Phenotyping Ethnic Differences in Body Fat Depots

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Declaration of Originality

The thesis has not previously been presented in any form to the University for assessment. I declare that all the materials contained in this thesis are my own work and any specific contribution or assistance is fully explained and appropriately referenced.

ABSTRACT

There are remarkable ethnic differences in the incidence of metabolic syndrome associated features; including insulin resistance, type 2 diabetes, hypertension and cardiovascular diseases. Studies have suggested that South Asians (SA) present an unfavourable body fat phenotype, which includes a pattern of elevated visceral adipose tissue (VAT), and liver fat content; depots strongly associated with the progression of metabolic dysregulation. However, there are a limited number of studies examining body fat composition by ethnicity.

The purpose of this thesis was to comprehensively phenotype VAT, abdominal subcutaneous adipose tissue (ASAT) and liver fat content in Caucasian (Cau), SA and Black African (BA) individuals from a large number of distinct populations. Here, I include data from three adult cohorts: the UK Biobank (n=9533) of mixed ethnicities, the DIRECT cohort (n=1553) of Cau pre-diabetic individuals and The West London Observation (TWLO) cohort (n=747) of mixed ethnicities. In addition, I present data from Pune Maternal Nutrition study (PMNS) cohort; comprising 423 young adults of SA descent in India.

Analyses of body fat phenotype in Cau pre-diabetic populations showed higher VAT (mean differences= 0.5 litre, $p<0.0001$) and liver fat content (mean differences= 0.6%, $p<0.0001$), but lower ASAT (mean differences= -0.2 litre, $p<0.0001$) compared to Cau from the general population (free-living). I also observed negative associations between VAT, ASAT, liver fat content and day to day physical activity in both pre-diabetic and general populations (pre-diabetic; VAT; r= -0.296, ASAT; r= -0.163, liver fat: r= -0.186 and general population; VAT; r= -0.185, ASAT: r= -0.374, liver fat: r= -0.139, $p<0.001$ for all).

Analysis of both the TWLO and UK Biobank revealed no differences in VAT or liver fat in SA in UK compared to other ethnic groups (TWLO; VAT: SA: 3.0 ± 1.6 litres, Cau: 3.3 ± 2.1 litres; liver fat: SA= 6.4 ± 11.1%, Cau= 6.5 ± 13.6%, p=ns - UK Biobank; VAT: SA: 3.6 ± 1.6 litres, Cau: 3.8 ± 1.5 litres;

liver fat: SA: 4.6 ± 4.6%, Cau: 4.2 ± 4.6%, p=ns). Analysis of both these cohorts also revealed a more favourable body fat phenotype with BA males presenting significantly less VAT than SA and Cau males ($p<0.05$ for both). Data from the PMNS cohort revealed high levels of VAT in 18 year old India-based SA population. A high proportion (58.7%) of these lean individuals also presented with the thin-outside fat inside (TOFI) phenotype (a ratio of VAT to ASAT).

A key finding is the lack of an unfavourable body fat phenotype in UK based SA. Therefore, the increased incidence of metabolic syndrome associated features in the SA population may arise via a mechanism unrelated to elevated levels of VAT or liver fat.

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This thesis is dedicated to my father, Hassan Alenaini, who passed away after brutal fight with cancer while holding on the dream that his little princess is becoming a Doctor. Dear Father, this PhD thesis is dedicated to you.

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List of Abbreviations

2D	2dimensional
3D	3dimensional
ALT	Alanine Amino Transaminase
ANCOVA	Analysis Of Covariance
ANOVA	One-Way Analysis Of Variance
ASAT	Abdominal Subcutaneous Adipose Tissue
AST	Aspartate Amino Transaminase
AT	Adipose Tissue
BA	Black African
BEI	Bioelectrical Impedance
BMI	Body Mass Index
Cau	Caucasian
CHD	Coronary Heart Diseases
cm	Centimetre
CT	Computed Tomography
CV	Coefficient of Variation
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
DICOM	Digital Imaging And Communications in Medicine
DIRECT	Diabetes Research on Patient Stratification
DNA	Deoxyribonucleic Acid
DXA	Dual-Energy X-Ray Absorptiometry
ENMO	Euclidean Norm Minus One
FFA	Free Fatty Acid

FFM	Fat-Free Mass
FM	Fat Mass
g	Gram
GWAS	Genome Wide Association Study
HbA1c	Glycated Hemoglobin
HDL	High-Density Lipoprotein
HTN	Hypertension
IFG	Impaired Fasting Glucose
IHCL	Intra Hepatocellular Lipid
IMCL	Intramyocellular Lipid
IMI	Innovative Medicine Initiative
IPAQ	International Physical Activity Questionnaire
kg	Kilogram
L	Litres
m	Metres
MET minutes	Metabolic Equivalent Minutes
mg/dL	Milligrams per Decilitre
MHO	Metabolically Healthy Obese
mmHg	Millimetres of Mercury
mmol/L	Millimole per Litre
MR	Magnetic Resonance
MREC	Multi-Centre Research Ethics Committee
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
ms	Millisecond
mU/L	Milliunits per Litre
NAFLD	Non-Alcohol Fatty Liver Disease

NASH	Non-Alcoholic Steatohepatitis
NASP	Non-Alcoholic Steato-Pancreatitis
NGT	Normal Glucose Tolerance
NHS	National Health Service
OGTT	Oral Glucose Tolerance Test
PD	Pre-Diabetic
PDFF	Proton Density Fat Fraction
PMNS	Pune Maternal Nutrition Study
PPM	Parts Per Million
PRESS	Point-Resolved-Spectroscopy
s.d.	Standard Deviation
SA	South Asian
SAT	Subcutaneous Adipose Tissue
SBP	Systolic Blood Pressure
SPSS	Statistical Package for The Social Sciences
SWS	Southampton's Women's Survey
T2D	Type 2 Diabetes
TAT	Total Adipose Tissue
TE	Echo Time
TG	Triglycerides
TOFI	Thin Outside Fat Inside
TR	Repetition Time
TWLO	The West London Observation Study
UK	United Kingdom
VAT	Visceral Adipose Tissue
WC	Waist Circumference
WHO	World Health Organization

WHR	Waist To Hip Ratio

Chapter One

Introduction

Chapter 1. Introduction

1.1 General introduction

Obesity is considered a 21st century epidemic in developed and developing countries (1, 2). Obesity has massive public health consequences; it is a strong risk factor for type 2 diabetes (T2D) (relative risk >3), coronary heart diseases (CHD) and hypertension (HTN) (relative risk 2-3) (3-5). It also shares a linear relationship with all causes of mortality (relative risk 1.05) (6). Obesity is a physiological dysfunction with environmental, genetic and endocrine aetiologies. Hence, there is a drive to recognise it as a medical condition (7, 8). Obesity is defined by increased fat accumulation, which adversely affects normal body functions (1, 9) and is characterised by a body mass index (BMI) higher than 30 (10-12). BMI is the ratio of an individual's weight (expressed in kilogram, kg) to height (expressed in meters squared, m^2), and BMI unit is kg/m^2 (13). The obesity BMI threshold (>30 kg/m^2) and overweight threshold (>25 kg/m^2) were based on extensive epidemiological studies demonstrating the relationship between BMI and mortality, which tends to be J or U shaped (14).

