, a moderate risk factor (relative risk 2–3) for coronary heart disease (CHD) and hypertension and shares a linear relationship with all causes of mortality. Obesity also increases the risk of atherosclerosis, obstructive sleep apnea, certain cancers, and osteoarthritis. Abdominal obesity in particular is an important component of the metabolic syndrome, a condition associated with insulin resistance, type 2 diabetes, hyperlipidemia, and cardiovascular disease (CVD).

Incidence of Obesity

Obesity incidence is increasing rapidly on a global scale. Based on body mass index (BMI), the World Health Organization (WHO) estimates that more than 1 billion adults worldwide are overweight (i.e. BMI ranging from 25 to 29.9); and of these, at least 300 million are obese (i.e. BMI of 30 and above). Over the past decade levels of overweight and obesity have increased on average between 10-40%. WHO recently stated that "the growth in the number of severely overweight adults is expected to be double that of underweight during 1995-2025" (WHO 1998). Crude projections, from extrapolating existing data, suggest that by the year 2025 levels of obesity could be as high as 45-50% in the USA, between 30-40% in
Australia, and Mauritius and over 20% in Brazil. Today it is believed nearly 25% of all Americans are obese\(^1\). These figures are mirrored in the UK, where excess weight affects 68% of adult men (44% overweight, 24% obese) and 60% of women (34% overweight, 26% obese)\(^2\). Furthermore, in England the prevalence of obesity has trebled in the last 25 years\(^3\). Recent data also show the continuing steep rise in the prevalence of childhood overweight and obesity, between the ages of 2 and 15 years, with boys averaging 21.8% (5% obese) and girls averaging 27.5% (7% obese)\(^4\). Other studies have put the number of children classified as obese closer to 12-16%\(^5\). The WHO (2007) estimated that approximately 22-million children under 5 years were overweight, with more than 75% of these children living in low and middle income countries, particularly in urban settings.\(^6\)

The financial burden of obesity in the UK, in terms of direct (health care) and indirect (work-related economic losses) costs, is also growing as a consequence. Recent estimates of the total annual direct cost of obesity (i.e. those individuals with a BMI >25) for the UK as a whole were £6 billion; £4.4 billion for obese (i.e. those individuals with a BMI >30) adults, £1 billion for overweight (i.e. those individuals with a BMI ranging from 25 to 29.9) adults, and £0.5 billion for childhood obesity (i.e. those individuals with a BMI >25)\(^7\). Of this estimated £6 billion, approximately £1 billion is spent on health care provision such as general practitioner consultations, in-patient / out-patient admissions and drug costs. The majority of the estimated annual £6 billion is spent on treating the consequences of obesity such as type 2 diabetes, cardiovascular disease, stroke and osteoporosis. The total annual indirect cost of obesity, estimated by the House of Commons Health Select Committee (HSC), is approximately £4 billion. This estimate is based on factors such as lost potential national output, loss of earnings from premature (obesity related) mortality and lost earnings owing to certified sickness. Additional costs as a result of obesity that have yet to be estimated include: the social care costs of obesity, the impact of lower wages for obese individuals in employment\(^8\), welfare costs for incapacity and unemployment benefit, as well as the potential lost earnings owing to lower employment (believed to be in excess of £10 billion).
In addition to the financial burden of obesity there is also the human cost to consider. It was estimated by the HSC that approximately 6.8% of all deaths were attributable to obesity and associated pathologies, a more recent study put the figure for England at 0.14% (certified cause of death) and 24.8% (underlying cause of death) between 1995 and 2006. The financial costs of obesity are clearly very high, but the smaller, yet significant healthcare costs demonstrate the burden placed on an increasingly stretched National Health Service (NHS). Obesity is one of the most profound public health problems today, and simplistic explanations based on excessive nutritional consumption or lack of physical activity may be inadequate to account for this dramatic and literal growth in obesity in our world population. As with other complex disorders, obesity is heavily influenced by genetic and environmental factors. The genetic makeup of some individuals may make them more prone to obesity. Twin studies showed that genes were responsible for 77% of the difference in BMI and waist circumference, and environmental influences accounted for the remaining 23%. While obesity appears to be a heritable trait, the genes contributing to it have been difficult to identify. Obesity is also a feature of at least 24 genetic disorders. However, the genetic influence of obesity has to be considered as related to two main processes: (1) the presence of a normal drive to eat despite low energy requirements, and (2) the susceptibility to overeating despite normal energy requirements. Appetite and the drive to eat may be affected by several genetically programmed metabolic pathways.

**Defining Obesity (BMI)**

The World Health Organization (2000) recommendations for the diagnosis of obesity are based on the body mass index (BMI). Originally named the Quetelet index in 1835 by a Belgian statistician (Adolphe Quetelet) as part of his theory of the average man (in *Sur l'homme et le développement de ses facultés, essai d'une physique sociale*), the body mass index (BMI) was a simple measure used to classify people's weight relative to an ideal weight for their height. Its high correlation to adiposity (measured by skinfold and hydrodensitometry) was explored by Keys in the early 1970s before Garrow proposed high levels of BMI to define grades of obesity with different categories. In the late 1980s BMI was adopted by the
International Dietary Energy Consultancy Group (IDECG) as an operational definition for the degree of a loosely defined term for a nutritional health hazard: ‘chronic energy deficiency’ (CED) in adults resulting from inadequate household food supply. This simple anthropometric index provided invaluable results for classifying both malnutrition and obesity, owing to its fundamental repeatable and valid components, which relate to the physical description of an individual or population. Supported by research, the usability of this index forms the basis of the World Health Organization (WHO) BMI cut-off points for classifying underweight, healthy weight, overweight and obese.

The fundamental principle behind BMI classifications and health is that nutritional status is linked to longevity and mortality\textsuperscript{43-46}. Individuals with low BMI values (18.5-22.5 kg/m\textsuperscript{2}) have an increased risk of death (mainly from respiratory diseases) compared to those with higher BMI (27.5 - 30 kg/m\textsuperscript{2}) values owing to their diminished immune status and low protection from fat stores during acute illness\textsuperscript{47,48}. Additionally, low BMI individuals are also prone to developing problems such as nutritional deficiency and osteoporosis. The optimal survival is achieved at a BMI of 22.5 - 25 kg/m\textsuperscript{2}. Conversely, individuals in the overweight (> 25 kg/m\textsuperscript{2})
and obese (> 30 kg/m²) categories are at the greatest risk of chronic diseases such as type 2 diabetes and cardiovascular diseases⁴⁴ as well as certain site specific cancers including colorectal and breast cancer⁴⁹,⁵⁰. A recent report from the Prospective Studies Collaboration with data from 57 prospective studies (based on 900,000 participants and > 66,000 deaths) confirms that obesity shortens lifespan by 3 years in individuals with moderate obesity (BMI 30-35 kg/m²) and by 10 years in individuals with extreme obesity (BMI 40-50 kg/m²). The increased mortality attributed to high BMI is mainly owing to ischaemic heart disease, stroke, diabetes and liver disease and is equivalent to years lost by lifetime smoking⁴⁴. BMI is often used as a measure of adiposity in large epidemiological studies due to its ease of use, simplicity and low cost in both time and money. However, owing to it being correlated with more direct measures of adiposity⁵¹,⁵² BMI is assumed to represent the degree of overall adiposity. It has never been a direct measure of adiposity – merely the index of assumed adiposity by right of it's the total fat mass contribution to an individual's weight. While an individual with increased adipose tissue will have a concomitant increase in weight, BMI does not distinguish between fat mass (FM) and fat free mass (FFM). Therefore, BMI only provides information about body volume and mass, with no information about body composition and shape.

1.2. Adiposity in Obesity

Although obesity means excess body fat, the current definition of obesity, using BMI, is based on body weight regardless of its composition. Increasingly the risks associated with excess adiposity have consistently been shown to be a function of regional fat distribution, rather than overall fat volume⁵³,⁵⁴ owing to the stronger associations with physiological and pathological processes⁵⁵-⁵⁸. These processes are intrinsically linked to the multifunctional nature of adipose tissue itself. Adipose tissue (AT) is primarily made up of lipid-filled adipocytes held together by a collagen fiber framework, and has many roles including mechanical cushioning, heat insulation, as well as being a major energy storage reservoir (in
the form of triglycerides\textsuperscript{59}. Additionally, adipose tissue is an active endocrine organ secreting adipocyte products such as leptin, (involved in appetite regulation), adiponectin (glucose regulation), free fatty acids (energy expenditure), plasminogen activation inhibitor-1 (blood clotting), interleukin-6 (IL-6), complement factor 3, adipin, tumour necrosis factor – alpha (TNF-\( \alpha \) ) (immune response). These adipocyte secretory products are necessary for a favourable metabolic profile. Disruption of the complex hormonal pathways that control the regulation of these homeostatic processes can therefore lead to pathophysiological events believed to be involved in both the development and progression of several obesity-related comorbidities such as CVD, hypertension and dyslipidemia\textsuperscript{60}.

An understanding of the complex nature of human body fat distribution is therefore essential in order to reassess the definition of obesity at the individual level.

**Body fat storage / distribution.**

Generally, in humans most adipose tissue (approximately 85\%) is located under the skin as subcutaneous adipose tissue (SAT) and a smaller (approximately 15\%) is located in the abdomen and referred to as intra-abdominal adipose tissue (IAAT) or visceral fat. In certain circumstances fat can also be stored in other (non-adipose tissue) parts of the body and is known as ectopic (‘out of place’) fat. These undesirable (ectopic) fat depots include the liver (hepatic), skeletal muscle, heart and pancreas, which can cause serious problems with carbohydrate and lipid metabolism\textsuperscript{61–63}.

**Intra-abdominal (Visceral) fat depot**

Intra-abdominal adipose tissue (IAAT), can be subdivided into intraperitoneal fat (mesenteric and omental fat) which drains into the portal circulation and retroperitoneal fat which drains into the systemic circulation\textsuperscript{64}. It has been proposed that excess IAAT (intra-abdominal adipose tissue) may indicate that an individual’s subcutaneous AT is unable to serve as an “energy sink” for a calorie surplus resulting from excess energy intake or reduced energy expenditure. Factors associated with preferential accumulation of IAAT include smoking, genetic susceptibility\textsuperscript{65}, and a maladaptive response to stress\textsuperscript{66}. This inability may cause fat to
accumulate in other places as a consequence (i.e. ectopic fat accumulation) with subsequent metabolic disruption. Excess IAAT may therefore be seen as a warning sign of increased risk of diabetes and cardiovascular disease. This deposition of excess fat in the upper part of the body (i.e. central or abdominal, android obesity) is more often associated with a constellation of comorbidities that include diabetes, insulin resistance, dyslipidaemia, hypertension and cardiovascular disease\textsuperscript{67-70}. This form of obesity is often simplistically attributed to an accumulation of intra-abdominal (visceral) fat, resulting in an increase in basic anthropometric measures (waist circumference, skinfolds etc.).

While there is much evidence supporting the notion that intra-abdominal fat accumulation is an important correlate of the features of the insulin-resistant syndrome\textsuperscript{71-73}, it has also been proposed that excessive IAAT is a marker of dysfunctional adipose tissue rather, than a cause of insulin resistance\textsuperscript{74}. In this scenario it's possible that when subcutaneous adipose tissue fails to act as an ‘energy sink', during excess energy intake or a sedentary lifestyle (or a combination of both), the IAAT compartment accumulates\textsuperscript{75}. It is not yet certain whether visceral adiposity causes insulin resistance (IR) or insulin resistance causes adiposity dysfunction owing to excessive release of non-esterified fatty acids (NEFA) which may impair insulin sensitivity\textsuperscript{76}.

With obesity, patterns of adipose tissue metabolism are altered, with impaired production of the ‘anti-insulin resistance’\textsuperscript{77} hormone - adiponectin and increased release of NEFAs and pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-\textalpha) and interleukin-6 (IL-6). Such a pattern may contribute to systemic insulin resistance. Evidence of this fat depot’s adverse metabolic effects is seen when IAAT is removed (i.e. omenectomy) - it results in decreased insulin and glucose levels in humans\textsuperscript{78}. Also, loss of IAAT following diet and exercise is associated with improvements in insulin sensitivity, blood pressure and circulating lipid levels\textsuperscript{79-81}. These metabolic relationships are particularly prominent in intra-abdominal adipose tissue, thus contributing to the heightened risk associated with abdominal pattern obesity\textsuperscript{82}. 
Subcutaneous fat depot

Subcutaneous adipose tissue (SAT) is believed to play an equally important role – albeit one in which the metabolic consequences are less clear. Under normal circumstances SAT depot is believed to act as an energy sink. The body's ability to deal with surplus energy intake (in excess of requirements) is believed to be owing to SAT function. When SAT function is impaired or altered, the excess fat is stored ectopically. While SAT has been linked to obesity-related insulin resistance and metabolic syndrome, human and animal studies have also indicated a possible protective role for this compartment. In humans, increased SAT in the leg is associated with decreased risk of altered glucose and lipid metabolism. Furthermore, in patients with high levels of IAAT, an increase in SAT correlated with reduced triglyceride content and a diminished susceptibility of metabolic syndrome. However, removal of SAT by liposuction does not result in improvement in any aspect of the metabolic syndrome. In mice, surgical transplantation of SAT into visceral compartments results in improved glucose metabolism (through improved insulin sensitivity of their liver and muscles) and a reduction in body mass and total fat mass.

Additional evidence of SAT's protective role is that it is the strongest predictor of plasma leptin levels, the fat-derived hormone that reduces appetite. Also, in patients treated with a drug (thiazolidinedione) to improve insulin sensitivity in type 2 diabetics, their total body fat mass increases – but primarily the SAT depot. Finally, in cases where there is no protective SAT mass (eg. lipodystrophy), severe insulin resistance and diabetes develops. In congenital lipodystrophy there is a failure to develop adequate adipose tissue storage and fat is redistributed and stored ectopically.

Abdominal subcutaneous adipose tissue (ASAT) in particular has a potential ‘diagnostic’ capacity. Since greater amounts of IAAT than ASAT have been associated with increased insulin resistance and the metabolic syndrome, it is possible to use a ratio of the two fat depots as an index of abdominal adiposity. From detailed measures of the respective abdominal fat depots a ratio of IAAT to abdominal subcutaneous adipose tissue (ASAT) can be used as a relative index of intra-abdominal fat accumulation. This ratio was shown to be
strongly related to disorders of glucose and lipid metabolism in obese participants. The metabolic parameters concerned being significantly higher in a more visceral group than a more subcutaneous group^106.

**Ectopic fat depots**

Whilst most cells have a small intracellular reserve of fat for either an immediate energy source or essential functions such as membrane structure and signalling, ectopic fat refers to the accumulation of fat in non-adipose cells (i.e. outside the classical adipose depots) in organs such as the liver, skeletal muscle, heart, kidneys and pancreas. The presence of fat in these non-adipose tissues overwhelms their normal clearance capacity and leads to tissue dysfunction (through “lipotoxicity”)^107 and subsequent specific metabolic risk. Ectopic fat the lipids accumulating within the muscle cells (intramyocellular lipids, IMCL) and the liver (intrahepatocellular lipids, IHCL)^108-111 in particular, has been linked to obesity, insulin resistance, type 2 diabetes mellitus. However, recent data have suggested that IHCL is more closely related to the development of insulin resistance than IMCL^112.

**Hepatic fat depot**

Fat storage in the liver in particular is associated with a range of metabolic complications^113-118 and is mostly accompanied by the metabolic syndrome^119-122. Excessive accumulation of fat in the liver has been proposed as a new potential component of the metabolic syndrome when it manifests as non-alcoholic fatty liver disease (NAFLD) owing to its ability to predict T2D risk and its association with various metabolic disturbances. NAFLD^123 is a broad condition with symptoms similar to that of alcohol-induced fatty liver damage but found in non-alcohol abusers. Symptoms of NAFLD are wide ranging and include benign fatty infiltration of the liver (hepatic steatosis), fatty infiltration and liver inflammation (non-alcoholic steatohepatitis - NASH), and fibrosis and cirrhosis, which can lead to liver failure. In a small minority, NAFLD can progress to hepatocellular carcinoma (HCC). NAFLD has been reported in approximately 30% of the adult population, but it also varies with race; being most common among Hispanics (45%), Caucasians (33%) and Afro-Caribbean (24%) populations^124. Although liver fat is commonly associated with obesity^125, it can also be present in normal
weight, non-obese (non-diabetic) individuals with risk factors including dyslipidemia, insulin resistance and abdominal obesity. Liver fat has also been linked to health risk factors, including hypertension, elevated insulin, elevated triglycerides, and low levels of HDL cholesterol.

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While the relationship of liver fat to metabolic risk, insulin resistance in particular, is clear, the mechanisms are not completely understood as the pathophysiology that leads to NAFLD has yet to be defined. Generally, it has been suggested that the adipocyte resistance to the antilipolytic effects of insulin and/or the exhaustion of adipose tissue storage capacity increases lipolysis rates and free fatty acid (FFA) delivery to the liver. This “overflow” of lipids from adipose tissue to the liver eventually exceeds the liver’s ability to secrete fatty acids in the form of VLDL, causing liver fat. Excess lipid storage in lean tissues, such as the liver, can lead to lipid-induced dysfunction (lipotoxicity) and lipid-induced programmed cell death (lipoapoptosis). Increased delivery of FFA to the liver, primarily from the intra-abdominal depot (IAAT), via the portal vein is believed to be responsible for hepatic insulin resistance and triglyceride accumulation in the hepatocytes, and increased synthesis and secretion of atherogenic lipoproteins. These findings are consistent with the strong relationship seen between IAAT and IHCL where there is an increased hepatic delivery of FFA as the visceral fat compartment expands. Additionally, liver fat content correlates with total and intra-abdominal adiposity, and liver enzyme abnormalities.

The release of lipids into the circulation in proportion to the individuals’ fat mass (adipose organ) may also increase the FFA flux to other non-adipose tissue even in the presence of “functional” adipose tissue. Obese individuals, with their increased fat mass, may become insulin resistant generating large quantities of atherogenic and diabetogenic FFA. However, the elevated outflow (“overflow”) of FFA does not entirely explain the development of NAFLD. It is also proposed that oxidative stress / cytokine action is a second step in the model. This second “phase” suggests that a reactive oxygen species can degenerate the excess lipids now stored in the liver, causing both hepatitis (liver inflammation) and hepatocyte damage or necrosis. It is also possible that excess liver fat storage can increase hepatic
production of bioactive materials such as tumour necrosis factor (TNF-alpha), which are also capable of damaging the liver\textsuperscript{144}. The blunting of a protective mechanism provided by plasma adiponectin (adipocyte-secreted protein) is believed to be an additional contributor to NAFLD progression. Adiponectin is produced exclusively by adipocytes (white and brown) and circulating levels fall in obesity. Adiponectin is involved in the modulation of insulin sensitivity\textsuperscript{145} and has an anti-inflammatory action in vascular function\textsuperscript{146-148}. Abdominal obesity in particular has been linked to low levels of plasma adiponectin\textsuperscript{149}, insulin resistance\textsuperscript{150} and fatty liver\textsuperscript{151,152}. Adiponectin acts as an anti-diabetic and anti-atherogenic adipokine in suppressing the production and function of TNF-alpha, as well as decreasing hepatic lipogenesis and increasing hepatic insulin sensitivity\textsuperscript{153}. Adiponectin may regulate fatty acid metabolism by activating AMP-activated protein kinase in hepatocytes\textsuperscript{154}, which stimulates B-oxidation and simultaneously inhibits lipogenesis. Depressed plasma adiponectin levels (as seen in obesity) may therefore lead to liver inflammation and liver fat accumulation by reducing insulin sensitivity, and damage through TNF-alpha action. The metabolic effect of insulin resistance, partly mediated by depressed plasma adiponectin levels, therefore contributes to increased fatty acid flux from adipose tissue to the liver and may induce the accumulation of fat in the liver. Elevated plasma glucose can further increase hepatic fat content through multiple pathways, resulting in overproduction of VLDL particles and leading to the characteristic dyslipidemia associated with type 2 diabetes. Thus, adiponectin may be a mediator between adipose tissue and the liver, influencing both glucose and lipid metabolites. Interestingly, animal studies\textsuperscript{144} have shown that administration of adiponectin to rodents with fatty liver has been shown to resolve the condition.

**Muscle depot**

Fat can be found stored within muscle cells - intramyocellular lipids (IMCL) as well as situated between the muscle bundles - intermuscular adipose tissue (extramyocellular lipid - EMCL). The IMCL storage reserve increases as a response to endurance training and physical activity, along with mitochondrial density and increased insulin sensitivity. This training response is linked to greater availability of substrate. However, increased amounts of this
(IMCL) ectopic depot are also seen in obese individuals or type 2 diabetics, and have been linked insulin resistance\textsuperscript{87,155}. IMCL measurements using magnetic resonance spectroscopy (MRS) have been found to correlate well with markers of insulin resistance and visceral adiposity\textsuperscript{156-159}, and have shown this depots accumulation to be associated with obesity and the development of skeletal muscle insulin resistance and T2D\textsuperscript{160,161}. This suggests a potential way that fat distribution could influence metabolic risk alongside IAAT and well-established risk factors for morbidity and mortality\textsuperscript{54}.

**Other ectopic stores**

While beyond the scope of this thesis, there are other ectopic fat depots that need mention for the purpose of context. These other depot sites are the pancreas and the heart. Owing to the difficulty in imaging the pancreas, this is an area of investigation that is still developing. Additional factors that influence the deposition of fat in these different locations include age, gender and ethnicity. These may also be important confounding factors in the relationship between adiposity stores and metabolic risk\textsuperscript{162}. Ethnicity is known to effect fat and AT distribution\textsuperscript{76,163}, and additionally the association of fat distribution with insulin sensitivity also varies by ethnicity\textsuperscript{164,165}. As an example South Asians, despite their slimness, are predisposed to fat disproportionately accumulating in the abdominal cavity\textsuperscript{166,167}, which is thought to increase insulin resistance. Genetic influences may also contribute to this complex area.
1.3. The Obesity Phenotype

For many decades Body Mass Index (BMI) has been the ubiquitous tool to define an individual as lean, overweight or obese, and also to get a surrogate measure of body adiposity. At population level BMI has served extremely well as a tool to uncover the relationship between obesity and some life-threatening disorders, however there is mounting evidence questioning its utility and applicability to various populations\textsuperscript{168}. Since its introduction, BMI has been known to have considerable limitations, especially at the extremes of BMI, in children, athletes and for different ethnic and age groups\textsuperscript{168,169}. Based on the assumption that changes in body weight reflected changes in body fat, BMI was proposed as an estimate of obesity. However, obesity is defined as excess body fat rather than body weight (regardless of its composition) and differences in body weight do not always go hand in hand with changes in body fat\textsuperscript{170}. For example, body builders and power athletes (rugby, boxing, wrestling, shot put etc) may have a low percentage body fat, but their BMI would classify them as overweight or obese because of their greater lean (muscle) mass. Variations in muscularity represent a major confounding factor and demonstrates the low sensitivity of BMI. In the case of mortality and ageing, it is well known that there is a greater loss of muscle mass (sarcopenic obesity) in women as they age compared to men. This increased loss of muscle in women exacerbates the misclassifications of BMI\textsuperscript{171,172}. Also, since ageing men lose less muscle than ageing women (i.e. that they suffer from sarcopenia less than women do), the men’s BMI should take this fact into account more. In men, there is an inverse relationship between muscular strength and mortality, which may be missed using BMI as a measure of adiposity\textsuperscript{173}.

In recent years there has been a slow, but inexorable move towards the acquisition of more detailed phenotypic information from overweight/obese individuals, both for scientific purposes, as well as to provide better advice to those trying to avoid or reverse the effects of increased body adiposity.
A more Accurate Assessment of Adiposity

Many techniques have been developed over the years to provide a more detailed measure of body adiposity than can be obtained using BMI. Most recently imaging techniques (CT and MRI) have been used to assess body fat content and distribution. The applicability of the former to the study of fat in human volunteers has been hampered by the fact that ionising radiation is required during the acquisition of the images, while the relative cost of a MRI examination has limited its applicability to large population studies. Despite this, magnetic resonance spectroscopy (MRS) and MRI have become the gold-standards in the field of obesity\textsuperscript{174-176}. Applying these advanced techniques to specific populations, for whom BMI may be particularly unreliable, will enable more accurate phenotyping of their potentially differing fat distributions. Such populations include: the metabolically healthy obese (MHO)\textsuperscript{177-179}, the metabolically obese-normal weight (MONW) and its linked sub-group the “thin on the outside-fat on the inside” (TOFI). While both MONW and TOFI individuals share a normal BMI (ie, 18-25 kg/m\textsuperscript{2}) and normal weight, the MONW individuals have a cluster of metabolic characteristics that may increase the risk of developing the metabolic syndrome. TOFI population is distinguished from the population in that TOFIs do not have any overt cardiometabolic disease (i.e. normal metabolic profile and normal insulin sensitivity) but may be at increased risk of developing obesity-related diseases.

Metabolically Healthy Obesity “Fat-Fit”

It has been reported, for well over two decades, that some obese participants do not present the common cardiometabolic risk factors of elevated blood pressure, elevated triglycerides, C-reactive protein, insulin resistance, elevated glucose levels/diabetes, or decreased HDL, despite their excess body fat\textsuperscript{177}. Research groups in Europe and the USA have estimated that a significant number of overweight/obese participants (20%-35%) are “metabolically healthy obese” (MHO) individuals\textsuperscript{180-183}. In this sub-phenotype, there is a clear dissociation in the well-established relationship between body fat content and metabolic risk factors; in that despite a BMI of > 30 kg/m\textsuperscript{2}, MHO individuals possess a favourable metabolic profile of: high insulin sensitivity, a healthy lipid profile (high HDL, low triglycerides), low visceral fat and no
hypertension. While there is little understanding of the complex factors that constitute this protective profile, it is believed to be related to the lower amounts of visceral fat in the presence of high total body adiposity. A related sub-phenotype has also been reported, known as the “fat-fit”. In this phenotype individuals, like the MHO, show a similar dissociation between body fat content and metabolic risk factors\(^\text{184-186}\) but whose increased activity levels may be providing the protection from metabolic disturbances associated with obesity.

**Metabolically-obese but normal-weight (MONW)**

Interestingly an opposite phenotype to the “MHO/Fat-Fit” has also been reported. In this phenotype, participants with a BMI <25 kg/m\(^2\) were seen to have disproportionately high levels of many risk factors associated with the metabolic syndrome. These participants have been defined as “metabolically-obese but normal-weight” (MONW)\(^\text{187}\). This syndrome (often in young individuals) is characterized by a normal body weight and BMI, but display a cluster of obesity-related abnormalities such as premature signs of insulin resistance, hyperinsulinemia and dyslipidaemia, high visceral fat, high liver fat and a high fat mass (usually greater than 30%)\(^\text{188,189}\). There is uncertainty about the factors involved in this phenotype, but it is believed that body composition and body fat distribution abnormalities may be important factors in their metabolic complications\(^\text{190}\). Several studies have shown that young MONW individuals have a lower fat-free mass and a tendency for a greater central (intra-abdominal) fat mass\(^\text{191,192}\). It has been hypothesized that MONW individuals may have decreased fat storage in adipose tissue – leading to greater ectopic storage\(^\text{130}\). Interestingly the MONW phenotype may be similar to phenotypes reported in different ethnic groups including Japanese and South Asian.

**Ethnicity – ‘Asian Indian’ Phenotype**

‘Asian Indians’ are often incorrectly grouped together as one homogeneous region on the basis that they share similar cultural, social and linguistic characteristics. South Asians (individuals from Pakistan, Bangladesh, Nepal and Sri Lanka) in particular represent an ethnic sub-group that exhibit the so called ‘Asian Indian Phenotype’, where individuals present with a ‘normal’ BMI, indicating less generalized obesity, but have a disproportionately large waist circumference owing to greater central (abdominal) obesity\(^\text{193-195}\).
With such a global increase in obesity and its related disease risks it needs to be remembered that prevalence of obesity in a population is crudely determined as the proportion above a BMI cut-off. This is thought to represent the number of individuals with excess amount of body fat\textsuperscript{196}. However, the relationship between BMI and total adiposity varies with different populations\textsuperscript{197} and South Asians in particular, have a different BMI-adiposity relationship compared to Caucasians and African Americans. A substantial proportion of South Asians are found to have an increased risk of type-2 diabetes (T2D) and CVD at BMI values lower than the proposed WHO cut-off point of 25 kg/m\textsuperscript{2} used to define in individuals as overweight\textsuperscript{198,199}. BMI can therefore differ significantly among populations of the same age, gender and body adiposity\textsuperscript{200}. As a consequence, revised BMI cut-off points to define obesity have been suggested for several ethnic groups including populations from South Asia, China and Native Australia\textsuperscript{201} to reflect the fact that CVD risk factors occur at a much lower BMI than those individuals of European origin\textsuperscript{201}. For instance individuals of Afro-Caribbean origin exhibit more subcutaneous fat for a given BMI compared to Caucasians\textsuperscript{76,202-204}, whereas Japanese populations are more prone to the accumulation of intra-abdominal fat\textsuperscript{199,205}.

South Asians have been identified as a particular at risk ethnic group as they are predisposed to insulin resistance, the metabolic syndrome and type 2 diabetes mellitus\textsuperscript{206-208}. South Asian men in particular may also be genetically predisposed to premature CVD\textsuperscript{209,210} as it is widely reported that this ethnic group has a significantly higher CVD mortality rate from the condition than other ethnicities\textsuperscript{211-214} along with increased CVD risk seen in childhood. In England and Wales, compared to the general population, mortality from CHD is 50% higher in South Asians\textsuperscript{214}. The pathology of this increased susceptibility is yet to be fully explained. Conventional risk factors, including type 2 diabetes and central obesity, insulin resistance parameters, or metabolic syndrome criteria (as currently defined) do not adequately explain their excess risk compared to other populations. One key factor is that current definitions of the metabolic syndrome by the National Cholesterol Education Program (NCEP) and the WHO give an inconsistent picture of cardiovascular disease risk when
applied to different ethnic groups within the UK. Rates of smoking, hypertension and levels of low-density lipoprotein (LDL) cholesterol tend to be similar or lower in South Asians, although diabetes is more prevalent\textsuperscript{215}. It is possible that with South Asians, their increased predisposition for central obesity and insulin resistance, accompanied by raised triglyceride and lowered high-density lipoprotein (HDL) cholesterol concentrations, are contributing factors to their elevated vascular risk\textsuperscript{207,210,216,217} and further categorize them as metabolically obese, normal weight individuals.

This ethnic group faces additional health risk as it has been found that migrants South Asians are more predisposed to developing CHD\textsuperscript{210,218}. With increasing numbers of young South Asians now settling abroad, there is a very strong possibility of increased obesity related health risks, along with the associated increased healthcare burden in this “at risk” population. The U.S. South Asian population grew 38\% (almost 15 times the national growth rate) in the years 2000-2005 and by 53\% in 2007 according to the American Community Survey of the U.S. Census Bureau. South Asians are among the fastest growing ethnic groups in the U.S. (3\textsuperscript{rd} largest ethnic group after Chinese Americans and Filipino Americans). In the UK, British South Asians make up approximately 6\% of the population (50\% of the UK’s non-European population) and therefore would benefit from focused research linked to their possible ethnic predisposition to health risk. However, it is unknown at this time if generational differences may be diluting or amplifying these existing risk predispositions.

A greater knowledge of South Asian adiposity may be key to understanding this ethnic group’s predisposition to numerous health risks owing to adiposity’s obvious commonality and strong causal relationship to many of the related factors (hypertension, CVD, type-2 diabetes etc). As with studies between Afro-Caribbean and Caucasian populations\textsuperscript{165}, where similar levels of BMI appeared to differentially affect the metabolic syndrome, differences in fat distribution partially explained some of the observed ethnic differences. It is proposed that this may also be the case with South Asians.
1.4. Body Composition Measurement

In order to accurately assess adiposity and obesity related health risk we must be able to accurately measure body composition and particularly fat distribution. With the exception perhaps of cadaver dissection, a ‘true’ value of body fat content is not attainable. For *in vivo* quantification of body composition, we must therefore rely on a criterion (reference) with high accuracy in order to be able evaluate and utilise other less accurate methods. However, because the human body can be quantified at numerous levels, the criterion can vary. Body composition assessment can be at the atomic level (carbon, calcium, potassium, and hydrogen); at the molecular level (water, protein, and fat); at the cellular level (total cell mass, extracellular fluid, and extracellular solids) and at the tissue or systems level (based on functional arrangement of 4 tissue types: connective, epithelial, muscular, and nervous). The choice of criterion should however, be chosen based on its avoidance of major assumptions and its degree of maximal precision. Depending on the criterion involved the numerous methods available for measuring body composition *in vivo* can be classified as either direct, indirect, and doubly indirect\(^\text{219}\).

1.4.1. Direct methods

Direct methods as indicated, directly measure amounts of chemical elements in the body, from which information about body components of interest can be obtained. Apart from cadaver studies\(^\text{220-227}\) an example is *in vivo* neutron activation analysis (IVNAA), where body content is measured at the elemental level\(^\text{228}\). After exposure to a neutron field, gamma output is measured as the cell nucleus relaxes and returns to its pre-exposed state. However, worldwide, very few laboratories use this method, and it is used mainly for clinical purposes. The expose to high levels of neutron radiation is a concern with this technique and therefore limits its use in large-scale population research.
1.4.1. Indirect methods

Indirect methods, such as, deuterium oxide dilution, dual-energy X-ray absorptiometry (DXA) and densitometry measure body components (fat mass, FM; fat-free mass, FFM; water etc.) and rely on certain assumptions that might not always be true. This component approach to body composition has developed over the years from a two-component model (2-C), where tissue is categorised as either fat mass or fat free mass, to multi-component models, where the various components measured depend on the methods used. Wang et al. summarised these methods in a proposed five-level model for body composition research (Figure 1.2).

Dilution based techniques use 2-C model to predict the FFM and FM components by measuring total body water (TBW) volume using an isotope. Based on the fact that water maintains a stable relationship to FFM dilution techniques can predict the FFM and FM (i.e, \( FM = \text{body weight} - \text{FFM} \)). This technique is limited in obesity studies as it is based on an assumed average proportion of TBW in FFM of 73% \(^{229}\), with 15-30% present in adipose tissue \(^{230}\). However, the FM proportion of TBW increases with obesity causing estimate inaccuracies \(^{231}\). Additionally, disease states such as diabetes further affect the variation in TBW distribution, increasing estimate error further.
Figure 1.2: The five levels of human body composition (adapted from Wang et al., 1992).

Basic Model
2-component

Multicomponent Models
A chemical four-component model is generally regarded as the best choice to measure body composition.\textsuperscript{232} In a four-component model the amount of minerals, protein, and water in the body is measured, and body fat (fourth component) is calculated by difference. The number of assumptions in such a four-component model is small, and consequently the possible bias is small. While the four-component model is recommended as the method of choice in population studies, it is expensive and time-consuming and few laboratories have the capacity for using it since densitometry or IVNAA, deuterium oxide dilution, and DXA must also be available for measurement of the component parts (i.e. compartments).

The standard model most widely used today is the four-component model comprising of adipose tissue, body cell mass, extracellular water and the skeleton. This model extended the basic 2-C underwater weighing (UWW) model to four components using more accurate measurements of body protein mass and bone mineral mass alongside assumed densities of body protein (1.34 kg/L) and bone mineral density (3.075 kg/L)\textsuperscript{233}. The two additional measurements of neutron activation analysis (NAA) for body protein mass and dual-energy DXA for bone mass can themselves be used to provide an accurate estimate of the body fat mass without the need for UWW measurement\textsuperscript{234-236}.

### 2-C Densitometric Model

Most widely available body composition methods are currently based on the simplest two-component (2C) chemical model with the human body partitioned into the fat mass (FM) and the fat-free mass (FFM)\textsuperscript{200,237}, where fat mass is estimated indirectly using body density. All such measurements focus on distinguishing between relatively inert components of the body and those that are metabolically more active. The fat mass (FM) component with a density very close to 0.900 g/mL has a relatively constant composition\textsuperscript{238-240} and consists primarily of triglyceride. The fat-free mass (FFM) component is estimated to be 1.100 g/mL\textsuperscript{241}, as there is no direct measurement available. However, there is known variability in the fat-free density measure\textsuperscript{242,243}, as verified by bone mineral density (BMD) and cadaver studies\textsuperscript{220,222,223,225}. Additionally, this measure was also found to vary with respect to ethnicity\textsuperscript{244}.  

Equation 1.1. \(^{245}\) for predicting percent body fat (\(\%BF\)) from body density is derived from the above principles.

\[
BF\% = \frac{1}{D_b} \times \left(\frac{d_f \times d_{fm}}{d_{ffm} - d_f} - \frac{d_f}{d_{ffm} - d_{fm}}\right) \times 100
\]  \hspace{1cm} (1.1)

\[
BF\% = \frac{495}{D_b} - 450
\]  \hspace{1cm} (1.2)

In equation 1.1:

\(D_f\) = density of fat mass (FM), \(D_{ffm}\) = density of fat free mass (FFM), \(D_b\) = Body density

In equation 1.2, Siri’s equation\(^ {246}\) for percent body fat is derived when the known respective values for FM and FFM densities, detailed earlier, are used.