In 2018, the World Health Organization (WHO) published data showing that 1.9 billion adults were overweight, and 650 million were obese (15). Overall, 39% of the world's adults population were overweight (39% male and 40% female), and 13% of the world's adult population was obese (11% male, and 15% female) (15). By 2030, it is estimated that 57.8% (3.3 billion people) of the world adult population will have a BMI of 25 kg/m^2 or higher (16, 17). Hence, the obesity-associated burden of disease is expected to rise in the forthcoming years. In developed countries, the number of adults who are overweight or obese often exceeds those who are normal weight (15). For example, in the United Kingdom (UK), the percentage of adults who are overweight (BMI = 25-29.9 kg/m^2) or obese (BMI = 30-39 kg/m^2) is 61% (65% male and 57% female) compared to 34% adult with normal BMI (31% males and 37% females, normal BMI ≥18.5-24.9kg/m^2), according to the Health Survey for England 2017 (18).

The global rise in obesity has been reported among all ethnicities, although there are variations. For example, in the United States, Mexican American males and females had the highest prevalence of overweight (BMI = 25-30 kg/m^2: 43% males and 34% females) compared to Black African (BA) (33% males and 26% females) and Caucasian (Cau) (from white ancestry including Americans: 41% males and 30% females), whereas BA males and females exhibited a higher prevalence of morbid obesity (BMI >40 kg/m^2: 7% males and 17% females) than Cau (4% males and 7% females), or Mexican Americans (5% males and 7% females) (19). In the UK, the South and East Asian group have lower BMI values than the Cau population despite showing greater susceptibility to developing T2D at lower BMI levels compared to Cau individuals (for the equivalent prevalence of T2D at 30 kg/m^2 in Cau, BMI equated to 22 kg/m^2 in South East Asians) (20).

The high global obesity figures are mirrored in the UK, where data from the Health Survey for England 2016 showed that 40% of males and 30% of females are overweight, and 26% of males and 27% of females were obese (21). The prevalence of overweight and obesity in adults in the UK has almost tripled since 1980 in both genders (22). The enormous increase in the prevalence of obesity in adults, in addition to its associated health disorders, comes with a high economic cost to society. The financial burden of overweight and obesity in the UK is growing. In 2007, direct healthcare costs of obesity were estimated to be £3.2 billion, and indirect costs at £4.4 billion (23). Direct obesity healthcare costs include general practitioner consultations, in-patient / out-patient admissions and drug costs, while indirect costs include treating the consequences of obesity such as T2D, cardiovascular diseases (CVD), stroke, lost potential national output and loss of earnings from premature mortality (24). The economic cost of obesity is high, accounting for 5% of the entire National Health Service (NHS) budget. The UK foresight projections calculated future cost models on elevated BMI in 2025 and 2050 with the increasing prevalence of overweight and obesity predicted by the model was projected to add £5.5 billion (at 2002 prices) to the annual total cost of the NHS by 2050 (25). The total attributable to

overweight and obesity by 2050 is predicted to be £9.7 billion in the UK alone (25).

1.2 Causes of obesity

At first glance, the cause of obesity seems simple; energy intake exceeds energy expenditure. Nevertheless, this simplistic view, which is widely held to be true, hides massive complexities inherent in how we acquire and use energy. Thus, the factors associated with the development of obesity are complex and multifaceted (1). Although there are many reasons why an individual may become obese, it is now generally accepted by health and other professionals that the current prevalence of obesity is primarily due to people's inherent biological susceptibility interacting or adapting with a changing environment that includes more sedentary elements and increased dietary abundance (26). Recently, obesity is increasingly recognised by medical societies and healthcare professionals as an endocrine disease, which is caused by health inequalities, genetic influence and social factors (27, 28).

The specific causes of obesity differ between population groups and across a person's life course, with the accumulation of excess fat being the result of a variety of causal pathways (1). This variability is an essential feature in that it points to a broad spectrum of different solutions. Indeed, the multifactorial condition of obesity is inherently unsuited to a 'one size fits all' approach, and the complexity of the aetiology of obesity is showing why this disorder is hard to treat.

Large numbers of studies indicated that obesity represents a disorder of energy homeostasis (defined as the stability of the physiological parameters such as glucose and calcium levels to maintain life) (29). For example, in a normal weight non-obese individual, the physiological homeostasis is functioning normally 'in a healthy balanced way' which results in well-maintained body weight, with population studies showing greater than 99.5%

agreement between an individual energy intake to energy expenditure (29). Therefore, in a perfectly balanced energy-control, obesity in healthy populations may be a consequence of regular, relatively small, cumulative imbalances (~100 calories/day). These imbalances may result from variations in different environmental factors (living environment, opportunities for physical activity, food and drink access and availability), psychological factors (stress, emotional eating, depression, sleep disorder), development (early life impact, mothers health) and socioeconomic drivers (food marketing, the price of food and drink, portion size), genetic and epigenetic factors (1). The obesity Foresight project produced a complex map representing the leading causes of obesity (25). Although the Foresight project focused primarily on the prevention of obesity, it offers a comprehensive approach for determining the causes of obesity based on robust scientific evidence from a wide range of disciplines to identify the influencing factors (25). The Foresight report concluded that the causes of obesity are highly complex and interchangeable, which can be grouped into genetic (biology), epigenetic (early life programming), and environmental factors (behaviour and socioeconomics) (25). Although a full discussion regarding obesity causes is beyond the scope of this thesis, the section below discuss in details the obesity causes which have relevance for the interpretation of later chapters in this thesis.

1.2.1 Obesity: epigenetic causes

As mentioned in Chapter 1 section 1.2, there are many factors involved in the development of obesity and its related disorders, including epigenetic, genetic and environmental factors. The epigenetic factors are the changes that affect the deoxyribonucleic acid (DNA) expression without changing the DNA sequence, such in early life programming (30). The early life environment (foetal malnutrition) has become an area of interest and a topic of investigation as a possible influence of adulthood obesity (31). Some of the variations in the prevalence of obesity-related metabolic disorders such as in the high prevalence of T2D and pre-diabetes (defined as fasting plasma glucose of 5.6–6.9 mmol/L and/or two hour post-challenge glucose of 7.8–11.0 mmol/L) in South Asian (SA) compared to Cau living in the same

geographical location opened the door for an early adult life investigation (32, 33). Currently, it is widely accepted that a relationship exists between foetal malnutrition and the development of obesity-related disorders (34, 35). However, many of the exact underpinning physiological mechanisms involved in understanding how obesity manifests from early life are still under investigation, with accumulating evidence supporting the negative impact of the mothers malnutrition or overnutrition on foetal development and adult life (31).

Human development during foetal life occurs to establish the basic organ systems, including homeostatic mechanisms, necessary for independent survival after birth (35). The mother's general health status usually establishes the intrauterine environment, and it is known to influence much of the foetal development, which includes the initial foetal survival and the development of vital organs (35). The mother's nutritional intake and metabolism are known to affect the intrauterine environment and disturbances in these components during critical stages of foetal development. This could cause major changes in homeostatic regulation, leading to more severe problems in later life such as metabolic dysregulation (36). Several theories have emerged to explain the relationship between unbalanced early life growth, obesity and metabolic dysregulation such as CVD including the birthweight hypothesis (37, 38). The birthweight hypothesis proposes that low weight at birth has an impact on infant early catch-up and leads to increased incidence of CVD (36). A systematic review on 39 papers by Kelishadi *et al.* regarding the effect of birth weight on the growth trajectory, reported that 79.6% of all CVD risk factors were reported in early catch-up studies (38).

During pregnancy, metabolic changes occur in lipid metabolism and the circulating levels of triglycerides (TG), cholesterol, fatty acids, and phospholipids. These changes contribute to conditions such as hyperlipidaemia, hyperphagia, lipogenesis, and increases in fat mass (FM) and body weight in the first and second trimesters of the pregnancy (39). In the 3rd trimester, changes in catabolic status due to the lack of adequate nutrition results in increases in TG, phospholipid, and cholesterol levels (40).