**Densitometry**

Densitometry refers to the general procedure of estimating body composition from body density, and is based on the assumption that the density of any material is the function of the proportion and densities of its components. Measurement of whole-body density (Db) for the 2-C model has traditionally been derived from hydrodensitometry (more commonly known as ‘underwater weighing’, but also called ‘hydrostatic weighing’) and is the classic approach to determining body composition, based on Archimedes’ principle. This principle states that when a body is immersed in water, it is buoyed up by a force, which is equivalent to the weight of the volume of water displaced. Therefore the weight of water displaced by a submerged individual is equal to their weight in air minus their weight in water. When this value is divided by the density of water it provides the individual’s gross volume. This volume must then be corrected for lung volume and gastrointestinal gas (usually taken to be 100mL). Ordinarily, the underwater weight is obtained when the individual has exhaled completely (residual volume), then this value must be subtracted from the gross volume instead (as indicated in equation 1.3, Chapter 2 - General Methods section ).
Although body volume for determination of body density has primarily been assessed by UWW and Archimedes’ principle, an alternative method using air displacement plethysmography (ADP) is available in the Bod Pod (Life Measurement Instruments Inc., Concord, California). The Bod Pod addresses some of the technical difficulties involved in water submersion and displacement measurement. While ADP makes corrections for surface area, lung volume and clothing it appears to be less accurate than UWW. However, ADP is prone to the same error as UWW when using equations to convert body density to percent body fat. Densitometry and any subsequently derived methods of predicting percentage body fat are therefore prone to similar errors owing to questions relating to constancy of the FFM density. Additionally, since the original values were derived from limited reference cadaver data from a small number of Caucasians, the equations employed are therefore specific to a Caucasian population. In some cases conversion formulae have subsequently been derived for different populations to improve accuracy. One such example is in Afro-Caribbeans, who were found to have a denser fat-free mass than Caucasians. There is, however, very little comparable information on the density of FM and FFM in the South Asian population, so it is unknown whether body composition values obtained via the 2-C model are truly accurate for this ethnic group. Owing to uncertainty regarding constancy of such values and the potential errors that can result from a reliance on densitometry, validation against medical imaging (MRI, CT, DXA) should improve accuracy.

1.4.1.3. Doubly indirect methods

Doubly indirect methods rely on a statistical association between easily measurable body variables and a measure of body composition, usually obtained by an indirect method. Examples of doubly indirect methods are anthropometry (multiple skinfold thicknesses), bioelectrical impedance analysis (BIA), waist circumference, and BMI-based prediction equations for the percentage of body fat. Thus, they are no more than a prediction based on mathematical functions, and the bias at an individual level, and at a population level, can be substantial. The mathematical functions employed normally involve regression analysis where other parameters such as age or sex may be included. Such
methods indirectly measure body density and body water and have been mainly validated against UWW. A good example is BIA for which equations are available to calculate total body water or fat free mass. Alternatively, the mathematical function may make use of known properties or ratios within tissues, for example the ratio of total body water to fat free mass is known to be constant at about 0.73. Therefore, if total body water is measured using a labelled water technique, fat free mass can be calculated (see dilution method earlier in this chapter).

However, to be accurate, these relatively easy to perform field methods require validation against a criterion method with minimal error and being doubly indirect they are vulnerable to the errors and assumptions associated with UWW as well as those from their own technique. The use of deuterium dilution method (D₂O) or DXA (with its rise in acceptance) as the criterion methods is improving the accuracy of these methods but underlying validity issues remain.

These indirect methods do not relate to the different proportions of fat in the different storage depots. Skinfold measurement assume that the compressed double layer of skin and subcutaneous adipose tissue is representative of an uncompressed single layer of adipose tissue; that skin thickness is either negligible or constant and that subcutaneous adipose tissue compresses predictably. However, it has been found that skin thickness varies between individuals and between sites, as well as that compressibility varies with age, gender, site and tissue hydration.

The BIA method has the same ‘criterion’ method related errors as well as the fact BIA devices tend to be population specific with generally poor characteristics of fit for a large heterogeneous population. BIA has been shown to overestimate individuals with low percent fat and underestimated individuals with high percent fat. The inclusion of anthropometric measures improves BIA fat prediction somewhat, but additional measurement errors from hydration, electrode placement, prior exercise and temperature can still influence the measurement.

1.4.2. Magnetic Resonance Imaging (principles)

Although there may be high correlations between many of the different measurement techniques – there is generally poor agreement between the imaging and non-imaging techniques. Medical imaging technologies, Computerised Tomography (CT) and Magnetic Resonance Imaging (MRI), provide more accurate estimation of different body tissues from cross-sectional images of the body. Adiposity can be quantified using multiple consecutive scans to determine areas of subcutaneous and
internal adipose tissue that can then be converted to volumes as the distance between consecutive slices is known. The underlying principles of this method are briefly outlined here.

MRI utilises hydrogen nuclei ($^1$H), principally from water and fat, as described above. Hydrogen is one of the most abundant NMR active nuclei in the body, and it is distributed widely throughout most tissues. This means the radio waves, emitted from $^1$H after excitation, are sufficient to be converted into a detailed image. The intensity of the signal is relative to the number of hydrogen atoms present, and this enables different tissue types to be identified. However, the number of $^1$H alone cannot distinguish between all tissues, fat and muscle for example, do not have markedly different amounts of hydrogen.

To differentiate between fat and muscle, a MR property known as ‘relaxation time’ ($T_1$) is used. This is the time it takes for the nuclei to release the energy they have absorbed from the applied radio frequency pulse, and return to their natural state. The $T_1$ for $^1$H in fat and muscle tissue are different, thus, $T_1$ can be used to distinguish these tissues from each other within an image.

The detection of different $T_1$ times can be maximised by adjusting the time interval between each radio frequency pulse (Time to repeat=TR) and the time to detect the induced signal (Time to echo=TE). This process is called the pulse sequence, and each specific sequence is developed to provide the optimum image, in the minimum scan time, for the particular tissues under investigation.

MRI images are acquired in ‘slices’, so the whole body or a particular section is scanned in a series of fixed width slices. The scan produces a series of cross-sectional images that together make up the body or segment. Each image must be analysed individually and then tissue area calculated. As the slices are of a known width the tissue relative volume or weight can also be calculated.

MRI has been validated for measurement of both muscle and fat content in phantoms, animals and human cadavers. It has been shown to accurately measure adipose tissue content in vivo, demonstrating good agreement with the values produced by dissection and chemical analysis.$^{256,257}$ MRI has also been shown to be reliable and reproducible, with the coefficient of variation ranging from 0.3 – 2.3% for reproducibility and approximately 2% for reliability.$^{257}$
Choice of Body Composition Measurement Technique

It is important to understand that anthropometric-based measures such as BMI, waist circumference (WC), and waist-to-hip-ratio (WHR) estimate fat mass at different locations and may therefore reflect different etiological perspectives, and thus do not assess identical phenomena. Waist circumference for instance, tends to reflect both total and abdominal adiposity; whereas BMI may represent fat-free mass or fat mass but does not account for the wide variation in body fat distribution at any given level of body size. As such, these indirect surrogate measures are at present insufficiently precise, as there can for example be individuals with a moderately high BMI that have a low fat mass; as well as individuals with a low BMI and a disproportionately high fat mass. Therefore, in order to develop more reliable and accurate surrogate measures to allow easier and more accessible methods to be used in field situations, an accurate quantification of body fat is an essential starting point. Advances in medical imaging means newer and more sophisticated techniques, such as magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and computerised tomography (CT) that offer high resolution and sensitivity, can now be used to measure specific tissues and importantly their regional distribution, for example subcutaneous and intra-abdominal fat.

Such issues underpin the importance of a sensitive assessment of the “excess fat mass” to better identify obesity and related health risks and also to fully investigate the extent to which generalized and/or regional obesity are associated with the different health risks seen.

It is clear to see that studies investigating the association between intra-abdominal adipose tissue and disease require an accurate and reliable measure of the different adipose depots. IAAT is a particular case in point. Owing to the considerable variability in IAAT for both men and women IAAT requires direct measurement for accurate quantification. Even within a narrow (2 cm) range of either sagittal diameter or BMI, there can be an approximate three-fold variation in total IAAT. Even though a similar level of BMI appears to affect metabolism or metabolic syndrome components differently between populations, differences in fat distribution partially explain some of these.

The purpose of the current research was to fully quantify adiposity in various populations with predicted phenotypic differences including a comparison between South Asian and Caucasian
participants, and also both lean and obese populations with documented differences in their physical fitness.

1.5. Aims of the thesis

The aims of this thesis are:

1. To examine the relationship between BMI and regional adiposity using whole body MRI & regional MRS.

2. To evaluate the accuracy of 2-C body composition measurement techniques (UWW, BodPod, bioimpedence, skinfold) for assessing adiposity in non-obese men.

3. To examine the relationship between BMI and adiposity in the MONW phenotype.

4. To examine the relationship between BMI and adiposity in the Fat-Fit phenotype.

5. To determine whether the relationship between BMI and adiposity differs with ethnicity (specifically in South Asians vs Caucasians).

Hypotheses

Study 1 hypotheses:

Hypothesis 1: 2-C methods (UWW, ADP, BIA, skinfolds) are a valid alternative to MRI for adiposity quantification.

Hypothesis 2: The Viscan BIA device is an accurate intra-abdominal adiposity proxy measurement method.

Study 2 hypotheses:

Hypothesis 1: The established TOFI index can identify individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m²).

Hypothesis 2: Established proxy (anthropometric) measures are able to successfully identify MONW individuals within a large cohort of healthy Caucasians.
**Study 3 hypothesis:**  Fitness results in reduced IAAT and ectopic fat, regardless of overall adiposity.

**Study 4 hypotheses:**

Hypothesis 1:  South Asians have greater internal and ectopic adipose stores than age and BMI matched Caucasians of the same gender.

Hypothesis 2: The established TOFI index can identify South Asian MONW individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m²).

Hypothesis 3: Established proxy (anthropometric) measures are able to successfully identify South Asian MONW individuals.
Chapter 2. General Research Methods and Procedures

This chapter describes the experimental detail of all the measurement techniques used for each investigation in the thesis.

Personal contribution to research presented in thesis.

Owing to the fact that there have been collaborative projects published from aspects of the data included in this thesis, it is necessary to outline my personal involvement and contribution to the work being presented. The background to the research environment is that my research was a sub-component of a larger ongoing research program in the group.

During my time as a PhD student I was directly involved in the scanning of all the participants included. My role was to set up the magnet for each of the participants:

- calibration of magnet with phantom
- participant consenting & metal checking
- scan room database entry and image transfer (for backup)
- explanation of procedure to participant (health & safety, protocol etc)
- repositioning (for each different scan in sequence) and centering of participant in magnet
- accompaniment during scan sequence (if necessary) – for extremely anxious individuals
- trained in voxel localization by radiographers

I also performed all the following:

- anthropometry : height, weight, skinfolds, limb and body segment girths
- Viscan measurements (abdominal BIA device)
- UWW, BodPod, BIA, measures (Bedford Campus lab)
- VO2 max tests at Hammersmith Hospital Unit
- Analysis of own data (with help from statisticians listed - where necessary)

I was not permitted to do the MRI analysis as data analysis was outsourced for reliability (see details in this chapter), but I was mentored and trained by Dr Louise Thomas to inform my work and understanding. While MR spectroscopy required one trained analyst (for reliability) I was trained MR spectroscopy analysis and my work quality checked by Dr. Thomas before I subsequently performed MRS analysis on my data and other studies when Dr Thomas was maternity leave. During that time I maintained the database used for data management and recruitment.

During the course of the research, I personally recruited approximately 110 participants using email, newspaper articles (with correspondence details listed), flyers and posters. The remaining subjects from the cohort presented here were from other studies in the group, to which I was granted access due to my contribution to data acquisition and analysis.
2.1. Ethics

Ethical approval for this study was obtained from the main Research Ethics Committee (REC) of Hammersmith and Charing Cross Hospital, London (Rec: 07Q04011/173) as well as additional site-specific assessment (SSA) ethical approval from Bedfordshire REC, Essex (Ref: 08/H0309/56) for the University of Bedfordshire site (Appendix 1). All volunteers gave informed written consent for the study. Informed consent was obtained as follows: The study was discussed with each volunteer individually to explain the study procedures in detail, the potential benefits of participation, any potential risks and the rational for the study itself. After this discussion the individual was given the study information sheet and the study consent form (Appendix 1). The volunteers were given the opportunity to ask questions about the study and were subsequently given at least 24 hours to consider whether they wished to participate. Any objection to participating was accepted, and the volunteers did not need to provide a reason for a refusal to take part. The participants were assured that they were not obliged to enrol in the study and additionally, should they wish to, they could withdraw from the study at any time without giving a reason and without penalty.

2.2. Participants

Inclusion criteria required that participants be ambulatory with no medical conditions that could potentially affect any of the variables under investigation. Only self-reported, healthy participants, without any diagnosed medical conditions, were enrolled in the studies. All participants completed detailed screening to ensure suitability for inclusion (Appendix 1) Detailed participant information appropriate to each study can be found in the relevant chapter.

2.2.1. Recruitment

All participants were recruited randomly from the general public using various advertising media resources (email, poster, published newspaper article contact, television program research contribution contact, commercial radio research interview contact etc.) as well as from local community groups, public festivals and from any existing research volunteer databases.
2.3. Assessment protocol

All participants were asked to attend the Robert Steiner MRI Unit (Hammersmith Hospital, London.) on the morning of their booked assessment. Participants were asked to fast from midnight the night before testing. The sequence of assessment was:

1. Check for completion of medical history and general information documents (sent to all volunteers in advance of first visit). Witnessed signing of consent and metal check documents (Appendix 1).

2. Participants asked to change into hospital issue theatre garments (‘scrubs’ = trousers and top) provided for them, retaining only underwear (pants). All jewellery/metal objects were to be removed.

3. Baseline anthropometric measures were then performed as well as, where indicated, additional body composition techniques.

4. MRI scan pre-check was completed to ensure that no metal was being taken into magnet area. This included a verbal confirmation from the participant, to myself and the senior radiographer, that their signed metal check was accurate. A final physical check (hair clips, removable dentures, jewellery etc) was also conducted.

5. MRI whole body scan & MRS of calf muscle and liver were performed.

6. Post-scan aerobic capacity test undertaken (in appropriate study only).

2.4. Anthropometric methods

Body mass (kg), height (cm), waist circumference (WC) (cm) and hip circumference (cm) were measured in each participant. From these values, BMI (kg/m²), waist-to-hip ratio (WHR) (waist/hip) and waist-to-height ratio (WHR) (waist/height) were calculated. To minimise measurement error and increase accuracy and reliability of anthropometric measures, I personally performed all measurements using established, standardised protocols, thereby reducing any potential error.

2.4.1. Standing height (Stature) measurement protocol

Standing height (stature) was measured against a wall-mounted stadiometer (Seca, Birmingham, U.K.) employing the stretch stature method\textsuperscript{265}. This method requires the participant to stand unaided.
(barefoot) looking straight ahead with their feet together and their heels, buttocks and upper part of
the back touching the measurement scale. The head when placed in the Frankfort plane need not be
touching the scale. The Frankfort plane is achieved when the orbitale (lower edge if the eye socket) is
in the same horizontal plane as the tragion (the notch superior to the tragus of the ear). When aligned
the vertex is the highest point on the skull. During positioning my hands were placed along the
participant’s jaw (under their mandible) with my fingers reaching to the mastoid process. Keeping the
head in the Frankfort plane, I stretched the participant by applying gentle upward lift through the
mastoid processes while the participant took (and held) a deep breath. The stadiometer headboard
was then lowered firmly on the vertex, gently compressing the participants’ hair as much as possible.
Stature was measured to the nearest 0.1 cm. This measurement was repeated a second time. Owing
to the fact that there can be diurnal variation in stature, with participants being taller in the morning
and shorter in the evening, all measures were taken in the mornings.

2.4.2. Weight measurement protocol

Weight was measured using a calibrated digital platform scale (TANITA electronic Scale WB-110MA,
Tanita Corporation, Tokyo, Japan) with participants wearing only light clothing (hospital issue theatre
‘scrubs’ – provided), barefoot, and with an empty bladder. Weight was recorded to the nearest 100 g.
The scale was standardized to zero before each use and regularly calibrated using standard
procedures.

2.4.3. Waist & Hip circumference measurement protocol

Standing waist circumference was measured at 4 different sites with a non-flexible anthropometric
tape, and recorded to the nearest 0.1cm. The sites included: midpoint (at the midpoint between the
lower costal margin and the iliac crest), the umbilicus (at the level of the umbilicus), the minimal waist
(the observed minimal waist as viewed anteriorly) and iliac crest (at the level of the iliac crest). In
addition, supine waist circumference was also measured at the level of the umbilicus. All waist
measurements were taken at the end of a normal expiration, with the tape held snugly against the
skin (ensuring its horizontal position), without pulling the tape too tightly so as to avoid compressing
the underlying tissues.
Hip circumference was measured in the standing position at the level of the greater trochanter and incorporated the maximal circumference over the buttocks. From these measures waist to hip ratio (WHR) and waist to stature ratio (WSR) were calculated.

2.4.4. Skinfolds measurement protocol

Prior to taking skinfold measurements the exact location of each skinfold was measured and marked using standardised guidelines\textsuperscript{267}, based on identifiable anatomical landmarks. This was to ensure accuracy and reproducibility of the measurements\textsuperscript{268}. The designated skinfold locations should ideally be marked because the measurements are made from multiple successive cycles of all sites. The rationale for ‘cycling’ the measurement sites is that subcutaneous adipose tissue at each site, once measured, must be allowed to ‘relax’ to its pre-test state so that successive measurements are not subject to cumulative tissue compression. This method ensures that all measures are made on fully “relaxed” tissue at identical anatomical locations.

The skinfold measures were taken on the right side of the body (irrespective of limb dominance) in accordance with standardised measurement sites and techniques\textsuperscript{267,269} using calibrated Harpenden skinfold calipers (CMS Instruments, London, U.K.). The accuracy of the calipers used was routinely checked using calibration blocks (precision cut brass blocks of known widths) and a high precision Vernier caliper set to a series given widths (10mm, 20mm, 30mm). Manufacturer recalibration was also an option had calibration errors were experienced. It must be mentioned that there is no consensus for body side recommendation in anthropometric research; mainly owing to the fact that the minor differences seen between left and right-sided measurements owing to handedness were so small compared to the measurement error involved as to be considered trivial\textsuperscript{270,271}. I chose to adopt the right-sided measures so as to align my work with the majority of large population studies (e.g. the National Health Examination Surveys – NHES, and the National Health and Nutrition Examination Surveys - NHANES\textsuperscript{272,273}) in the United States, which form the basis of worldwide reference data, and took measurements on the right side. However, in Europe and also in many developing countries the general practice is to take measurements on the left side as recommended by the International Biological Program\textsuperscript{274}. Skinfolds for the chosen sites were made using the flat surfaces of my thumb.
and forefinger to pull a fold of skin away from the underlying muscle. Care was taken to ensure the fold was not under tension or compression at the point where it was to be measured and in line with standard practice the calipers were applied perpendicular to the fold being measured. Constant tension of the calipers was made 1 cm from my fingers and at a point where the edges of the skinfold were parallel (a depth about equal to the thickness of the fold). Each fold was then held during the measurement process and readings were made to the nearest 0.1mm four seconds after the complete release of the caliper trigger. After all the required sites had been measured once, the sequence was repeated twice more, in the same order, and the mean of the three readings was recorded. Values that differed from each other by more than +/- 10% were repeated until this criterion was met. If there was any doubt about the presence of muscle tissue, the participant was asked to make a voluntary contraction of the muscle involved to ensure that only subcutaneous adipose tissue was being measured.

In the past a number of different skinfold profiles have been used, with up to 10 sites in some profiles. The International Society for the Advancement of Kinanthropometry (ISAK) defines a standard skinfold profile as incorporating 7 skinfolds sites. The 7 sites measured are detailed on the following pages. The skinfold values measured can be summed to provide simplistic comparative values or they can be incorporated into equations to compute estimates of percentage adiposity. The exact number of skinfold values required for adiposity prediction varies depending on which particular equation is being employed. There are over 100 equations for predicting adiposity from skinfold measurements, based on requirements of different numbers of skinfold sites as well as different combinations of skinfold sites. This diversity is owing to the fact that the equations are population-specific and are based on the criterion value used in their specific validation study.

In my study I used a four site equation suitable for both males and females. The Durnin and Womersley sum of four skinfolds formula uses skinfold values from biceps, triceps, subscapular and suprailiac (iliac crest) sites. Like many others, this formula was validated using densiometry so it only predicts total body density (TBD) from the sum of four skinfolds. The computed value for TBD can then be converted to a predicted percentage body fat (%BF) value using Siri's equation.
Equations.

Where $\sum 4$ = sum of 4 skinfolds : bicep, tricep, subscapular, iliac.

Body density = $1.1610 - 0.0632 \log \sum 4$ (men)

Body density = $1.1581 - 0.0720 \log \sum 4$ (women)

% Body fat (BF) (Siri, 1956) = [(4.95 / body density) − 4.5] x 100

Skinfold sites and measurements.

1. Triceps

This skinfold was raised on the marked posterior mid-acromiale-radiale line (approximately midway between the top of the shoulder and the elbow joint). The fold is vertical and parallel to the line of the upper arm. With the participants arm relaxed, the skinfold was taken on the most posterior surface of the arm over the triceps muscle.

2. Subscapular

Participants were asked to stand with their arms by their sides. The inferior angle of the scapula was palpated to determine the lowermost tip of the shoulder blade. The skinfold was raised 2 cm along a line running laterally and obliquely downwards from the landmark (approximately 45°) following the natural fold lines of the skin.

Figure 2.0.A Triceps skinfold measure.

Figure 2.0.B Subscapular skinfold measure.
3. **Biceps**

This skinfold was raised on the mid-acromilae-radiale line so that the fold ran vertically i.e. parallel to the axis of the upper arm. The participant stood with the arm relaxed, the shoulder joint slightly externally rotated and the elbow extended. The fold was located on the most anterior aspect of the right arm.

![Figure 2.0.C Biceps skinfold measure.](image)

4. **Supraspinale (Iliocristale)**

This fold was raised approximately 5-7 cm above the anterior superior iliac spine on a line from the anterior axillary border to the spinale. The fold runs diagonally (medially) following the natural cleavage lines of the skin.

![Figure 2.0.D Supraspinale skinfold measure.](image)

5. **Iliac Crest (Suprailiac*)**

With the participants arm abducted to 90 degrees to the horizontal and the trunk erect, the most lateral superior edge of the ilium was palpated and the identified edge of the ilium was marked in line with the imaginary line made to the mid-line of the axilla. This skinfold was raised immediately at this landmark above the iliac crest. The fold is directed diagonally (anterior and downward) in line with the natural fold of the skin.

![Figure 2.0.E Iliac Crest skinfold measure.](image)

* NB. This was referred to as the Suprailiac by Durnin and Womersley.
6. Abdominal

This is a vertical* fold raised 2 cm lateral (right) to the centre of the omphalion (mid-point of the navel). The initial grasp is firm and broad since often the underlying musculature is poorly developed. This is a standard guideline to avoid any underestimation of the thickness of the subcutaneous layer of tissue267.

* NB. Vertical fold used for Jackson & Pollock equations; horizontal fold used with other equations.

Figure 2.0.F Abdominal skinfold measure.

7. Medial Calf

With the participant either seated or with the foot on a box (knee at 90°) and with the calf relaxed, the vertical fold was raised at the pre-marked location, on the most medial aspect of the calf at its maximal circumference.

Figure 2.0.G Medical Calf skinfold measure.
2.5. Indirect Adiposity quantification: 2-C model methods

Participants had their body composition measured by 2C model methods on one day and were scanned by MRI on a separate day within the same week following identical preparation (i.e. fasted from midnight on night before test and no vigorous exercise in previous 48 hours). As all 2C model methods were tested on the same day, it was important to ensure underwater weighing (UWW) was the last technique used as excess moisture on the skin can affect the accuracy of the other measurements. The testing order was as follows: skinfolds, bioelectrical impedance (Tanita, Bodystat Quadscan and 1500 devices), air displacement plethysmography (ADP / BodPod) and UWW. Protocols for use of all devices were as per manufacturers guidelines. All 2-C measures were conducted at the Sport and Exercise Science laboratory, University of Bedfordshire (Polhill, Bedford Campus), Hertfordshire.

2.5.1. Bioelectrical impedance analysis (BIA) using Tanita (BC-413 MA)

Participant information (gender, height and age) was entered manually into the device via the main control panel according to the manufacturers guideline. Wearing minimal clothing the participant was required to step onto the platform at the base of the device, carefully placing their feet on the electrode plates. The participant was asked to stand still while a measurement of weight was acquired. The participant then grasped the handles of the Tanita device, one in each hand, ensuring a tight grip around the electrodes of the handles. While standing still, with their arms by their side slightly abducted from the body, an unperceivable micro-current was passed through the hands and feet around the body, for approximately 5 seconds. Body composition data from the device printout were recorded on each participant’s data sheet.
Figure 2.1 Tanita BC-413 MA impedance analyser
2.5.2. Bioelectrical impedance analysis (BIA) using Bodystat devices

These bioelectrical impedance devices (Bodystat 1500 and Bodystat Quadscan 4000) were used according to the manufacturer’s instructions (Bodystat, Douglas, Isle of Man.). The participant was laid in a supine position for a standardised 5 minutes prior to testing to ensure body fluids had static and stable. During this time the participant was prepared for measurement. Disposable, single-use sensor (proximal) electrodes were placed on the dorsal surface of the wrist with the upper border of electrode bisecting the styloid process of the ulna and radius, and on the dorsal surface of the ankle with the upper border of electrode bisecting the medial and lateral malleoli. Source (distal) electrodes were placed at the base of the second or third metacarpal-phalangeal joints of the hand and foot as per the manufacturers guidelines (Appendix 2).
The electrode sites were cleaned with an alcohol wipe and were shaved to remove excess hair (if required) to maximise skin contact and conductivity. The participants’ arms and legs were comfortably abducted (35-40°) as recommended, ensuring no contact between the thighs, and the arms and trunk to prevent any “short circuit” of the path of the electrical current, and affecting the impedance value. I then manually entered baseline participant information (height, weight, gender, age, and self-reported activity level) into the device setup according manufacturer guidelines. One setup was complete I activated the device to allow delivery of a micro-electrical current through the participant (approximately 5 seconds duration). As with other devices, results from the device were recorded on each participant’s data sheet. Due to the fact that a micro-current is used in such BIA devices, a contraindication to use is if the participant has a pacemaker or is pregnant. These conditions were exclusion criteria in the study.
2.5.3. Bioelectrical impedance analysis (BIA) of the trunk (VISCAN™)

Abdominal fat content was measured using a VISCAN body fat analyser. During measurements, participants were instructed to lie supine wearing the theatre garments provided for MRI scanning. The standard test position was supine, with arms by sides (slightly abducted, elbows flexed at 90 degrees, with hands interlocked and resting on lower portion of rib cage), with clothing moved to allow full visualisation of the umbilical area (i.e. no undergarments impinging on test area. Participants were instructed to relax and breathe normally. Arm positioning was standardised to aid repeatability and prevent participants from placing their hands behind their neck to support their head and thereby potentially distorting resting abdominal dimensions by unnecessary potential elevation of rib cage and visceral (abdominal) region or contraction of abdominal muscles.

Protocol

- According to the manufacturer’s (Tanita Corporation, Tokyo, Japan) guidelines, the fully charged base unit was placed over the exposed umbilical area.
- With the power on, the infrared positioning light was aligned at the navel, and participant gender was selected on integral control panel.
- On activating the device ‘start’ button, the participant waist circumference was measured non-invasively using the ViScan measurement system comprising an infrared beam projected over
the waist at the umbilical sagittal plane, detected by eight in-built infrared sensors on either side of the base unit. The result was then displayed on the unit control panel.

- With the participant still supine, impedance was measured, using the ViScan system. The electrode belt holding four sensors (essentially a tetrapolar impedance method involving two pairs of injecting and sensing electrodes manifested as a wireless measurement “belt”) was then placed, in the sagittal plane, on the exposed midriff at the umbilicus, which had been lightly dampened (using moistened paper towel) to ensure optimum contact (Figure 2.5). Correct positioning of the electrode belt was ensured using systems infrared (laser line) positioning line, which was then confirmed by a short auditory beep.

For participants with a very large waist circumference (> 130 cm), where the base unit could not be placed over the abdomen – the electrode belt was placed on the abdomen as usual (waist < 130 cm), and then the base unit was positioned near, but not over the individual being tested. The “waist circumference > 130cm mode” was selected. The trunk and visceral fat measurements were taken as before and transmitted wirelessly to the base unit for display.

- The manufacturers dual frequency BIA technology then allows the electrical signal to be passed through the abdominal area providing the visceral and trunk fat results (displayed on unit control panel).

- These ViScan abdominal body composition values are derived from extrapolation of impedance measures (at 6.25 KHz and 50 KHz) using inbuilt software.
ViScan abdominal body composition values are sub-divided into: total abdominal adiposity (i.e. IAAT + ASAT), expressed as % trunk fat (range 0-75%), whereas IAAT is expressed as “visceral fat” (arbitrary units ranging from 1 to 59). The ViScan also rates these 2 measures using arbitrary band ratings of “low”, “average” and “high” for % trunk fat and “average”, “high” and “very high” for visceral fat.

2.5.4. Underwater weighing (UWW)

Underwater weighing (UWW) determines body fat values from body density. Body density (D) is the ratio of body mass to body volume  (ie. Mass / Volume). It is based on the whole-body principle of Archimedes, which states that an object’s loss of weight in water equals the weight of the volume of water it displaces. All participants were required to wear minimal clothing, i.e. tight fitting pair of swimming briefs along with a weighted diving belt to stop the participant from floating to the surface of the tank, this is particularly important for individuals with more adipose tissue, as excess fat will make
the participant more buoyant. (Care is taken to exclude the additional weight of the diving belt from all final participant values and calculations.)

Before the underwater measurement was made, each participant had their dry weight and lung volume (forced vital capacity - FVC) measured. FVC was measured using a calibrated volume displacement spirometer (Vitalograph Gold Standard 2150, Vitalograph Ltd. Buckingham, U.K.) according to the manufacturers established protocol guidelines. The spirometer was calibrated before all testing sessions using a known volume (3L) calibration syringes. (The 3L calibration syringe is regularly volume checked using a calibrated water displacement method in addition to being serviced and checked by the supplier – Cranlea Ltd, Birmingham, UK.)

Figure 2.6. Vitalograph Gold Standard 2150 used for spirometry.

UWW Test procedures:

1. An appropriate bathing suit was to be worn (i.e. one that did not add to buoyancy by trapping air - preferably nylon).

2. Participants were asked to have showered before the procedure in order to be devoid of excess body oils. Additionally, they were asked to have urinated and defecated, if possible, as well as to have removed all jewellery.

3. Normal hydration was requested and participants were asked to be 3-12 hours post-absorptive state (in this case fasted from midnight before test).
4. The underwater weighing tank used should ideally be as small and as controlled as possible. The temperature was maintained between 33-36 °C, as the density of the water is determined based on its temperature (e.g. 38 °C water density = 0.99296).

5. Participants were weighed pre-immersion (dry land weight) in as little clothing as possible.

6. A weight (diving) belt was attached to each participant to assist submersion.

7. The participant’s underwater weight was recorded several times. Each weight was measured after a deep, full expiration. Participants needed to be fully submerged and at residual volume (5-10 seconds). The movement of the apparatus under the water was kept to an absolute minimum at the time of recording and UWW was recorded to the nearest 0.01 kg.

8. On exiting the UWW tank, the water temperature is recorded as well as the weight of the diving belt and accessories (e.g. chains used etc) to ensure the participants weight was the only one recorded.

On entering the filled UWW tank care was taken to ensure participants did not disrupt the water unduly, and also avoided contact with the suspended seat attached to the load cell. In preparation for testing each participant was familiarised with the UWW technique by instructions to slowly submerge themselves fully under water and gently using their hands to eliminate any trapped air on the skin, hair and swimsuit.
Before measurements were undertaken each participant was recommended to practice the technique of fully exhaling before they submerged their head under water. Participants were then asked to practice gradually expelling any residual air left in their lungs while fully submerged. Once competent with this technique participants were required to sit on the suspended “button” chair - this too required familiarisation before the participant could balance on the seat. Once comfortable and balanced on the seat, the participant was instructed to repeat the exhalation and submersion process, avoiding contact with the sides of the tank, so not to produce a false underwater weight. During the seated submersion trials (once they had fully expelled any remaining air in their lungs) participants were instructed to remain still for as long as they could comfortably do so (approximately 10 seconds maximum) in order to allow the underwater weight to be recorded with minimal fluctuation.

Once the weight was recorded the participant was instructed to ascend from under the water so they were not holding their breath unnecessarily long and instructed to breathe normally between trials. This process was repeated until 3 readings were within 100g of each other. The mean of 3 final values was used as the underwater weight. The recorded values were used along with the participants’ pre-test, dry (land) weight in the calculation of body density (used to estimate body composition) using established formulae (1.3):
\[ BD = \frac{\text{mass (g)}}{\text{volume (cm}^3\text{)}} \]  

\[ BD = \frac{\text{mass of body in air (g)}}{\text{Mass of body (g) – mass of body in water (g)}} \]

\[ BD = \frac{\text{mass of body in air (g)}}{\text{Mass of body (g) – mass of body in water (g) – residual volume}} \]

\[ \text{Density of water} \]

2.5.5. Air displacement plethysmography – ADP (BodPod®)

Before the measurement procedure all participants were instructed to completely void their bowels and bladder. In line with the manufacturers guidelines (to standardise the exclusion of trapped air in body hair) all participants were requested to remove all items of jewellery and to wear only a tight fitting swimsuit and swimming cap. Baseline calibrations of the empty chamber with and without a standard phantom (40.995 L metal calibration cylinder) were performed before each participant as per the manufacturers guidelines. Once weighed on the integrated (calibrated) digital weighing scales, the participant was seated in the BodPod® (ADP) chamber and instructed to sit in the centre of the seat resting their back against the rear wall of the chamber with their feet in the centre of the base of the BodPod, with legs apart and their hands on their lap and instructed to remain still and breath normally. For safety reasons each participant was shown the emergency stop button (located inside the chamber), which releases the door if required for any reason. With the chamber door shut the test initiation activates magnetic door locks. Following each 50 seconds measurement, the BodPod chamber door was opened fully in order to avoid any potential feelings of claustrophobia by the participant. During these standardised door open intervals, participants were asked if they are ready.
for the next test and happy to proceed. Once confirmed the door was gently shut and the second test was undertaken.

If the repeat measurements disagreed by more than 150 mL, a third test was performed. If no two tests were in agreement by 150 mL, the entire process including calibration was repeated until two test values met the required accuracy. The resultant (accepted) test values were averaged and used in the calculation of raw body volume. The calculation of a body volume, height and weight must be used to estimate body surface area. Predicted thoracic gas volume was also used in this calculation. Body surface area is required to account for the negative volume produced by the isothermal air surrounding the surface of the body. According to manufacturers guidelines, thoracic gas volume (TGV) was also accounted for owing to the isothermal air in the lungs and airway. Raw body volume, surface area artefact and TGV are used to produce a corrected body volume. This resultant value was used to estimate body density, which was then used to estimate percentage body fat.

Figure 2.7  General arrangement of Bod Pod® front (test) chamber containing participant and rear (reference) chamber separated by a diaphragm mounted in common wall. Electronic oscillation of the diaphragm during testing produces minute (± 0.5cm H₂O) complimentary pressure changes in the chambers allowing derivation of an unknown volume from the direct pressure measurement.
2.6. Direct Adiposity quantification: assessment by MRI and MRS

2.6.1. MR setup protocol

On a single visit, participants underwent total body MRI scanning and in vivo proton (1H) magnetic resonance spectroscopy (MRS) of their liver and their calf muscles.

Calibration

The MR equipment was calibrated each day according to manufacturers guidelines using a standardized phantom to detect drifts in measurements. Additionally, equipment servicing and upgrading was performed regularly.

Participant positioning & safety.

Participants lay in a prone position with arms straight above the head, and were scanned from fingertips to toes. All participants were screened (Appendix 1) before scanning to ensure no metallic items were taken into magnet field – to avoid personal injury as well as any radiographic artefacts that
could compromise the MR images and the subsequent image analysis. Standard health and safety protocols were followed for all scanning. These included:

1. Participants being provided with hospital issue theatre garments to wear during scanning to ensure removal of all personal clothing that might pose a risk / alter scan images,

2. Participants being instructed to lie very still during scanning to avoid image artefact. Sandbags and foam pads were used to support / brace limbs and ease muscle tension or unnecessary discomfort.