Studies that have investigated the different rates of growth have concluded that it is essential for the growth rate to remain constant throughout the different stages of early life, to minimise the risk of adverse health outcomes in later life (This topic will be discussed further in Chapter 3 in the Discussion 3.4) (41).

Evidence on the impact of early life programming on metabolic health and cardiovascular function come from previously documented famine and feast periods (39). During the Dutch Hunger Winter (1944-1945), offspring exposed to the famine in early gestation were at higher risk for the development of obesity, increased systolic blood pressure (SBP) and diastolic blood pressure (DBP), as well as a premature presentation of coronary artery disease (39). Interestingly, offspring exposed in late gestation were more likely to develop metabolic abnormalities such as impaired glucose tolerance (42). These studies highlighted that the organs and systems affected in adulthood often reflected the period at which the biological insult occurred during gestation (39). Interestingly, data from another famine in Saint Petersburg known as the Leningrad famine (1941–1944) show conflicting results. Offspring exposed to the Leningrad famine *in utero* showed no increase in blood pressure, atherogenic lipid profile or impaired glucose tolerance in later life (39). These variations in outcomes could be attributed to the fact that individuals from the Dutch Hunger Winter were well nourished before and after the famine, therefore helping to drive catch-up growth, accelerated catch-up growth associated with increased propensity to metabolic and CVD (39). In contrast, after the Leningrad famine, food remained scarce, so offspring exposed to the famine was born into a nutritionally deprived environment that matched their experiences *in utero*. Thereby supporting the role of a 'Predictive Adaptive Response' which is that foetal adaptations to scarcity become maladaptive only when affected individuals are later exposed to an environment of plenty (for example from rural to urban areas, or from developing to developed countries) (39).

Neel in 1962 (43) proposed the thrifty gene hypothesis which states that individuals who live in an environment characterised by unstable food supply (scarce) would maximise their probability of survival by maximising the

storage of surplus energy (43). From this point, genetic selection would favour the energy-conserving phenotype in such an environment. However, the selected genetic variations that were favoured for survival during malnutrition (scarce) would become unfavourable when nutrition is improved. This hypothesis assumes that the common genetic variants of the thrifty genes predispose to metabolic syndrome. In 1992, another thrifty hypothesis was proposed by Barker and Hales named as Barker's hypothesis and stated that biological insults that occur in the uterus are likely to account for the development of metabolic alteration (44). Babies who experience an insult preconception (intrauterine through the mother's malnutrition status) may have adapted to poor nutrition by reducing energy expenditure and becoming 'thrifty'. These metabolic adaptations, which lead the foetus to survive through scarce environment intrauterine successfully, showed beneficial when individuals have poor nutrition yet experienced the same early development environment during childhood and adulthood. However, with increased food intake, these metabolic adaptations lose their beneficial effect and lead to increased risk of metabolic syndrome in adulthood (45).

It has been proposed that foetal growth restriction (defined as a condition in which a baby has a smaller birth weight (less than 10th percentile) of those born at the same gestational age) and low birth weight may serve as a marker of adverse environmental influences for hampering growth, eventually over generations, leading to glucose intolerance and T2D (46, 47). Early catch-up growth (following foetal growth restriction), which is long viewed as an essential recovery from the deleterious effects of poor growth on development and health, is now recognised as a risk factor for insulin resistance, obesity and T2D (48). Some others proposed that this is the difference between an environment of scarce (in the uterus) and an environment of excess (after birth) that leads to metabolic dysregulation (36) (please refer to the discussions in Chapter 3 as we return to this topic to explain the results for Chapter 3).

1.2.2 Obesity: genetic causes

Through the years, many studies have suggested that gene modifications might be the primary cause underpinning the prevalence of obesity. In 1949,

it was noticed by chance that T2D mice who had a mutation in a certain gene (ob), despite similarity at birth with non-mutant mice, were predisposed to excessive eating and rapid weight gain by 4-fold than non-mutant mice during lifetime (49). In the mid-1990s, this gene was discovered by Friedman *et al.* as the gene responsible for leptin production. Leptin is a hormone produced by adipose tissue (AT) and plays an important role in appetite regulation by sending signals to the brain to stop eating (50). Leptin discovery helped in recognising fat as an endocrine organ (51). In humans, leptin deficiency or resistance causes uncontrolled food intake and severe weight gain, such as in rare congenital cases of leptin deficiency or lipoatrophy, which is a condition where there is a lack of fat for leptin production (51). Leptin supplements will reduce food intake and induce weight loss in some, but not all obese individuals, which is probably because not all obese individuals are characterised by leptin deficiency or resistance (52).

Although a full discussion regarding the importance of genetics on obesity is beyond the scope of this thesis, **Table 1.1** lists some genes and their associated phenotypes.

Table 1.1 List of some genes identified to contribute to BMI and their associated phenotypes. BMI; Body Mass Index.

Protein	Gene	Associated phenotype
Leptin	LEP	Morbid obesity caused by leptin deficiency Affect regulation of body weight and stimulating energy expenditure
Leptin Receptor	LEPR	Morbid obesity caused by leptin receptor deficiency. Affect regulation of satiety and energy expenditure.
Pro-opiomelancotrin	POMC	Early-onset obesity in children. Affect appetite regulation
Fat mass and obesity-associated	FTO	Obesity and increased fat mass. Affect neural function and appetite regulation.
Melanocortin receptor	MC4R	Found in numerous appetite-controlling centres in the hypothalamus.

Adopted twin studies suggested high hereditability in obesity, which is responsible for up to 45-75% of an individual variation in BMI (53). However, this contribution might be overestimated due to limited interpretation of data from twin pair studies (54) in addition to using BMI as a tool for assessing body fatness which showed weak sensitivity on an individual level (55). Moreover, high genetic susceptibility to BMI is usually translated in a relatively small clinical significant though adult life (~ 0.3 - 0.5 kg/m^2) (56, 57). For example, the FTO gene, one of the most well studied, only has a small impact on BMI variations of 0.1 – 0.4 kg/m^2 (58). Not only identifying certain genotypes that lead to a risk of obesity, but it is also crucial to consider the contribution of gene-gene interaction, gene-environment interaction and gene-behavioural interaction (59). Studies on the incidence of obesity in pets and their owners demonstrated a similarly strong correlation between the owner and the pet weight (60), which indicates a strong environmental bias in the estimation of genetic heritability.

The Pima Indians are a subgroup of Native Americans who live in Southern Arizona in the USA, and they exhibit one of the highest prevalence of obesity and T2D in the world and higher than the general US population (75% and 64% in females and males respectively)(61). The incidence of T2D in Pima Indians over ten years found to be 19-fold higher than White American (62). Interestingly, a group of the Pima Indians who live in semi-rural areas of Mexico make one fifth of the prevalence of T2D in the whole Pima Indian subgroup in the USA (T2D prevalence; 6.9% in Mexican Pima Indian, 38% in US Pima Indian, and 2.6 % in White American) (63). This example demonstrates that even at genetically susceptible population for developing associated obesity diseases; the environment has a noticeable impact on disease development (**Figure 1.1**) (63, 64).

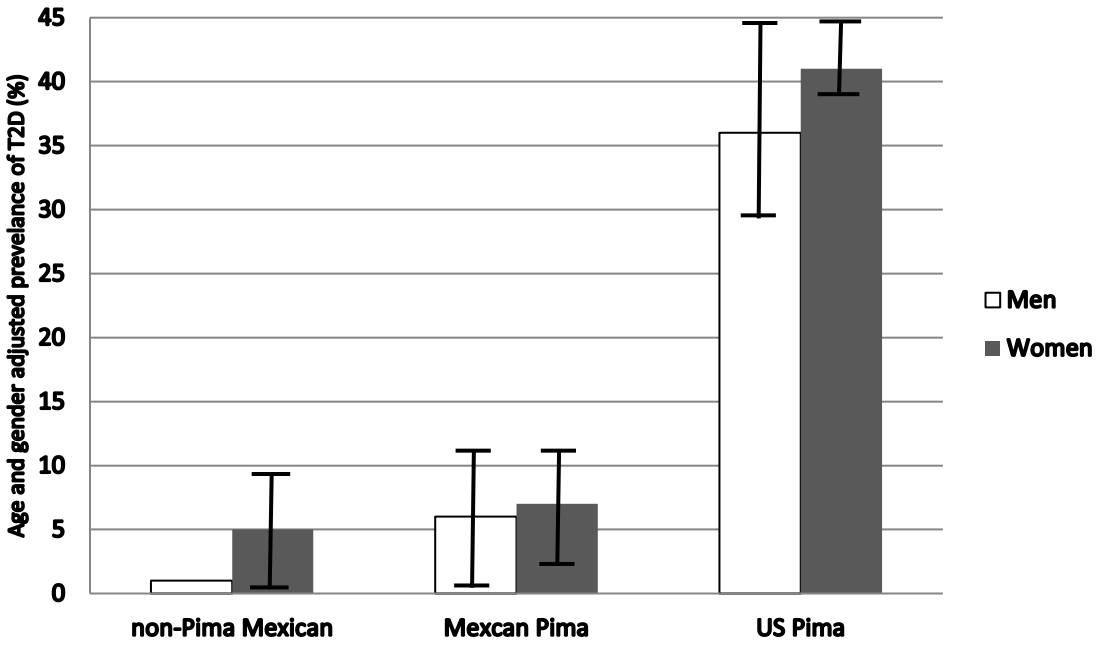

Figure 1.1 Age-adjusted prevalence of type 2 diabetes in non-Pima Mexicans, Mexican Pima Indians, and the U.S. based Pima Indians. Data presented as ± 95 confidence interval, US; United States of America. Adjusted from Leslie O. Schulz et al. 2006 (63).

1.2.3 Obesity: environmental causes

Determining the environmental causes of obesity such as food consumption, energy intake and energy expenditure are highly complex. Wang *et al.* (65) investigated the association between adherence to healthy diet consumption and genetic predisposition to obesity and found that after following a healthy diet, weight loss was stronger in the genetically highly predisposed to obesity group (65). This concludes that environmental circumstances may diminish the effect of strong genetic predisposition to develop obesity.

It is well known that there are enormous advantages of consuming a healthy diet, however achieving such is highly challenging in an environment that is obesogenic where it is common to promote cheap, large portions of dense energy diets and sedentary lifestyles via prolonged desk jobs and passive commuting (66, 67). However, not every individual who experiences an obesogenic environment is obese. Additionally, there is an emerging phenotype of metabolically healthy obese individuals, commonly known as metabolically healthy obese (MHO) or fat-fit (68). This subgroup showed

increased cardiorespiratory fitness (as measured by achieving the physical activity guidelines) compared to unfit obese counterparts (69, 70). This increase in fitness level is associated with reduced abdominal adiposity in particular visceral adiposity, which is the fat accumulation inside the abdomen (see Chapter 1 section 1.3.2 Visceral Adipose Tissue) (71, 72). Despite the benefits of increased fitness in maintaining a healthy metabolic profile, Chritou *et al.* showed that increased adiposity is associated with an adverse metabolic risk independently of fitness measures (73).

Moreover, some studies showed that MHO subjects have a lower risk for developing CVD and metabolic diseases compared to the metabolically unhealthy obese but, at the same time having a higher rate of CVD and metabolic diseases compared to normal-weight individuals (74). Since then, evidence shows conflicting data regarding the existence of MHO (75). The MHO phenotype might not present metabolic or CVD risks, but this does not necessitate a decrease in mortality (76). A recent follow-up study over 30 years published in 2018 showed that individuals with increased adiposity, despite maintained metabolic health, are eventually transformed into individuals with unhealthy metabolic profiles (77).

A study compared MHO with insulin resistance versus MHO with insulin sensitivity found that MHO with insulin sensitivity had significantly lower visceral adipose tissue (VAT) area (138 ± 27 cm^2 in MHO insulin-sensitive versus 316 ± 91 cm^2 in MHO insulin resistant) but similar subcutaneous adipose tissue area (935 ± 124 cm^2 in MHO insulin-sensitive versus 890 ± 110 cm^2 in MHO insulin resistant) (78). Similarly, other studies showed lower visceral adiposity in MHO when compared to obese metabolically unhealthy (78-81) or weight-matched individuals (82). Importantly, MHO is characterised by lower visceral adiposity, smaller adipocytes and a reduced inflammatory profile compared to metabolically unhealthy obese individuals (77, 78). Visceral adiposity is associated with an increased predisposition to metabolic and CVD risk (83). It has been suggested that MHO cannot be seen as healthy despite no evidence of metabolic disease since subjects with MHO will still encounter other obesity-related comorbidities such as chronic pain and cancers (84). Taking together, this evidence suggests that MHO

does not exist, as MHO is merely experiencing a delayed-onset of metabolic diseases (77).

Reducing abdominal adiposity via obesity management (weight loss via lifestyle modifications or obesity surgeries) can improve overall metabolic profiles, including glycaemic control, T2D and CVD risk in particular (85). Obesity is a preventable public health issue, which means the money spent on treating obesity could be saved through a better understanding of the condition, designing better treatments and improving prevention and early detection.

1.3 Adiposity in obesity

Fat or AT is a profoundly different component between individuals and within an individual (86). The terms fat and AT are often used interchangeably, but it is important to understand the distinction (87). AT consists of 80% fat (TG), and the rest is made up of water, proteins and minerals (86). Fat and AT are distinct with different compartments, and their taxonomic separation is important for any assessment of body mass and associated metabolic outcomes (86).

AT is located in distinct depots in the human body, the main compartment making up approximately 85% of total AT is subcutaneous adipose tissue (SAT) forming an extended layer surrounding the entire body, the most important subdivisions to note are abdominal and glutofemoral (**Figure 1.2**).