3. Ear defenders were worn at all times during scanning – manufacturers headphones or in-ear foam plugs,

4. All cables in contact with participant were shielded and positioned according to strict MR safety guidelines to provide maximum protection.

5. Participants were given an emergency buzzer to hold at all times to alert operating staff to any problems. As well as being fully instructed on scanning procedures beforehand – participants were reassured and communicated with at all times using a built-in open channel audio system (microphone and speaker) between the scanner room and the operators observation room (equipped with behind magnet video capability to monitor all aspects of the procedure).

6. All staff entering the magnet area were appropriately trained in magnet safety and duly observed all departmental MR safety protocols.

Figure 2.9 Philips Achieva 1.5T MRI scanner used in all studies.
2.6.2. MR assessment of adiposity

MRI of total body and regional adipose tissue

Whole-body magnetic resonance (MR) images were obtained using a 1.5T multinuclear scanner (Phillips Achieva™, Philips Medical Systems, Best, Netherlands) using established protocols previously described in detail by Thomas et al. (1998). The images were acquired using a whole body axial T₁-weighted spin echo sequence and a Q body coil, without respiratory gating (typical parameters: repetition time (TR) 560ms; echo time (TE) 18ms; slice thickness 10mm; interslice gap 10mm; flip angle 90 degrees; number of excitations 1). Initially coronal and sagittal whole body views were obtained to allow planning of the transverse images (Figure 2.10).

![Sample coronal and sagittal MR images](image)

Figure 2.10. Sample coronal and sagittal MR images. (Original image.)

Transverse images were then acquired as nine equal stacks of 12 slices at the isocentre of the magnet. A typical dataset is shown in Figure 2.11.
MRI Adipose tissue quantification (data analysis methods)

As part of this research I was mentored and trained by Dr Louise Thomas in the use the commercially available software specifically designed for image analysis (SliceOmatic: Tomovision, Montreal, Quebec, Canada). This extensive training was to inform both my work and my understanding. Using this software, each image was segmented into subcutaneous and internal adipose tissue, and the relevant pixels coded accordingly. This process is summarised in Figure 2.12. However, since my data and research was part of a much larger research program involving many projects, I was not permitted to perform the MRI analysis and the data analysis was outsourced for reliability purposes. All outsourced images were quantified, using the above method, by an independent data analysis company (Vardis Group, London, U.K.), who were blinded to the specific participant and project details.
Figure 2.12 The grey scale image was initially processed using mathematical morphology to segment the subcutaneous and internal fat. These were labelled (Tagged) with specific colour codes for each depot. In these studies, subcutaneous fat was coded green and internal fat coded red. Manual editing was then applied to remove pixels incorrectly assigned to fat, such as those arising from motion or bowel contents.

The adipose tissue volumes (cm$^3$) of each compartment were calculated by summing the number of pixels and multiplying by the known pixel dimensions (in cubic centimetres). Adipose tissue volume for the whole body was then calculated by multiplying the adipose tissue volumes of each slice by the sum of the slice thickness (10 mm) and interslice distance.

Total and regional adipose tissue volumes were subsequently recorded in litres (L). These consisted of total adipose tissue (TAT), subcutaneous adipose tissue (ASAT) and internal adipose tissue (TIAT). These depots were further refined to enable measurement of abdominal adipose tissue depots. The abdominal region was defined as the images between the slice containing the femoral heads to the slice containing the top of the liver or the bottom of the lungs, usually between 15–17 images in most individuals$^{279}$. The adipose tissue within the abdomen was therefore separated into intra-abdominal adipose tissue (IAAT) and abdominal subcutaneous adipose tissue (ASAT). Outside the abdominal
regions, adipose tissue was separated into non-abdominal subcutaneous adipose tissue (NASAT) and non-abdominal internal adipose tissue (NAIAT) as previously described by members of research group\textsuperscript{279}. An additional quantification of abdominal adiposity as a whole, was derived from the sum of IAAT and ASAT, and termed “trunk” fat.

In summary:

1. Total Adipose tissue = Subcutaneous AT + internal AT

2. Subcutaneous AT (SAT) = abdominal subcutaneous AT (ASAT) + non-abdominal subcutaneous AT (NASAT): SAT = ASAT + NASAT.

3. Total internal AT (TIA) = intra-abdominal AT (IAAT) + non-abdominal internal AT (NAIAT):
   \[
   \text{Internal} = \text{IAAT} + \text{NAIAT}.
   \]

4. Total Trunk AT = IAAT + ASAT.

Regular quality control duplicate datasets were also used to verify the accuracy of the outsourced image analysis. This involved internal reanalysis of randomly chosen sets of data - the results of which were then compared to the respective outsourced data results. In these datasets adipose tissue areas were estimated by a single trained observer (Dr. Louise Thomas) to minimise operator error (and variability) and maximise reliability. The coefficient of variation of these methods is low, ranging from <1\% for total fat to 5\% for visceral fat\textsuperscript{280}.

Adiposity data were expressed in three ways;

1) \textit{Absolute volume (L)}: adipose compartment volume in litres;

2) \textit{Percentage of body mass (%kg)}: the total mass of each deposit (kg) was obtained by multiplying its volume in litres with the density of fat, which was taken to be 0.9gm/cm\textsuperscript{3}\textsuperscript{281}. Percentage adiposity values for each fat compartment were calculated by dividing the body mass in kg by the appropriate fat store (% adiposity = fat (kg) / body mass (kg) x 100);

Absolute volume provides information on the total volume of an adipose compartment, %kg data is useful as it includes information about fat mass relative to lean body mass.
MRS of liver and muscle adipose tissue

During the same scanning session, \(^1\)H MR spectra were also acquired at 1.5T, using a flexible body coil. Participants were positioned supine, arms resting by their side, and legs supported with the hips slightly flexed using a foam wedge (to prevent lumbar discomfort from flat surface of scanning table) as in Figure 2.13.

Liver MR Spectroscopy: \(^1\)H MR spectra were acquired at 1.5 T from the right lobe of the liver using a PRESS (point-resolved spectroscopy) sequence (TR 1500 ms/ TE 135 ms) without water saturation and with 128 signal averages. The right lobe was chosen owing to its larger size and its ease of imaging owing to it being closer to the centre of the magnet. As part of my research and to inform my work I underwent extensive training with experienced members of the research team (radiologists: Julie Fitzpartick and Giuliana Durighel, and senior researcher - Dr Louise Thomas) in order to be able to position participants in the magnetic field correctly and locate the voxel in the area of interest accurately. Transverse images of the liver were acquired and used to ensure accurate positioning of the (20x20x20 mm) voxel in a representative region of the liver, avoiding blood vessels, the gall bladder, and fatty tissue. Typical images and corresponding spectra from participants with high and low levels of liver fat infiltration are shown in figure 2.14
Muscle Spectroscopy: Intramyocellular lipids (IMCL) were measured in the soleus (S-IMCL) and tibialis (T-IMCL) muscles by obtaining proton MR spectra (\(^1\)H MRS) from the respective muscles of the left calf of all participants, using the same PRESS sequence described above. For each of my data measurements I placed the voxel in a representative region of tissue that avoided visible streaky fat and main blood vessels.

Figure 2.15 Typical *in vivo* \(^1\)H magnetic resonance spectra from soleus muscle Cho, choline-containing compounds (carnitine + glycerophosphocholine); Crto, creatine + phosphocreatine; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid.
This ability to select volumes if interest at different regions within a given tissue is a distinct advantage to using MRS for such analysis. While there are other methods for measuring muscle lipids, such as electron microscopy and morphometry\textsuperscript{282-284} or biochemical assay\textsuperscript{285-287}, these and invasive and require a tissue biopsy. MRS has a high correlation (0.934) with EM morphometry, thus negating such invasive procedures; but has a low correlation (0.47) with biochemical, which additionally has less sensitivity to distinguish IMCL from EMCL\textsuperscript{288}.

A typical image and corresponding spectrum obtained from the soleus muscle of the left leg is shown in figure 2.15. Lipid resonances (IMCL) were quantified with reference to the total muscle creatine signal, in accordance with protocols previously published by the research team\textsuperscript{289}.

The coefficient of variation for these methods is approximately 7\% for IHCL\textsuperscript{290} and 2\% for IMCL\textsuperscript{291}. MRS data (IHCL, S-IMCL and T-IMCL) were presented as the geometric mean, while statistical analysis was performed on log\textsubscript{10}-transformed variables, owing to the positively skewed distribution of these data-sets\textsuperscript{292}.

**MRS analysis**

Following acquisition I then analysed the $^1$H MR spectra for my research subjects to determine the concentration of both IHCL and IMCL using spectral fitting applications (jMRUI / AMARES). The MRUI (jMRUI = Java based version) software is a graphical user interface (GUI) that allows time-domain analysis of *in vivo* MR data. Quantification of the reconstructed signals was performed in the time-domain. AMARES\textsuperscript{293}, as included in the jMRUI software package\textsuperscript{294}, uses Fourier analysis to fit all relevant metabolites using established prior knowledge in the software setup. I used the AMARES algorithm to resolve the components of my acquired spectra based on the stored prior knowledge for the tissue and metabolites. AMARES is essentially a non-linear least square, fitting algorithm operating in the time domain and allows the inclusion of a large amount of prior knowledge through a so-called singlet approach. Intrahepatocellular lipids (IHCL) were measured relative to liver water content, according to previously published research\textsuperscript{295} and were expressed as a percentage ratio of the CH\textsubscript{2} lipid peak area relative to the water peak area after correction for T\textsubscript{1} and T\textsubscript{2} relaxation.
Typical examples of the fitting process applied to the liver and soleus muscle are shown in figures 2.16. A and 2.16. B respectively.

Figure 2.16. $^1$H spectrum of the liver (A) and soleus muscle (B) processed by AMARES.


### 2.7. Fitness Assessment: VO$_2$max

Aerobic fitness was assessed via oxygen consumption (VO$_2$) measurement using an automated, breath-by-breath gas analyser (Metalyser 3B, CORTEX Biophysik GmbH, Leipzig, Germany), this is an on-line cardiopulmonary gas exchange system. This ‘indirect calorimetry’ is used to calculate values for oxygen uptake from the difference between ambient gas composition and the expirate. The system comprises three major components: gas analysers for carbon dioxide (infrared) and oxygen (paramagnetic), a turbine or ‘triple-V’ sensor for recording gas flow (and hence volume) and a central processing unit (CPU) and a monitor to integrate and display graphical information in real time.
Calibration

Prior to every trial the gas analysers were calibrated using known concentrations of CO₂ and O₂ (Cranlea Ltd, Birmingham, UK) and the flow-volume sensor was calibrated using a 3-litre syringe (Cranlea Ltd, Birmingham, UK). The system contains hardware to allow the continuous measurement of ambient temperature and barometric pressure and automatically corrects values, made for expired air, to standard temperature (0 °C), barometric pressure at sea level (101.3 kPa) and dry gas (STPD). Measurements are all made at BTPS (body temperature and pressure saturated) under ambient conditions where gas water vapour saturation varies with changes in temperature, pressure and moisture in the air. BTPS measurements are converted to STPD. The gas is dried to remove water vapour pressure from the measurement, allowing greater ease of comparison.
Validation

The accuracy of the computerized, breath-by-breath, metabolic system (Cortex Metalyser 3B) used was periodically compared against the criterion Douglas bag method, which is considered the “gold standard” for cardiopulmonary gas exchange assessment, in order to check the validity of the on-line system in determining a number of cardiopulmonary parameters. The Douglas bag method is an historic manual measurement method whereby large impermeable (non-diffusing) collection bags, linked in series, are used to collect expired gases over a known time period (eg. 1 minute). Subsequently, gas fractions of oxygen and carbon dioxide are measured offline from the contents of these collection bags using calibrated gas analysers (for O₂ and CO₂) for each respective gas. At the same time, expired gas volumes in these collection bags are measured using a dry gas meter and vacuum pump to ensure matching of the gas fractions with the ventilatory volumes. The summary coefficients of variation (i.e. the differences between Cortex Metalyser 3B and Douglas bags) in this study were: VE 1.40%, VO₂ 0.60%, VCO₂ 0.10%, RER 1.20%. Most certifying organizations tolerate a maximal error of 4% in VO₂ determination. The British Association of Sport and Exercise Sciences (BASES) advises that less than a 2% difference for VO₂ between Douglas bag and an on-line gas analysis system is acceptable. The Cortex (Metalyser 3B) system used in this research was therefore deemed accurate and acceptable.

VO₂max

Maximal aerobic power, or VO₂max, is the upper limit of the criterion standard of cardiorespiratory fitness, maximal oxygen uptake and is the maximum rate at which an individual can extract oxygen and then transport and utilize it in tissues while breathing air, at sea level. In vivo, there exists a finite reactant supply and thus, at some stage, as external power is increased, there will be no further rise in VO₂. Thus, VO₂ is a measure of 1) the maximal energy output by aerobic processes and 2) the functional capacity of the circulatory system. Criteria for attainment of VO₂max are generally accepted as being:

1) an increase in VO₂ <100 mL (2mL kg⁻¹ min⁻¹) between the final 2 workloads, and
2) an increase in HR <5 bpm between the final 2 workloads, (or final heart rate within 10 beats min⁻¹ of maximum value or predicted age-related maximum : 220-age)
3) a final respiratory exchange ratio (RER) greater than 1.15. VO$_{2\text{max}}$ must, by definition, cause volitional exhaustion between 9 and 15 minutes. If the plateau in criteria 1) is not achieved (or the test lasts less than 9 minutes or more than 15 minutes) then the resulting value is referred to as VO$_2$ peak. This is deemed a satisfactory index of aerobic power as there is usually less than 3% difference between a ‘max’ and a ‘peak’ value. However, it is now becoming widely accepted that attainment of a true VO$_{2\text{max}}$ value requires a further verification phase (test) as in many cases individuals being tested may not exhibit a clearly definable VO$_2$ plateau, despite giving a maximal effort. It is well known that there are also concerns about using the term VO$_{2\text{max}}$ when the exercise test does not incorporate the largest possible proportion of muscle mass (e.g. in cycling tests as compared to treadmill tests). With the exception of elite cyclists, most individuals undertaking a cycle test may experience a greater degree of local muscle fatigue during their maximal exertion and not elicit a true VO$_{2\text{max}}$. In such cases where the primary criterion (1. listed above) for VO$_{2\text{max}}$ is not attained / evident (i.e. absence of definable VO$_2$ plateau), secondary criteria (i.e. 2) and 3) listed above) would be used for confirmation. These secondary criteria have been criticised on the basis that these can in cases be satisfied at exercise intensities as low as 73% of VO$_{2\text{max}}$. The physiological reasons for this greater degree of fatigue may be linked to the fact that cycling may require a greater force output than running and may cause greater fast twitch fibre recruitment – with a corresponding increased lactate production (through glycolytic metabolism) compared to the oxidative slow twitch fibres. Additionally, there may be reduced blood flow in the legs during cycling compared to running, thus further increasing the local fatigue.

Resulting values for VO$_{2\text{max}}$ (or VO$_2$ peak) are generally expressed as absolute terms (L. min$^{-1}$). As there is a strong positive relationship between body size and absolute values (L. min$^{-1}$), these can be divided by body mass to yield a relative value (mL.kg$^{-1}$ min$^{-1}$) in an attempt to overcome the effects of differences in body mass when comparing values. In weight bearing sports / activities the relative value is more commonly used and in non-weight bearing sports / activities the absolute value is more often used. Maximal oxygen consumption (VO$_{2\text{max}}$) was expressed in absolute terms (L.min$^{-1}$) and relative terms (mL.kg.min$^{-1}$).
Heart rate (HR) measurement

Heart rate was measured before, during and after exercise tests. Heart rate (HR) was monitored using a Polar (Polar Electro, Finland), telemetry system (chest strap transmitter & watch monitor) as well as by a telemetry receiver linked to the on-line gas analyser (Metalyser 3B, CORTEX Biophysik GmbH, Leipzig, Germany). The manufacturer quotes accuracy for heart-rate measurement of 1% per minute for steady-state conditions. The resultant heart rate data was digitally processed in real-time and integrated simultaneously with the on-line gas analysis data using inbuilt proprietary software (Metasoft 3, CORTEX Biophysik GmbH, Leipzig, Germany).

Figure 2.18 Integrated cardiopulmonary exercise testing components used.

VO$_2$max protocol

Cardiovascular fitness was measured with an incremental (ramp) exercise protocol. The incremental cycle test was performed using a calibrated electronically braked ergometer (Lode Corival, Lode, Germany) employing an automated incremental increase in workload of 15 watts / min until voluntary exhaustion. All participants started from 50 watts. Participants were requested to maintain a constant pedal frequency of 60-70 rpm throughout the test (rpm rate displayed to participant on cycle ergometer digital display). To aid the timing of the cycling tempo, a digital metronome with an acoustic beep (set to 60 beats / minute) was also used so that participants could maintain the requested
constant pedal frequency more easily than just viewing the ergometer visual feedback display. Standardised (scripted) verbal cues were given to all participants to encourage a maximal effort.

Cycle ergometry was the chosen mode of exercise:

1) owing to limited available space in hospital location for a treadmill installation and use,
2) to accommodate participants that may be unfamiliar with treadmill use (and therefore require prior familiarization sessions) and to avoid unnecessary risk,
3) to accommodate participants that may be non-exercisers, or participants of a very low fitness level who could be disadvantaged owing to skill needed for effective treadmill use.

With all tests there are safety concerns, but with maximal fitness testing these relate to the fact that the test is difficult and stressful. Many individuals with chronic disease or disability, do not achieve a 'true' VO2max. Instead, they reach a point at which they cannot continue because of limiting factors such as mental fatigue, fear, lack of motivation or symptoms such as chest pain and light-headedness. In these cases, the individuals are said to have reached 'symptom-limited exhaustion' and this is referred to as VO2 peak. A further issue with cycle tests, as opposed to treadmill tests, is that individuals can often reach a point of peripheral fatigue, before central (cardiorespiratory) fatigue that prevents them reaching a 'true' VO2max. This can happen when the test workloads are increased by too big an increment at any point. This is due to the fact that cycling demands a greater power output than running, which causes increasing recruitment of more fast twitch (glycolytic) fibres as the test progresses. This increasing recruitment of predominantly glycolytic metabolism results in increased lactate production, leading to fatigue, decreasing power output and reduced blood flow the legs. This series of events often happens before long before any limitations in the supply of oxygen. In such cases the final value should be referred to as VO2 peak. Additionally, it is the job of the test supervisor to observe the person exercising at all time in case the participant experienced discomfort or showed signs they were becoming unwell during a test. In the absence of cardiac (ecg) monitoring, observing for signs of poor perfusion (cyanosis or pallor), ataxia, dizziness or discomfort would be a priority. Standard guidelines do exist for detailing ‘absolute’ and ‘relative’ indications for stopping a test.
2.8. Statistical Analysis

Statistical calculations were performed using SPSS software (version 17.0, SPSS Inc, Chicago, IL, U.S.A.) throughout this thesis. Statistics appropriate to each study can be found in the relevant chapter. Additional statistical advice and support was provided during aspects of the thesis by Caroline Dore (MRC Clinical Trials Unit, London. UK) and Mr. Peter Sheard (Faculty of Health and Social Sciences, University of Bedfordshire, Beds. UK).
Chapter 3. Comparison of body composition techniques.

3.1. Introduction

One of the key issues in the quantification of adiposity is the method of measurement. While many studies have reported that different populations have greater body fat depots than others, the studies have used a variety of measurements – not all of which give the greatest accuracy and may therefore have limited applicability. Studies exclusively measuring body composition with anthropometric measures such as waist-to-hip ratio (WHR) and body mass index (BMI) could potentially miss valuable data owing to low predictive accuracy. For instance using a methodology such as MRI, which enables accurate quantification of regional adipose tissue depots such as internal fat, may influence the outcome of a clinical study. This is particularly the case for certain populations in whom it is known that simple anthropometric measures under predict adiposity.

As an example, despite its convenience, BMI fails to account for the composition of body weight, which is comprised of mainly fat, lean tissue and bone mineral. Previous studies have confirmed that the relationship between BMI and relative body adiposity varies considerably by age, gender, and race/ethnicity. Additionally, the waist-to-hip ratio is also a poor indicator of regional adipose tissue distribution since it is influenced by a number of factors such as frame size and skeletal muscle mass.

Since obesity is technically characterized by excess fat mass, a measure of whole body adiposity (% body fat) only provides an estimate of relative adiposity or leanness. Such proxy measures do not account for the heterogeneity that is inherent in human body composition, especially since body fat and its distribution are known risk factors for metabolic abnormalities. The risks associated with excess adiposity have consistently been shown to be a function of regional fat distribution, rather than overall fat volume owing to the stronger associations with physiological and pathological processes.

While various methodologies exist to measure overall body adiposity, available methods for measuring regional adiposity are more limited. Simple anthropometry is often used as a predictor of
abdominal adiposity, for example, an increased waist circumference has been shown to be an effective predictor of abdominal obesity.\textsuperscript{321}

Abdominal obesity relates to excess adiposity at the abdomen and is known to predispose individuals to diabetes and cardiovascular disease.\textsuperscript{83} Adipose tissue in the abdomen is found in several compartments, the ones that can typically be measured using MRI include abdominal subcutaneous adipose tissue (ASAT) and intra-abdominal adipose tissue (IAAT). Elevated IAAT, (also referred to as "visceral fat") is associated with insulin resistance, dyslipidemia, systemic inflammation, diabetes, hypertension, myocardial infarction, and all-cause mortality.\textsuperscript{85,91,322,323} While proxy measures such as waist circumference may reflect actual measured abdominal adiposity it is not in itself a direct measure of either ASAT or IAAT and may not be sufficiently sensitive to detect changes in abdominal body composition.\textsuperscript{324} This is further complicated by that fact that abdominal subcutaneous and intra-abdominal fat compartments may expand independently of each other and there is no simple metric relationship between IAA\textsuperscript{325-327}.

Magnetic resonance imaging (MRI) and computed tomography (CT) are the ‘gold standard’ methods for measurement of ASAT and IAAT.\textsuperscript{176} However, both are high cost techniques, labour intensive, non-portable and of limited availability for wide application.

This study aimed to assess the accuracy of several different whole body composition methods (devices) to accurately measure/predict adiposity with reference to the different AT depots as well as an assessment of regional body composition measurement methods. For the comparisons made, MRI-based techniques of body fat distribution and ectopic fat content were used as the gold standard criterion measurement.

Therefore in this chapter I have compared body fat measures derived from hydrodensitometry, bio-impedance and anthropometry, to measures of adiposity from MRI in a heterogeneous group of male and volunteers. MRI-based techniques were used to determine the criterion range of all adiposity stores for all participants in the cohort. The study was divided into 2 parts:
Part A – Comparison of general (total) body composition methods

Part B – Comparison of abdominal adiposity measurement methods

In doing so the study aimed to use assess the feasibility of these different devices (techniques) to provide accurate, and relatively more affordable measures of adiposity.

Objectives:

1. Quantify adiposity stores in a cohort of healthy volunteers using MRI and MRS.

2. Part A: To evaluate the accuracy of four different 2-C body composition measurement techniques (UWW, BodPod, BIA, skinfolds) in a group of 21 non-obese men (10 South Asian, 11 Caucasian) against the criterion (gold standard) magnetic resonance imaging (MRI).

3. Part B: Assess the accuracy of abdominal adiposity proxy measurement methods to predict regional adiposity derived from MRI in a group of 74 adults Caucasians (40 females and 34 males).

Study Hypotheses:

Hypothesis 1: 2-C methods (UWW, ADP, BIA, skinfolds) are a valid alternative to MRI for adiposity quantification.

Hypothesis 2: The Viscan BIA device is an accurate (internal) abdominal adiposity proxy measurement method.
3.2. Methods

Recruitment issues.

During Part A of this study, involving comparison of multiple 2-C devices (UWW, BodPod, BIA, and skinfolds), there was a low participant number (N= 21) as there was extreme difficulty recruiting sufficient willing volunteers to all tests conducted. The low number recruited was due to several factors. The first major obstacle to recruitment was participant reluctance to give up half a day (at maximum – including travel) for the battery of tests that were involved. Whilst most of these individuals would volunteer for the MRI component, the additional measurement techniques were usually the limiting factor. This may have been because participants perceived there was intrinsic benefit in the medical (MRI) scanning component as opposed to the other ‘non-medical’ techniques. However, it is widely accepted that with UWW a major limitation is that it requires a highly motivated participant who is willing to don bathing attire, complete effective spirometry, is willing and capable of exhaling prior to submersion in a water tank and can be confident in that tank for a period of approximately 30 seconds. In some cases, it was discovered that subjects that were successfully recruited and willing, subsequently found they were not comfortable with or were unable to successfully complete the submerged component. Another aspect of the additional techniques that hampered recruitment was that obese individuals are less inclined to want to undertake such testing that requires minimal attire, let alone, close fitting swim-type costumes that are necessary for the UWW and ADP measurements (to prevent air being trapped that may influence results adversely). The ADP measurement, whilst not involving water, involves sitting in a close fitting sealed chamber, which can feel claustrophobic (despite the chamber having a perspex viewing window) and may feel especially so for large framed and obese individuals. The state of undress and need for close fitting, minimal clothing with the addition of a swim cap (to prevent air trapped in hair confounding results) can put volunteers off.
Participants

Written, informed consent was acquired from all volunteers. Ethical approval permission for this study was obtained from the Research Ethics Committee of Hammersmith and Queen Charlotte's and Chelsea Research Ethics Committee Hospital, London (Rec: 07Q04011/19). In total, 84 volunteers (44 male: 34 Caucasian, 10 South Asian; 40 female Caucasian) were recruited. All participants were aged 20-70 years. Self-reported exclusion criteria included participants suffering from chronic disease including diabetes, cardiovascular or liver disease, metabolic conditions, claustrophobia, and anyone taking prescribed medication and women on the contraceptive pill.

Anthropometric measurements

Body mass (kg), height (cm), midpoint waist circumference (WC) (cm) and hip circumference (cm) were measured in each participant as detailed in the General Research Methods and Procedures section (Chapter 2). From these values, BMI (kg/m$^2$), waist-to-hip ratio (WHR, waist/hip) and waist-to-height ratio (WHtR, waist/height) were calculated. As previously mentioned, BMI grouping corresponded to the following ranges; 1: 18.5<25 kg/m$^2$, 2: 25<30 kg/m$^2$, 3: 30<40 kg/m$^2$, 4: 40+ kg/m$^2$.

Measures of Adipose Tissue Content

Part A

On a single visit, a sub-group of 21 male participants, underwent: whole body MRI scanning, total body fat measured using bioelectrical impedance (BIA), air displacement plethysmography (ADP or the BodPod), underwater weighing (UWW) and skinfold anthropometry according to the methods described in Chapter 2.

Part B

On a single visit, a sub-group of 74 male and female participants (33 males, 40 females), underwent: whole body MRI scanning, abdominal body fat measured using an abdominal adiposity device (Viscan BIA), and anthropometry according to the methods described in Chapter 2.
Statistical Analysis

Part A

In order to assess the validity of the five predictor variables were compared to the criterion. The relationship between MRI, ADP, BIA UWW and SKF percent body fat estimates was examined using repeated measures ANOVA to assess the differences of the mean percentage body fat scores both within and between Caucasian and South Asian male groups. Correlation coefficients and multiple linear regression analysis were used to assess the linear relationship between the criterion and predictor variables and agreement between body composition estimates was examined by calculating the 95% limits of agreement as explained by Bland-Altman. The use of multiple regression allowed for the prediction of MRI body fat from one or more predictor variables. Potential bias between MRI percentage body fat and the predictor variables was assessed using residual plots. For all analysis the alpha level set for statistical significance was P<0.05, using SPSS software (version 17.0 SPSS, Chicago, IL, USA). Statistical advice was provided during this study by Mr. Peter Sheard (Faculty of Health and Social Sciences, University of Bedfordshire, Beds. UK).

Part B

Agreement between methods of umbilical waist measurement (i.e. ViScan vs. manual measurement) was assessed according to Bland-Altman and systematic bias between methods was assessed via paired sample t-test. As the units of the ViScan and MRI were not the same, Bland-Altman plots could not be constructed to compare the different measures of abdominal adipose tissue compartments. Pearson’s correlation coefficients were calculated to assess the association between MRI derived abdominal fat compartments and VISCAN BIA estimates. Significant differences in MRI derived total abdominal fat between the ViScan trunk fat bandings and between IAAT and the ViScan visceral fat bandings were assessed using a one-way ANOVA. Associations between other anthropometric measures and MRI derived abdominal fat compartments were also assessed using Pearson’s correlation coefficients. All statistical analyses were performed using SPSS software (version 17.0 SPSS, Chicago, IL, USA). Level of significance was set at p<0.05.
3.3. Results

3.3.1. PART A. Assessment of 2 Component (2C) measurement systems

Descriptive Statistics

This aspect of the study compared five 2-component (2C) model methods of estimating percentage body fat in 21 Caucasian and South Asian male participants. The group characteristics are summarized in Table 3.0. The Caucasian participants were older, taller (1.79 m ± 0.08 m vs 1.70 ± 0.04 m, p=0.003) heavier (83.85 kg ± 9.30 vs 73.21 kg, p=0.025) and had a greater BMI compared to the South Asian participants.

Table 3.0. Characteristics of Caucasian and South Asian participants included in technique comparison study.

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=21)</th>
<th>Caucasian (n=11)</th>
<th>South Asian (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± sd</td>
<td>range</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.24 ± 9.94</td>
<td>31.00</td>
<td>33.36 ± 11.67</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 ± 0.08</td>
<td>0.25</td>
<td>1.79 ± 0.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.78 ± 11.15</td>
<td>49.80</td>
<td>83.85 ± 9.30</td>
</tr>
<tr>
<td>BMI (kg m²)</td>
<td>25.81 ± 25.81</td>
<td>13.19</td>
<td>26.19 ± 3.34</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.
### Table 3.1 Comparison of adiposity measurements by five different methods (using six devices) in Caucasian and South Asian participants.

<table>
<thead>
<tr>
<th>Method</th>
<th>Caucasian (n=11)</th>
<th>South Asian (n=10)</th>
<th>Between groups difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinfolds</td>
<td>19.57 ± 1.48</td>
<td>26.53 ± 1.86</td>
<td>6.95 ± 2.37* P = 0.010</td>
</tr>
<tr>
<td>BIA-Bodystat</td>
<td>15.98 ± 1.23</td>
<td>19.01 ± 1.54**</td>
<td>3.03 ± 2.37 P = 0.144</td>
</tr>
<tr>
<td>BIA-Tanita</td>
<td>16.08 ± 1.51</td>
<td>22.00 ± 1.89**</td>
<td>5.92 ± 2.42* P = 0.026</td>
</tr>
<tr>
<td>ADP</td>
<td>20.36 ± 1.97</td>
<td>30.09 ± 2.47</td>
<td>9.73 ± 3.16 P = 0.007</td>
</tr>
<tr>
<td>UWW</td>
<td>18.91 ± 1.81</td>
<td>27.64 ± 2.27</td>
<td>8.74 ± 2.91* P = 0.008</td>
</tr>
<tr>
<td>MRI</td>
<td>17.18 ± 1.88</td>
<td>30.29 ± 2.35</td>
<td>13.11 ± 3.01* P = 0.000</td>
</tr>
</tbody>
</table>

Data expressed as means ± standard error, results of a repeated measures ANOVA. Where BIA – bioelectrical impedance, ADP - air displacement plethysmography, UWW - underwater weighing and MRI - magnetic resonance imaging. * = between groups mean difference is significant (P<0.05), ** = within groups significant mean difference compared to MRI (P<0.05).

MRI was taken to be the criterion, based on its acceptance as a direct method to measure body fat content and distribution. Within group comparison of Caucasians revealed no significant mean difference between the MRI and the five other methods of measuring adiposity (Figure 3.1). ADP overestimated MRI percent body fat by the greatest degree (3.19%), while both BIA devices, Bodystat and Tanita provided the closest measurement to MRI in the Caucasian group, underestimating percent body fat by 1.20% and 1.10%, respectively. UWW was ranked as the third closest measure to MRI, and skinfolds were fourth closest.

However, within the South Asian group, there were significant differences between body fat measured by MRI and both BIA devices (Tanita and Bodystat). In contrast to the Caucasian group, in this population, BIA (Bodystat and Tanita) significantly underestimated % body fat by 11.27% and 8.29%, respectively. Unlike the Caucasian group, ADP exhibited the least difference to MRI percent body fat with a 0.20% underestimation, UWW was the second closest measure to MRI and skinfolds were ranked third.

Comparing the different methods for measuring body fat content, all methods measured a higher percentage body fat in South Asian compared to Caucasian participants. Indeed, significant
differences in percentage body fat content between South Asian and Caucasian participants were detected using skinfolds (p=0.004), BIA-Tanita (p=0.026), BodPod (p=0.002) HW (p=0.016) and MRI (p=0.0001). Whereas no significant differences could be detected using BIA-Bodystat. The greatest measurable difference in % body fat between South Asian and Caucasian was obtained using MRI measured adiposity (Table 3.1). There was significantly less variation in body fat percentage measured by the different techniques in Caucasian compared to South Asian participants (8.30 ± 2.82 vs 12.95 ± 3.93, p<0.01), figure 3.0B.

**Figure 3.0A. Total body fat content of Caucasian and South Asian male participants measured by 6 different devices using 5 different techniques (all subjects).**

Data expressed as mean and SD.

UWW – underwater weighing

MRI – magnetic resonance imaging

BIA – bioelectrical impedance analysis

BodPod (ADP) – air displacement plethysmography
Figure 3.0B. Total body fat content of Caucasian and South Asian male participants measured by 6 different devices using 5 different techniques (grouped by ethnicity).

Data expressed as mean and SD.

UWW – underwater weighing

MRI – magnetic resonance imaging

BIA – bioelectrical impedance analysis

BodPod (ADP) – air displacement plethysmography
Figure 3.1 Mean differences and 95% confidence intervals for 2C devices (SKF, BODYSAT (BIA), TANITA (BIA), ADP, HW and MRI) minus MRI

Mean differences and 95% confidence intervals (CI) between MRI and the five predictor estimates of percent body fat are shown. The mean MRI % body fat value was subtracted from the mean % body fat value of each 2C device to produce a mean difference for both ethnic groups, the dashed zero line indicates MRI measured % body fat. The error bars representing the 95% confidence interval show that ADP in the Caucasian group is close to being significantly different from MRI, because the error bars only just cross zero. (a = Caucasian, b = South Asian)

In the Caucasian group, all estimates of % body fat, with methods other than BIA (Bodystat and Tanita) overestimated % body fat compared to that measured by MRI. In contrast, in South Asian participants % body fat was underestimated by all 2C methods.

Correlation analysis

In the Caucasian participants, all 2-C methods (other than Bodystat), had a significant, positive linear relationship with MRI (Table 3.2). The strongest positive, linear relationship (r= 0.878, P≤0.0001), between ADP and MRI, was also found in this group.

In the South Asian participants, all 2C methods, other than Tanita (BIA), had a significant, positive linear relationship with MRI. The strongest positive, linear relationships with MRI were demonstrated
by the South Asian comparisons with tricep skinfold thickness \( r = 0.852, P = 0.004 \) and the skinfold analysis method \( r = 0.821, P = 0.007 \). In the South Asian group, skinfold thickness measurements (bicep SKF, tricep SKF and subscapular SKF) all had stronger correlations \( r = 0.807 \) with MRI % body fat compared to the Caucasian group correlation \( r = 0.647 \). However, suprailiac skinfold thickness correlation with MRI body fat was similarly low in both ethnic groups (Caucasian: \( r = 0.647, P = 0.020 \) and South Asian: \( r = 0.610, P = 0.041 \)).