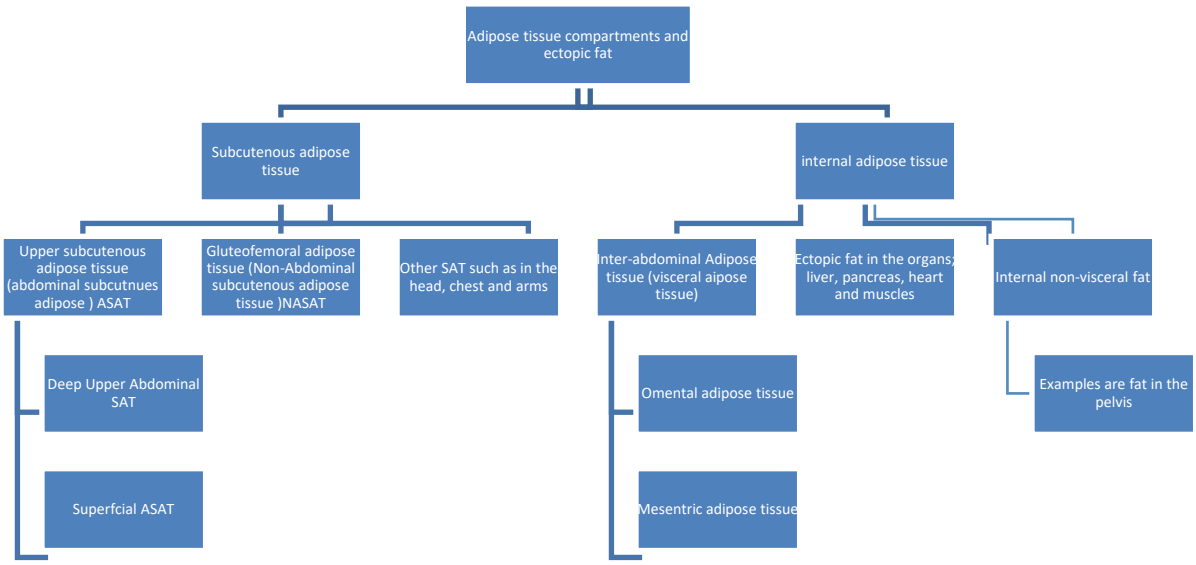

Figure 1.2 Diagram for the distribution of adipose tissue compartments and ectopic fat.

Closer examination of the abdominal subcutaneous adipose tissue (ASAT) (via radiological imaging studies) revealed further divisions; superficial and layers of upper ASAT which are separated by a facia layer (88). Of particular importance is intra-abdominal adipose tissue or VAT, which is located internally in the abdomen and divided into omental AT (located around upper abdominal organs such as liver and pancreas) and mesenteric AT (located around lower abdominal organs such as the colon) (83). Besides these AT compartments, fat can be stored inside the organs such as in the liver, pancreas and kidneys (89). Despite that, AT compartments and ectopic fat might seem to be separated anatomically, but they appear to work in a controlled manner and govern metabolic regulation. Research has shown that there is a considerable variation in the association between distinct AT compartments or fat depots and metabolic risk, with some AT and ectopic fat shown to be independent risk factors for disease development (90, 91).

1.3.1 Visceral adipose tissue (VAT)

VAT is considered a metabolically active component of total body AT, which imposes distinct biochemical features that influence several normal and pathological processes in the human body (83). The VAT is characterised by a high number of large adipocytes as well as increased vascularity and a rich blood supply, which makes it more metabolically active (greater fat storage and release), leading to a higher lipid turnover than other fatty tissues (92). Studies have shown that larger adipocytes are more insulin resistant compared to small adipocytes (92). This might explain the strong association between increased VAT and insulin resistance observed in many studies (93-95). Moreover, the amount of VAT is a significant risk factor determining the variations in systemic insulin resistance (96), Abate *et al.* studied the relationship between insulin sensitivity, measured via a euglycemic clamp, and visceral adiposity assessed by MRI in 39 healthy males, and demonstrated a significant negative association between VAT and insulin sensitivity (97). Furthermore, increased VAT is a risk factor for developing T2D, and a predictor of CVD, independent of total body fat percentage, BMI or SAT (95, 98) (**Figure 1.3**).

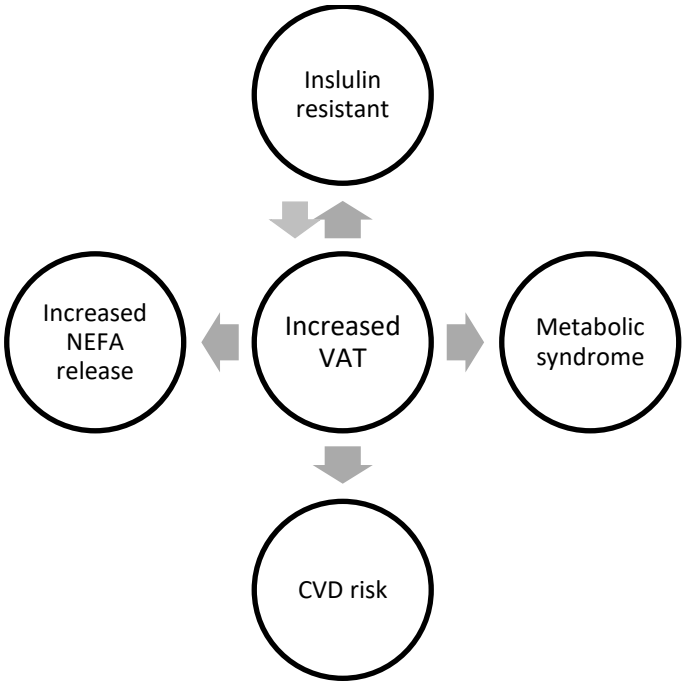

Figure 1.3 Increased VAT potential metabolic outcomes. VAT: Visceral Adipose Tissue; CVD: cardio metabolic risk, NEFA; non-esterified fatty acid.

Studies have showed that increased amount of VAT has been more closely linked with metabolic disorders than SAT (93), and a predictor of CVD independent of SAT (99). This might be due to the anatomical location of VAT, and the fact that free fatty acid (FFA) drains directly into the liver through the portal vein (96). This portal drainage of visceral AT provides direct hepatic access for the FFA and adipokines, which are highly secreted by visceral adipocytes (96, 100). Despite the evidence supporting the notion that VAT is a driver of insulin resistance syndrome (93, 101), others proposed that increased accumulation of VAT is a marker of dysfunctional AT, rather than a cause of insulin resistance (102). Therefore, it is unclear whether visceral adiposity causes insulin resistance or insulin resistance causes adiposity dysfunction through an excessive release of NEFAs, which impairs insulin sensitivity and increased oxidative stress (103). Whether VAT is a cause or a result of insulin resistance, its presence in excess amounts is a marker of metabolic dysfunction. Indeed, surgical removal of VAT resulted in improving metabolic profile in humans (104, 105). Importantly, excess visceral adiposity is reversible, and its reduction can have an excellent effect in diminishing cardiovascular and metabolic syndrome risks. Indeed, lifestyle modifications (diet and exercise) when leading to loss of VAT, even without weight loss, showed improvement in insulin sensitivity and circulating lipid levels (106, 107).

1.3.2 Subcutaneous adipose tissue (SAT)

In an energy-balanced setup, SAT primary function is acting as an energy sink with an expanding ability to store excess energy intake (91). When SAT function is impaired or altered, the excess fat is stored in VAT or lean tissues (91). Upper and lower body SAT exhibits opposing association with obesity-associated metabolic disorders and CVD risk (91). In addition, deep abdominal SAT showed similar association to VAT with obesity-associated metabolic disorders, while superficial abdominal SAT is shown to be benign (108).

Interestingly, studies performed on mice showed that transplantation of VAT to SAT location improves metabolic functions (109). The authors concluded that adipose depots own a “residence memory” and autologous transplantation of visceral fat to subcutaneous sites (chest or thigh) offers metabolic advantages (109). However, the removal of visceral fat alone did not improve the metabolic profile in mice which indicates that AT compartments are intrinsically different from each other not only anatomically but also on a functional and physiological level (109). In human studies, liposuction of large quantities (>9 kg) of subcutaneous abdominal fat results in large reductions in waist circumference (WC) (>12 cm), but showed no effect on cardiovascular risk factors (110). By contrast, surgical removal of <1 kg of VAT results in substantial improvements in oral glucose tolerance, insulin sensitivity, fasting plasma glucose and insulin levels than in control patients (in proportion to the baseline) despite similar overall weight loss (104). Interestingly, surgical removal of SAT did not result in improvement in metabolic syndrome (110) with a few limited studies have linked the SAT to the development of obesity-related insulin resistance (97, 111).

SAT, mainly in the lower body, plays an essential role in maintaining normal AT function, and it showed an opposing association with CVD across wide ranges of age and BMI (112, 113). A lower amount of body SAT is associated with lower total cholesterol and LDL cholesterol and lower TG (114, 115). A study of 27,000 participants from 52 countries were investigated to measure the association between obesity and risk of myocardial infarction, an independent association was found between the larger hip circumference and lower risk of myocardial infarction (116). Moreover, in 623 participants from the Hoorn study (117), increased SAT in the leg was associated with decreased risk of altered glucose in males (standardized beta coefficient for fasting glucose= -0.2, 95% confidence interval -0.4 to -0.1, standardized beta coefficient for post-load glucose= -0.1, 95% confidence interval -0.3 to 0.1) and females (standardized beta coefficient for fasting glucose= -0.2, 95% confidence interval -0.4 to -0.2, standardized beta coefficient for post-load glucose= -0.3, 95% confidence interval -0.4 to -0.1) (118).

Interestingly, in subjects with increased VAT, an increase in SAT correlated with reduced TG content and lower susceptibility to developing the metabolic syndrome (119). In mice studies, the surgical transplantation of SAT into visceral areas lead to improved glucose metabolism (through improved insulin sensitivity of their liver and muscles) (120), decreased body weight and reduction in total FM (121). In T2D patients who were treated with thiazolidinedione to improve insulin sensitivity showed increased total body mass which was primarily SAT (via the increased proliferation of subcutaneous adipocytes) accompanied with improved insulin sensitivity (122). Furthermore, in some cases where there is no protective SAT (e.g. lipodystrophy), severe insulin resistance and diabetes developed (123-125). For example, in congenital lipodystrophy, there is a failure to develop adequate AT storage and fat is consequently stored ectopically (125).

Indeed, a detailed study of AT content and distribution led to the realisation of the great importance of AT distribution (91, 126). For example, 'thin outside fat inside' (TOFI) who are normal weight subjects (BMI 18.5 < 25 kg/m^2) but characterised with potential increased risk for developing metabolic diseases due to increased VAT accumulation (72). TOFI is estimated to affect 12% to 13% of the general white population, who have a normal BMI (72). Furthermore, a prominent feature of the TOFI phenotype that they exhibit higher ectopic fat in the liver and muscles than normal BMI non-TOFIs, this emphasises the importance of AT distribution and ectopic fat in determining metabolic risks (72).

1.3.3 Ectopic fat

Ectopic fat refers to the accumulation of fat (TG) in lean tissues which lead to disturbing their normal function (i.e. normal clearance capacity) and may lead to tissue dysfunction (via lipotoxicity) and subsequent metabolic risk (127). Ectopic fat can be found in organs such as the liver, pancreas, heart, muscles and kidneys (102).

1.3.3.1 Liver fat

Accumulation of liver fat is a result of fat that builds up in the liver (more than 5% of liver cells), not from alcohol abuse, that can damage the liver and lead to serious complications known clinically as non-alcohol fatty liver disease

(NAFLD) or fatty liver. Fatty liver is a condition with a broad spectrum and its symptoms are similar to that of alcohol-induced fatty liver damage but found in individuals who do not abuse alcohol; therefore males were consuming < 30 g ethanol per day and females <20 g ethanol per day (128). Despite having no symptoms, fatty liver has been reported to affect between 20% - 30% of the general population (129), but it also varies with ethnicity, with high prevalence among Hispanic (45%), Cau (33%) and BA (24%) populations (130). Generally, the presence of increased fatty liver is higher in obese populations (odds ratio, 7.2; 95% confidence interval, 5.3-9.8) (131). However, the coexistence of the two conditions is not necessary. The prevalence of fatty liver or NAFLD in the general population increases in male sex (odds ratio, 1.4; 95% confidence interval, 1.1-2), elevated alanine amino transaminase (ALT) (odds ratio, 5.66; 95% confidence interval, 4-8), fasting plasma glucose ≥126 mg/dL (odds ratio, 2.08; 95% confidence interval, 1.4-3.05), total cholesterol ≥240 mg/dL (odds ratio, 1.5; 95% confidence interval, 1.1-2.1), and TG ≥150 mg/dL (odds ratio, 1.8; 95% confidence interval, 1.3-2.4) (131).

Although fatty liver is associated with the global rise in obesity and affects 70% to 80% of the obese population, it can also be found in non-obese individuals with elevated abdominal adiposity, dyslipidaemia and insulin resistance (128, 132, 133). The presence of fatty liver in non-obese individuals and its associated metabolic disorders such as T2D makes it a potential biomarker for the prediction and detection of various clinical endpoints (134). For example, in a longitudinal study on 906 non-diabetic subjects at baseline from the Insulin Resistance Atherosclerosis cohort, elevated aspartate amino transaminase (AST) and ALT (both markers of NAFLD) were significant predictors of T2D after adjusting for the percentage of body fat, WC, TG, impaired glucose tolerance and insulin sensitivity (135).

Fatty liver has been associated with many health risk factors, such as HTN elevated TG, low levels of high-density lipoprotein (HDL) cholesterol and elevated insulin, as an umbrella marker of risk (132, 136, 137). Owing to its strong association with the metabolic syndrome, it has been proposed as both the hepatic manifestation of the metabolic syndrome (138) and as a

component for metabolic syndrome owing to its role in the progression of T2D and CVD (139-141). Sixty per cent of patients with fatty liver meet the National Cholesterol Education Programme Adult Treatment Panel-III criteria for the metabolic syndrome (142). Similarly, fatty liver was also shown to be a predictor of early alterations in cardiovascular function in the absence of HTN, T2D, morbid obesity and increased WC (143-146). Interestingly, Kim *et al.* reported that fatty liver is associated with carotid artery calcifications independently of the traditional risk factors, including visceral adiposity (147).

The fatty liver starts with simple steatosis (fat accumulates in 5% of liver hepatocytes), but when markers of inflammation manifest, it progresses to non-alcoholic steatohepatitis (NASH) (148). Scarring tissue appearance in the hepatocytes as a result of inflammation is a further stage of the fatty liver spectrum, and it is known as 'liver fibrosis' (148). In the fibrotic stage, the liver function is limited but not fully inhibited. Prolonged inflammation in the fatty liver, left untreated, results in liver cirrhosis where the liver function is impaired. Persistent cirrhosis leads to liver failure. Despite its benign beginning, fatty liver is a condition that, if left untreated, can lead to life-threatening conditions such as liver failure and hepatocellular carcinoma (149). Today, in parallel with the global increase in unhealthy lifestyle choices, NASH has become the third indication for liver transplantation (from 1.2% in 2001 to 9.37% in 2009), with NASH recipients of transplant sharing features of older age, higher BMI and interestingly, mostly female (47% female versus 29% male) (150).

Fatty liver and it progression rates to further liver conditions are unpredictable, which has necessitated the development of multiple screening and monitoring techniques. Individuals with fatty liver have few or no presentable symptoms, albeit occasional complaint of fatigue in the early stages of the disease, this silent nature of the condition makes it more crucial to identify an accurate assessment tool for diagnosis and treatment (151).