Table 3.2 Correlation coefficients \( (r) \) of five 2C model methods of estimating % body fat (including four individual skinfold sites) compared to the criterion MRI measure.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficients ( (r) )</th>
<th>( \text{Caucasian (n = 11)} )</th>
<th>( \text{South Asian (n = 10)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI vs Skinfolds</td>
<td>0.721</td>
<td>0.821</td>
<td>P = 0.006</td>
</tr>
<tr>
<td>MRI vs BIA-Bodystat</td>
<td>0.508</td>
<td>0.666</td>
<td>P = 0.666</td>
</tr>
<tr>
<td>MRI vs BIA-Tanita</td>
<td>0.767</td>
<td>0.529</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>MRI vs ADP</td>
<td>0.878</td>
<td>0.796</td>
<td>P ≤ 0.0001</td>
</tr>
<tr>
<td>MRI vs UWW</td>
<td>0.808</td>
<td>0.748</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>MRI vs Bicep SKF</td>
<td>0.584</td>
<td>0.807</td>
<td>P = 0.030</td>
</tr>
<tr>
<td>MRI vs Tricep SKF</td>
<td>0.556</td>
<td>0.852</td>
<td>P = 0.038</td>
</tr>
<tr>
<td>MRI vs Subscapular SKF</td>
<td>0.626</td>
<td>0.810</td>
<td>P = 0.020</td>
</tr>
<tr>
<td>MRI vs Suprailiac SKF</td>
<td>0.647</td>
<td>0.610</td>
<td>P = 0.016</td>
</tr>
</tbody>
</table>

*\( \text{= P}<0.05, **=P<0.01.\)

Where BIA – bioelectrical impedance, ADP - air displacement plethysmography, UWW - underwater weighing and MRI - magnetic resonance imaging

There was also no significant correlation between Bodystat (BIA), tricep skinfold thickness and bicep skinfold thickness with MRI % body fat in the Caucasian group (table 3.2). Therefore, these variables were excluded from the subsequent multiple regression analysis (Table 3.3). This was also the case in the Asian Indian group for Tanita (BIA) and suprailiac skinfold thickness.
Regression analysis

Regression analyses using a stepwise multiple regression produced, one model in the Caucasian group and two models in the South Asian group (Table 3.3). In the Caucasian participants ADP % body fat could predict 77.1% of the variance in MRI % body fat. The estimated coefficient of determination for the population (adjusted r²) reveals that 74.6% of the variance in MRI % body fat is associated with changes in the variable ADP in the population tested. The final equation produced from this was MRI% BF = 0.757 + (1.76 × ADP% BF)

Table 3.3 Regression analyses to predict MRI % body fat in Caucasian and South Asians.

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept (A)</th>
<th>Slope (B)</th>
<th>r</th>
<th>r²</th>
<th>Adjusted r²</th>
<th>s.e.e</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. (ADP)</td>
<td>0.757</td>
<td>1.76</td>
<td>0.878</td>
<td>0.771</td>
<td>0.746</td>
<td>3.274</td>
<td>0.000</td>
</tr>
<tr>
<td>South Asian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. (Tricep SKF)</td>
<td>17.327</td>
<td>0.926</td>
<td>0.852</td>
<td>0.726</td>
<td>0.687</td>
<td>3.11</td>
<td>0.004</td>
</tr>
<tr>
<td>2. (Tricep SKF)</td>
<td>0.757</td>
<td>0.716</td>
<td>0.962</td>
<td>0.926</td>
<td>0.901</td>
<td>1.75</td>
<td>0.000</td>
</tr>
<tr>
<td>(UWW)</td>
<td>0.476</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where s.e.e = standard error of the estimate

In the South Asian group, two models were produced: the first including tricep skinfold thickness as a predictor. This could predict 68.7% of the variance in MRI % body fat resulting in the prediction equation: MRI% BF = 17.327 + (0.926 × tricep SKF). Interestingly the s.e.e was 3.11, similar to that produced in model 1 for the Caucasian group. The adjusted r² in the South Asian participants was greatly improved by the addition of the predictor variable UWW. In the revised equation combining tricep skinfold thickness and HW, 90.1% of the variance in MRI could be accounted for. The improved s.e.e (1.75) suggests that this model was the best predictor for MRI % body fat.
**Agreement between measurement methods**

To further assess the validity of the models produced by the multiple regression analyses, Bland-Altman\textsuperscript{329} plots were employed. Residual body composition scores of the included devices were analysed against those of MRI to determine the 95% limits of individual agreement. Agreement between MRI and ADP in Caucasian participants showed a mean difference (MRI-ADP %BF) of -3.19 ± 3.60 %, producing % body fat 10.39% below and 4.02% above that of the MRI measurement, suggesting significant agreement between the two methods.

Figure 3.2  Bland-Altman plot showing bias of agreement between Caucasian MRI and ADP %BF scores (difference between MRI and ADP against their mean). Central line is group mean difference and the outer lines represent ± 2 standard deviations.
Similarly the Bland-Altman plot (Figure 3.3) for agreement between UW and MRI shows a mean difference (MRI-UWW %BF) of $4.05 \pm 3.99\%$). Therefore, UW results in a % body fat values $3.93\%$ below and $12.04\%$ above that of MRI measured % body fat.

Figure 3.3. Bland-Altman plot showing bias of agreement between MRI and HW %BF scores (difference between MRI and UWW against their mean). Central line is group mean difference and the outer lines represent $\pm 2$ standard deviations.
Reliability of 2C devices and MRI

Repeated measurements of the different methods of measuring % body fat had a typical error less than 0.71% BF. After MRI, both BIA devices tested had the lowest coefficient of variation (COV), suggesting they were the most accurate methods, and could be used to reliably detect the small differences/changes.

Table 3.4. Mean typical error of measurement (TEM) and mean TEM as coefficient of variation for each 2C device (95% limits of agreement)

<table>
<thead>
<tr>
<th>Device</th>
<th>CoV (%) and (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIA-Tanita</td>
<td>2.8% (2.2-3.6%)</td>
</tr>
<tr>
<td>BIA-Bodystat</td>
<td>2.2% (1.8-2.9%)</td>
</tr>
<tr>
<td>ADP</td>
<td>4.0% (3.2-5.3%)</td>
</tr>
<tr>
<td>UWW</td>
<td>4.0% (3.2-5.2%)</td>
</tr>
<tr>
<td>MRI</td>
<td>1.0% (0.6-2.1%)</td>
</tr>
</tbody>
</table>
3.3.2 PART B - Assessment of abdominal adiposity measurement device – Viscan BIA.

Descriptive Statistics

The characteristics of the population used to assess the validity of the Viscan analyser to measure abdominal fat content compared to MRI measurements of visceral fat are summarised in Table 3.5. Taking the study population as a whole, body mass, and height were significantly greater in the male participants (P<0.001), whereas %BF was significantly greater in the females (P<0.001), with no other significant gender differences in the group (P≥ 0.06). Participant characteristics and summary body composition compartments (MRI and ViScan) are shown in table 3.5, by gender and BMI group (lean vs. overweight/obese).

Pearson's correlation coefficients relating to MRI derived abdominal fat compartments in the whole population are shown in table 3.6. The ViScan derived % trunk fat most strongly associated with MRI derived total abdominal fat (IAAT + ASAT) expressed as a percentage of body weight (r=0.938, P<0.001), explaining 88% of the variance in total abdominal fat. This relationship is shown in figure 3.4A. Lower correlations were shown with total abdominal adiposity and manual anthropometric measures that singularly explained between 23% and 68% of the inter-individual variance. ViScan derived % trunk fat was the strongest single correlate with ASAT (r=0.884, P<0.001), explaining 78% of the inter-individual variance (this relationship is shown in figure 3.4B). Other anthropometric measures individually explained between 16% and 72% of the variance.
Table 3.5: Measured participant characteristics and body composition compartments, split by BMI (lean group represents BMI ≤ 25 kgm$^{-2}$, the overweight/obese group represents individuals with a BMI above 25 kgm$^{-2}$).

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th></th>
<th>Overweight/obese$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males (n=13)</td>
<td>females (n=18)</td>
<td>males (n=21)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>36.7 ± 13.0</td>
<td>29.3 ± 10.4</td>
<td>42.1 ± 14.6</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>71.0 ± 7.2</td>
<td>58.8 ± 5.4$^*$</td>
<td>91.4 ± 11.8</td>
</tr>
<tr>
<td>BMI (kgm$^{-2}$)</td>
<td>22.7 ± 2.0</td>
<td>21.5 ± 1.8</td>
<td>29.2 ± 3.3</td>
</tr>
<tr>
<td>Mid waist circumference (cm)</td>
<td>81.4 ± 6.5</td>
<td>71.8 ± 5.1$^*$</td>
<td>97.0 ± 12.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.07</td>
<td>0.76 ± 0.04$^*$</td>
<td>0.92 ± 0.07</td>
</tr>
<tr>
<td>Truncal fat (mm)</td>
<td>15.3 ± 4.0</td>
<td>13.8 ± 4.9</td>
<td>21.1 ± 7.9</td>
</tr>
</tbody>
</table>

**MRI**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>total body fat (%)</td>
<td>19.8 ± 5.7</td>
<td>28.1 ± 6.4$^*$</td>
</tr>
<tr>
<td>total abdominal fat (IAAT + ASAT) (kg)</td>
<td>4.5 ± 1.8</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td>ASAT (l)</td>
<td>3.1 ± 1.3</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>IAAT (l)</td>
<td>1.9 ± 1.1</td>
<td>0.9 ± 0.4$^*$</td>
</tr>
<tr>
<td>IAAT:ASAT ratio</td>
<td>0.65 ± 0.35</td>
<td>0.23 ± 0.07$^*$</td>
</tr>
<tr>
<td>IHCL (%)</td>
<td>0.8 ± 1.0</td>
<td>0.4 ± 0.7</td>
</tr>
</tbody>
</table>

**ViScan (BIA)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ViScan % trunk fat (%)</td>
<td>20.2 ± 5.2</td>
</tr>
<tr>
<td>ViScan visceral fat (no units)</td>
<td>6.8 ± 1.8</td>
</tr>
</tbody>
</table>

WHR= waist hip ratio. Truncal skinfold measurements calculated as mean of repeated skinfold measures at 4 sites, namely subscapular, supraspinalae, iliac crest and abdominal; IAAT, intra-abdominal adipose tissue; ASAT, abdominal subcutaneous adipose tissue; IHCL, intra-hepatocyte lipid.

$^*$ Significant gender difference within the same BMI group (P<0.05)  
$^\dagger$ All variables significantly different from lean group of the same gender (P<0.05)
Table 3.6: Pearson’s correlation coefficients for associations with MRI abdominal fat compartments.

<table>
<thead>
<tr>
<th></th>
<th>IAAT</th>
<th>ASAT</th>
<th>IHCL</th>
<th>Total abdominal fat (kg)</th>
<th>Total abdominal fat (% of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANTHROPOMETRY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist (mid)</td>
<td>0.844</td>
<td>0.690</td>
<td>0.630</td>
<td>0.821</td>
<td>0.651</td>
</tr>
<tr>
<td>Waist (umbilicus)</td>
<td>0.805</td>
<td>0.765</td>
<td>0.582</td>
<td>0.861</td>
<td>0.697</td>
</tr>
<tr>
<td>Waist (supine umbilicus)</td>
<td>0.796</td>
<td>0.773</td>
<td>0.597</td>
<td>0.860</td>
<td>0.698</td>
</tr>
<tr>
<td>Hip</td>
<td>0.613</td>
<td>0.846</td>
<td>0.461</td>
<td>0.844</td>
<td>0.670</td>
</tr>
<tr>
<td>WHR</td>
<td>0.783</td>
<td>0.400</td>
<td>0.601</td>
<td>0.583</td>
<td>0.480</td>
</tr>
<tr>
<td>WSR</td>
<td>0.82</td>
<td>0.798</td>
<td>0.606</td>
<td>0.889</td>
<td>0.825</td>
</tr>
<tr>
<td>BMI</td>
<td>0.702</td>
<td>0.843</td>
<td>0.520</td>
<td>0.875</td>
<td>0.733</td>
</tr>
<tr>
<td>Trunkal skinfold thickness</td>
<td>0.592</td>
<td>0.773</td>
<td>0.473</td>
<td>0.799</td>
<td>0.762</td>
</tr>
<tr>
<td><strong>VISCAN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ViScan % Trunk fat</td>
<td>0.688</td>
<td>0.884</td>
<td>0.447</td>
<td>0.899</td>
<td>0.938</td>
</tr>
<tr>
<td>Viscan Visceral fat</td>
<td>0.731</td>
<td>0.622</td>
<td>0.567</td>
<td>0.725</td>
<td>0.742</td>
</tr>
</tbody>
</table>

Abbreviations: Waist (mid), midpoint between lower rib and greater trochanter; Waist (umbilicus), waist circumference at the level of the umbilicus; Waist (umbilicus supine), waist measured at the level of the umbilicus with the participant laying supine; WHR, waist-hip ratio; WSR, waist-stature ratio; truncal skinfold, mean of repeated skinfold measures at 4 sites, namely subscapular, supraspinalae, iliac crest and abdominal. IAAT, intra-abdominal adipose tissue; ASAT, abdominal subcutaneous adipose tissue; IHCL, intra-hepatocyte lipid. All variables correlated at a significance of P<0.001 unless otherwise stated.
Figure 3.4A: Relationship between MRI derived total abdominal adipose tissue (IAAT + ASAT) expressed as a % of body weight, and % trunk fat as measured by the ViScan™ Measurement system

As well as attempting to quantify abdominal fat compartments, the ViScan also categorises individuals into bandings of adiposity both for % trunk fat ("low", "average" or "high") and for visceral fat ("average", "high" or "very high"). How these categories relate to MRI measured adipose tissue compartments are represented as boxplots in figure 3.5 for both total abdominal fat (figure 3.5A) and IAAT (figure 3.5B). Following one way ANOVAs there was a significant difference in MRI derived total abdominal fat between the three ViScan categories of % trunk fat (P<0.001), with the "low" group significantly less than the "average" group (P<0.001) and the "average" group significantly less than the "high" group (P<0.001). There was also a significant difference between ViScan visceral fat categories in terms of MRI derived IAAT (P<0.001). However, following post-hoc tests IAAT was only significantly different between the "average" and
the “high” groups (P<0.001) with no differences seen between the “high” and the “very high” groups.

**Figure 3.5:** Resultant boxplots to show the ViScan derived banding of individuals in terms of abdominal fat compartments and MRI derived abdominal adipose tissue (n=74)

**Figure 3.5A:** ViScan categorised percentage trunk fat

![ViScan trunk fat rating boxplot](image)

**Figure 3.5B:** Viscan categorised visceral fat.

![Viscan visceral fat rating boxplot](image)
A further factor that was investigated in reference to the comparison between MRI and ViScan measures of visceral fat was the presence of fat in the liver. Participants were divided into two groups according to their liver fat content; High liver fat >2% (n=30) and Low liver fat <2% (n=44). The correlation between MRI and ViScan measures of visceral fat was stronger in the participants with low liver fat content (r=0.83, p<0.001) compared to those with high liver fat content (r=0.69, p<0.001).
3.4. Discussion

Part A - Assessment of 2-Component (2C) measurement systems

There have been very few studies comparing how conventional 2-C methods for measuring body composition compare with reference methodology (such as MRI) in different ethnic groups. The 2C devices tested in this part of the study (Part A) were less sensitive in detecting difference between groups, generally overestimating Caucasian adiposity and underestimating South Asian adiposity (depicted in Figure 3). BodPod (ADP) was closest to MRI values in South Asians. BIA was better at measuring % body fat in Caucasians while all other devices overestimated. Both BIA devices tested would be unsuitable for estimating South Asian adiposity, owing to the significant difference between their values and MRI. The remaining 2C devices could be valid tools.

It is acknowledged that the rather modest sample size in this part of the study makes generalization of the prediction equations somewhat limited. A larger sample population that considers obesity level and type would be required to confirm the prediction accuracy and enable generalisation to the wider community. However, there is no escaping the fact that even in this healthy, non-obese group there was considerable disagreement between the methods tested.

When all participant data are considered (Figure 3.0A) the disagreement is however not as great as when the group are split by ethnicity (Figure 3.0B). The apparent polarisation of the results by ethnicity clearly demonstrates the limitations of assuming such different devices can be used for interchangeably for all groups.

Possible reasons for the lack of agreement in methods point to the principles of 2-C models. The main criticism of the 2-C methods in body composition is that they are *doubly indirect* methods (discussed in Chapter 1) and are therefore based on assumptions that may not be entirely correct. The main assumption is that the fat-free mass (FFM) component is estimated to be 1.100 g/mL\(^2\) as there is no direct measurement available. Unsurprisingly, there is known variability in this fat-free density measure\(^2\) as verified by bone mineral density (BMD) and cadaver studies\(^2\). Additionally, this measure was also found to vary with respect to ethnicity\(^2\). Within commercial measurement methods, like some of those tested in this study, the algorithms used to quantify % body fat were derived from a small number of Caucasians, and as such the equations employed are specific to the Caucasian population\(^\)\(^2\). There is very little comparable
information on the density of fat and fat free tissues in the different ethnic populations, so it is unknown whether body composition values obtained via the 2C model are truly accurate for these groups.

While it is well known that impedance based measures are influenced by age, gender and body water distribution\textsuperscript{219}, body build is also believed to be a confounding factor. It has been shown in various studies that the contribution of the limbs to total body impedance is disproportional to the amount of water in these segments\textsuperscript{330,331}. Population groups are known to differ in build. Malays and Chinese have relative short arms and short legs compared to their body height\textsuperscript{332} and Indians and Australian Aboriginals have relatively long legs\textsuperscript{333}. Some studies report the impact of relative leg length on the relationship between BMI and \%BF\textsuperscript{334-337}. The same holds for slenderness as more slender individuals are reported to have less bone mass, less connective tissue and less muscle mass for the same height, resulting in higher \%BF at the same BMI as a more stocky build person\textsuperscript{336,337}. Also within ‘homogeneous’ ethnic populations, the bias in predictive body composition has been reported to depend on body build factors\textsuperscript{336}.

The errors seen in predicting body composition in this study may be linked to variability in body build between ethnic groups\textsuperscript{338}, as variation in the relative distribution of weight and impedance amongst the limbs and trunk may confound body impedance algorithms that basically treat the body as a single cylinder. The interrelated variation in body fat distribution also becomes a factor as this impacts on body geometry and is seen to vary with different ethnic groups and populations.

The use of 2-C methods to accurately measure adiposity is problematic owing to the fact that such devices make indirect measurements of combined adipose depots that are not constant and vary differently between individuals and ethnic groups. Accepting the fact that 2C methods can measure adiposity reasonably satisfactorily, the problem arises when they cannot account for ethnic / racial differences. There have already been specific correction factors developed for black participants based on their higher fat-free mass\textsuperscript{244} but none yet exist for South Asians. The poor reliability of field devices to quantify adiposity points to the development of better ethnic specific corrections or algorithms. The fact that such affordable devices cannot yet distinguish between various internal adipose depots is a problem that will not be easily solved. Such methods are
more sophisticated than purely anthropometric surrogates of adiposity (BMI, circumference measures and their respective ratios) but are not significantly improving the quantification of adiposity for accurate health risk reduction. A prime example being the larger areas of visceral adiposity seen in Japanese men compared to Caucasian men with the same waist circumference\textsuperscript{205}.

**Part B - Assessment of Abdominal adiposity measurement device**

In this part of the study (Part B) comparisons between the ViScan and MRI were undertaken across a range of adiposity (i.e. obese and non-obese) to fully examine the influence of adiposity on ViScan measurement. In this study ViScan derived percentage trunk fat strongly and significantly related to TA\textsubscript{AT} and AS\textsubscript{AT} in both lean and overweight/obese individuals. Furthermore, ViScan derived “visceral” fat correlated significantly with IA\textsubscript{AT} but the strength of this relationship was much weaker in overweight/obese individuals. Similarly, the ViScan significantly overestimated waist circumference compared to manual measurement.

In this cohort of adults ranging in body size, the ViScan appears to systematically overestimated waist circumference in comparison to manual measurement (both standing and supine) by 4-6cm. This is far in excess of the within-participant variation of manual waist measurement, shown to be in the order of 5-9mm\textsuperscript{339}. This difference in waist circumference measurement between methods is likely explained by the fact that the ViScan is in reality a predicted measure using a patented “waist circumference calculator” technology. This method calculates a waist circumference of the human participant on the basis of the abdomen width, itself determined by the distance of each side of the abdomen from the two infrared sensors either side of the base unit. Waist girth is more than likely then calculated based on a correlation between abdomen width and waist circumference stored in the memory of the machine. It is unclear as to the potential impact this demonstrated error in waist prediction will have on the BIA derived body composition of the trunk by the ViScan. Previous models of assessing whole body fat from BIA have used anthropometric data such as height and body mass to improve the predictive accuracy and reproducibility\textsuperscript{340} and one could speculate that
waist circumference is incorporated into the ViScan prediction equations to potentially give an estimate of the cross sectional area of the trunk (e.g. crudely as waist circumference squared).

The ViScan method is a direct measurement of the transimpedance at the abdominal region, and therefore should better reflect the immediate local tissue compartments, particularly fat. In terms of total abdominal fat, % trunk fat as derived by the ViScan did show an excellent relationship to MRI total abdominal fat. A strong relationship between ViScan measured % trunk fat, and DXA derived FM of the trunk has been reported previously by authors associated with the manufacturers. My study represents the first time this association has been independently confirmed against the “gold standard” for adipose tissue measurement, MRI. However, it is difficult to directly compare methods like for like for two reasons; the ViScan does not quantify adipose tissue in terms of absolute mass or volume, while MRI analysis cannot express abdominal fat as a % of trunk weight because the non-adipose weight of the trunk is not measured by this technique.

The use of transimpedance may have advantages over traditional segmental multifrequency BIA (eg Tanita device used in Study A comparing BIA devices) which has been used by various authors to estimate regional fat mass, almost exclusively utilising predictive equations based on DXA. This traditional use of BIA is dependent on modelling the body segments (e.g. the trunk) as distinct cylinders, and utilising the relationship between the resistance of these cylinders and the FFM of the segment. Fat mass of the segment is then derived by subtraction of FFM from total mass, assuming a constant hydration of FFM. Moreover, FFM (and therefore FM) in the trunk are often estimated, by difference, from the FFM and FM of the total body and extremities. Additionally, the measurement of trunk composition is heavily influenced by electrode placement, which has differed between authors.

If it is assumed, via a prediction model, that the transimpedance is only influenced by the IAAT and ASAT compartments in the abdominal region then this is assuming the other non-adipose structures are constant. This may not take into account differences in other factors such as musculature, tissue hydration, or intestinal contents (gaseous or solid). It is also unclear how big an influence gastrointestinal (GI) or bladder contents have on measurements as in my study all participants were
measured following an overnight fast and having voided their bladder. This is a standard procedure with BIA devices, as additional fluid volume will potentially influence conductance and the subsequent measurement.

In this study a significant relationship was also found between the ViScan measures and IHCL (Table 3.8), suggesting a possible influence of organ mass/composition on transimpedance. Moreover, measurement of visceral fat using the ViScan may relate to increased liver volume. I found that participants with elevated liver fat content had a lower correlation between the MRI and ViScan measures of visceral fat. Since increasing liver fat content is closely associated with increasing liver volume\textsuperscript{176}, it may be inferred that the greater contribution of liver to the abdominal contents also affects the ability of ViScan to accurately measure visceral fat. I am not suggesting, based on this correlation that the ViScan can be used to detect/measure liver fat in any way. Moreover, liver fat helps support the notion that liver size may be influencing the prediction of abdominal adipose tissue compartments when using transimpedance. Further studies using MRI or CT need to be undertaken to investigate the influence structural differences, tissue hydration and musculature have on transimpedance measurements using the ViScan or similar devices.

Assuming constancy of non-adipose components, the ratio of ASAT:IAAT may itself influence the measurement as increased ASAT would lead to “deeper” IAAT and hence could influence its contribution to the transimpedance. In this study ASAT:IAAT ratio was significantly higher in women than men (P<0.001), as has demonstrated by other authors\textsuperscript{57,345,346}. This gender influence on abdominal adipose tissue compartments may need to be taken into account when interpreting transimpedance. Although the operator must select the participant’s gender as part of the ViScan measurement, it is unclear how this is factored into the interpretation of the subsequent transimpedance.

Taking this argument further, it is likely the absolute amount of ASAT (litres) is exhibiting an influence on the contribution that the deeper IAAT may have on the transimpedance; hence influencing the ability of the ViScan to predict visceral adiposity. In this study the cohort was separated according to obesity status and by ASAT and I observed weaker relationships in the obese participants and those with higher ASAT. This finding is particularly relevant when
assessing abdominally obese individuals, particularly female abdominally obese using the ViScan. In very obese individuals with large abdominal adiposity it may be increasingly difficult to distinguish IAAT and ASAT as these compartments may be “bridged” with each other anatomically, a further explanation for increasing difficulty of interpreting BIA in the very obese.

Another possible influence on the ViScan’s ability to predict adipose tissue compartments may be in the relative placement of the electrodes. Nagal et al. (2008)\textsuperscript{347}, in a similar model to the ViScan, noted that the electrode placement would determine how much ASAT influences the impedance, and that to determine deeper structures there should be a greater distance between injecting and sensing electrodes. In the ViScan system, the distance between electrodes is fixed, being housed within a measurement “belt” placed at the level of the umbilicus. Nevertheless, the relative positions of these electrodes, compared to anatomical landmarks may be slightly different in the abdominally obese compared to leaner individuals, i.e. a leaner individual (with a smaller waist circumference) will have electrodes spanning proportionally more of the abdomen that an abdominally obese individual (with a larger waist circumference). Linked to this is the fact that there are reported gender differences observed in abdominal fat distribution that may influence IAAT and ASAT measures. In men there is believed to be a steady increase in IAAT from 5cm below L4-L5 to 10cm above L4-L5, whereas in women, total IAAT peaks below or at L4-L5\textsuperscript{346,348}.

Taking all these factors into account, the ViScan may be a better predictor of IAAT in leaner individuals than in the abdominally obese. In the current cohort, this would explain the greater inconsistency in the categorising of individuals in terms of IAAT compared to categorising individuals in terms of total trunk fat. This potential influence of relative adiposity on prediction using the ViScan is one factor that has not been highlighted in the original Japanese development study\textsuperscript{349} as these measurements were conducted mainly on lean individuals. As discussed here, reasons for differences could be firstly that leaner individuals would tend to have lower ASAT, and hence any IAAT is anatomically nearer to the surface electrodes; and secondly a lower waist girth could mean the placement of the electrodes is more evenly spread across the whole abdomen.
In conclusion, the ViScan measurement system may be a simple and useful tool for the estimation of total abdominal adiposity. However, the use of this system to distinguish visceral fat (i.e., IAAT) remains limited. More studies are needed to investigate the reliability of this measurement, and more importantly the ability of the ViScan to detect changes in abdominal fat compartments. Nevertheless, the ViScan system may still prove to be a useful motivational device for the health practitioner, despite limitations as a diagnostic or monitoring tool.

**Conclusion**

**Part A.** Findings from this part of the study have demonstrated that, when compared to the criterion (MRI), the 2-C body composition measuring devices tested were less accurate. Groups looking for a more available and cheaper alternative to MRI for percentage adiposity quantification, would need to give additional consideration to the ethnicity of their test population. In this study (Part A), Caucasian adiposity was overestimated (up to 3%) and Asian adiposity was underestimated (up to 11%) by the 2-C devices. Based on a sample size (N=21), the hypothesis for this current study: that 2-C methods (UWW, ADP, BIA, skinfolds) are a valid alternative to MRI for adiposity quantification is rejected.

**Part B.** Findings from this part of the study have demonstrated that a BIA device (ViScan) developed for measuring abdominal adiposity was not able to conclusively measure intra-abdominal adipose tissue (IAAT) in obese males and females. The hypothesis for this current study: that the Viscan BIA device is an accurate intra-abdominal adiposity proxy measurement method is rejected.
Chapter 4. The “Thin Outside Fat Inside” (TOFI) phenotype.

4.1 Introduction

Body mass index (BMI) is the current benchmark for obesity classification, but like all anthropometric measurements, it only offers a proxy measure of body adiposity. Furthermore, at any given body size, there is significant variation in adipose tissue content and distribution which cannot be predicted by standard anthropomorphic characteristics; such as skin-fold measurements, body mass index (BMI), or waist-to-hip ratio (WHR). Measuring body fat content and distribution directly is essential as the contribution of adipose tissue to disease is differentially related to disease. As mentioned in chapter 1, deposition of adipose tissue internally within the abdomen, or in lean tissues such as the liver (hepatic fat, IHCL) and muscle (intramyocellular lipids, IMCL) is associated with greater risk of metabolic disease such as, type 2 diabetes, obesity and insulin resistance, more than elevated stores of subcutaneous adipose tissue.

In research, surrogate measurements of adipose tissue content are common, particularly of ‘central fat or abdominal fat’, which is often taken to be a proxy of visceral fat content. Waist circumference (WC) is widely used and often quoted in papers as a measure of abdominal fat. While easily obtainable, it is unable to distinguish between IAAT and abdominal subcutaneous adipose tissue (ASAT) deposition. Similarly, many publications report abdominal fat content using DXA, which while providing more information than waist circumference, still cannot distinguish between different abdominal adipose tissue depots. Given their different contributions to disease there is an essential need for any measurement of abdominal fat content.

Recently it has become apparent that there are populations in which the risk of metabolic disease cannot be adequately explained from measures of adiposity alone. Indeed there have been several reports of lean individuals (BMI < 25 kg/m²) who demonstrate reduced insulin sensitivity, increased abdominal adiposity, a more atherogenic lipid profile, and raised blood pressure, similar
to that usually observed in overweight or obese individuals. These changes are usually accompanied by reduced physical activity and a low VO\textsubscript{2}max. The combination of these factors is thought to predispose these individuals to an increased risk of type 2 diabetes and CVD\textsuperscript{354-356}. These individuals have been referred to as being ‘metabolically obese but normal-weight’ (MONW)\textsuperscript{177,187}. The etiology of this phenotype is not fully understood, but may in part be related to adipose tissue distribution and ectopic fat content. Further understanding of this phenotype is important since it poses a potentially serious hidden risk.

This chapter describes the relationship between anthropometric measurements, individual adipose stores and ectopic fat in a heterogeneous group of male and female Caucasian volunteers. MRI-based techniques were used to determine the “normal” range of abdominal adiposity stores (IAAT and ASAT) in a defined healthy active subset of the cohort. In doing so the study aimed to use the ratio of intra-abdominal (IAAT) and subcutaneous (ASAT) adipose tissue to generate a potential clinical index of abdominal obesity, which could be used in future to identify individuals at increased metabolic risk.

Objectives:

1. Quantify adiposity stores in a large cohort of healthy Caucasian volunteers using MRI and MRS.
2. From the large cohort, identify a healthy, active, control sub-group in order to establish a normal range of abdominal adipose tissue (IAAT/ASAT ratio).
3. Identify MONW individuals within the main cohort’s lean (BMI 18.4 – 25 kg/m\textsuperscript{2}) category based on the clinical index developed and assess the relationship between their adiposity and established proxy (anthropometric) measures.

Study 2 Hypotheses:

Hypothesis 1: The established TOFI index (identify) can identify individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m\textsuperscript{2}).

Hypothesis 2: Established proxy (anthropometric) measures are able to successfully identify MONW individuals within a large cohort of healthy Caucasians.
4.2. Methods

Participants
Written, informed consent was acquired from all volunteers. Ethical approval permission for this study was obtained from the Research Ethics Committee of Hammersmith and Queen Charlotte’s and Chelsea Research Ethics Committee Hospital, London (Rec: 07Q04011/19). In total, 477 Caucasian volunteers (243 male, 234 female) were recruited. All participants were aged 17-71 years. Self-reported exclusion criteria included participants suffering from chronic disease including diabetes, cardiovascular or liver disease, metabolic conditions, anyone taking prescribed medication and women on the contraceptive pill. Volunteers underwent anthropometric assessment, total body MRI scanning and in vivo proton (¹H) magnetic resonance spectroscopy (MRS) of liver and calf muscle.

Anthropometric measurements
Body mass (kg), height (cm), midpoint waist circumference (WC) (cm) and hip circumference (cm) were measured in each participant by myself, as detailed in the General Research Methods and Procedures section (Chapter 2). From these values, BMI (kg/m²), waist-to-hip ratio (WHR, waist/hip) and waist-to-height ratio (WHtR, waist/height) were calculated. As previously mentioned, BMI grouping corresponded to the following ranges; 1: 18.5<25 kg/m², 2: 25<30 kg/m², 3: 30<40 kg/m², 4: 40+ kg/m².

MRI scanning: Total body and regional adipose tissue content
On a single visit, participants underwent total body MRI scanning and in vivo proton (¹H) magnetic resonance spectroscopy (MRS) of their liver and their calf muscle as described in the General Research Methods and Procedures chapter (Chapter 2).

The adiposity data obtained was expressed as previously described (Chapter 2 - section 2.5.1.3).

MRS of liver and muscle fat
During the same scanning session, ¹H MR spectra were also acquired at 1.5T, and analysed using methods described earlier (Chapter 2).
Control Group – “normal” range of abdominal adiposity.

A healthy, active control group was identified within the main cohort in order to classify the ‘normal’ fat distribution. The following criteria were used to define Caucasian individuals as healthy, active, controls:

1) Absence of disease/metabolic condition;
2) BMI: 18.5 < 25 kg/m$^2$ (WHO guidelines$^1$);
3) WC: male ≤ 94 cm, female ≤ 80 cm (WHO guidelines$^1$);
4) WHR: male ≤ 0.90, female ≤ 0.80$^{357}$;
5) Age: male (18-50 yr), female (18-39 yr): the younger age group in female participants was chosen in order to eliminate the effects of menopause from our control group, as there is a significant increase in obesity related-metabolic disorders after menopause, which has been linked to alterations in body adiposity, notably an increase in IAAT$^{358}$.
6) Activity level: sedentary participants were excluded following physical activity classification, determined using Baecke$^{359}$ and/or I-PAQ$^{360}$ questionnaires (Appendix 2). Both tests calculate weekly physical activity, classifying individuals into low, moderate or high categories based on specific criteria; I-PAQ assesses the duration and frequency of walking, moderate intensity and vigorous intensity activity. Participants with a high or moderate score in the “healthy” definition were included, as this level of activity was considered sufficient to maintain cardiovascular health while individuals reported in the “low” physical activity group from either test were excluded.

The advantage to using such validated activity scoring methods is that each has a robust checklist for assessing categories of activities and therefore cover the spectrum of intensities that make up discreet aspects of everyday living. The two questionnaires also encompass a “scripted” protocol for verbal instructions to ensure reliability, as well as to ensure interviewers assess the activity pattern accurately. The I-PAQ has additional benefits in that it has been translated into several languages and has telephone ‘scripts’ too for non face-to-face interviews too. Choosing the appropriate activity assessment method was critical so that the reliability was high and the scoring was relatively straightforward in terms of ease of calculating and subsequent logging. The physical activity assessments I used also require the researcher to complete them using a
structured interview technique (hence the need for a standard questioning script and protocol) rather than the participant completing it themselves.

**Statistical Analysis**

Gender differences were analysed using the Student’s t-test. Significance was taken as p<0.05. All data are presented as mean ± standard deviation. Data which were found not to be normally distributed (IHCL and IMCL) are presented as a geometric mean, while statistical analysis was performed on log_{10} transformed variables. Associations between variables were investigated using Pearson partial correlation r values. The statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). Statistical advice was provided during this study by Caroline Dore (MRC Clinical Trials Unit, London, UK).
4.3. Results

Descriptive Statistics

The mean age of all participants was 37yrs (range 18 – 71yrs) with 25.0% of all participants classified as overweight (32.6% of men, 17% women, BMI: 25<30), 24.7% qualifying as obese (26.8% of men, 22.6% of women, BMI: 30<40) and 2.9% morbidly obese (2.1% of men, 3.8% of women, BMI: 40+). Gender specific characteristics are shown in Table 4.0. Males had a significantly greater weight, WC, height and WHR, than females (P < 0.001 for all). Overall, female participants were found to have significantly more TAT, SAT, ASAT, NASAT, and significantly lower levels of internal, IAAT and a lower IAAT/ASAT ratio than males participants (P < 0.001 for all). Ectopic fat content in both the liver and soleus muscle were also found at significantly lower levels in female compared to male participants (p<0.001). There were no significant differences in the tibialis muscle.
### Table 4.0 Gender specific variable data.

<table>
<thead>
<tr>
<th></th>
<th>MALE (n=243)</th>
<th>FEMALE (n=234)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>40.3 ± 13</td>
<td>17 - 70</td>
<td>34.5 ± 12.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.6 ± 16.2</td>
<td>59.0 - 146.6</td>
<td>71.4 ± 17.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 4.8</td>
<td>18.6 - 44.5</td>
<td>26.2 ± 6.6</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>95.4 ± 13.3</td>
<td>70.0 - 131</td>
<td>81.4 ± 13.8</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>103.5 ± 8.4</td>
<td>85.4 - 136</td>
<td>102.3 ± 10.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179 ± 7.3</td>
<td>143 - 199</td>
<td>165.2 ± 6.6</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.07</td>
<td>0.75 - 1.11</td>
<td>0.8 ± 0.07</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.53 ± 0.08</td>
<td>0.37 - 0.85</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>IHCL*</td>
<td>6.8 ± 14.0</td>
<td>0 - 89.6</td>
<td>2.8 ± 8.5</td>
</tr>
<tr>
<td>S-IMCL*</td>
<td>15.5 ± 9.7</td>
<td>2.9 - 100.1</td>
<td>11.5 ± 6.9</td>
</tr>
<tr>
<td>T-IMCL*</td>
<td>6.3 ± 3.8</td>
<td>0.25 - 30.5</td>
<td>6.7 ± 4.1</td>
</tr>
<tr>
<td>TAT (l)</td>
<td>24.9 ± 10.9</td>
<td>6 - 67.7</td>
<td>31.2 ± 15.9</td>
</tr>
<tr>
<td>SAT (l)</td>
<td>18.6 ± 8.4</td>
<td>4.2 - 58.2</td>
<td>26.6 ± 13.5</td>
</tr>
<tr>
<td>ASAT (l)</td>
<td>5.3 ± 3.0</td>
<td>0.7 - 20.2</td>
<td>7.6 ± 4.9</td>
</tr>
<tr>
<td>NASAT (l)</td>
<td>13.3 ± 5.6</td>
<td>1.3 - 38</td>
<td>19.0 ± 8.8</td>
</tr>
<tr>
<td>Internal (l)</td>
<td>6.3 ± 3.3</td>
<td>0.7 - 15.8</td>
<td>4.6 ± 2.8</td>
</tr>
<tr>
<td>IAAT (l)</td>
<td>3.5 ± 2.1</td>
<td>0.2 - 9.4</td>
<td>2.3 ± 1.8</td>
</tr>
<tr>
<td>NAIAT (l)</td>
<td>2.8 ± 1.4</td>
<td>0.5 - 7.9</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Trunk (l)</td>
<td>8.8 ± 4.7</td>
<td>1.0 - 25.5</td>
<td>9.9 ± 6.4</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.7 ± 0.3</td>
<td>0.18 - 1.64</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Mean and range variable data. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intrahepatocellular lipid; IMCL: intramyocellular lipid (S - soleus, T - tibialis); Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. * MRS data (IHCL (M: 234, F: 169), S-IMCL (M: 239, F: 179) and T-IMCL (M: 239, F: 178)) is presented as the geometric mean, while statistical analysis was performed on log_{10} transformed variables. All data are presented as mean ± SD. Male versus female data were analysed by Student’s t-test.
### Table 4.1 BMI group specific summary measures in male and female volunteers

<table>
<thead>
<tr>
<th>BMI group</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n) number</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Age (y)</td>
<td>34.4 ± 12</td>
<td>42.5 ± 12.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 ± 6.8</td>
<td>87.3 ± 9.0</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.8 ± 6.4</td>
<td>96.0 ± 7.6</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>96.4 ± 5.1</td>
<td>103.8 ± 4.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.0 ± 6.3</td>
<td>178.4 ± 7.4</td>
</tr>
<tr>
<td>WHR</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.5 ± 0.01</td>
<td>0.5 ± 0.01</td>
</tr>
<tr>
<td>IHCL*</td>
<td>0.9 ± 2.1</td>
<td>6.8 ± 14.4</td>
</tr>
<tr>
<td>S-IMCL*</td>
<td>13.5 ± 12.0</td>
<td>15.2 ± 7.0</td>
</tr>
<tr>
<td>T-IMCL*</td>
<td>4.9 ± 2.9</td>
<td>6.3 ± 3.4</td>
</tr>
<tr>
<td>TAT (L)</td>
<td>16.1 ± 6.0</td>
<td>25.0 ± 7.1</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>12.2 ± 4.5</td>
<td>18.3 ± 4.9</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>3.0 ± 1.2</td>
<td>5.1 ± 1.8</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>9.2 ± 3.7</td>
<td>13.2 ± 3.4</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>3.9 ± 2.0</td>
<td>6.7 ± 3.1</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>2.0 ± 1.1</td>
<td>3.8 ± 1.9</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>2.0 ± 0.9</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>Trunk (L)</td>
<td>4.9 ± 2.2</td>
<td>8.9 ± 3.1</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.6 ± 0.3</td>
<td>0.8 ± 0.4</td>
</tr>
</tbody>
</table>

WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intrahepatocellular lipid; IMCL: intramyocellular lipid (S - soleus, T - tibialis); Adipose tissue deposits are presented as volume in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: visceral; NAIAT: non-abdominal internal. * MRS data: (IHCL (M: 234, F: 169), S-IMCL (M: 239, F: 179) and T-IMCL (M: 239, F: 178)) is presented as the geometric mean. BMI grouping corresponds to the following BMI ranges; 1: 18.5<25, 2: 25<30, 3: 30<40, 4: 40+. All data is presented as mean ± SD.
Figure 4.0 Abdominal subcutaneous (ASAT), intra-abdominal adipose tissue (IAAT) and IAAT/ASAT ratio by BMI group in male and female volunteers.

BMI group specific variation in abdominal subcutaneous (ASAT) (A) and intra-abdominal adipose tissue (IAAT) (B) in male and female volunteers. Adiposity stores are presented in litres. The ratio of IAAT/ASAT is also presented by BMI groups (C). The graphs present the fitted mean curve, and the 2.5th and 97.5th centiles, calculated assuming normal errors.
Correlation Analysis

Gender-specific correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) are shown in Table 4.2 (men) and Table 4.3 (women). Apart from a few exceptions, all variables correlated with each other to a significant degree (p<0.01). In male participants, WC was the variable, which correlated to the greatest degree with individual adiposity stores (TAT: $r = 0.92$, SAT: $r = 0.88$, ASAT: $r = 0.85$, NASAT: $r = 0.87$, Internal: $r = 0.80$, IAAT: $r = 0.82$, NAIAT: $r = 0.71$, Trunk: $r = 0.92$, p<0.01, Table 4.2), while in female participants BMI had the strongest correlation with individual adiposity stores (TAT: $r = 0.95$, SAT: $r = 0.94$, ASAT: $r = 0.94$, NASAT: $r = 0.93$, Internal: $r = 0.85$, IAAT: $r = 0.84$, NAIAT: $r = 0.78$, Trunk: $r = 0.95$, p<0.01, Table 4.3).

Intrahepatocellular lipid (IHCL) correlated most strongly with WC in male participants ($r = 0.71$, p<0.01, Table 4.2) and WHtR in female participants ($r = 0.64$, p<0.01, Table 4.3). In male participants, WC was also the strongest correlate of S-IMCL ($r = 0.50$, p<0.01) and T-IMCL ($r = 0.39$, p<0.01) (Table 4.2), while in females S-IMCL and T-IMCL correlated most strongly with BMI (S-IMCL: $r = 0.45$, T-IMCL: $r = 0.27$, p<0.01 for both, Table 4.3). Intra-abdominal adipose tissue was the depot, which correlated most strongly with ectopic fat in both genders (Male IAAT: IHCL $r = 0.72$, S-IMCL $r = 0.47$, T-IMCL $r = 0.42$, p<0.01 for all, Table 4.2; Female IAAT: IHCL $r = 0.72$, S-IMCL $r = 0.51$, T-IMCL $r = 0.32$, p<0.01 for all, Table 4.3). In both male and female participants, weight, BMI, WC and WHtR correlated more strongly with each other than individual percentage adiposity stores (Tables 4.2 and 4.3).
Table 4.2 Linear correlation analysis between anthropometric measurements, lipid stores and body fat stores in male participants.

<table>
<thead>
<tr>
<th>MALE</th>
<th>AGE</th>
<th>ANTHROPOMETRIC VARIABLE</th>
<th>ECTOPIC FAT STORE</th>
<th>ADIPOSITY DEPOT (LITRES)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IHCL</td>
<td>T-IMCL</td>
</tr>
<tr>
<td>n = 243</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.288**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.378**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.478**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>0.311**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-0.124</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.510**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHIR</td>
<td>0.493**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHCL</td>
<td>0.405**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-IMCL</td>
<td>0.389**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-IMCL</td>
<td>0.314**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>0.376**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>0.271**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASAT</td>
<td>0.231**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NASAT</td>
<td>0.280**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal</td>
<td>0.550**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAAT</td>
<td>0.548**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAIAT</td>
<td>0.501**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>0.396**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.460**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are presented as Pearson correlation r values. Bold typeface indicates a significant correlation; shaded boxes indicate the anthropometric variable with the strongest correlation. ^ Statistical analysis of IHCL (M: 234), S-IMCL (M: 239) and T-IMCL (M: 239) performed on log10 transformed variables; * = p<0.05, ** = p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intrahepatic cellular lipid and IMCL: intramyocellular lipid (S - soleus, T - tibialis); Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
Table 4.3 Linear correlation analysis between anthropometric measurements, lipid stores and body fat stores in female participants.

<table>
<thead>
<tr>
<th>FEMALE</th>
<th>AGE</th>
<th>ANTHROPOMETRIC VARIABLE</th>
<th>ECTOPIC FAT STORE</th>
<th>ADIPOCYTE DEPOT (LITRES)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 234</td>
<td>Age</td>
<td>Weight</td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.276**</td>
<td>0.357**</td>
<td>0.945**</td>
<td>0.862**</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>0.826**</td>
<td>0.892**</td>
<td>0.350**</td>
</tr>
<tr>
<td>WC</td>
<td>0.477**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.456**</td>
<td>-0.261</td>
<td>0.114</td>
<td>-0.208*</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td>0.462**</td>
<td>0.734**</td>
<td>0.832**</td>
</tr>
<tr>
<td>WHtR</td>
<td></td>
<td>0.477**</td>
<td>0.501**</td>
<td>0.622**</td>
</tr>
<tr>
<td>IHCL</td>
<td>0.501**</td>
<td>0.397**</td>
<td>0.450**</td>
<td>0.436**</td>
</tr>
<tr>
<td>S-IMCL</td>
<td>0.371**</td>
<td>0.196*</td>
<td>0.265**</td>
<td>0.278**</td>
</tr>
<tr>
<td>T-IMCL</td>
<td></td>
<td>0.461**</td>
<td>0.946**</td>
<td>0.927**</td>
</tr>
<tr>
<td>TAT</td>
<td>0.292**</td>
<td>0.959**</td>
<td>0.951**</td>
<td>0.854**</td>
</tr>
<tr>
<td>SAT</td>
<td>0.256**</td>
<td>0.959**</td>
<td>0.944**</td>
<td>0.834**</td>
</tr>
<tr>
<td>ASAT</td>
<td>0.227**</td>
<td>0.945**</td>
<td>0.937**</td>
<td>0.845**</td>
</tr>
<tr>
<td>NASAT</td>
<td>0.266**</td>
<td>0.946**</td>
<td>0.927**</td>
<td>0.803**</td>
</tr>
<tr>
<td>Internal</td>
<td>0.424**</td>
<td>0.821**</td>
<td>0.850**</td>
<td>0.772**</td>
</tr>
<tr>
<td>IAAT</td>
<td>0.461**</td>
<td>0.796**</td>
<td>0.839**</td>
<td>0.787**</td>
</tr>
<tr>
<td>NAIAT</td>
<td>0.322**</td>
<td>0.773**</td>
<td>0.777**</td>
<td>0.657**</td>
</tr>
<tr>
<td>Trunk</td>
<td>0.302**</td>
<td>0.943**</td>
<td>0.949**</td>
<td>0.879**</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.446**</td>
<td>0.053</td>
<td>0.122</td>
<td>0.232**</td>
</tr>
</tbody>
</table>

All data are presented as Pearson correlation r values. Bold typeface indicates a significant correlation; shaded boxes indicate the anthropometric variable with the strongest correlation. ^ Statistical analysis of IHCL (F: 169), S-IMCL (F: 179) and T-IMCL (F: 178) performed on \( \log_{10} \) transformed variables; * = p<0.05, ** = p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intrahepatic cellular lipid and IMCL: intramyocellular lipid (S - soleus, T - tibialis); Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
The TOFI Phenotype

In an attempt to refine the phenotype of individuals classed as MONW, the TOFI (Thin on the Outside Fat on the Inside) index was derived in order to identify individuals as having the most adverse fat distribution using MRI imaging. The index derived was based on the ratio of intra-abdominal to abdominal subcutaneous adipose tissue (IAAT/ASAT). This ratio was plotted against BMI. The values measured in the control group established a representative “normal” range of abdominal adiposity.

Participants in the main group with a BMI range of 18.5 < 25 kg/m² were then classified as TOFI if their IAAT/ASAT ratio was two standard deviations above the mean of healthy control individuals. The gender specific variable data from healthy, active individuals used to define the thin-on-the-outside fat-on-the-inside (TOFI) phenotype (Table 4.4) shows the mean IAAT/ASAT ratio for healthy Caucasian individuals was 0.59 (male) and 0.25 (female). Two standard deviations above the mean IAAT/ASAT of healthy individuals (+2 SD male: 1.04, female: 0.45) were used to define the cut-off for TOFI classification. Thus individuals with a BMI 18.5 < 25kg/m² with a IAAT/ASAT ratio above 1.0 (males: aged 18-50yr) and 0.45 (females: aged 18-39yr) were classified as TOFI; this corresponds to 14.0% of men (15/106) and 12% of women (17/132) in the main cohort.
Table 4.4. Gender specific variable data for healthy, active individuals.

<table>
<thead>
<tr>
<th></th>
<th>Male (n = 57)</th>
<th>Female (n = 54)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>32 ± 10</td>
<td>17 - 50</td>
<td>26 ± 5</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>73.2 ± 7.3</td>
<td>59 - 91.6</td>
<td>59.5 ± 5.5</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>22.6 ± 1.6</td>
<td>18.6 - 25</td>
<td>21.7 ± 1.6</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>81.3 ± 5.3</td>
<td>70.5 - 90.5</td>
<td>71.6 ± 4.3</td>
</tr>
<tr>
<td><strong>Hip (cm)</strong></td>
<td>96.4 ± 5.3</td>
<td>85.4 - 107</td>
<td>95.3 ± 5.1</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>179.7 ± 6.4</td>
<td>164 - 192.8</td>
<td>165.5 ± 5.0</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.8 ± 0.01</td>
<td>0.76 - 0.9</td>
<td>0.8 ± 0.01</td>
</tr>
<tr>
<td><strong>WHtR</strong></td>
<td>0.5 ± 0.01</td>
<td>0.37 - 0.53</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>*<em>IHCL</em></td>
<td>11.0 ± 6.4</td>
<td>2.85 - 40.96</td>
<td>8.3 ± 5.6</td>
</tr>
<tr>
<td>*<em>S-IMCL</em></td>
<td>4.7 ± 2.4</td>
<td>1.31 - 12.48</td>
<td>5.2 ± 2.3</td>
</tr>
<tr>
<td>*<em>T-IMCL</em></td>
<td>0.8 ± 2.3</td>
<td>0.0 - 17.36</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td><strong>TAT</strong></td>
<td>14.3 ± 4.5</td>
<td>6 - 26.2</td>
<td>20.1 ± 4.9</td>
</tr>
<tr>
<td><strong>SAT</strong></td>
<td>11.0 ± 3.4</td>
<td>4.2 - 20.4</td>
<td>17.6 ± 4.2</td>
</tr>
<tr>
<td><strong>ASAT</strong></td>
<td>2.7 ± 1.2</td>
<td>0.7 - 6.2</td>
<td>4.4 ± 1.3</td>
</tr>
<tr>
<td><strong>NASAT</strong></td>
<td>8.4 ± 2.4</td>
<td>3.5 - 14.4</td>
<td>13.2 ± 3.1</td>
</tr>
<tr>
<td><strong>Internal</strong></td>
<td>3.3 ± 1.5</td>
<td>0.7 - 7.7</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td><strong>IAAT</strong></td>
<td>1.6 ± 0.9</td>
<td>0.2 - 3.7</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td><strong>NAIAT</strong></td>
<td>1.7 ± 0.7</td>
<td>0.5 - 4.1</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td><strong>Trunk</strong></td>
<td>4.3 ± 1.9</td>
<td>1.02 - 9.58</td>
<td>5.4 ± 1.5</td>
</tr>
<tr>
<td><strong>IAAT/ASAT</strong></td>
<td>0.6 ± 0.2</td>
<td>0.21 - 1.41</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intrahepatocellular lipid; IMCL: intramyocellular lipid (S - soleus, T - tibialis); Adipose tissue deposits are a percentage of body mass (%kg); TAT: total adipose tissue; SAT: subcutaneous AT; ASAT: abdominal subcutaneous AT; NASAT: non-abdominal subcutaneous AT; Internal: total internal AT; IAAT: intra-abdominal AT; NAIAT: non-abdominal internal AT. * MRS data (IHCL (M: 57, F: 51), S-IMCL (M: 57, F: 51) and T-IMCL (M: 57, F: 51)) is presented as the geometric mean while statistical analysis was performed on log₁₀ transformed variables. All data are presented as mean ± SD. Male versus female data analysed by Student’s t-test.
Table 4.5. Gender specific variable data for TOFI and non-TOFI individuals.

<table>
<thead>
<tr>
<th></th>
<th>MALE</th>
<th>FEMALE</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-TOFI (91)</td>
<td>TOFI (15)</td>
<td>p</td>
<td>non-TOFI (115)</td>
<td>TOFI (17)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>34.6 ± 11.8</td>
<td>46.8 ± 12.5</td>
<td>&lt;0.001</td>
<td>30.0 ± 8.9</td>
<td>40.5 ± 15.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.0 ± 7.2</td>
<td>77.5 ± 6.8</td>
<td>&lt;0.05</td>
<td>60.9 ± 6.7</td>
<td>59.8 ± 5.5</td>
</tr>
<tr>
<td>BMI</td>
<td>22.7 ± 1.5</td>
<td>23.5 ± 1.6</td>
<td>0.076</td>
<td>22.0 ± 1.8</td>
<td>22.0 ± 2.2</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.4 ± 6.5</td>
<td>87.8 ± 8.3</td>
<td>&lt;0.05</td>
<td>73.4 ± 6.5</td>
<td>76.2 ± 9.7</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>96.2 ± 5.1</td>
<td>99 ± 5.4</td>
<td>0.10</td>
<td>96.2 ± 6.0</td>
<td>94.2 ± 8.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.1 ± 6.9</td>
<td>181.8 ± 7.2</td>
<td>0.16</td>
<td>166.3 ± 5.8</td>
<td>165.0 ± 8.6</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.05</td>
<td>0.89 ± 0.07</td>
<td>0.11</td>
<td>0.76 ± 0.05</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.4 ± 0.15</td>
<td>0.44 ± 0.15</td>
<td>0.4</td>
<td>0.44 ± 0.06</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>IHCL*</td>
<td>0.86 ± 1.3</td>
<td>3.0 ± 4.6</td>
<td>&lt;0.001</td>
<td>0.33 ± 0.52</td>
<td>3.76 ± 10.64</td>
</tr>
<tr>
<td>S-IMCL*</td>
<td>12.8 ± 11.5</td>
<td>19.8 ± 12.0</td>
<td>&lt;0.05</td>
<td>9.25 ± 4.74</td>
<td>15.7 ± 10.1</td>
</tr>
<tr>
<td>T-IMCL*</td>
<td>4.9 ± 3.0</td>
<td>6.8 ± 2.8</td>
<td>&lt;0.05</td>
<td>5.88 ± 2.95</td>
<td>8.3 ± 4.1</td>
</tr>
<tr>
<td>TAT</td>
<td>15.8 ± 6</td>
<td>19.5 ± 4.6</td>
<td>&lt;0.05</td>
<td>21.3 ± 5.1</td>
<td>23.8 ± 6.6</td>
</tr>
<tr>
<td>SAT</td>
<td>12.2 ± 4.7</td>
<td>12.6 ± 2.7</td>
<td>0.76</td>
<td>18.4 ± 4.4</td>
<td>18.9 ± 5.4</td>
</tr>
<tr>
<td>ASAT</td>
<td>3.0 ± 1.2</td>
<td>3.2 ± 0.8</td>
<td>0.47</td>
<td>4.7 ± 1.6</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td>NASAT</td>
<td>9.3 ± 3.8</td>
<td>9.4 ± 2.1</td>
<td>0.89</td>
<td>13.8 ± 3.1</td>
<td>14.3 ± 4.4</td>
</tr>
<tr>
<td>Internal</td>
<td>3.6 ± 1.7</td>
<td>6.9 ± 2.1</td>
<td>&lt;0.001</td>
<td>2.8 ± 1.0</td>
<td>4.9 ± 1.5</td>
</tr>
<tr>
<td>IAAT</td>
<td>1.8 ± 1</td>
<td>3.8 ± 1.1</td>
<td>&lt;0.001</td>
<td>1.2 ± 0.5</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>NAIAT</td>
<td>1.8 ± 0.7</td>
<td>3.1 ± 1.1</td>
<td>&lt;0.001</td>
<td>1.7 ± 0.6</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>Trunk</td>
<td>4.7 ± 2.1</td>
<td>7.0 ± 1.8</td>
<td>&lt;0.001</td>
<td>5.8 ± 1.9</td>
<td>7.0 ± 2.2</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.6 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>&lt;0.001</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Anthropometric, and adiposity variable data for male individuals (BMI 18.5<24) and female volunteers (BMI 18.5<24) classified as TOFI or non-TOFI. All data are presented as mean ± SD. * MRS data is presented as the geometric mean while statistical analysis was performed on log_{10} transformed variables. Data analysed by Student’s t-test.

Significantly greater S-IMCL depots were observed in TOFI female volunteers. However, there was no difference between IHCL in either gender (Male IHCL: p=0.59; Female IHCL: p=0.95). Participants classified with the TOFI phenotype based on their IAAT, could not be predicted from BMI, WC, WHR or WHtR or trunk fat since there was no significant difference in these variables between TOFI and non-TOFI healthy participants (Table 4.5).
Interestingly, MRI phenotyping also revealed participants with an identical waist circumference, and overall trunk fat content, who could therefore not be distinguished from anthropometric phenotyping, had different levels of IAAT. For instance, the images shown in Figure 4.1, shows two individuals both with 12.8 litres of trunk fat. Participants A as 4.6 litres of IAAT, while participants B has 6.3 litres.

Figure 4.1 Anthropometric variation in abdominal adiposity.

WHR was the anthropometric variable, which correlated most with the IAAT/ASAT ratio in both men (r = 0.37, p<0.01) (Table 4.2) and women (r =0.34, p<0.01) Table 4.3).
The male individuals shown in this figure are of similar age, BMI and percentage body fat but have different levels of visceral fat and therefore different disease risks. Participant (a) is a TOFI (BMI 25·8 kg/m$^2$; 3·3 litres of visceral fat); participant (b) is a healthy volunteer (BMI 26·5 kg/m$^2$; 2·2 litres of visceral fat).
4.4. Discussion

There are well established differences in adipose tissue content and distribution between male and female participants. In this chapter my results confirmed these findings, with females demonstrating significantly higher percentage total and subcutaneous fat stores than the males. Male participants also had significantly greater IAAT compared to females. There were also gender differences in ectopic fat content, with male participants having significantly higher levels of IHCL and S-IMCL compared to females. Ectopic fat depots in the liver and skeletal muscle are implicated in the pathogenesis of insulin resistance, a key feature of the metabolic syndrome. The exact mechanism by which ectopic fat accumulation affects tissue and organ function is unknown, but possible theories include physical compression, altering local secretory profiles, and lipotoxicity. The strong association between increased ectopic fat storage and obesity, type 2 diabetes mellitus, and insulin resistance is well established.

Anthropometric variables as markers of fat deposition

Anthropometric measurements are easily obtainable, inexpensive, and commonly used determinants of both obesity and the metabolic syndrome. In this study, I found that anthropometric variables generally correlated with total and subcutaneous stores better than with internal depots or ectopic fat stores. Anthropometric variables such as WC and WHtR can give little indication of the proportion of IAAT or ASAT in seemingly “lean” participants and are therefore inappropriate for classifying individuals that may be at increased metabolic risk within a “normal” BMI range. Methods of measuring total abdominal obesity such as Viscan and DXA offer a faster, less expensive alternative to the MRI protocol implemented here. However, while there is no doubt a strong correlation between abdominal fat and metabolic risk, these methods are unable to differentiate between subcutaneous and internal abdominal adipose stores. This was clearly illustrated in Figure 4.1. These two individuals have an identical amount of trunk fat and yet have entirely different amounts of IAAT and ASAT.
In this study the use of a ratio of IAAT and ASAT (IAAT/ASAT) is proposed to identify individuals at potentially increased metabolic risk. The study determined the range of IAAT and ASAT in healthy individuals in order to define the limits by which individuals with a disproportionally elevated IAAT, or TOFI (Thin Outside Fat Inside) phenotype could be identified. When considering the larger cohort, this healthy control group represents 10-20% of individuals; the same proportion of healthy individuals in the normal population. The use of ASAT, as opposed to whole body SAT, allows a more accurate comparison of these two abdominal fat depots. For ease of reference, IAAT/ASAT developed ratios of greater than 1.0 in Caucasian male participants and 0.45 in Caucasian female participants are proposed to define this phenotype.

Correlation analysis revealed relatively weak associations between the IAAT/ASAT ratio that was used to define TOFI individuals and other physiological characteristics. In fact, age, a non-anthropometric variable, provided the strongest correlation to IAAT/ASAT. Furthermore, there was a notable lack of statistical difference in anthropometric, adiposity and ectopic fat depots between TOFI and non-TOFI individuals, except for changes in internal fat depots. These data suggest that MRI analysis is currently the only means of successfully identifying individuals with a disproportionately high amount of intra-abdominal fat. Given the previously reported significant positive relationship between liver fat and IAAT content and the fact that individuals with a phenotype opposite to that of the TOFI (i.e., FOTI, fat outside, thin inside), have reduced intra-abdominal and IHCL compared to weight matched individuals, it was surprising that a significant difference in IHCL deposition was not detected between TOFI and “non-TOFI” individuals. This discrepancy could potentially be due to the fact that the cohort had a small number of individuals in the TOFI group, and the fact that the analysis of “non-healthy” individuals was limited to those within the age and BMI range used to define the cut-off values. It could also be possible that elevated IAAT is a precursor to later metabolic changes, which might include increased deposition of IHCL. However, as this is a cross-sectional study, only a subsequent longitudinal can confirm this.

Incidence of ‘metabolically-obese but normal-weight’ has been reported to be between 13 and 18%, which is similar to the incidence of TOFI found in this study (12% women, 14% men). When the proposed IAAT/ASAT cut-off values were applied to individuals with an increased BMI (greater than the 18.5<25 kg/m² range) it was found that 16% of females and 23% of males were identified as TOFI. This increase in male classification is likely to be a reflection of the increased proportion and deposition of visceral
adipose tissue at a higher BMI in males. Disproportionate levels of IAAT in overweight and obese participants are also thought to be problematic. Indeed, obese participants with a disproportionate accumulation of visceral fat have been reported to have an increased incidence of disorders of glucose and lipid metabolism. Further work will be required to characterise healthy control individuals within increased BMI ranges to accurately define those with excessive IAAT.

In this study, the TOFI phenotype was attributed to 12-13% of Caucasian volunteers that fall within a normal BMI range. Additional studies may also reveal the applicability of the TOFI index to additional ethnic populations given the established differences in body fat distribution between racial groups. Currently, the TOFI index provides a quantitative means of comparing intra-abdominal fat deposition. Clearly, the utility of the TOFI phenotype to classify this “at risk” group of individuals will only be fully realised once it has been correlated with markers of the metabolic syndrome. Further work is required to determine the physiological basis for the wide variation in the abdominal fat partitioning reported here. The mechanism is likely to be complex, with a multitude of genetic, environmental and age related determinants. However, the TOFI index provides an effective means of classification; addressing what is emerging as the key phenotype for metabolic risk.

In summary, this study used the ratio of IAAT and ASAT in a defined “healthy” subset of this cohort to define the TOFI sub-phenotype - a potential means of evaluating abdominal obesity and identifying individuals at increased metabolic risk. Therefore the hypothesis (1) for this current study: that the established TOFI index (identify) can identify individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m²) is accepted.

Additionally, the findings demonstrate that anthropometric measurements such as waist circumference (WC) and waist to height ratio (WHtR) are not appropriate for classifying the TOFI phenotype in a seemingly ‘lean’ individuals (i.e. in normal BMI range 18.5<25 kg/m²). The hypothesis (2) for this current study: that established proxy (anthropometric) measures are able to successfully identify MONW individuals within a large cohort of healthy Caucasians is rejected.
Chapter 5. Aerobic fitness and adiposity.

5.1. Introduction

There are instances in which excess body fat is not associated with metabolically adverse profiles such as increased risk of insulin resistance, glucose intolerance, type 2 diabetes and CVD\textsuperscript{85,370,371}. There is increasing evidence to support the existence of ‘metabolically normal obesity’, the phenotypic opposite of the MONW individuals discussed in Chapter 4. The phenotype is typically referred to as metabolically healthy obese (MHO)\textsuperscript{177,180-183} and describes individuals with high body fat, but normal insulin sensitivity, blood pressure, high HDL and low plasma triacylglyceride (TAG) levels\textsuperscript{179,372}.

A similar phenotype, known as “fat-fit”, similar to the MHO has also been identified, but in this case it includes a measure of physical activity\textsuperscript{185,373}. Blair and colleagues pioneered the notion that physical activity reduces the risk of chronic disease in the ‘fat-fit’ \textsuperscript{374}. In an early study, they found that cardiovascular disease risk was lower in 3,217 men who were fat, fit and active compared to 2,182 men who were fat, unfit and inactive\textsuperscript{375}. Cardiovascular and all-cause mortality were also lower in men who were fat, fit and active than 1,852 men who were normal-weight, unfit and inactive\textsuperscript{375}. In later studies, Blair and colleagues assessed physical fitness instead of physical activity because fitness can be more accurately measured and because fitness is largely the product of aerobic activity\textsuperscript{374,376}. An interesting example of this phenotype is seen in Japanese Sumo wrestlers, who despite their elevated total fat content, have low levels visceral adiposity accompanied by high insulin sensitivity\textsuperscript{377,378}.

It is unclear whether the MHO and fat-fit are indeed describing a single phenotype, since reports of this phenotype vary somewhat owing to there being no clear consensus regarding how they should be defined. Some authors rely on absence of (or presence of only one) cardiometabolic risk factors including elevated blood pressure, elevated TAG, C-reactive protein, insulin resistance, elevated glucose levels/diabetes, or decreased HDL\textsuperscript{379} while others rely solely on insulin sensitivity\textsuperscript{380}. Studies of fat-fit individuals tend to include a measure of physical activity or assessment of fitness, while studies focused on MHO tend not to include any.
The relevance of these phenotypes demands attention, especially in light of the evidence that fat-fit individuals have substantially lower mortality risk than normal-weight unfit individuals\textsuperscript{185,381}. Indeed Lee et al.\textsuperscript{184} have shown that physical fitness reduces or eliminates the risks associated with obesity. Indeed with increasing levels of physical activity or fitness there is a linear reduction in all-cause mortality\textsuperscript{382}. How much and what type of physical activity an obese individual might need to undertake to become ‘fit-fat’ is unclear. Mortality was reported to be 20-30\% lower at a threshold exercise energy expenditure of about 4200 kJ (1000 kcal) per week, with even lower mortality above 4200 kJ per week.

Measurement of cardiorespiratory fitness.

Both physical and health-related fitness are complex attributes, and thus there is no single measurement method for these parameters. Moreover, different methods are used to measure different components of fitness, such as motor skills, muscular strength, and agility. Cardiorespiratory fitness is one of the most important components of health-related fitness. It is usually measured by indirect calorimetry in a laboratory setting as maximal aerobic power or maximal oxygen uptake (\textit{VO}_2\text{max}), referring to the maximum rate at which an individual can take up and utilize oxygen while breathing air at sea level during heavy dynamic exercise\textsuperscript{383}. Owing to difficulties in assessing a true plateau in maximal oxygen uptake, an allied measure of \textit{VO}_2peak is also used (i.e. the highest rate of oxygen uptake achieved during heavy dynamic exercise). Cardiorespiratory fitness can also be estimated from peak power achieved on a cycle ergometer, total time on a standard treadmill test, or submaximal tests by estimating age-predicted values from the heart rate response\textsuperscript{301,384-386}. (See Chapter 2 for full protocols and rationale.)

The aim of this chapter study was to refine the MHO phenotype to include accurate measures of fitness in addition to accurate MRI/MRS measures of adiposity and ectopic fat content.

Objectives:

1. Identify 4 phenotypes relating to adiposity and fitness:
   1.1 Slim, fit and active (the slim-fit),
   1.2 Slim, unfit and inactive (the slim-unfit),
   1.3 Fat, fit and active (the fat-fit)
   1.4 Fat, unfit and inactive (the fat-unfit).
2. Investigate whether fat-fit results in a ‘healthier’ body composition than slim-unfit.

Study 3 Hypothesis:

Hypothesis: Fitness results in reduced IAAT and ectopic fat, regardless of overall adiposity.
5.2. Methods

Participants
Fifty male volunteers aged 34–56 years were recruited. Hammersmith Hospital Research Ethics Committee approved this study and all participants gave written informed consent (REC reference number 04/Q0403/87). The study protocol conformed to the ethical guidelines outlined in the 1975 Declaration of Helsinki.

Adiposity, fitness and activity
Waist circumference (minimal), fitness level and exercise habit were subsequently used to distinguish and categorize the participants as being: “fat”, “slim”, “fit” and “unfit”. Waist circumference was measured (as described in Chapter 2 – General Methods) using an inelastic tape, at the narrowest part of the torso, in the horizontal plane, and values ≤90 cm were used to identify and classify “slim” men and values ≥100 cm were used to identify and classify “fat” men.

Aerobic fitness was assessed by oxygen consumption measurement using a calibrated online gas analysis system during an incremental cycling test. A full description is given in the General Research Methods and Procedures chapter (Chapter 2).

Maximal oxygen consumption (VO₂ max) was expressed in absolute terms (L·min⁻¹) and using previously established age-specific norms (Appendix 2). Men who scored ‘very poor’, ‘poor’, ‘fair’ or ‘average’ were classified as “unfit”; and men who scored ‘good’, ‘very good’ or ‘excellent’ were classified as “fit”. In the knowledge that there could potentially be some very unfit, overweight participants being tested, I ensured that established safety guidelines were observed to ensure no unnecessary risk was encountered. As maximal fitness testing is stressful, it can be necessary to terminate testing under certain circumstances. Such circumstances would include dizziness, pain or significant discomfort, or other overt signs of becoming unwell as a consequence of increasing workload. During the course of this study, exercise tests were terminated at 80% of age-predicted maximum heart rate in three “fat-unfit” participants, to reduce their risk of cardiovascular injury. This decision was made based on their initial assessment and their physiological response to the early stages of their cycle tests. In these three cases,
the participants’ VO₂ max was subsequently estimated (using standardised methods) by extrapolating their 80% submaximal heart rate and oxygen uptake values. During these tests cardiac (3 lead ECG) monitoring was utilised to record heart rate data and to provide additional safety criteria necessary in clinical exercise testing. As a registered nurse (intensive care speciality), experienced exercise physiologist, and Advanced Life Support qualified practitioner, I was able to provide the necessary appropriate support for such test scenarios and be able to recognise test termination points as required. The fitness tests were also undertaken in the hospital MR imaging unit where trained medical practitioners (who were part of the wider research team) were regularly in attendance to provide medical cover for research programs, and would routinely be informed that such exercise testing was taking place.

In this study physical activity was assessed using the Five-City Project Physical Activity Recall Questionnaire \(^{389}\) (in addition to the previously mentioned Baecke questionnaire). These validated questionnaires ensured that an accurate and standardised method was used for this important data, by avoiding any ambiguity in participant questioning. I employed these, interviewer probe-type format methods, to minimise the potential for over-reporting. Only unfit men who reported no regular moderate or vigorous activity in the previous two years and fit men who reported taking part in at least 60 minutes of vigorous aerobic activity per week in the previous two years were included in the study. This amount of activity is the minimum amount recommended to develop and maintain cardiorespiratory fitness \(^{390}\). Moderate-intensity activities are those \(\approx 4\) METs and vigorous-intensity activities are those \(\geq 6\) METs, where 1 MET is equivalent to the energy expended at rest \(^{391,392}\).

**MRI scanning: Total body and regional adipose tissue content**

On a single visit, participants underwent total body MRI scanning and in vivo proton magnetic resonance spectroscopy (\(^1\)H MRS) of their liver and their calf muscle. A full description is given in the General Research Methods and Procedures chapter (Chapter 2).

**Statistical methods**

Descriptive characteristics were compared using univariate general linear model analysis of variance (GLM-ANOVA) with Bonferroni post hoc tests. Magnetic resonance imaging and spectroscopy data were
compared using general linear model analysis of covariance (GLM-ANCOVA) with Sidak post hoc tests. Age and height were the covariates in the comparisons of the slim-fit, the slim-unfit, the fat-fit and the fat-unfit. Age, height and BMI were the covariates in the comparisons of the fit-and-active and the unfit-and-inactive men. Multiple linear regression was used to investigate if simple measures of fitness ($\text{VO}_2\text{ max}$), adiposity (waist circumference) and activity (years of exercise) could predict total and regional adiposity measurements. The probability of $F$ was used to retain ($<.05$) or remove ($\geq.10$) predictors in backwards elimination models. Only one measure of fitness, adiposity and activity was used in each regression model because there was evidence of collinearity when two measures were included. All data were analysed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). All $p$ values presented are two-tailed.
5.3. Results

Table 5.1 presents the descriptive characteristics of four different phenotypes identified for this study. Based on the results of the waist circumference and fitness testing there were: 13 slim-fit individuals, 12 slim-unfit, 13 fat-fit, and 12 fat-unfit. Their characteristics are presented in Table 5.1.