There have been several hypotheses proposed to explain why fat is deposited in lean tissues such as the liver (103), including the overflow hypothesis (152). The overflow hypothesis suggests that adipocytes become

resistant to the effects of insulin or the exhaustion of AT storage capacity increases the lipolysis rates and the release of FFA delivery to the liver (153). This 'overflow' of FFA from the AT to the liver eventually exceeds the liver's ability to produce fatty acids in the form of VLDL, causing fat to accumulate in the liver (153). Surplus lipid in the liver can cause lipid-induced dysfunction (lipotoxicity) and lipid-induced programmed cell death (lipoapoptosis, via ceramide overproduction) (154). This is consistent with the association between VAT and fatty liver (132); hepatic delivery of FFA increases as VAT compartment expands (155). Indeed, increased liver fat content correlates with total and visceral adiposity (139). The release of excess lipids to the circulation is proportion of the overall individuals' FM, which increases the FFA flux to other non-adipose tissue (153). However, the 'overflow' hypothesis of excess FFA does not fully explain the development of excess liver fat accumulation as the condition exists in non-obese individuals. It has been suggested that oxidative stress and cytokine action are the second step in the overflow hypothesis (156, 157).

Furthermore, in fatty liver, FFA and triacylglycerol metabolites (fatty acyl-CoA, diacylglyceride and ceramides) accumulate (158). Diacylglyceride can activate protein kinase C, which phosphorylates insulin receptors, thereby inhibiting insulin signalling transduction and ultimately increasing hepatic glucose production. Indeed, fatty acids can induce intracellular inflammation by generating oxidative stress (158).

To date, the exact mechanism of how increased adiposity, in particular, visceral adiposity, induces fatty liver has not been resolved. Furthermore, it is unclear why some individuals progress from fatty liver toward further stages of liver diseases and life-threatening disorders, while others do not. Currently, there are no specific drugs for fatty liver except lifestyle modifications through reducing calorie consumption and increased energy expenditure. With the global obesity epidemic, it is of great importance to understand the mechanism behind ectopic liver fat accumulation and its role in the development of metabolic diseases. The deep understanding and assessment of the contribution of ectopic fat in the liver will allow for precise

monitoring of healthy metabolic status and allow new insights for prevention, prediction and treatment of NAFLD.

1.3.3.2 Pancreatic fat

Less is known about the human pathophysiology consequences of lipid accumulation in the pancreas, sometimes referred to as intra-pancreatic cellular lipids or fatty pancreas, in particular with regards to metabolic syndrome (159, 160). Deposition of fat within pancreatic cells showed a link with a higher risk of T2D (161, 162), and is thought to follow a similar progression to NASH in the liver and is termed non-alcoholic steato-pancreatitis (NASP) (163).

β-cells, the insulin production site, are usually present with other endocrine cells in the Islets of Langerhans which are scattered throughout the pancreas (159). It has been suggested that excess ectopic pancreatic fat accumulation deteriorates β-cell function, exocrine function and insulin secretion (164). Pancreatic fat usually starts appearing several years before the diagnosis of T2D and has been proposed as a potential marker to identify individuals at risk, in particular in the case of pre-diabetes (please refer to Chpater 3 for an in-depth discussion on pre-diabtes) (165). However, it remains of a clinical challenge because the quantification of pancreatic fat is challenging due to the size and the location of the pancreas.

The increased accumulation of lipids in the pancreas may arise through several different mechanisms including the local release of FFA, TG metabolic accumulation, oxidative stress and release of pro-inflammatory factors and cytokines production, all which have been shown to stimulate β-cell injury (159). In obese individuals, increased lipolysis contributes to high levels of circulating NEFA (166) subsequently, various mechanisms including the formation of reactive long-chain fatty acyl-CoAs and toxic metabolites, such as ceramide, may contribute to the decline of β-cell mass (167, 168). In animal studies, adipocyte expansion (increase in the size), and TG accumulation increased in parallel in both exocrine and endocrine pancreatic regions (169). This phenomenon leads to eventual β-cell dysfunction, leading

to lipoapoptosis and impaired insulin secretion as the animals develop pre-diabetes and T2D (169, 170).

β-cell impairment was thought to be irreversible, however, recently, it has been suggested that pancreatic β-cell function and recovery is possible. Elegant work by Roy Taylor's group suggested that remission of T2D might be possible, by reduction in liver and pancreas fat, and therefore β-cells in the pancreas may recover and produce the right amount of insulin again allowing for the remission of T2D and pre-diabetes in some but not all cases (171).

1.3.3.3 Other ectopic fat

While beyond the scope of this thesis, other ectopic fat should be mentioned for context. These other ectopic fat sites are the muscle, the heart and the kidneys. For example, muscle fat can be found between the muscle fibres (inter-muscular) or inside the muscle cells (intra-muscular) also known as intramyocellular lipid (IMCL) content which serves as an energy response for training. As seen in all ectopic fat depots, excess IMCL in obese individuals showed an association with T2D and insulin resistance (172).

1.4 Quantifying obesity

The most widely used method for obesity diagnosis is BMI which was originally named the Quetelet index after its discovery in 1835 by Adolph Quetelet (1796-1874). He was a Belgian statistician, and this index was included as part of his theory of the average man from his classic book (A Treatise on Man and the Development of his Faculties). The BMI was a simple measure used to classify people's weight relative to an ideal weight for their height. In 1972, Ancel Keys explored the high correlation between BMI and adiposity (measured by skinfold and hydrodensitometry) and concluded that BMI usage is preferable in all population at all times (173). Since then, it has been accumulating evidence on the usability of BMI as a simple anthropometric index due to its fundamental repeatable and valid components that relate to the physical description of an individual or population (174, 175).

WC was another proposed method for central obesity assessment. Studies have shown a strong association between the metabolic syndrome and elevated abdominal size measured via WC, and it is estimated that for each 11 cm increase in WC, there is an 80% increased risk for developing the metabolic syndrome (176). A prospective study of 714 non-diabetic participants found that after five years, 19.5% of the participants had developed the metabolic syndrome with elevated abdominal adiposity measured via WC was the best predictor (176). Therefore, central obesity is taken as an essential measurement for the diagnosis of metabolic syndrome; however, there are ethnic-gender disparities in determining the most appropriate WC threshold. The reason behind this is that different ethnic groups develop metabolic syndrome at a different level of abdominal adiposity (177, 178). The WC threshold for the diagnosis of metabolic syndrome in Cau, Middle Eastern and BA males is 10 cm higher than that suggested in Asian and Latin American males (177). For females, the WC threshold to diagnose metabolic syndrome is similar between Cau, Asian, Middle Eastern, BA and Latin American populations (177). One of the major problems for the diagnosis of the metabolic syndrome as well as with obesity is the applicability of appropriately defined threshold or biomarkers for different ethnicities, in particular, those at high risk for developing metabolic disease (20, 177).

Currently, the WHO obesity classification using BMI defines undernutrition or underweight as <18.5 kg/m^2, normal weight as 18.5–24.9 kg/m^2, overweight as 25–29.9 kg/m^2, obesity as ≥30 kg/m^2, and ≥40 kg/m^2 is considered morbid obesity (**Figure 1.4**). The fundamental health principle behind using BMI categories is that nutritional status is linked to longevity and mortality (179, 180). Individuals with a low BMI (underweight or undernutrition; less than 18 kg/m^2) have a higher risk of mortality and infectious diseases compared to those with higher BMI (27.5 – 30 kg/m^2) values owing to their diminished immune status and low protection from fat stores during acute illness (181).