Slim-fit and slim-unfit individuals differed only in measures of fitness; there were no significant differences in any adiposity measures. In addition to reduced fitness, fat-unfit individuals also had a significantly greater waist circumference compared to fat-fit participants (p<0.05).

Maximal oxygen uptake was significantly lower in unfit men. Self-reported vigorous activity ranged from 2–13 hours per week and exercise history ranged from 2–33 years in the slim-fit and the fat-fit. Slim-fit and fat-fit men took part in aerobic exercise or a mixture of aerobic and resistance exercises. The slim-unfit and the fat-unfit reported no regular moderate or vigorous activity in the last two years.
Table 5.1 Characteristics of the slim-fit, slim-unfit, fat-fit and fat-unfit groups

<table>
<thead>
<tr>
<th></th>
<th>Slim-fit (n=13)</th>
<th>Slim-unfit (n=12)</th>
<th>Fat-fit (n=13)</th>
<th>Fat-unfit (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43 ± 5</td>
<td>43 ± 7</td>
<td>46 ± 6</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.7 ± 7.9</td>
<td>75.9 ± 6.5</td>
<td>112.6 ± 15.7*</td>
<td>104.8 ± 12.3*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 1.7</td>
<td>23.5 ± 1.5</td>
<td>33.7 ± 4.2*</td>
<td>33.0 ± 3.9*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>83.7 ± 4.89</td>
<td>83.9 ± 4.3</td>
<td>110.8 ± 8.9*</td>
<td>114.2 ± 9.3*</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>99.0 ± 5.1</td>
<td>98.7 ± 3.5</td>
<td>115.6 ± 8.5*</td>
<td>110.8 ± 5.6*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.86 ± 0.04</td>
<td>0.85 ± 0.03</td>
<td>0.96 ± 0.04*</td>
<td>1.01 ± 0.04*†</td>
</tr>
<tr>
<td>VO₂ max (L·min⁻¹)</td>
<td>3.87 ± 0.56</td>
<td>2.84 ± 0.42*</td>
<td>3.97 ± 0.32†</td>
<td>3.18 ± 0.77*‡</td>
</tr>
<tr>
<td>VO₂ max (mL·kg⁻¹·min⁻¹)</td>
<td>48.1 ± 7.6</td>
<td>36.7 ± 4.8*</td>
<td>36.0 ± 4.3*</td>
<td>30.25 ± 6.4*</td>
</tr>
<tr>
<td>Vigorous activity (h·wk⁻¹)</td>
<td>6.0 ± 3.2</td>
<td>-</td>
<td>5.5 ± 2.9</td>
<td>-</td>
</tr>
<tr>
<td>Years of exercise</td>
<td>16 ± 9</td>
<td>-</td>
<td>17 ± 13</td>
<td>-</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. *Significantly different to slim-fit after adjustment for age and height, p<0.05. †Significantly different to slim-unfit after adjustment for age and height, p<0.05. ‡Significantly different to fat-fit after adjustment for age and height, p<0.05.

Body Composition

Differences in adiposity between the 4 groups are summarised in Table 5.2.

**Slim-fit vs slim-unfit:** There were no significant differences in adiposity in either subcutaneous or internal depots between the slim-fit and the slim-unfit. The ratio of IAAT/ASAT (TOFI index) was significantly higher in the slim-unfit group. There were no significant differences in ectopic fat between the slim-fit and the slim-unfit groups.

**Fat-fit vs fat-unfit:** There were no significant differences in TAT, SAT or ASAT between the fat-fit and fat-unfit groups. However total internal adipose tissue, as well as IAAT and NAIAT were significantly higher in the fat-unfit compared to the fat-fit. The IAAT/ASAT was also significantly higher in the fat-unfit group. Similarly, IHCL was significantly elevated in the fat-unfit compared to the fat-fit, despite no significant differences in IMCL.
Table 5.2 Total and regional fat distribution in the slim-fit, slim-unfit, fat-fit and fat-unfit groups

<table>
<thead>
<tr>
<th></th>
<th>Slim-fit (n=13)</th>
<th>Slim-unfit (n=12)</th>
<th>Fat-fit (n=13)</th>
<th>Fat-unfit (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT (L)</td>
<td>15.2 ± 4.0</td>
<td>17.6 ± 5.6</td>
<td>36.6 ± 11.2*†</td>
<td>38.3 ± 6.6*†</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>12.2 ± 3.4</td>
<td>12.7 ± 3.7</td>
<td>28.8 ± 10.4*†</td>
<td>26.2 ± 4.9*†</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>3.0 ± 1.4</td>
<td>3.2 ± 1.2</td>
<td>9.2 ± 4.0*†</td>
<td>8.1 ± 1.8*†</td>
</tr>
<tr>
<td>TIAT (L)</td>
<td>3.0 ± 1.1</td>
<td>4.8 ± 2.3</td>
<td>7.8 ± 1.5*†</td>
<td>12.1 ± 3.7*†‡</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>1.4 ± 0.8</td>
<td>2.6 ± 1.4</td>
<td>4.7 ± 1.1*†</td>
<td>7.5 ± 2.2*†‡</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>1.5 ± 0.4</td>
<td>2.3 ± 1.1</td>
<td>3.1 ± 0.6*</td>
<td>4.5 ± 1.8*†‡</td>
</tr>
<tr>
<td>IAAT:ASAT</td>
<td>0.5 ± 0.2</td>
<td>0.8 ± 0.3*</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.3*†</td>
</tr>
<tr>
<td>IHCL</td>
<td>0.4 ± 0.5</td>
<td>1.5 ± 1.4</td>
<td>5.5 ± 6.1</td>
<td>26.1 ± 24.2*†‡</td>
</tr>
<tr>
<td>S-IMCL</td>
<td>8.3 ± 3.6</td>
<td>11.0 ± 7.3</td>
<td>15.4 ± 5.7</td>
<td>14.7 ± 10.7</td>
</tr>
<tr>
<td>T-IMCL</td>
<td>5.0 ± 2.7</td>
<td>6.3 ± 2.6</td>
<td>8.3 ± 7.3</td>
<td>9.3 ± 5.4</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD.
*Significantly different to slim-fit after adjustment for age and height, p<0.05.
†Significantly different to slim-unfit after adjustment for age and height, p<0.05.
‡Significantly different to fat-fit after adjustment for age and height, p<0.05.

TAT - total adipose tissue; SAT – subcutaneous; ASAT - subcutaneous abdominal; TIAT - Total internal; IAAT - Intra-abdominal adipose tissue; NAIAT - Non-abdominal internal; IAAT/SAAT (L). IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data were analysed as log data. Values are mean±SD.

Fat vs slim: Interestingly, when the adiposity data are presented as a percentage of body weight, rather than in absolute volumes (as shown in Table 5.2), the proportion of internal fat was actually lower in the fat-fit compared to the slim-unfit group (Figure 5.1).
The percentage of IAAT was higher in the slim-unfit compared to the slim-fit though this did not reach significance (p=0.06). This was significantly higher in both the fat-fit and fat-unfit. (Figure 5.2).

Figure 5.1. Internal fat expressed as a percentage of body weight in 13 slim-fit men, 12 slim-unfit men, 13 fat-fit men, and 12 fat-unfit men. Data are presented as means ± standard deviations. *Significantly different to slim-fit after adjustment for age and height, p<0.05. †Significantly different to slim-unfit after adjustment for age and height, p<0.05. ‡Significantly different to fat-fit after adjustment for age and height, p<0.05.

Figure 5.2. Intra-abdominal adipose tissue (IAAT) expressed as a percentage of body weight in 13 slim-fit men, 12 slim-unfit men, 13 fat-fit men, and 12 fat-unfit men. Data are presented as mean ± standard deviation. *Significantly different to slim-fit after adjustment for age and height, p<0.05. †Significantly different to slim-unfit after adjustment for age and height, p<0.05. ‡Significantly different to fat-fit after adjustment for age and height, p<0.05.
Combining the data from the fit group (slim-fit and fat-fit) as well as data from the unfit groups (slim-unfit and fat-unfit), created two groups comprised of 26 fit-and-active men and 24 unfit-and-inactive men. Comparing these two categories showed the amount of IAAT and the amount of IHCL were significantly lower in the fit-and-active compared to the unfit-and-inactive, independent of age, height and BMI (Figure 5.3).

Figure 5.3. The amount of IAAT (top) and intrahepatocellular lipids (IHCL, bottom) in 26 men who were fit-and-active and 24 men who were unfit-and-inactive. Data are presented as mean ± standard deviation. *Significantly different to fit-and-active after adjustment for age, height and BMI, p<0.01.

A number of significant predictors of total and regional fat were identified in multiple linear regression models (Table 5.3). For example, waist circumference and years of exercise explained 84% of the variance in total fat, waist circumference and VO₂ max explained 70% of the variance in IAAT, and waist circumference explained 25% of the variance in IHCL. Regression coefficients indicated that a 10 cm
increase in waist circumference was associated with a 7.3 litre increase in total fat and a 1.3 litre increase in IAAT. In contrast, a 0.5 L-min\(^{-1}\) increase in VO\(_2\) max was associated with a 0.5 L reduction in IAAT.

Table 5.3 Fitness, adiposity and exercise history as predictors of total and regional fat in multiple linear regression models

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Significant predictor variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant</td>
</tr>
<tr>
<td>TAT (L)</td>
<td>-44.02</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>-35.29</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>-15.01</td>
</tr>
<tr>
<td>TIAT (L)</td>
<td>-5.44</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>-5.02</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>-0.42</td>
</tr>
<tr>
<td>IAAT:ASAT</td>
<td>1.44</td>
</tr>
<tr>
<td>S-IMCL</td>
<td>-3.59</td>
</tr>
<tr>
<td>T-IMCL</td>
<td>-4.31</td>
</tr>
<tr>
<td>IHCL</td>
<td>-37.52</td>
</tr>
</tbody>
</table>

Values are unstandardised coefficients derived from all participants. Predictors were included in the model if the probability of \(F\) was <.05. Outcome variables were measured in litres apart from IAAT/ASAT and IHCL. TAT - total adipose tissue; SAT – subcutaneous; ASAT - subcutaneous abdominal; TIAT - Total internal; IAAT - Intra-abdominal adipose tissue; NAIAT - Non-abdominal internal; IAAT/ASAT (L). IHCL - intrahepatocellular lipid; IMCL - Intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data were analysed as log data.
5.4. Discussion

In this chapter, I have shown that intra-abdominal adipose tissue and liver fat are lower in men who are fat, fit and active than men who are fat, unfit and inactive. This may help explain why the risk of chronic disease is lower in the ‘fat-fit’ than the ‘fat-unfit’\textsuperscript{184}. It is important to explain how it is possible to be fat, fit and healthy because weight loss is difficult to achieve\textsuperscript{393}.

Several investigators have compared intra-abdominal adipose tissue in fit men and unfit men of different waist circumference or BMI\textsuperscript{394-396}. Wong and colleagues\textsuperscript{395} obtained five to seven computed tomography (CT) images bordered by the L4–L5 and L3–L4 vertebral disc spaces and found that IAAT was lower in 169 fit men than 124 unfit men of a given BMI (fit men were those in the highest two quintiles and unfit men were those in the lowest two quintiles of treadmill time). Lee and colleagues\textsuperscript{394} obtained a single CT or MR image at L4–L5 and found that IAAT was lower in 147 men who were fat and fit (BMI 26.9±3.2 and highest 40% of treadmill times) than 56 men who were fat and unfit (BMI 32.1±3.5 and lowest 20% of treadmill times). Fit men also had lower triglyceride and higher HDL cholesterol levels for a given waist circumference or amount of IAAT. Arsenault and colleagues\textsuperscript{396} obtained a single CT image at L4–L5 and found that IAAT was lower in 58 fit men than 58 unfit men matched for BMI (fit men were above the 50th percentile and unfit men were below the 50th percentile in submaximal cycling tests). The cardiovascular disease risk factor profile of fit men was also better than that of unfit men matched for BMI. Physical activity was not assessed in any of these studies and the authors could only speculate that physical activity was associated with lower levels of IAAT.

Most fit men are active\textsuperscript{375}, but around 2-40% of sedentary men perform well in fitness tests\textsuperscript{375,397}. ‘Naturally fit’ men were excluded from my study because the available evidence suggests that fitness does not reduce the risk of chronic disease in sedentary men. For example physical activity and physical fitness were assessed in 4,999 middle-aged men in the Copenhagen Male Study and coronary heart disease rates were comparable in those who were fit and sedentary and those who were unfit and sedentary during 17 years of follow-up\textsuperscript{397}. In fact, coronary heart disease rates fell across fitness quintiles in active men and these data suggest that the protective effects of physical fitness are mediated through physical activity.
Men who were fat, fit and active were carefully identified in this study. Waist circumference was measured because large studies suggest it is a stronger predictor of disease than general adiposity\textsuperscript{398,399} and was part of the standard anthropometry protocol adopted in such studies within the research group. There is however, no consensus on waist circumference thresholds\textsuperscript{400}, but a male waist circumference of 100 cm is associated with the accumulation of IAAT\textsuperscript{401}. A waist circumference of 90 cm or less was used to define slim individuals to avoid misclassification. Maximal oxygen consumption is the ‘gold standard’ measure of aerobic fitness and ‘fit’ and ‘unfit’ men in my study were defined with reference to healthy, untrained men of the same age from established normative values\textsuperscript{402}. These normative values used were themselves largely derived from cycling tests and VO\textsubscript{2} max was expressed independent of body weight to avoid penalising heavier individuals. Vigorous activity is known to be recalled with greater accuracy\textsuperscript{403} and only fit men who reported taking part in at least 60 minutes of vigorous activity per week in the last two years were recruited.

Waist circumference is believed to be a surrogate measure of IAAT\textsuperscript{404} and it has been suggested that waist circumference is a better predictor of cardiovascular disease risk than aerobic fitness\textsuperscript{405}. Waist circumference was associated with IAAT in the present study, but there were no significant differences in waist circumference between the fat-fit and the fat-unfit despite significant differences in IAAT content. Waist circumference (cm) and VO\textsubscript{2} max (L\textperiodcentered min\textsuperscript{-1}) explained 70\% of the variance in IAAT in the present study and these findings suggest that waist circumference and aerobic fitness should be assessed in clinical practice.

This is the first study of liver fat in men who were fat, fit and active. The expansion of adipocytes is associated with the accumulation of lipid in non-adipose cells in the liver, skeletal muscle and pancreas (ectopic fat), but some individuals with ‘metabolically benign’ obesity appear to have much lower levels of ectopic fat\textsuperscript{406}. One possible explanation for the lower levels of IAAT and liver fat observed in the fat-fit than the fat-unfit in my study is that ectopic fat may be accumulating when there is lipid ‘overflow’ from adipocytes along with concomitant storage in non-adipose depots\textsuperscript{407,408}. This overflow may be less likely to occur in the fat-fit participants owing to a capacity to store excess fat in insulin-sensitive subcutaneous adipose tissue\textsuperscript{408} as a consequence of them being ‘metabolically healthy’. Stefan and colleagues\textsuperscript{406} found that ectopic fat was significantly lower in 31 obese individuals who were insulin sensitive than 96 obese individuals who were insulin resistant (obesity was defined as a BMI $ \geq $30 and insulin sensitivity was
assessed during oral glucose tolerance tests). Ectopic fat accumulation may also be less likely to occur in those individuals whose lean tissue is able to maintain and/or increase fat oxidation to accommodate normal and/or increased triglyceride levels\textsuperscript{407}, such as the fat-fit. The presence of IMCL in fit men in the present study is probably a normal training adaptation that allows fat to serve as a readily available fuel\textsuperscript{409}.

It is interesting that the ratio of IAAT to ASAT (TOFI index – Chapter 4) was lower in the slim-fit and the fat-fit than the slim-unfit and the fat-unfit, and as discussed in previous chapters, IAAT is associated with greater risk of chronic disease than subcutaneous abdominal adipose tissue\textsuperscript{410}. Furthermore, authors from the Framingham Heart Study suggest that subcutaneous abdominal adipose tissue may be a ‘protective fat depot’ in those with higher levels of IAAT\textsuperscript{411}. In men in the highest tertile of IAAT, for example, the prevalence of high triglycerides was 52.7\% in those with the greatest levels of subcutaneous abdominal adipose tissue and 64.4\% in those with the lowest levels of subcutaneous abdominal adipose tissue (p=0.03 for trend). Data from the Framingham Heart Study also indicates that IAAT levels are lower in those who lead a healthy lifestyle, including those who are physically active and those who follow dietary guidelines\textsuperscript{412}.

Cross-sectional data cannot be used to infer causality, but several randomised, controlled trials have found that endurance training is accompanied by preferential reductions in IAAT\textsuperscript{413}. IAAT and liver fat vary considerably in healthy adults\textsuperscript{279,290} and the findings should be confirmed in a larger study with greater statistical power. This study of Caucasian men cannot be generalised to women, older adults and other groups. Blair\textsuperscript{414} recently suggested that weight loss was the wrong goal because physical activity is beneficial to health whether or not an individual loses weight.

The findings in this study have showed that intra-abdominal adipose tissue and liver fat are lower in men who are fat, fit and active than men who are fat, unfit and inactive. The hypothesis for this current study: that fitness results in reduced IAAT and ectopic fat, regardless of overall adiposity, is accepted.
Chapter 6. The “Asian Indian Phenotype” / (MONW)

6.1. Introduction

As discussed in Chapter 4, a significant proportion of lean individuals (BMI < 25 kg/m²) have been referred to as ‘metabolically obese but normal-weight’ (MONW) as a result of their increased abdominal adiposity. An example of this can be seen in the ‘Asian Indian Phenotype’ where individuals despite a body mass index (BMI) within the normal range have been reported as having increased waist circumference (WC) and waist-to-hip ratios (WHR). These individuals consequentially have an increased risk of type 2 diabetes and CVD similar to more overweight or obese individuals. This population evidences the shortcomings of the BMI with respect to ethnic differences.

Although BMI is considered by many to be a useful population-level measure of obesity, it does not capture variations in fat distribution and it does not correspond to the same degree of adiposity or health risk across different individuals or populations. There is growing evidence that the relationship between BMI and adiposity (body fat percent) varies considerably between populations of different ethnicities. This ethnicity based variation poses an additional problem since the majority of data used to establish BMI cut-off points (for increased risk of adverse health outcomes: overweight (BMI ≥ 25.0) and obese (BMI ≥ 30.0)) were collected from Caucasian populations. This multiplication of potential inaccuracies could potentially explain criticisms of BMI as a poor indicator of body adiposity in racial/ethnic minorities and individuals with a large body build. For example individuals of Polynesian ancestry have reduced body fat and increased muscle mass at a given BMI compared to Caucasian participants. Conversely, Asians from Asia-Pacific regions including Chinese, Koreans, South Asians, Japanese and Singaporean have higher body fat levels at any given BMI compared to Caucasians.

South Asians, in particular consistently exhibit the greatest adiposity differences compared to Caucasians, with up to 5% higher body fat at any BMI value. This culminates in increased risk of type 2 diabetes and CVD at relatively low BMI values. Since South Asians exhibit elevated adiposity at a lower body weight compared to Caucasians, BMI cannot be regarded as an accurate indicator of adiposity in South Asians. As a consequence, the number of individuals who are classified as obese (and
subsequently at risk of related comorbidities) will be vastly underestimated when using unadjusted BMI classifications.

An increased incidence of type-2 diabetes and impaired glucose tolerance has been reported in South Asians compared to Caucasian populations. Given the strong correlations between insulin resistance, visceral adipose tissue and ectopic fat content that have been reported in Caucasian populations, it is wholly possible that in South Asians, their additional proneness to visceral fat accumulation, is a major part of the mechanism underlying their greater propensity to develop type-2 diabetes and related morbidities at comparatively lower BMI values than other ethnic groups.

Most previous studies to measure South Asian adiposity have relied on indirect measurements, such as waist-to-hip ratio (WHR) and body mass index (BMI), few have applied direct imaging studies to accurately map whole body adipose tissue content and distribution (see Table 6.0).

The aim of this study was to develop a detailed phenotype of South Asian adiposity compared to Caucasians, and specifically to determine whether TOFI-like characteristics underlie the increased metabolic risk traditionally associated with this population.

Objectives:

1. Quantify adiposity stores (across all BMI) in a cohort of healthy South Asian male and female volunteers using MRI and MRS.
2. Identify MONW South Asian individuals (BMI 18.4 – 25 kg/m²) based on the clinical index developed in Chapter 4 and assess the relationship between their adiposity and established proxy (anthropometric) measures.
3. Compare the South Asian and Caucasian MONW groups to assess the extent of ethnic differences in adiposity stores and abdominal adipose tissue (IAAT/ASAT ratio).
Study 4 Hypotheses:

Hypothesis 1: South Asians have greater internal and ectopic adipose stores than age and BMI matched Caucasians of the same gender.

Hypothesis 2: The established TOFI index can identify South Asian MONW individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m$^2$).

Hypothesis 3: Established proxy (anthropometric) measures are able to successfully identify South Asian MONW individuals.
<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Participants</th>
<th>Measurement Technique</th>
<th>Metabolic measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anthropometry</td>
<td>Body water dilution</td>
</tr>
<tr>
<td>1991</td>
<td>Cruickshank</td>
<td>314: 107(A), 101(C), 106(AfrC)</td>
<td>UWW</td>
<td>-</td>
</tr>
<tr>
<td>1994</td>
<td>Dhawan</td>
<td>200 M: 83(A), 87(C), 30(A_mig)</td>
<td>BMI, WHR</td>
<td>-</td>
</tr>
<tr>
<td>1996</td>
<td>Bose</td>
<td>(A) vs (C)</td>
<td>BMI, WC</td>
<td>-</td>
</tr>
<tr>
<td>1999</td>
<td>Banerji</td>
<td>20 (A) (M)</td>
<td>Skf, WC.</td>
<td>22 slices</td>
</tr>
<tr>
<td>1999</td>
<td>Chandalia</td>
<td>44: 21(A), 23(C)</td>
<td>Skf</td>
<td>Y</td>
</tr>
<tr>
<td>1999</td>
<td>Forouhi</td>
<td>40: 20(A), 20(C)</td>
<td>BMI, WC</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Forouhi</td>
<td>141</td>
<td>BMI, WC</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Raji</td>
<td>24: 10M (5A, 5C), 12F (7A, 7C)</td>
<td>BMI, WC</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>Abate</td>
<td>140 M: 79(A) 61(C)</td>
<td>Skf x9</td>
<td>Y</td>
</tr>
<tr>
<td>2005</td>
<td>Bhat</td>
<td>141(A) (M)</td>
<td>BMI, WC</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Tillin</td>
<td>3,662:</td>
<td>BMI, WC</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Goel</td>
<td>168 A: (94M, 74F).</td>
<td>Skf x7</td>
<td>(n=171)</td>
</tr>
<tr>
<td>2009</td>
<td>Deepa</td>
<td>2350 (A); 1096(M), 1254(F)</td>
<td>BMI, WC</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.0 Summary table of South Asian adiposity studies published detailing the participant numbers, measurement techniques used and proxy measures (if any) used.

Key:


Metabolic measurements. OGTT – oral glucose tolerance test, NEFA – non-esterified fatty acids, PAI-1 – plasminogen activator inhibitor-1, CHD – coronary heart disease, eu-clamp – euglycaemic clamp
6.2. Methods

Participants
Written, informed consent was acquired from all volunteers. Ethical approval permission for this study was obtained from the Research Ethics Committee of Hammersmith and Queen Charlotte’s and Chelsea Research Ethics Committee Hospital, London (Rec: 07Q04011/19). In total, 68 South Asian (49 male, 19 female) and 192 Caucasian (99 male, 93 female) age and BMI matched volunteers were recruited.

Self-reported exclusion criteria included participants suffering from chronic disease including diabetes, cardiovascular or liver disease, metabolic conditions, anyone taking prescribed medication and women on the contraceptive pill. Volunteers underwent anthropometric assessment, total body MRI scanning and in vivo proton $^1$H MRS of liver and calf muscle. Ethnicity was determined by self-report and all parents and grandparents were required to be South Asian decent and European Caucasian for the South Asian and Caucasian participants, respectively.

Anthropometric measurements
Body mass (kg), height (cm), midpoint waist circumference (WC) (cm) and hip circumference (cm) were measured in each participant as detailed in the General Research Methods and Procedures section (Chapter 2). From these values, BMI (kg/m$^2$) and waist-to-hip ratio (WHR, waist/hip) were calculated. BMI grouping corresponded to the following ranges; 1: 20.5<25 kg/m$^2$, 2: 25<30 kg/m$^2$, 3: 30<40 kg/m$^2$, 4: 40+ kg/m$^2$.

MRI scanning: Total body and regional adipose tissue content
On a single visit, participants underwent total body MRI scanning as described in the General Research Methods and Procedures chapter (Chapter 2). The adiposity data obtained was expressed as previously described (Chapter 2 - section 2.5.1.3).

MRS of liver and muscle fat
During the same scanning session, $^1$H MR spectra were also acquired on a 1.5T scanner and analysed using methods previously described (Chapter 2).
Statistical Analysis

Differences relating to gender and/or ethnicity were analysed using one-way ANOVA. Significance was taken as $p<0.05$. All data are presented as mean ± standard deviation. Data which was found not to be normally distributed (IHCL and IMCL) is presented as a geometric mean, while statistical analysis was performed on $\log_{10}$ transformed variables. Associations between variables were investigated using Pearson partial correlation $r$ values. The statistical analysis was performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).
6.3. Results

Of the participants included in this part of the study 32.4% of the South Asians were classified as overweight (34% of men, 26.3% women, BMI: 25<30), 19.1% qualifying as obese (16% of men, 26.3% of women, BMI: 30<40) and none as morbidly obese (BMI: 40+).

**Gender Differences**

**Male South Asian vs Female South Asian comparison**

Gender specific characteristics are shown in Table 6.1. South Asian males were significantly taller (P < 0.001), heavier (P < 0.001) and younger (p=0.028) than the South Asian females. Despite this, the only difference between the groups that could be detected using anthropometry was the WHR, which was significantly lower in the female group.

Significant differences in body composition could be measured using MRI. South Asian female participants were found to have significantly higher levels of adipose tissue, predominantly reflecting increases in the subcutaneous depots, with significant increases in TAT, SAT, ASAT, NASAT, (P < 0.0001 for all). While the IAAT/ASAT ratio was significantly lower in female, compared to male participants (P < 0.01), there were no gender differences in any of the internal adipose tissue depots (including IAAT, TIAT or NAIAT). There was a small but significant difference in some of the ectopic fat depots, T-IMCL was higher in female compared to the male South Asian participants, though there was no difference in S-IMCL. While there were no significant differences in ectopic fat content in the liver, there was a trend of elevated IHCL in the male group, similar to the gender differences found in Caucasian participants in Chapter 4.
## Table 6.1. Gender differences in South Asian participants.

<table>
<thead>
<tr>
<th></th>
<th>South Asian Male (n=49)</th>
<th>South Asian Female (n=19)</th>
<th>SAM vs SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td><strong>Range</strong></td>
<td><strong>Mean ± SD</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>28.41 ± 6.65</td>
<td>19 - 44</td>
<td>33.11 ± 10.0</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>77.3 ± 11.4</td>
<td>57.6 - 102</td>
<td>65.7 ± 14.8</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>26.0 ± 3.5</td>
<td>20.4 – 34.3</td>
<td>25.6 ± 5.1</td>
</tr>
<tr>
<td><strong>WC</strong></td>
<td>89.4 ± 10.9</td>
<td>70.5 - 110.4</td>
<td>85.9 ± 9.8</td>
</tr>
<tr>
<td><strong>Hip</strong></td>
<td>100.2 ± 6.0</td>
<td>88.5 - 112.0</td>
<td>100.9 ± 10.7</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td>172 ± 6.75</td>
<td>155 – 192</td>
<td>160.5 ± 5.4</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.89 ± 0.06</td>
<td>0.73 – 0.99</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td><strong>WHtR</strong></td>
<td>0.52 ± 0.07</td>
<td>0.42 - 0.70</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td><strong>IHCL</strong></td>
<td>4.05 ± 7.68</td>
<td>0 – 48.07</td>
<td>2.61 ± 5.13</td>
</tr>
<tr>
<td><strong>S-IMCL</strong></td>
<td>12.77 ± 6.84</td>
<td>3.31 - 32.51</td>
<td>10.95 ± 7.85</td>
</tr>
<tr>
<td><strong>T-IMCL</strong></td>
<td>5.01 ± 2.34</td>
<td>1.08 - 11.69</td>
<td>6.88 ± 2.97</td>
</tr>
<tr>
<td><strong>%TAT</strong></td>
<td>26.9 ± 6.66</td>
<td>14.7 - 44.8</td>
<td>38.8 ± 7.0</td>
</tr>
<tr>
<td><strong>%SAT</strong></td>
<td>21.4 ± 5.4</td>
<td>12.2 – 35.1</td>
<td>33.5 ± 6.3</td>
</tr>
<tr>
<td><strong>%ASAT</strong></td>
<td>6.3 ± 2.2</td>
<td>2.8 – 12.0</td>
<td>9.4 ± 2.6</td>
</tr>
<tr>
<td><strong>%NASAT</strong></td>
<td>15.1 ± 3.5</td>
<td>8.9 - 23.4</td>
<td>24.7 ± 4.0</td>
</tr>
<tr>
<td><strong>%TIAT</strong></td>
<td>5.4 ± 1.9</td>
<td>2.4 – 9.7</td>
<td>5.3 ± 1.8</td>
</tr>
<tr>
<td><strong>%IAAT</strong></td>
<td>2.9 ± 1.3</td>
<td>0.7 – 6.0</td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td><strong>%NAIAT</strong></td>
<td>2.6 ± 0.7</td>
<td>1.4 – 4.2</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td><strong>IAAT/ASAT</strong></td>
<td>0.47 ± 0.19</td>
<td>0.19 - 1.55</td>
<td>0.26 ± 0.11</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. Data analysed by one-way Anova: SAM: South Asian Male; SAF: South Asian Female. Fat stores are presented as a percentage of total body weight. WC – waist circumference; WHR – waist-to-hip ratio; WHtR - waist-to-height ratio; IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data were analysed as log transformed data; TAT - total adipose tissue; SAT – subcutaneous; ASAT - subcutaneous abdominal; NASAT - subcutaneous non-abdominal; TIAT - Total internal; IAAT - Intra-abdominal adipose tissue; NAIAT - Non-abdominal internal; IAAT/ASAT (L). All data are presented as mean ± SD.
Ethnic Differences

Male South Asian vs Male Caucasian comparison

Since Male participants had been matched for age and BMI, unsurprisingly there were no differences in these variables. Interestingly there were significant differences in weight and height, with South Asian males being significantly lighter (<0.0001) and shorter (<0.0001) compared to the Caucasian participants (Table 6.2). No other differences in body composition were detected using anthropometry. However, phenotyping using MRI and MRS showed several striking differences. South Asian males were significantly ‘fatter’ with significant increases in all subcutaneous depots compared to the Caucasian male group. Specifically there were increases in TAT, SAT, ASAT and NASAT (p<0.0001) compared to Caucasian males. There were no significant differences in any of the internal adipose tissue depots measured.

Similarly there were no ethnic differences in muscle fat content between male and female participants. However South Asian males had significantly higher levels of IHCL compared to the Caucasian group (p=0.017).
Table 6.2. Comparison between South Asian Male vs Caucasian Male Participants.

<table>
<thead>
<tr>
<th></th>
<th>South Asian Male (n=49)</th>
<th>Caucasian Male (n=99)</th>
<th>SAM vs CM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>28.41 ± 6.65</td>
<td>19 - 44</td>
<td>30.19 ± 6.51</td>
<td>19 - 44</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>77.3 ± 11.4</td>
<td>57.6 - 102</td>
<td>82.95 ± 12.3</td>
<td>62.4 - 117</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>26.0 ± 3.5</td>
<td>20.4 – 34.3</td>
<td>25.7 ± 3.6</td>
<td>20.1 – 35.2</td>
</tr>
<tr>
<td><strong>WC</strong></td>
<td>89.4 ± 10.9</td>
<td>70.5 - 110.4</td>
<td>90.4 ± 10.3</td>
<td>70.0 -113.0</td>
</tr>
<tr>
<td><strong>Hip</strong></td>
<td>100.2 ± 6.0</td>
<td>88.5 - 112.0</td>
<td>101.3 ± 7.4</td>
<td>87.0 -119.0</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td>172 ± 6.75</td>
<td>155 – 192</td>
<td>179.4 ± 6.63</td>
<td>166 - 193</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.89 ± 0.06</td>
<td>0.73 – 0.99</td>
<td>0.89 ± 0.06</td>
<td>0.75 – 1.02</td>
</tr>
<tr>
<td><strong>WHtR</strong></td>
<td>0.52 ± 0.07</td>
<td>0.42 - 0.70</td>
<td>0.51 ± 0.06</td>
<td>0.38 - 0.64</td>
</tr>
<tr>
<td><strong>IHCL</strong></td>
<td>4.05 ± 7.68</td>
<td>0 – 48.07</td>
<td>3.37 ± 7.03</td>
<td>0 – 43.86</td>
</tr>
<tr>
<td><strong>S-IMCL</strong></td>
<td>12.77 ± 6.84</td>
<td>3.31 - 32.51</td>
<td>13.11 ± 6.89</td>
<td>3.10 – 38.97</td>
</tr>
<tr>
<td><strong>T-IMCL</strong></td>
<td>5.01 ± 2.34</td>
<td>1.08 - 11.69</td>
<td>6.10 ± 7.42</td>
<td>1.48 – 72.36</td>
</tr>
<tr>
<td><strong>%TAT</strong></td>
<td>26.9 ± 6.6</td>
<td>14.7 - 44.8</td>
<td>22.1 ± 7.4</td>
<td>7.8 – 43.8</td>
</tr>
<tr>
<td><strong>%SAT</strong></td>
<td>21.4 ± 5.4</td>
<td>12.2 – 35.1</td>
<td>17.0 ± 5.8</td>
<td>6.2 – 31.5</td>
</tr>
<tr>
<td><strong>%ASAT</strong></td>
<td>6.3 ± 2.2</td>
<td>2.8 – 12.0</td>
<td>4.7 ± 2.2</td>
<td>1.1 – 11.2</td>
</tr>
<tr>
<td><strong>%NASAT</strong></td>
<td>15.1 ± 3.5</td>
<td>8.9 - 23.4</td>
<td>12.3 ± 3.9</td>
<td>1.7 – 20.6</td>
</tr>
<tr>
<td><strong>%TIAT</strong></td>
<td>5.4 ± 1.9</td>
<td>2.4 – 9.7</td>
<td>5.1 ± 2.5</td>
<td>1.3 – 13.2</td>
</tr>
<tr>
<td><strong>%IAAT</strong></td>
<td>2.9 ± 1.3</td>
<td>0.7 – 6.0</td>
<td>2.7 ± 1.6</td>
<td>0.4 – 7.9</td>
</tr>
<tr>
<td><strong>%NAIAT</strong></td>
<td>2.6 ± 0.7</td>
<td>1.4 – 4.2</td>
<td>2.4 ± 1.0</td>
<td>1.0 – 6.2</td>
</tr>
<tr>
<td><strong>IAAT/ASAT</strong></td>
<td>0.47 ± 0.19</td>
<td>0.19 - 1.55</td>
<td>0.59 ± 0.27</td>
<td>0.18 - 1.54</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. Data analysed by one-way Anova: SAM: South Asian Male; CM: Caucasian Male; AF. Fat stores are presented as a percentage of total body weight. WC – waist circumference; WHR – waist-to-hip ratio; WHtR - waist-to-height ratio; IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data were analysed as log transformed data; TAT - total adipose tissue; SAT – subcutaneous; ASAT - subcutaneous abdominal; NASAT - subcutaneous non-abdominal; TIAT - Total internal; IAAT - Intra-abdominal adipose tissue; NAIAT - Non-abdominal internal; IAAT/ASAT (L). All data are presented as mean ± SD.
Female South Asian vs Female Caucasian comparison

Age and BMI matched groups of South Asian and Caucasian were compared. As with the previous comparison in male participants, female Asians were significantly shorter (p=0.016) than their Caucasian counterparts, although interestingly there were no differences in weight in the female group. Unlike the findings in the Male comparison, several significant differences could be detected between South Asian and Caucasian female participants using standard anthropometric measurements. Waist circumference, WHR and WTHR were all significantly greater in the South Asian female group. Again there were significant differences in adiposity measurable by MRI, and as in the male comparison, the differences were restricted to the subcutaneous depots. Female South Asian participants had significantly higher levels of TAT, SAT, ASAT and NASAT (p<0.001) compared to Caucasian females.

There were no significant differences in IMCL in either the soleus or tibialis muscles. However, as in the male participants, South Asian females had significantly higher levels of ectopic fat deposited within their livers (p=0.037).
Table 6.3. Comparison between South Asian and Caucasian Female participants.

<table>
<thead>
<tr>
<th></th>
<th>South Asian Female (n=19)</th>
<th>Caucasian Female (n=93)</th>
<th>SAF vs CF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age</td>
<td>33.11 ± 10.0</td>
<td>21 - 49</td>
<td>32.18 ± 9.07</td>
<td>21 - 49</td>
</tr>
<tr>
<td>Weight</td>
<td>65.7 ± 14.8</td>
<td>44 - 93.3</td>
<td>67.2 ± 11.9</td>
<td>47.7 – 105.1</td>
</tr>
<tr>
<td>BMI</td>
<td>25.6 ± 5.1</td>
<td>18.1 - 34.7</td>
<td>24.5 ± 4.0</td>
<td>18.6 - 34.5</td>
</tr>
<tr>
<td>WC</td>
<td>85.9 ± 9.8</td>
<td>68.6 - 98.0</td>
<td>78.8 ± 10.8</td>
<td>62.3 – 114.4</td>
</tr>
<tr>
<td>Hip</td>
<td>100.9 ± 10.7</td>
<td>84.5 - 117.5</td>
<td>100.3 ± 8.6</td>
<td>85.0 - 119.0</td>
</tr>
<tr>
<td>Height</td>
<td>160.5 ± 5.4</td>
<td>152.5 – 171</td>
<td>165.6 ± 6.97</td>
<td>145.5 - 182</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84 ± 0.06</td>
<td>0.77 - 0.98</td>
<td>0.79 ± 0.06</td>
<td>0.61 - 0.99</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.54 ± 0.06</td>
<td>0.44 - 0.61</td>
<td>0.48 ± 0.06</td>
<td>0.38 - 0.66</td>
</tr>
<tr>
<td>IHCL</td>
<td>2.61 ± 5.13</td>
<td>0 – 17.97</td>
<td>0.78 ± 1.98</td>
<td>0 – 16.7</td>
</tr>
<tr>
<td>S-IMCL</td>
<td>10.95 ± 7.85</td>
<td>3.45 - 38.38</td>
<td>10.40 ± 5.63</td>
<td>2.8 – 39.2</td>
</tr>
<tr>
<td>T-IMCL</td>
<td>6.88 ± 2.97</td>
<td>2.40 – 10.79</td>
<td>6.4 ± 3.6</td>
<td>0.96 – 19.1</td>
</tr>
<tr>
<td>%TAT</td>
<td>38.8 ± 7.0</td>
<td>24.0 - 51.0</td>
<td>34.8 ± 8.0</td>
<td>20.3 - 54.5</td>
</tr>
<tr>
<td>%SAT</td>
<td>33.5 ± 6.3</td>
<td>21.2 - 44.7</td>
<td>30.0 ± 6.9</td>
<td>17.0 – 47.3</td>
</tr>
<tr>
<td>%ASAT</td>
<td>9.4 ± 2.6</td>
<td>4.8 – 15.3</td>
<td>8.3 ± 2.8</td>
<td>3.0 – 15.8</td>
</tr>
<tr>
<td>%NASAT</td>
<td>24.7 ± 4.0</td>
<td>16.4 - 30.7</td>
<td>21.7 ± 4.5</td>
<td>13.6 - 36.6</td>
</tr>
<tr>
<td>%TIAT</td>
<td>5.3 ± 1.8</td>
<td>2.8 – 10.3</td>
<td>4.8 ± 2.0</td>
<td>1.9 - 11.6</td>
</tr>
<tr>
<td>%IAAT</td>
<td>2.5 ± 1.1</td>
<td>1.0 – 5.3</td>
<td>2.2 ± 1.3</td>
<td>0.7 - 6.4</td>
</tr>
<tr>
<td>%NAIAT</td>
<td>2.8 ± 0.8</td>
<td>1.8 – 5.1</td>
<td>2.6 ± 0.9</td>
<td>1.1 – 6.7</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.26 ± 0.11</td>
<td>0.14 - 0.55</td>
<td>0.28 ± 0.15</td>
<td>0.09 - 0.97</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. Data analysed by one-way Anova: South Asian Female; CF: Caucasian Female. Fat stores are presented as a percentage of total body weight. WC – waist circumference; WHR – waist-to-hip ratio; WHtR - waist-to-height ratio; IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data were analysed as log transformed data; TAT - total adipose tissue; SAT – subcutaneous; ASAT - subcutaneous abdominal; NASAT - subcutaneous non-abdominal; TIAT - Total internal; IAAT - Intra-abdominal adipose tissue; NAIAT - Non-abdominal internal; IAAT/SAAT (L). All data are presented as mean ± SD.
Correlation Analysis

Correlation analysis of Asian Indian male data resulted in a similar pattern of $r$ values to that seen in Caucasian counterparts, with WTHR and WHR the best representatives of adiposity stores (Chapter 4, Table 4.3). Unlike Caucasian males, abdominal adipose stores in South Asian males were most strongly correlated to WTHR rather than WC. There was a large reduction in correlation between BMI and internal fat stores (%TIAT, %IAAT, %NAIAT) in South Asian women, with age providing the best marker for these adipose deposits (Table 6.4).
Table 6.4. Pearson correlation analysis between anthropometric measurements, lipid stores and percentage body fat stores in South Asian male participants.

<table>
<thead>
<tr>
<th>MALE SOUTH ASIAN</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECOTOPIC FAT STORES</th>
<th>PERCENTAGE ADIPOSITY STORES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 49</td>
<td>Age</td>
<td>Weight</td>
<td>BMI</td>
</tr>
<tr>
<td>Weight</td>
<td>0.526**</td>
<td>0.456*</td>
<td>0.895**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.456**</td>
<td>0.393**</td>
<td>0.828**</td>
</tr>
<tr>
<td>WC</td>
<td>0.696**</td>
<td>0.895**</td>
<td>0.934**</td>
</tr>
<tr>
<td>Hip</td>
<td>0.279</td>
<td>0.910**</td>
<td>0.927**</td>
</tr>
<tr>
<td>Height</td>
<td>0.358*</td>
<td>0.554**</td>
<td>0.213</td>
</tr>
<tr>
<td>WHR</td>
<td>0.780**</td>
<td>0.796**</td>
<td>0.701**</td>
</tr>
<tr>
<td>WHIR</td>
<td>0.660**</td>
<td>0.856**</td>
<td>0.855**</td>
</tr>
<tr>
<td>IHCL</td>
<td>0.382*</td>
<td>0.614**</td>
<td>0.557**</td>
</tr>
<tr>
<td>S-IMCL</td>
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<td>0.532**</td>
<td>0.529**</td>
</tr>
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<td>0.441**</td>
<td>0.344*</td>
<td>0.279</td>
</tr>
<tr>
<td>%TAT</td>
<td>0.286</td>
<td>0.584*</td>
<td>0.640**</td>
</tr>
<tr>
<td>%SAT</td>
<td>-0.067</td>
<td>0.553**</td>
<td>0.629**</td>
</tr>
<tr>
<td>%ASAT</td>
<td>0.048</td>
<td>0.624**</td>
<td>0.729**</td>
</tr>
<tr>
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<td>0.469**</td>
<td>0.521**</td>
</tr>
<tr>
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<td>0.28</td>
</tr>
<tr>
<td>%IAAT</td>
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<td>0.362*</td>
</tr>
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<td>0.135</td>
<td>0.119</td>
</tr>
<tr>
<td>NA/SA</td>
<td>0.691**</td>
<td>0.052</td>
<td>0.001</td>
</tr>
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</table>

Abbreviations: WC – waist circumference; WHR - waist-hip ratio; WHIR - waist-to-height ratio; IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S-soleus, T-tibialis); Fat stores were analysed as a percentage of body-weight; TAT - total adipose tissue, SAT - subcutaneous, ASAT - subcutaneous abdominal, NASAT - subcutaneous non-abdominal, TIAT - Total internal, IAAT - Intra-abdominal adipose tissue, NAIAT - Non-abdominal internal, IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S-soleus, T-tibialis); IHCL and IMCL data are presented as the logarithm of MRS readings; IA/SA = IAAT/ASAT; Bold typeface indicates a significant correlation; The shaded boxes indicate the anthropometric variable which most highly correlates with individual ectopic fat or adiposity stores; * = p<0.05, ** = p<0.01.
Table 6.5 Pearson correlation analysis between anthropometric measurements, lipid stores and percentage body fat stores in South Asian female participants.

<table>
<thead>
<tr>
<th>FEMALE SOUTH ASIAN</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>EC TOPIC FAT STORES</th>
<th>PERCENTAGE ADIPOSITY STORES</th>
</tr>
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<tr>
<td></td>
<td>n = 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.015</td>
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<tr>
<td>BMI</td>
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</tr>
<tr>
<td>WC</td>
<td>0.16</td>
<td><strong>0.755</strong></td>
<td><strong>0.855</strong></td>
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<tr>
<td>Hip</td>
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<td><strong>0.947</strong></td>
<td><strong>0.934</strong></td>
</tr>
<tr>
<td></td>
<td><strong>0.865</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.051*</td>
<td>0.252</td>
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<tr>
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</tr>
<tr>
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<td>0.283</td>
<td>-0.023</td>
<td>-0.395</td>
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<tr>
<td>WHR</td>
<td>0.341</td>
<td>0.578*</td>
<td>0.789**</td>
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<tr>
<td></td>
<td><strong>0.952</strong></td>
<td><strong>0.774</strong></td>
<td>-0.464</td>
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<tr>
<td></td>
<td>0.372</td>
<td></td>
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<tr>
<td>IHCL</td>
<td><strong>0.617</strong></td>
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<td>0.32</td>
</tr>
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<td></td>
<td>0.256</td>
<td>-0.208</td>
<td>-0.289</td>
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<td></td>
<td>0.598</td>
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<td>S-IMCL</td>
<td>0.48</td>
<td>0.174</td>
<td>0.308</td>
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<tr>
<td></td>
<td>0.418</td>
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<td>-0.388</td>
</tr>
<tr>
<td></td>
<td>-0.387</td>
<td>0.548</td>
<td></td>
</tr>
<tr>
<td>%TAT</td>
<td><strong>0.520</strong></td>
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<tr>
<td>%SAT</td>
<td>0.161</td>
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<td><strong>0.897</strong></td>
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<tr>
<td></td>
<td><strong>0.605</strong></td>
<td><strong>0.865</strong></td>
<td>-0.057</td>
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<tr>
<td></td>
<td><strong>0.244</strong></td>
<td>0.551</td>
<td></td>
</tr>
<tr>
<td>%ASAT</td>
<td>0.237</td>
<td><strong>0.856</strong></td>
<td><strong>0.920</strong></td>
</tr>
<tr>
<td></td>
<td><strong>0.898</strong></td>
<td><strong>0.891</strong></td>
<td>-0.106</td>
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<tr>
<td></td>
<td><strong>0.863</strong></td>
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<tr>
<td>%NASAT</td>
<td>0.109</td>
<td><strong>0.787</strong></td>
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<tr>
<td></td>
<td>0.358</td>
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<tr>
<td>%TIAT</td>
<td><strong>0.794</strong></td>
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</tr>
<tr>
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<td>-0.341</td>
<td>-0.371</td>
<td>0.404</td>
</tr>
<tr>
<td></td>
<td>0.073</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%IAAT</td>
<td><strong>0.828</strong></td>
<td>0.128</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>0.352</td>
<td>0.168</td>
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</tr>
<tr>
<td>%NAIAT</td>
<td><strong>0.523</strong></td>
<td>0.101</td>
<td>-0.292</td>
</tr>
<tr>
<td></td>
<td>-0.642*</td>
<td>-0.256</td>
<td>0.367</td>
</tr>
<tr>
<td></td>
<td>-0.221</td>
<td></td>
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<tr>
<td>IA/SA</td>
<td><strong>0.778</strong></td>
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<td>-0.137</td>
</tr>
<tr>
<td></td>
<td>-0.213</td>
<td>-0.453</td>
<td>-0.348</td>
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<td>0.343</td>
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<td>0.395</td>
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<td></td>
<td>0.189</td>
<td>-0.07</td>
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<tr>
<td></td>
<td>-0.091</td>
<td><strong>0.854</strong></td>
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</tr>
<tr>
<td></td>
<td><strong>0.557</strong></td>
<td></td>
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</tbody>
</table>

Abbreviations: WC – waist circumference; WHR - waist-hip ratio; WHtR - waist-to-height ratio; IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S-soleus, T-tibialis); Fat stores were analysed as a percentage of body-weight; TAT - total adipose tissue, SAT - subcutaneous, ASAT - subcutaneous abdominal, NASAT - subcutaneous non-abdominal, TIAT - Total internal, IAAT - Intra-abdominal adipose tissue, NAIAT - Non-abdominal internal, IHCL - intrahepatocellular lipid; IMCL - intramyocellular lipid (S - soleus, T - tibialis); IHCL and IMCL data are presented as the logarithm of MRS readings; IA/SA - IAAT/ASAT; Bold typeface indicates a significant correlation; The shaded boxes indicate the anthropometric variable which most highly correlates with individual ectopic fat or adiposity stores; * = p<0.05, ** = p<0.01.
TOFI Analysis

The mean IAAT/ASAT ratio was significantly higher in Caucasian males compared to that measured in South Asian participants (IAAT/ASAT: Caucasian male 0.59 ± 0.27 vs. Asian male: 0.47 ± 0.19 p=0.0077), despite the range being similar in both groups. There were no significant differences in IAAT/ASAT between South Asian and Caucasian female participants. Interestingly, applying the TOFI cut-off discussed in Chapter 4 to this South Asian population, only one female and no male participants would be defined as TOFI.
6.4. Discussion

Ethnic effects
South Asian Indian men and women have a higher incidence and mortality rate from CVD than Caucasians\textsuperscript{211,217}. The pathology of this increased susceptibility is yet to be fully elucidated; however for any proposed value of BMI, South Asians have a higher degree of adiposity, abdominal obesity and a lower muscle mass than Caucasians\textsuperscript{443}. This was confirmed in this study where I showed that age and BMI matched male and female South Asian participants had significantly higher levels of subcutaneous adipose tissue compared to a Caucasian population. The mechanism behind this apparent increase in adiposity is unclear, although there have been studies which would suggest that these differences occur as early as infancy with South Asian newborn infants demonstrating increased fat deposition in all abdominal adipose compartments compared to Caucasian infants\textsuperscript{444}. British South Asian children (10-11 years old)\textsuperscript{445,446} and adolescents (13-16 years old)\textsuperscript{447,448} have also been found to be significantly more insulin resistant than Caucasians despite similar adiposity levels, further supporting early risk development prior to obesity.

These findings are in agreement with previous body composition studies using computerised tomography (CT) which have shown that South Asian Indian men have lower muscle mass and more body fat than Caucasian counterparts\textsuperscript{442}. Interestingly although the authors report that visceral fat is high in their South Asian population, they didn’t directly compare it with measurements from a Caucasian population. Their assertion that the increased insulin resistance in South Asians is related to increased visceral adipose tissue may therefore be incorrect. Indeed I found a significant increase in total percentage adiposity and subcutaneous depots in South Asians compared Caucasians counterparts, but no significant differences in internal adipose tissue depots. This suggests that the differences in adiposity between Caucasian and South Asians are limited to subcutaneous fat deposits as opposed to internal adipose stores. Therefore, the increased insulin resistance and metabolic risk in South Asians does not appear to be linked to an internal adiposity, suggesting ethnic differences in the pathogenicity of individual adipose stores.

A greater understanding the changes in body composition is essential if we are to understand the mechanism behind the increased metabolic risk in this population. The rapid increase in the
prevalence of type 2 diabetes and cardiovascular disease in India and in South Asian migrant populations has been associated with rapid urbanization, dietary changes and increasing obesity. Type-2 diabetes in South Asians is also accompanied with a higher risk of complications, particularly cardiovascular and renal, compared to Caucasians.

Although the research in this thesis does not address the underlying mechanism responsible for type-2 diabetes in the South Asian population, the results suggest that research should perhaps refocus from visceral to the involvement of subcutaneous fat as a potential basis. Indeed, data published by Chandalia et al. revealed South Asian male participants have larger subcutaneous abdominal adipocytes compared to age and weight matched Caucasian controls which was negatively correlated with glucose disposal.

Another potential mechanism may well be related to the elevated levels of liver fat found in both male and female South Asians compared to the Caucasian controls. Indeed it has been suggested that in the development of insulin resistance and type 2 diabetes, accumulation of lipids outside the classical adipose tissue depots may be the critical factor. As previously mentioned (Chapter 1) lipid accumulation in non-adipose cells such as the liver may impair the normal housekeeping function through a process known as ‘lipotoxicity’. Ectopic storage of excess lipids in the liver, pancreas and muscle has been proposed as the causative link between fat distribution and the metabolic syndrome.

There were no ethnic differences in IMCL in male participants, and no difference in S-IMCL in female participants, which would suggest that perhaps IMCL is not a key mechanism. Indeed Forouhi et al. reported that while there was an association between IMCL and insulin sensitivity and obesity in Caucasians participants, consistent with the idea that IMCL mediates the effect of obesity on insulin sensitivity, this is not the case in Asian participants. They suggested that in the absence of a relationship between insulin sensitivity and IMCL in South Asians another mechanism must underlie the high insulin resistance observed in this population.

An alternative mechanism is the elevated IHCL observed in my Asian population. Recent studies have shown that 25–50% of patients with fatty liver subsequently became diabetic, suggesting that hepatic steatosis may have a direct causative effect in the development of diabetes. This steatosis has
also been linked to the development of CVD\(^{472}\), suggesting that non-alcoholic fatty liver disease is an independent risk factor for metabolic disease. There have been several studies assessing the incidence of fatty liver in South Asian populations\(^{473-475}\), which have reported an incidence of NAFLD from 8.7% (Kolkata, India) to as high as 32% (Chennai, India) and 32.6% (Ragama, Sri Lanka).

Despite elevated IHCL being a feature of the TOFI phenotype, it appeared that TOFI classification was not useful in identifying ‘at risk’ participants within the Asian population, in part because ‘disproportional’ deposition of visceral adipose tissue does not appear to be a key component of the Asian phenotype. Perhaps measurements of IHCL alone would be sufficient to identify individuals ‘at risk’ of developing metabolic disease within the Asian population, although prospective longitudinal studies would be essential to determine if IHCL was a driving factor, or secondary to the development of impaired glucose tolerance and related metabolic changes.

The hypothesis for this current study: South Asians have greater internal and ectopic adipose stores than age and BMI matched Caucasians of the same gender is rejected as not all aspects were true.

The hypothesis for this current study: the established TOFI index can identify South Asian MONW individuals with adverse fat distribution within the normal BMI range (18.5<25 kg/m\(^2\)) is rejected.

Differences in body composition in male participants in particular were not readily measurable using anthropometric measurements. Given the significant differences in abdominal subcutaneous adipose tissue content, it is somewhat surprising that these could not be detected from measures of waist circumference. This reinforces the need for accurate direct measurements of body composition for studies of this nature. The hypothesis for this current study: established proxy (anthropometric) measures are able to successfully identify South Asian MONW individuals is rejected.
Chapter 7. Summary

The original stimulus for the present thesis was the observation that in the health, medical and insurance industries, body mass index (BMI) can be an ambiguous diagnostic for obesity in that it can potentially classify a power athlete as obese and also indicate a normal body weight individual as healthy when they that may actually be at significant risk of metabolic dysfunction. Unanswered questions surrounding the accuracy of industry standard body composition measuring devices as well as how being obese and fit might be acceptable captured the imagination and provided a focus for this work to be carried out.

The work central to the thesis sought to add further insight in this area by investigating the variation of body fat distribution in healthy volunteers using whole body MRI techniques. A series of studies were undertaken to explore further the relationship between BMI and adiposity in the adult.

Findings from the present thesis have demonstrated that:

1. When whole cohort data were compared to MRI adiposity data there was no significant difference between the measures derived. However when the cohort was divided by ethnicity (Asian vs Caucasian) differences were more apparent. Caucasian adiposity was overestimated by up to 3% and Asian adiposity was underestimated by up to 11%. Bodpod would be best suited to measuring Asian adiposity and BIA devices would be best suited to measuring Caucasian adiposity.

2. An abdominal transimpedance device (ViScan) based in BIA technology developed for measuring abdominal adiposity was tested. The Viscan measurement system was not able to conclusively measure intra-abdominal adipose tissue (IAAT) adiposity in obese males and females. It appeared to be better at measuring abdominal subcutaneous adipose tissue (ASAT). Additionally, the ViScan measurements appeared to be influenced by organ volumes – in particular the liver.
3. The ‘Thin on the Outside – Fat on the Inside’ (TOFI) phenotype can be defined using the ratio of IAAT and ASAT (IAAT/ASAT). The resulting TOFI index provides a quantitative means of comparing intra-abdominal fat deposition and thereby identifying “at risk” individuals. In Caucasians, cut-off values of >1.0 in males and >0.45 in females are proposed for TOFI definition. Additionally, anthropometric measurements such as waist circumference (WC) and waist to height ratio (WHtR) are not appropriate for classifying the TOFI phenotype. This is because these surrogates generally correlated more with total and subcutaneous adipose tissue stores than internal or ectopic depots.

4. IAAT and liver fat are lower in men who are fat, fit and active than in men who are fat, unfit and inactive. These ‘metabolically healthy’ individuals have the capacity to store excess fat in insulin-sensitive abdominal subcutaneous adipose tissue (ASAT) and this may help explain why the risk of chronic disease is lower in the ‘fat-fit’ than the ‘fat-unfit’. As a consequence, aerobic activity and the pursuit of physical fitness may be more appropriate goals in the battle against chronic disease than weight loss.

5. Asian Indian males were found to be significantly ‘fatter’ with significantly higher subcutaneous fat depots compared to similar Caucasian males. Given the increased metabolic risks seen in the Asian population, increased IAAT measures were not found to be significantly higher. If these results could be replicated in a larger cohort, there might be merit in assuming that the metabolic risks this group are prone to may be linked to dysfunctional ASAT and not IAAT as was initially thought. An exercise study with metabolic data would be beneficial in testing this theory. Additionally, the TOFI classification was not useful in identifying ‘at risk’ individuals in the Asian group. Also, waist circumference measurements did not identify Asian males that had significantly elevated ASAT. However, elevated liver fat stores were seen in Asian males and females compared to Caucasians. Liver fat may therefore be a potential ‘at risk’ identifier in this ethnic group.
Obesity is a complex, multi-faceted issue that is not fully accounted for in the simplicity of the BMI. There are issues in many BMI related population studies. In particular, BMI, and other anthropometric proxies (WC, WHR) do not measure body fat directly, which questions the validity of these methods. Further error is introduced with some of these proxies – for instance Wang\textsuperscript{339} reported that he found 14 different descriptions of the waist measurement site.

Another major limitation in BMI related population studies is their use of different gold standards for the definition of excess adiposity. As shown in this thesis, it is clear that some techniques to measure body composition are suboptimal for measuring adiposity. For this reason MRI and other high quality (and high cost) imaging methods are still the best method for health risk based research. While beyond the remit of this thesis, DXA for example cannot accurately differentiate internal fat stores (IAAT from ASAT) and has an additional radiation exposure risk.

BMI may be an inexpensive, non-invasive measure of obesity for predicting the risk of related complications, but its accuracy is limited by its dysregulation with adiposity. While obesity means excess body fat, the current definition of obesity using BMI is based on body weight regardless of its composition. The studies in this thesis have highlighted that fact that there are several different sub-populations of individuals for whom BMI does not tell the whole story. The Fat-Fit, the TOFI and the Asian Indian are specific phenotypic examples of these sub-populations. This is evidence of the fact that BMI should not be considered as the only measure of obesity.
7.1. Limitations (What went wrong)

Recruitment.

During the research for this thesis there were limitations. One of the most significant of these was the lack of South Asian volunteers. Whilst study 1 was limited by a small sample size (N= 21) the lack of Asian participants is not a new issue. Other research teams have identified this as a potential obstacle. To put this in context, whilst was recruiting for my studies I was fortunate enough to be working as part of a team that had significant media exposure based on the MR adiposity work being performed. TV and journalist exposure to the work was very helpful for marketing purposes. Any articles written or TV shows screened would carry a contact detail for the team. This was a deliberate move to develop a database of willing participants for the research group. I developed a simple web based response questionnaire that could be automated and completed by prospective volunteers. The information would populate our database with relevant information, including a metal check, magnet suitability etc.

When this was interrogated regularly the percentage of South Asian respondents was approximately 4%. Of that 4%, 50% would lose contact after first follow-up phone call to discuss testing dates availability, and a further 50% again would either not be available or change their mind. From 2000 respondents we would invariably get 2-4 successful South Asian tests.

Contact with BBC Asian radio to increase public interest of our recruitment and to engage with the South Asian community never paid dividends.

Distribution of Asian specific volunteer flyers at solely South Asian events (e.g. London & Birmingham Mela festivals) failed to stimulate new volunteers to come forward.

Contact with local places of worship to help engage the community elders etc was unsuccessful, as was contacting and visiting South Asian GP clinics.

In Chapter 2, I highlighted the additional problems with recruiting to the multiple 2-C techniques comparison study, in which UWW and BodPod both presented significant obstacles to recruitment of volunteers. An unwillingness / discomfort with being a confined space for the measurements as well as being in a state of undress (swimwear etc) were the main issues.
Funding

Several charities (including a South Asian charity) were approached for research funding in response to their annual bid announcements. The funds were to enabling payment for volunteers in case that would encourage a greater project take-up. Unfortunately none of these were successful.

Documentation.

Diet diary completion was an area where participants became less compliant. It is well known that 7 day food diaries are more accurate for assessing all 3 macronutrients (CHO, fats, proteins) than 3 day diaries; but the reality is that participants get bored after 3 days and tend to forget to complete the rest usually.
7.2. Future Directions

The relatively small number of participants included in the comparison of 2-C measurement techniques made generalization of results beyond the study limited to a small BMI range. It would be desirable to have a much larger cohort that represented obese individuals too as well as other ethnicities. This would help in the development of better ethnic specific algorithms for these 2-C devices.

Asian adiposity and health risk appears to be significantly more complex than just BMI and adipose stores. Since the onset and progression of diabetes and other obesity-related diseases are also linked to family history and ethnicity, the larger picture of genetic influence in this group requires greater detail of metabolic data included to support the adiposity findings in this thesis. This should also be accompanied by genetic markers a well if possible. Some potential candidates might include blood samples for: Cholesterol, Triglycerides, Cholesterol (Total, HDL, LDL), Glucose, Insulin  HOMA-IR, C-Peptide, PAI-1, IL-18 (plasma interleukin-18) linked to insulin resistance, Adiponectin (serum) - HMW (high molecular weight) 12- to 18-mer, Leptin, ALT, A1C (%) - Glycosylated Haemoglobin, TFTs, SHBG, Apolipoprotein A and B.

The participant of body build was mentioned as a potential influencing factor in conjunction with 2-C measurements (Chapter 3). The addition of additional measurements to BMI has been muted. The possibility of combining somatotype (accurate body geometry) data with adiposity measures may yield greater predictive accuracy than BMI. Also, since adiposity measures are a 'snapshot' of the individuals status at the time of their scan, there is often a lack of historical information regarding the individuals, dietary habits, activity levels, birthweight etc., that might give a better indication of how they came to be the way they are.

Exercise is a validated way of reducing the risk of diabetes and coronary heart disease. While numerous studies have reported significant associations between fitness measures (physical activity and cardiorespiratory), insulin resistance and most other separate components of the metabolic syndrome, very few have quantified the resulting regional fat loss using MRI and none have involved the British South Asian population. Previous research measured the effect of regular aerobic exercise on visceral adiposity and showed (in females) that there was a preferential loss of visceral
adiposity. This area would benefit from a project assessing the dose response of regular moderate aerobic exercise / or high intensity, short duration exercise on intra-abdominal fat and total adiposity in a group of South Asians. With increased interest in exercise prescription for at risk groups, the Asian population has had little such attention or uptake.

A study of free-living activity monitoring (in conjunction with diet diaries) study would be effective in quantifying the degree of habitual activity, or lack of it, in South Asians. This could take the form of an intervention study baseline period using micro activity monitors (eg. Actiheart, actigraph etc). However these are expensive (approx. £500 each) and would therefore require funding support.
References


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Physiological study.

Boden, G. Effects of free fatty acids on gluconeogenesis and glycogenolysis. (MAGMA, 2001).


Thomas, E.L. Muscle triglyceride metabolism during alternating intensity exercise in humans. Rico


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Appendix 1
Information Sheet for Research Participants

Study title:
Characterising the functional phenotype of Asian Indian adiposity.

Invitation paragraph.
You are being invited to take part in a research study. Before you decide 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. Ask 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.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

We will be happy to let you have a copy of the leaflet entitled ‘Medical Research and You’ - published by Consumers for Ethics in Research (CERES). This leaflet gives further information about medical research and looks at some questions you may want to ask.

PART ONE

What is the purpose of this study?
The purpose of this study to measure the differences in regional body fat distribution in British South Asian and Caucasian males. We believe that British South Asian males’ abdominal fat (internal and subcutaneous) distribution is different from that of Caucasians and behaves differently (when an individual performs exercise) which has a direct effect on health. The fat content of the liver and calf muscle will be measured by magnetic spectroscopy (MRS), the total body fat by magnetic resonance imaging (MRI). This study is also part of an academic qualification (PhD).

Why have I been chosen?
You have been asked to participate because we believe you may be able to help us with this research as you are in good health, are not taking part in regular exercise and are a member of the ethnic groups being researched. You will be one of 180 patients participating in this study.

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 do decide to take part you are still free to withdraw at any time and without giving a reason. Also, your doctor may withdraw you from the study at any time if he/she feels it is not in your best interest. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What are the possible disadvantages and risks of taking part?
Some of the procedures in this study, such as the recording of your weight, height and blood pressure present no risk to you. Other procedures, such as the insertion of a needle into your arm in order to take blood samples, can cause mild discomfort. We will offer you local anaesthetic before inserting the needle in order to minimise this if necessary. It is also possible to develop a small amount of bruising at the site of insertion of the needle; to minimise this we will apply firm pressure to your arm for 2-3 minutes after removing the needle.

Magnetic resonance imaging (MRI) does not involve any radioactivity or radiation and is free from side effects, provided that you are not wearing any metal jewellery or a watch (which can come loose or break in the magnet). You will be asked to remove your jewellery and watch before the scans take place. We will give you an alarm bell to sound at any time if you are upset or worried during the examination; and if the procedure does not suit you for any reason it can be stopped at any time.

What are the possible benefits of taking part?
The information we get from this study may help us to better understand ethnic differences in body fat distribution and hopefully identify effective exercise therapy guidelines to improve the health of those at risk within this ethnic group. This would have an enormous impact on the health and productivity of this population.

**Who will interpret the images?**
All the images from this study will be read by a qualified person, usually a radiologist, who holds a Hammersmith Hospital Trust clinical contract and is designated by the trust as competent to report that study. The only exception would be if you do not want such a qualified person to look at the images. If this is the case, please let us know on the consent form which you will be asked to sign. Images may also be analysed by other qualified persons for data analysis.

**What if new information becomes available during the study?**
Sometimes during the course of a research study, new information becomes available about the condition that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

**What happens when the research study stops?**
At the end of the research study we will thank you for your participation, answer any questions you may have, and let you know when we hope to be able to inform you of the results.

**What if something goes wrong?**
If you suffer an adverse event or deterioration in health as a result of your participation in this study, appropriate compensation will be paid to you through the Imperial College School of Medicine’s “No Fault” Compensation Scheme.

**PART TWO**

**What will happen to me if I take part and what will I have to do?**
All participants will be asked to attend the Robert Steiner Magnetic Resonance Imaging (MRI) Unit at Hammersmith Hospital on 1 occasion. Before attending you should fast for 10-12 hours overnight. You should not have anything to eat on the morning of the study and you should only drink water. Any tea, coffee or snacks will invalidate the results. We will ask you to refrain from drinking alcohol on the day before the study.

(i) **The assessments during the study visit will include:**
- A physical examination with measurements of body weight, blood pressure, body circumferences and a fitness assessment at each visit.
- Blood samples. These are taken to measure the levels of fats (lipids) in your blood and to look at chemical indicators of your health status. During the visit, approximately 12 teaspoonfuls of blood will be taken for analysis (after an overnight fast).
- During the study the total body fat content and the content of fat in the liver and in the calf muscle will be scanned by non-invasive, painless techniques. These MRI scans will be carried out at the Hammersmith Hospital.

**Main Visit:** At your visit to the MRI Unit (following a 10-12 hour overnight fast) you will have a short clinical assessment during which the study researcher will ask you a few questions about your general health (including the medications you normally take) and will measure your pulse, blood pressure, height, weight, waist and hip circumferences and skinfold thickness. A fasting blood sample will be taken for the measurement of cardiovascular risk factors (including cholesterol) and other lipoproteins (to estimate the contents of individual fatty acids in circulating fats), insulin and glucose. Some liver function tests will also be performed. The total amount of blood taken will be approximately 60 mL (about 12 teaspoons). All this will take about 20 minutes. With your permission (on one occasion only) we will also take a sample of DNA from blood or saliva to look for changes in your genes or chromosomes that may be involved in the how the body controls appetite, body
composition, fat and sugar levels. This will enable us to see what effect the changes may have on how and where your body stores fat. *(These samples will be under the guardianship of Professor J. Bell and will be stored for a maximum period of 10 years. During this period, as with all other study data, all personal details will be fully anonymised in compliance with medical research data protection policies.)*

We will perform magnetic resonance scans of your liver, calf and whole body. These scans are safe and do not involve any radioactivity or radiation. These scans may NOT be suitable for you if you suffer from claustrophobia. You will go into the magnet and will be asked to lie still for the study. You will hear an intermittent knocking noise during part of the procedure, and you will experience no discomfort. As this noise can be loud ear defenders will be provided for your comfort and safety. We will be able to talk to you while you are in the scanner through an intercom. We will give you an alarm buzzer to sound at any time in case you are upset or worried during the examination and if the procedure does not suit you for any reason it can be stopped at any time. The length of examination varies, but is generally about 40 minutes in total, including change of positions. Scans performed will include: (i) a liver MRS scan (approx’ 10mins), (ii) a leg MRS scan (approx’ 10 minutes), and (iii) a total body MRI scan (approx’ 20 minutes).

**Procedures for liver, calf muscle and total body fat scanning.**

Before starting the scanning we will ask you to complete a short checklist to identify whether you have any metal parts in your body (for example, metal heart valve, artificial joint, cardiac pacemaker etc.) which will stop us performing the scan.

- We will initially scan (MRS) your liver. For this you will be asked to lie on a scanner bed and then be moved into the magnet and asked to lie still for the study. The length of the examination varies, but is generally 10 minutes.
- Once the liver scan is complete, we will scan (MRS) your left calf muscle. You will remain lying on the bed and we will get you to place your left leg in a small coil. We will ensure you’re comfortable by placing some foam pads against your legs. You will still have the alarm buzzer to sound if you need us to attend to you for any reason. The scan will take approximately 10 minutes.
- Lastly we will scan your whole body to measure the amount and distribution of fat in your body, this generally takes 15-20 minutes. All care will be as with previous scans.

Following the scans we will ask you to perform a short (20 minutes maximum) exercise test on a stationary bicycle to measure your aerobic capacity. This procedure will involve you cycling at a set rate at increasing difficulties (every 2 minutes) while wearing a heart rate monitor to measure your heart rate and a (open ended) mouthpiece attached to a computer to measure the amount of air you breathe in and out. These combined measurements will tell us how well your heart and lungs are performing and how fit you are. A trained specialist (researcher) will be with you to ensure your safety and assist you. You will be free to stop the test at any stage if you feel discomfort or pain or simply do not wish to continue. Additionally the researcher may ask you to stop before you intend to if he feels for any reason you do not need to continue the test. This test may make you breathe hard and sweat slightly towards the end. After a brief rest (10 mins) you will proceed to the scanning area. *In some cases (if you prefer) it may be possible to perform this fitness test on another day before your scanning day if or any reason you are not able to do all measures in the one visit.*

During the study you will be asked to record and submit a 7 day food and activity diary (using forms provided) via email or by return post in prepaid envelopes provided. Telephone & email support will also be provided for you if needed.

**Will my taking part in this study be kept confidential?**

If you consent to take part in the study we may need access to your medical records. All information, which is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

We will inform your GP of your participation in this study. We will also inform all other doctors treating you (for example, hospital specialists), unless you have any objection to us doing so.
What will happen to the results of the research study?
When the study has been completed, we hope to be able to publish the results of the study in a scientific journal. Individual patients will not be identifiable from the information in any publication.

Who is organising and funding the research?
The study is organized and funded by the Medical Research Council (MRC).

Who has reviewed the study?
This research study has been reviewed by the Charing Cross Hospital Research Ethics Committee in accordance with local regulations.

Contact for Further Information

Mr. John McCarthy,
Robert Steiner MR Unit
Imaging Science Dept
MRC Clinical Sciences Centre
Hammersmith Hospital
Imperial College London
Du Cane Rd
London W12 0HS

john.mccarthy@imperial.ac.uk

Mob. 07910516422

You will be given a copy of this information sheet and a signed consent form to keep.

Thank you for taking the time to read this information sheet. We would appreciate it very much if you could help us with this important study.
Dear Mr J McCarthy*

**Full title of study:** Characterising the functional phenotype of Asian Indian adiposity in the young adult.

**REC reference number:** 06/Q0411/173

The REC gave a favourable ethical opinion to this study on 28 March 2007.

Further notification(s) have been received from local site assessor(s) following site-specific assessment. On behalf of the Committee, I am pleased to confirm the extension of the favourable opinion to the new site(s). I attach an updated version of the site approval form, listing all sites with a favourable ethical opinion to conduct the research.

**R&D approval**

The Chief Investigator or sponsor should inform the local Principal Investigator at each site of the favourable opinion by sending a copy of this letter and the attached form. The research should not commence at any NHS site until approval from the R&D office for the relevant NHS care organisation has been confirmed.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

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**06/Q0411/173 Please quote this number on all correspondence**

Yours sincerely,

Carli Sager
Committee Co-ordinator

Email: carli.sager@imperial.nhs.uk

Enclosure: Site approval form

Copy to: Prof J. Bell, Medical Research Council (MRC)
04 July 2008

Mr John McCarthy
Senior Lecturer - Sport & Exercise Physiology
University of Bedfordshire
School of P.E. and Sport Sciences
Park Square Campus
Luton, Beds.
LU1 3JU

Dear Mr McCarthy

Full title of study: Chacterising the functional phenotype of Asian Indian adiposity in the young adult.
REC reference number: 06/Q0411/173
SSA reference number: 08/H0309/56

Thank you for your application to conduct the above research as local Principal Investigator for University of Bedfordshire. We can confirm that the application was received on 04 July 2008.

An assessment of the suitability of the local Principal Investigator, site and facilities will be made by this Committee. We will notify the main Research Ethics Committee Charing Cross Research Ethics Committee within 25 days of receiving your application whether or not there is any objection to the research being conducted locally.

It is the responsibility of the main REC to confirm the favourable opinion for each research site, taking account of the advice from site-specific assessors. The main REC will notify the decision to the Chief Investigator for the study and provide a list of approved sites (on form SF1). It is the responsibility of the Chief Investigator to notify the local Principal Investigator at each site.

Yours sincerely

Mrs Jenny Austin
Committee Co-ordinator

Email: jenny.austin@nhs.net

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England
Charing Cross Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

<table>
<thead>
<tr>
<th>REC reference number:</th>
<th>06/Q0411/173</th>
<th>Issue number:</th>
<th>1</th>
<th>Date of issue:</th>
<th>17 July 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chief Investigator:</td>
<td>Mr John McCarthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full title of study:</td>
<td>Characterising the functional phenotype of Asian Indian adiposity in the young adult.</td>
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</tbody>
</table>

This study was given a favourable ethical opinion by Charing Cross Research Ethics Committee on 28 March 2007. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Post / Position</th>
<th>Research site</th>
<th>Site assessor</th>
<th>Date of favourable opinion for this site</th>
<th>Notes (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr John McCarthy</td>
<td>Senior Lecturer - Sport &amp; Exercise Physiology</td>
<td>University of Bedfordshire</td>
<td>Bedfordshire Research Ethics Committee</td>
<td>17/07/2008</td>
<td></td>
</tr>
</tbody>
</table>

Approved by the Chair on behalf of the REC:

[Signature]

[Name]

(1) The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.
Dear Dr Goldstone

Full title of study: Magnetic resonance imaging and spectroscopy for body composition analysis

REC reference number: 07/Q0411/19

Thank you for your letter of 26 June 2007, responding to the Committee's request for further information on the above research.

The further information has been considered on behalf of the Committee by the Chair.

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.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application</td>
<td></td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Investigator CV</td>
<td></td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Protocol</td>
<td>1</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Covering Letter</td>
<td></td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Letter from Sponsor</td>
<td></td>
<td>06 March 2007</td>
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</table>
Questionnaire: Int. Physical Activity

<table>
<thead>
<tr>
<th>Item</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter of invitation to participant</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>GP/Consultant Information Sheets</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Participant Information Sheet</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Participant Consent Form</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Response to Request for Further Information</td>
<td>26 June 2007</td>
</tr>
<tr>
<td>Check list for contra-indications</td>
<td></td>
</tr>
<tr>
<td>Dietary Card</td>
<td></td>
</tr>
<tr>
<td>Advert - Poster</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Advert - Newsletter</td>
<td>27 February 2007</td>
</tr>
<tr>
<td>Advert - Email</td>
<td>27 February 2007</td>
</tr>
</tbody>
</table>

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.


Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicalReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

07/Q0411/19 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

Chairman

Email: Twigley@hhnt.org

An advisory committee to London Strategic Health Authority
STUDY PROTOCOL:
Magnetic resonance imaging and spectroscopy for body composition analysis.

PRINCIPAL INVESTIGATOR
Dr. A.P. Goldstone

BACKGROUND

Increased body fat is associated with significant adverse effects on health. The associated risks of obesity include insulin resistance, type 2 diabetes mellitus, high blood pressure, cardiovascular disease and high blood fat levels. A cluster of metabolic changes are associated with obesity (‘syndrome X’): resistance to insulin-stimulated glucose uptake by muscle and liver, glucose intolerance, elevated insulin levels, increased blood triglyceride (fat) concentration, decreased high density lipoprotein (HDL)-cholesterol concentration and high blood pressure, that have their origin in insulin resistance.

High blood glucose and insulin levels, insulin resistance in blood vessels and high fat and cholesterol contribute to the development of heart disease, and hypertension, through effects on blood clotting, formation of fat deposits in blood vessels and the contraction of blood vessels.

Central or visceral obesity (in the abdomen around the internal organs), high amounts of liver or muscle fat are especially associated with increased insulin resistance, glucose intolerance, high lipids, heart disease and mortality. A number of products secreted by adipose tissue, including some known as ‘adipocytokines’, have been found to influence insulin action and affect blood vessels and blood clotting. They are therefore candidates for the link between increased adiposity, insulin resistance, sugar diabetes and heart disease.

These products released from fat cells into the blood include adiponectin, IL-6, tumour necrosis factor-alpha (TNFa), IL-1a, IL-8, MCP-1, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin, visfatin and retinol binding protein-4.

However the links between particular fat depots (visceral, muscle and liver), these circulating products and the development of insulin resistance has not yet been fully characterised. We do not know which of the various fat depots have the greatest effect on insulin resistance and blood markers of increased cardiovascular and metabolic risk. We do not know which regional fat depots are particularly associated with the development of diabetes mellitus.

Previous work by our Group has developed methods for whole body multi-slice human magnetic resonance (MR) imaging to quantitatively measure total and regional adipose volumes in adults to measure individual fat deposits, including...

visceral, muscle and liver fat content, in different diseases, patients with genetic abnormalities, on different diets and with different levels of physical activity. There is great variation in the fat distribution between individuals that cannot be easily predicted by their overall size.

For example we have found that non-diabetic adult females and males with Prader-Willi syndrome (PWS), a genetic disease causing profound hunger and obesity from childhood due to a defect on chromosome 15, have reduced visceral fat and surprisingly low insulin and blood fat levels. An increasing number of genetic studies have shown various gene markers and variations to be associated with obesity and crude measurements of fat distribution in the general population.

**OBJECTIVE**

The purpose of this cross-sectional study is to develop novel biomarkers for obesity, insulin resistance, diabetes mellitus and cardiovascular risk by:

(i) scanning a large cohort of control subjects, both with and without type 2 diabetes mellitus, of both gender and of various BMI, to measure total, visceral and subcutaneous, liver and muscle fat to allow correlation with blood measurements of factors involves in insulin resistance, metabolic and cardiovascular risk such as adipocytokines (including insulin, homeostasis model assessment of insulin resistance (HOMA-IR), leptin, adiponectin, IL-6, TNFa, IL-1a, IL-8, MCP-1, PAI-1, resistin, visfatin, retinol binding protein-4, CRP and lipids), collected when fasted and after drinking a sugary drink (oral glucose tolerance test);

(ii) using the genetic obesity syndrome, Prader-Willi syndrome (PWS), as a model of extreme obesity associated with abnormal fat distribution, to investigate whether their reduction in visceral fat, low insulin and blood fat are also associated with reduced liver and muscle fat, and altered blood levels of adipocytokines and other circulating markers of metabolic and heart disease risk, and examine if there are differences in those patients with PWS who have diabetes mellitus compared to those that do not;

(iii) examining whether abnormal fat distribution is seen in other patients with childhood-onset morbid obesity and genetic causes of obesity;

(iv) collecting and store DNA samples to allow analysis of the influence of genetic variants and mutations on total and regional adiposity.

**SUBJECTS**

Inclusion criteria

- Age 18-80
- Male or female
- Healthy volunteers; patients with impaired glucose tolerance, diabetes mellitus, cardiovascular disease, hyperlipidaemia or fatty liver; patients with genetic causes of obesity including Prader-Willi syndrome, or severe childhood-onset obesity.
- Normal weight, overweight and obese subjects
Exclusion criteria

- Medical or psychological condition or social circumstances which would interfere with ability to participate reliably in the study.
- Body mass index less than 16 kg/m$^2$ (underweight)
- Women who are pregnant.
- Patients with claustrophobia, as they may find the confined conditions within the MR scanner uncomfortable.
- Pacemaker, metal implant, clips, implanted device, shrapnel or bullet, metal in eyes that precludes magnetic resonance imaging.
- Patients with Cushing’s syndrome or untreated clinical hypothyroidism.

No. of subjects

580 in total: 300 non-diabetic, 200 diabetic, 40 Prader-Willi syndrome, 40 other genetic causes of obesity

**PROTOCOL**

Subjects will attend the Robert Steiner MRI Unit at the Hammersmith Hospital after an overnight fast. They will be asked to refrain from taking strenuous exercise and drinking alcohol for twenty-four hours before the visit.

**BLOOD SAMPLING**

Blood (up to 100ml in total) will be taken by venepuncture before and 2 hours after oral administration of a drink containing 75g glucose. Plasma and serum will be assayed for routine clinical chemistry, lipids (total, HDL and LDL cholesterol), triglycerides and free fatty acids, glucose, thyroid function; hormones, regulators of appetite, adipocytokines and markers of insulin resistance and blood clotting, including insulin, leptin, adiponectin, IL-6, TNFa, IL-1a, IL-8, MCP-1, PAI-1, resistin, visfatin, retinol binding protein-4, CRP and tPA-Ag. Assays will be performed by the Dept. of Chemical Pathology at Hammersmith Hospitals and by commercial kits for radio-immunoassay and ELISA, including the Lincoplex multiplexed immunoassay system.

**DNA SAMPLING**

Blood or saliva will be taken, with specific consent, to extract DNA to enable confirmation or exclusion of PWS and determination of molecular class by the appropriate regional cytogenetics laboratory (by SNRPN methylation and FISH analysis); examination of genetic abnormalities causing obesity (using array comparative genomic hybridisation for chromosomal abnormalities, and DNA sequencing of candidate genes such as MC4R, POMC, leptin receptor, SIM1); and polymorphisms or mutations associated with obesity, diabetes mellitus, fat distribution and body composition (using PCR based SNP analysis), in collaboration with Prof. Philippe Froguel, Dept. of Genomic Medicine, Imperial College, Hammersmith Hospital.

**ANTHROPOMETRY**

Baseline anthropometric measurements of height, weight, waist and hip
circumference, skinfold thickness, blood pressure, and bio-electrical impedance analysis of percentage body fat. The latter technique is a painless, safe procedure which involves either lying on a couch with sticky pads placed on a hand and foot, or standing on a metal platform for 1 minute so that the body's electrical resistance can be measured.

MAGNETIC RESONANCE SCANNING

Subjects will be in the MR scanner for up to 1 hour. Scanning will be performed on either the Philips 3.0 Tesla or Philips 1.5 Tesla MR scanners in the Robert Steiner MR Unit, or the Philips 3.0 Tesla research MR scanner in the Neonatal Unit, at the Hammersmith Hospital. None of the magnetic resonance imaging techniques to be used employs ionising radiation or intravenous contrast agents and all techniques mentioned may be performed during a single imaging session.

Subjects lie supine or prone in the scanner and are automatically moved through the scanner. While in the scanner volunteers will have access to a buzzer to sound an alarm, and will be able to hear and respond to instructions from the scanning console. Whole body anatomical MR scanning will be performed to determine total and regional fat volumes, and magnetic resonance spectroscopy (MRS) performed to measure lipid content in internal organs, such as liver (IHCL) and muscles (IMCL), such as soleus and tibialis.

QUESTIONNAIRES

Assessment of individual activity levels and dietary habits will be performed by completion of the International Physical Activity Questionnaire (IPAQ) and a 3 day food diary. Clinical information on medications, past medical history, family history of diabetes, obesity and cardiovascular disease, smoking and alcohol intake will also be recorded. This will enable assessment of the contribution from physical activity and diet towards body fat distribution, cardiovascular and metabolic risk.

OUTCOME MEASURES

PRIMARY OUTCOMES

Total, visceral and subcutaneous obesity, liver and muscle fat content.

SECONDARY OUTCOMES

Circulating bio-markers of insulin resistance, obesity and cardiovascular risk, including insulin, HOMA-IR, lipids, CRP, leptin, adiponectin, PAI-1, IL-6, resistin, visfatin, MCP-1, retinol-binding protein 4, tPA-Ag.

Reported average level of physical activity.

Reported 3 day caloric and macronutrient intake.
SAFETY AND PROTECTION OF VOLUNTEERS

The MRI scanner contains a very strong magnet. Therefore, individuals may not be able to have the MRI if they have any type of metal implanted in their body, for example, any pacing device (such as a heart pacer), any metal in their eyes, or certain types of heart valves or brain aneurysm clips.

There is not much room inside the MRI scanner. Some people may be uncomfortable if they do not like to be in close spaces ("claustrophobia"). This is therefore an exclusion criterion for recruitment to the study and they will have an opportunity to get used to the procedure during the dummy infusion. If, however, subjects experience discomfort within the scanner despite the measures taken to ensure patient comfort, the patient may request immediate cessation of the procedure with withdrawal from the scanner by ringing the patient alarm bell.

The MRI produces a “hammering noise” but subjects wear earplugs and headphones to prevent discomfort or damage to hearing.

The risks of placing a needle to draw blood from a vein include minor discomfort at the site of the puncture; possible bruising and swelling around the puncture site; rarely, infection or faintness during the procedure.

FINANCING AND INSURANCE

The study will be funded using core funding from the Molecular Imaging Group, Imaging Sciences Department, MRC Clinical Sciences Centre, and Section of Genomic Medicine, Imperial College at Hammersmith Hospital. All participants will be covered by the Imperial College "no fault" indemnity scheme.
Participant Consent Form

Title of Project:

**Characterising the functional phenotype of Asian Indian adiposity.**

Name of Researcher: MR JOHN McCARTHY

Please initial each box

I have read and understand the information sheet “for Research Participants” for the above study and have had the opportunity to ask questions.

I have received enough information about the study and satisfactory answers to all my questions.

I understand that sections of any of my medical notes and images may be looked at by responsible individuals from our research collaborators or from regulatory authorities where it is relevant to my taking part in research. I give my permission for these individuals to have access to my records and images.

I agree for a DNA sample to be taken and stored to look for changes that may be involved in obesity, diabetes, appetite, and how much fat and muscle is in the body.

I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting my future medical care.

I agree to take part in the above study.

__________________________  ________________  __________________
Name of Participant       Date            Signature

__________________________  ________________  __________________
Name of Person taking consent (if different from researcher) Date            Signature

__________________________  ________________  __________________
Researcher                  Date            Signature

1 for volunteer; 1 for researcher; 1 to be kept with hospital notes
Participant Safety Checklist Form

Please check the following list carefully, answering all appropriate questions. Please do not hesitate to ask staff, if you have any queries regarding these questions.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac pacemaker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any major surgery (cardiac bypass / orthopaedic, etc.)</td>
<td></td>
<td></td>
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<tr>
<td>Mechanical heart valve</td>
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<td></td>
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<td>History of foreign body in eye</td>
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<td></td>
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<tr>
<td>Occupation as metal worker, grinder, welder</td>
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<td></td>
</tr>
<tr>
<td>Metallic implant, metal prosthesis, orthopaedic plates, screws</td>
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<td>Aneurysm clip/haemostatic clip</td>
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<tr>
<td>Coloured contact lenses</td>
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<tr>
<td>Interventional radiological devices</td>
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<tr>
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<td>IUCD</td>
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<tr>
<td>Implantable pumps / neurostimulators</td>
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<td>Are you wearing a watch?</td>
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<tr>
<td>Are you wearing any jewellery?</td>
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<tr>
<td>Is there anything in your pockets such as keys, etc.?</td>
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<td></td>
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<tr>
<td>Are you susceptible to claustrophobia?</td>
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If the answer to any of the above questions is “YES” please give details.

NB: A device not known to be safe must be assumed to be unsafe. In the case of devices operated by microprocessors (e.g. implantable pumps) device malfunction caused by field effects or circuitry must be considered. Other devices such as ventricular shunts, orthodontic braces may be safe, but may degrade image quality significantly. Images may also be degraded by metal containing tattoos or cosmetics. (see AJR 1988; 151: 811)

I have understood the above questions and have marked the answers correctly.

Signed: ........................................... (participant)  Date: .....................

Signed: ........................................... (MR Unit Personnel)  Date: .....................
Patient Registration Form

Please complete this form and e-mail it to john.mccarthy@imperial.ac.uk

PATIENT DETAILS:

Surname: __________________________ First name: __________________________

Address: __________________________ Home telephone No: __________________________

Work telephone No: __________________________

Postcode: __________________________ Mobile telephone No: __________________________

Date of Birth: __________________________ Place of Birth: __________________________

Marital status: Single/Married/Widowed/Divorced/Separated (please circle)

Religion: __________________________

NHS number (if known): __________________________ Hospital number (if known): __________________________

E-mail address: __________________________

ETHNIC ORIGIN (Please circle letter of alphabet that applies):

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<th>Black or Black British</th>
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<tbody>
<tr>
<td>A British</td>
<td>M Caribbean</td>
</tr>
<tr>
<td>B Irish</td>
<td>N African</td>
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<tr>
<td>C Any other white background</td>
<td>P Any other black background</td>
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<td>H Indian</td>
</tr>
<tr>
<td>E White and Black African</td>
<td>J Pakistani</td>
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<tr>
<td>F White and Asian</td>
<td>K Bangladeshi</td>
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G Any other mixed background L Any other Asian background

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<th>Other Ethnic Groups</th>
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<tr>
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<tr>
<td>Name:</td>
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<td>Relationship to you:</td>
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| Address: |
| Home telephone No: |

| Work telephone No: |

| Postcode: |
| Mobile telephone No: |
PATIENT INFORMATION SHEET: FOR OBESE MEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Study Title: The pathogenesis of non-alcoholic fatty liver disease in obesity

Introduction

You are being invited to take part in a research study. Your participation in this study is entirely voluntary and your medical care will not be affected if you decide not to participate. Before you decide whether or not to participate 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 should you wish. Ask 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.

We will be happy to let you have a copy of the leaflet entitled ‘Medical Research and You’ published by Consumers for Ethics in Research (CERES). This leaflet gives more information about medical research and looks at questions you may want to ask.

What is the purpose of the study?

It is known that people with obesity have a greater risk of accumulating fat in their liver. The mechanism of fat accumulation is not known. In a small amount of cases such fat accumulation can lead to swelling and scarring of the liver.

The increased **fat accumulation in the liver** may be related to the rate at which the liver secretes fat and the way in which the body handles a hormone called insulin.

We do not know if the fat accumulation in the liver is out of proportion to the fat accumulation elsewhere. This study will help answer the above questions.

The study duration is 3 years but you will only be asked to attend on 4 occasions. These attendances will all be within a 12-week time period.

Why have I been chosen?

You have been asked to participate because we believe you may be able to help us with this research as you are in good health and of above average weight and **your ultrasound scan / liver biopsy showed you to have fat in your liver**. You will be one of 60 patients participating in this study.

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 do decide to take part you are still free to withdraw at any time and without giving a reason. Also, your doctor may withdraw you from the study at any time if he/she feels it is not in your best interest. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.
What will happen to me if I take part and what will I have to do?

You will be asked to attend the Metabolic Day Ward at St Mary’s Hospital on 3 occasions and the Robert Steiner Magnetic Resonance Imaging (MRI) Unit at Hammersmith Hospital on 1 occasion during a 12 week time period. Before each attendance at the Metabolic Day Ward, you should fast for 10-12 hours overnight. You should not have anything to eat on the morning of the study and you should only drink water. Any tea, coffee or snacks will invalidate the results. We will ask you to refrain from drinking alcohol on the day before the study.

**Visit 1:** At your first attendance to the Metabolic Day Ward, following a 10-12 hour overnight fast, you will have a short clinical assessment during which the study doctor will ask you a few questions about your general health (including the medications you normally take) and will measure your pulse, blood pressure, height, weight, waist and hip circumferences and skinfold thickness. A fasting blood sample will be taken for the measurement of cardiovascular risk factors (including cholesterol) and other lipoproteins (to estimate the contents of individual fatty acids in circulating fats), insulin and glucose. Some liver function tests will also be performed. The total amount of blood taken will be approximately 15 mls (about 3 teaspoons). All this will take about 20-30 minutes. You will then be given a snack and a drink.

**Visit 2:** Your second attendance to the Metabolic Day Ward will be for an intravenous glucose tolerance test (IVGTT). This study will examine the way your body handles the hormone insulin. You will be asked to come to the Metabolic Day Ward at St Mary’s Hospital in the morning for about three hours following a 10-12 hour overnight fast. A small plastic needle will be placed into a vein in each forearm. The right arm will be placed in a heated box that will warm up the right hand. Blood samples will be taken through the plastic needle in the warm arm and between 25-50mls of a concentrated glucose solution will be given through the plastic needle in the left arm. Small amounts of blood will be taken at regular intervals throughout the test (the total amount will be 120mls – less than one quarter of a pint). The blood samples will be taken over approximately three hours.

**Visit 3:** Your third attendance to the Metabolic Day Ward will be for the kinetic study. This will examine the rate at which fat is secreted by the liver. This is important because the increased fat accumulation in the liver in non-alcoholic fatty liver disease may be related to the rate at which the liver secretes fat. You will be asked to arrive on the Metabolic Day Ward at St Mary’s Hospital in the morning following a 10-12 hour overnight fast. You will be encouraged to include at least 150g carbohydrate in your diet during the three days prior to your visit (you will be advised how to do this) and should abstain from very strenuous exercise and excess alcohol for the same period. During the study day, you will be asked to remain lying on a bed throughout the 6-hour procedure and will remain fasting (you may consume water). A meal will be given to you on completion of the procedure. You will have two small cannulae (plastic needles), one in the forearm and one in the back of the hand, using some local anaesthetic and these will be the only needles involved (subsequent blood tests will be taken through one of the cannulae and will not therefore hurt). One of these cannulae will be used to infuse a solution containing the non-radioactive isotopes [1-13C]-leucine and [1-13C]-palmitate into your arm for 6 hours. [1-13C]-leucine is a specially tagged version of one of the building blocks of
protein normally present in your blood and is used to measure the lipid turnover. [1-13C]-palmitate is a fatty acid present in the blood and is used to measure the fatty acid turnover. Both have been many times and are very safe. The other intravenous cannula will be used to take regular blood samples every hour throughout the 6-hour infusion. This hand will be in a hot box which blows out hot air to maintain a temperature of 40°C. The amount of blood that will be taken if you participate in the study is 90 mls (less than one quarter of a pint). One of the blood tests, performed at the end of the study, involves an injection of a small amount of the anticoagulant heparin. At single small doses administered in this way, it is not known to have side effects.

Visit 4 (MRI): On one occasion you will be asked to attend the Robert Steiner MRI Unit at the Hammersmith Hospital after a 6 hour fast (you should not eat anything during this time but can drink water and take any medication you would normally take). We will perform magnetic resonance scans of your liver, calf and whole body. These scans are safe and do not involve any radioactivity or radiation. Before starting the scanning we will ask you to complete a short checklist. This will ensure that you do not have any magnetic implant (such as a cardiac pacemaker) which will stop us performing the scan. You will go into the magnet and will be asked to lie still for the study. You may hear a knocking noise during part of the procedure, and you will experience no discomfort. We will give you an alarm bell to sound at any time if you are upset or worried during the examination and if the procedure does not suit you for any reason it can be stopped at any time. The length of examination varies, but is generally about 40 minutes. Once the liver scan is complete, we will scan your left calf muscle. You will remain lying on the bed and we will get to place your left leg in a small coil. We will ensure you are comfortable by placing some foam pads against your legs. You will still have the alarm bell to sound if you need us for any reason. The scan will take approximately 20 minutes. You will then have a MR scan of your whole body to measure the amount and distribution of fat in your body. This scan will take approximately 30 minutes.

What are the possible disadvantages and risks of taking part?

Some of the procedures in this study, such as the recording of your weight, height and blood pressure present no risk to you. Other procedures, such as the insertion of cannulae (plastic needles) into your arm in order to take blood samples, can cause mild discomfort. We will offer you local anaesthetic before inserting the cannulae in order to minimise this. It is also possible to develop a small amount of bruising at the site of insertion of the cannulae; to minimise this we will apply firm pressure to your arm for 2-3 minutes after removing the cannulae.

Magnetic resonance imaging does not involve any radioactivity or radiation and is free from side effects, provided that you are not wearing any metal jewellery or a watch (which can come loose or break in the magnet). You will be asked to remove your jewellery and watch before the scans take place. We will give you an alarm bell to sound at any time if you are upset or worried during the examination and if the procedure does not suit you for any reason it can be stopped at any time.
What are the possible benefits of taking part?

The information we obtain from this study will further our understanding of non-alcoholic fatty liver disease, tell us more about the risks associated with accumulating fat in the liver, and help develop treatments for the condition. Yourself and other patients with non-alcoholic fatty liver disease may benefit from this in the future. If your liver tests are abnormal, or there is any evidence of any other liver disease, we will refer you to a specialist in liver disease for further investigation.

What if new information becomes available during the study?

Sometimes during the course of a research study, new information becomes available about the condition that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

What happens when the research study stops?

At the end of the research study we will thank you for your participation, answer any questions you may have, and let you know when we hope to be able to inform you of the results.

What if something goes wrong?

If you suffer an adverse event or deterioration in health as a result of your participation in this study, appropriate compensation will be paid to you through the Imperial College School of Medicine’s “No Fault” Compensation Scheme.

Will my taking part in this study be kept confidential?

If you consent to take part in the study we may need access to your medical records. All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

We will inform your GP of your participation in this study. We will also inform all other doctors treating you (for example, hospital specialists), unless you have any objection to us doing so.

What will happen to the results of the research study?

When the study has been completed, we will talk to and also write to you informing you of the results of the study. We hope to be able to publish the results of the study
in scientific journals. You will not be identifiable from the information in any publications.

Who is funding the research?

The research is being funded by the Novo Nordisk UK Research Foundation. The doctor conducting the research will not receive any payment for including you in this study.

Who has reviewed the study?

St Mary’s Local Research Ethics Committee has reviewed the study.

Who can I contact to find out more about the study?

If you have any further questions please contact:

Dr Sanjeev Mehta
Department of Endocrinology & Metabolic Medicine, 2nd Floor, Mint Wing, St Mary’s Hospital, London.
Tel: 020 7886 6120 (Work); 07961 839052 (Mobile)

You will be given a copy of this information sheet and a signed consent form to keep.

Thank you for taking the time to read this information sheet. We would appreciate it very much if you could help us with this important study.
CONSENT FORM

Title of Project: THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE IN OBESITY

Name of Researcher: DR SANJEEV R MEHTA

Please initial box

1. I confirm that I have read and understand the information sheet “For Obese Men With Non-Alcoholic Fatty Liver Disease (NAFLD)” for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from [company name] or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

_________________________  ___________________  ____________________
Name of Patient          Date                        Signature

_________________________  ___________________  ____________________
Name of Person taking consent (if different from researcher) Date                        Signature

_________________________  ___________________  ____________________
Researcher                Date                        Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes
Appendix 2
Asian Males

Free health scans

Volunteers (unpaid) needed:

- This cutting edge research into body fat and health is being done to measure ethnic differences in internal fat storage. This work may help identify and possibly limit future health problems in Asians - related to obesity, diabetes, high blood pressure and heart disease.

- Aged 20 - 40 yrs
- Average Fitness level
- Healthy & Inactive

When: Now (2009)

07910516422 / john.mccarthy@imperial.ac.uk

When: Now (2009)
Asian Males Needed

Free health scans

Volunteers needed:
This cutting edge research into body fat and health is being done to measure ethnic differences in internal fat storage. This work may help identify and possibly limit future Asian health problems related to obesity, diabetes, high blood pressure and heart disease.

To date very little work has been done with the Asian community - your help is greatly appreciated and needed.

You should be:
• Aged 20 - 40 yrs
• Average fitness level
• Healthy & Inactive

When: Now (2009)

Where: Hammersmith Hospital (White City Tube).

Time: 2 hour / visit

Other: Two hour visit for health screen = total body MRI scan, fitness test & diet analysis. Potential for follow-up study (exercise program option).

Contact:
07910516422 / john.mccarthy@imperial.ac.uk
<table>
<thead>
<tr>
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<th>D.O.E.</th>
<th>DVD #</th>
<th>REGION SCANNED</th>
<th>ETHICS #</th>
<th>PRINCIPAL INVESTIGATOR</th>
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**HOME ADDRESS**

_______________________________________________________

**POST CODE**

____________________________

**TELEPHONE (H) (M)**

____________________________

**EMAIL**

_______________________________________________________

**G.P. NAME**

____________________________

**ADDRESS**

_______________________________________________________

**POST CODE**

____________________________

**TELEPHONE**

____________________________

**STUDY**

_______________________________________________________

**Checklist**

- Reported
- Anthropometry
- BVI
- ViScan
- Saliva
- Bloods
- VO₂ Max
- Diet Diary
- Medical History Q
- Physical Activity Q

**Date**

______/______/______
Fat Imaging Data Sheet........(ctd.)

Name ___________________________ Hospital Number ___________________________

Ethnicity (see guide) __________________________________________________________

1. Asian or Asian British : Indian, Pakistani, Bangladeshi, Other Asian background
2. Black or Black British: Caribbean, African, Other Black background
3. Chinese or Chinese British: Chinese, Other Chinese
5. White: British, Irish, Other White background
6. Other: Other Ethnic background

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<td>Weight (kg)</td>
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<td>Height (cm)</td>
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<td>Blood Pressure (BP)</td>
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<td>Hip (cm)</td>
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<td>VO₂ (ml/kg/min)</td>
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DIET Type (Basic) : Standard, Vegetarian, Vegan, (Mediterranean etc) / Other........

Activity: Athlete [ ] Fit [ ] Active [ ] Sedentary [ ]

Details:

ACTIVITY : <1, 1-3, 3-5, >5 hrs/ wk

EXERCISE (level): Lo__Mod__Hi__ V.Hi_______

(type/freq/dur):__Aero / Resist /, _______mins/ sess,______day/wk

BLOOD RESULTS

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<td>TG (mmol/L)</td>
<td>0 – 2.0</td>
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<tr>
<td>HDL (mmol/L)</td>
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<tr>
<td>LDL (mmol/L)</td>
<td>2.0 – 5.0</td>
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Signed
Name

Birth weight: _________________________ Pre-term / Term / + Term

Delivery: standard / cesarean / forceps

Smoker / Non-smoker

2
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE  
(August 2002)  

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT  

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)  

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.  

Background on IPAQ  
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.  

Using IPAQ  
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.  

Translation from English and Cultural Adaptation  
Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.  

Further Developments of IPAQ  
International collaboration on IPAQ is on-going and an International Physical Activity Prevalence Study is in progress. For further information see the IPAQ website.  

More Information  
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?
   
   _____ days per week

   □ No vigorous physical activities ➔ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?
   
   _____ hours per day
   _____ minutes per day

   □ Don’t know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.
   
   _____ days per week

   □ No moderate physical activities ➔ Skip to question 5
4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day
_____ minutes per day

☐ Don’t know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

☐ No walking ➔ Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours per day
_____ minutes per day

☐ Don’t know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

_____ hours per day
_____ minutes per day

☐ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating.
PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

2. Do you feel pain in your chest when you do physical activity?

3. In the past month, have you had chest pain when you were not doing physical activity?

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

7. Do you know of any other reason why you should not do physical activity?

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

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**YES to one or more questions**

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

**NO to all questions**

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

**DELAY BECOMING MUCH MORE ACTIVE:**
- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

**NOTE:** If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

**NAME** ____________________________________________________________

**SIGNATURE** ______________________________________________________

**DATE** ____________________________________________________________

**SIGNATURE OF PARENT** _____________________________________________

**WITNESS** _________________________________________________________

**Signature of Parent** or GUARDIAN (for participants under the age of majority)

**Note:** This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

continued on other side...
Baecke Activity Questionnaire

1. what is your main occupation? ……………..never / seldom / sometimes / often / always
2. At work I sit…………………………………never / seldom / sometimes / often / always
3. At work I stand………………………………never / seldom / sometimes / often / always
4. At work I walk ……………………………never / seldom / sometimes / often / always
5. At work I lift heavy loads……………………never / seldom / sometimes / often / always
6. After working I am tired……………………never / seldom / sometimes / often / very often
7. At work I sweat…………………………never / seldom / sometimes / often / very often
8. In comparison with others of my age I think my work is physically……………..much lighter / lighter / as heavy / heavier / much heavier
9. Do you play sport……yes / no
   a. if yes:
      i. which sport do you play most frequently?
         - how many hours a week?………………..< 1 / 1 - 2 / 2 - 3 / 3 - 4 / > 4
         - how many months a year?……………..< 1 / 1 - 3 / 3 - 6 / 6 - 9 / > 9
      ii. If you play a 2nd sport:
         - Which sport is it?
         - how many hours a week?………………..< 1 / 1 - 2 / 2 - 3 / 3 - 4 / > 4
         - how many months a year?……………..< 1 / 1 - 3 / 3 - 6 / 6 - 9 / > 9
10. In comparison with others of my age I think my physically activity during leisure time is……..much less / less / the same / more / much more
11. During leisure time I sweat……………………never / seldom / sometimes / often / very often
12. During leisure time I play sport………………never / seldom / sometimes / often / very often
13. During leisure time I watch television………..never / seldom / sometimes / often / very often
14. During leisure time I walk…………………never / seldom / sometimes / often / very often
15. During leisure time I cycle…………………never / seldom / sometimes / often / very often
16. How many minutes do you walk and / or cycle per day to and from work, school and shopping?
   ……………………………………………………………..< 5 / 5 – 15 / 15 - 30 / 30 - 45 / > 45
ViScan validation data sheet

Scan number________________

Date ___________________ Subject name_________________________________

D.O.B ________________ Age ____________ Ethnicity _________________

Anthropometry

Weight (M1)_________ Height (H1) _________________ Hip (G17)_________

Waist(s): (mid point)(G16) _______ (umbil) _______ (min) _______ (iliac) _______

Flexed bicep girth (G15)______________ Calf girth (G12)______________

Humorus epicondyle (B6)___________ Femoral epicondyle (B8)___________

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VISCAN

Waist _________________ over 130cm used [] Manual supine waist_________

Trunk % body far _______________ Visceral fat area _______________

Trunk fat rating _______________ Visceral fat rating _______________

Somatotype

Endomorphy rating _______ Mesomorphy rating _______ Ectomorphy rating_________

BVI (Body Volume Index):

Total vol._________ Litres  Neck vol:_________ Abdom vol:_________

Waist : _________ cms  Hips: _________ cms
Fitness Norms in the Population

The aerobic fitness charts below show how fitness (Vo2 Max) changes with age (6 to 85 years). VO2max is the amount of oxygen taken up and used by muscles. The higher, the more aerobically fit you are. Each line on the charts separates the different fitness categories. The charts allow you to compare your fitness level with the fitness of different age groups. These norms are based on results of numerous studies* conducted in North America and Europe and apply to most of the industrial world.

* Shvartz E, R.C. Reibold. *Aerobic Fitness Norms For Males And Females Aged 6-75: A review.* Aviation, Space and Environmental Medicine, 61:3-11,1990
Fitness Categories

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Appendix 3

List of related publications.


Appendix 4

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