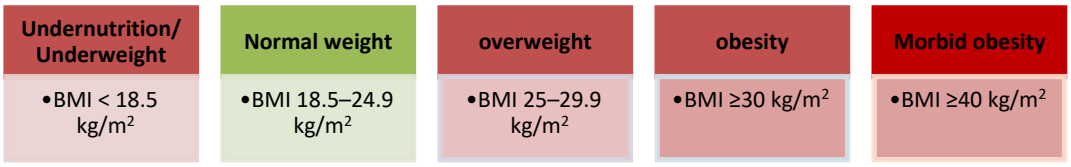

Figure 1.4 BMI Categorization and correspondent values based on WHO report 2001. BMI; body mass index, WHO; world health organisation.

Furthermore, low BMI individuals are at risk of developing conditions such as nutritional deficiency and osteoporosis (182). The optimal BMI category for survival is between 22.5 - 25 kg/m^2 (183, 184). Individuals who are overweight (>25 kg/m^2) or obese (30 kg/m^2) are at higher risk of non-communicable diseases as well as certain types of cancer (185). A report from the Global BMI Mortality Collaboration published in 2016 with data from 239 prospective studies in four continents (based on 10,625,411 participants and 385,879 deaths) showed that overweight (BMI 25<30 kg/m^2) and obesity grade I (BMI 30<35 kg/m^2) were associated with all-cause mortality (hazard ratio 1·1-1.2, 95% confidence interval 1·1-1·2 for BMI 25-<30·0 kg/m^2 and hazard ratio 1·6, 95% confidence interval 1·4-1·5 for BMI 30<35 kg/m^2) in a steep relationship across Europe, North America, East Asia, Australia and New Zealand (180). The Global BMI Mortality Collaboration 2016 analysis was restricted to never smokers and individuals who did not have a pre-existing chronic disease in order to eliminate cofounding bias (180).

BMI is commonly used in epidemiological studies because it is easily obtainable and has low cost in money and time. Despite BMI being correlated with a more direct measure of obesity (186), it is assumed to represent the degree of overall adiposity, and it remains a score rather than objectively measured FM (or FM related metabolic disturbance). BMI does not distinguish between FM and fat-free mass (FFM) (187). Therefore, BMI only provides a surrogate measure of body fatness, with no information regarding body composition or fat distribution (187). This is important since it has been established that obesity-related disorders may be a function of body fat

distribution rather than just total fat content (188). Moreover, since the inception of BMI, it has been known to have certain limitations particularly regard to its use in children, athletes, and for different ethnicities and age groups (187, 189). For example, using BMI for body builders and power athletes (rugby, boxing, wrestling etc.) who may have little body fat percentage, but their BMI would classify them as overweight or obese owing to their greater muscle mass (190).

Moreover, loss of muscle mass such as that observed in sarcopenia can also make BMI assessment inaccurate (191). Furthermore, BMI cut-offs based on Cau individuals result in misclassifications of BMI in non-Cau ethnicities (192, 193). In a large cross-sectional study from the UK Biobank, including 490,288 participants (3.9% non-white) to assess the relationship between BMI and the prevalence of T2D in multi-ethnic groups, it was demonstrated that at the same level of obesity (>30 kg/m^2), non-white individuals were 2 up to 4 fold more likely to develop T2D (20).

As highlighted in section 1.3, adiposity in obesity, an accurate assessment of body composition vitally important. Historically, the most accurate method was cadaver dissection, but this is not feasible for the clinical quantification of body compositions. Depending on the criterion involved, the numerous methods available for measuring body composition *in vivo* can be divided into different categories (194). First, the direct body composition methods which include total body weighing, and chemical dissection (194). The second method of determining body composition involves techniques such as MRI, CT, dual-energy X-ray absorptiometry (DXA) and body density (194). There are also indirect methods, including skinfold, ultrasound and bioelectrical impedance (BEI or referred to as BIA) (194).

1.4.1 Direct methods

Direct body criterion methods of body composition assessment are those that directly measure the amount of chemical elements in the body (195). Cadaver studies are the most prominent example of direct body composition assessment (196, 197). In another method, *in vivo* neutron activation analysis

is carried out, where body content is measured at the elemental level (86). After exposure to a neutron field, gamma output is measured. However, this is rarely used due to the exposure to high levels of neutron radiation (86).

1.4.2 Criterion methods

Indirect methods of body composition assessment include deuterium oxide dilution, DXA and densitometry to measure FM, FFM, water, and rely on certain assumptions. This approach to assessing body composition using a two-component model (2-C), where tissue is either FM or FFM. More recently, the multi-component model was used where the components measured depend on the method used (86, 194).

1.4.2.1 Magnetic resonance imaging (MRI)

The importance of accurately measuring adiposity, both for scientific purposes (accurate assessment of obesity-related diseases) as well as providing better recommendations for preventing or reversing the effect of increased body adiposity, has led to the development of various advanced techniques to provide a more detailed measure of body adiposity than can be obtained using BMI. Imaging techniques such as computed tomography (CT) and MRI, have been utilised to assess body fat content and distribution (197) accurately. The applicability of CT has been limited by the fact that ionising radiation is required during the acquisition of the images, while MRI studies have been limited by the relatively high cost of the examination. Despite this, MRI has become the gold standard for the measurement of body fat distribution, owing to its accuracy and non-invasive nature (198-200). Although MRI measurements of body fat require technical and anatomical knowledge, applying this technique to a large population is useful to accurately assess fat content and distribution in relation to fatness such as age, gender, lifestyle, ethnicity and genetic make-up. Therefore, MR quantification of AT and ectopic fat was the method used throughout the thesis and discussed in details.

1.4.2.2 MR principles

Although there may be high correlations between many of the different measurement techniques – there is generally a poor agreement between the imaging and non-imaging techniques (197). Diagnostic medical imaging technologies, including CT and MRI, provide a more accurate estimation of different body fat tissues from cross-sectional images of the body. Adiposity can be quantified using multiple contiguous scans to determine the area of subcutaneous and internal AT that can be converted to volumes as the distance between continues slices in known. Below is a brief description of the underlying principles of this method.

MRI, also known as Magnetic Resonance (MR), utilises hydrogen nuclei (^{1}H) mainly from water and fat. Hydrogen atoms are the most abundant nuclear magnetic resonance active nuclei in the body and are distributed widely throughout most tissues. This means the radio waves emitted from ^{1}H after MR excitation are sufficient to be converted into a detailed image. The intensity of the signal acquired is relative to the number of hydrogen atoms present and allows different tissue types to be quantified. However, the number of ^{1}H alone cannot distinguish between all tissues - fat and muscle, for instance, do not have markedly different amounts of hydrogen.

To enable the differentiation between fat and muscle, an MR property known as spin-lattice 'relaxation time, T1' is employed. T1 relaxation time is the time required for the nuclei to release the energy they have absorbed from the applied radiofrequency pulses, and return to their natural equilibrium state. Based on the fact that the T1 for ^{1}H in fat and muscle tissues are different, T1 can be used to differentiate these two tissues from each other within an image.

The measurement of different T1 times can be maximized by manipulating the time interval between each radiofrequency pulse (known as the time to repeat or repetition time TR), and the time required to detect the induced signal known as echo time (TE). This process is called the pulse sequence, from inducing the radiofrequency wave until detecting the echo that contains

the MR signal. Specific pulse sequences have been developed to provide the optimum image contrast, in the minimum scan time, for each particular tissue under investigation.

MRI images are either acquired in ‘slices’ where the whole body or particular section is scanned in a series of fixed-width 2dimensional (2D) slices or as a whole 3dimensional (3D) volume acquisition (201). For the 2D slice method, each MR scan consists of a series of cross-sectional images that together make up the body or the segment required. Each image must be analysed individually (via various techniques), and then the tissue areas can be calculated. Since the slices are of known thickness, the relative tissue volumes can be calculated. MRI has been validated for measurements of fat content in phantoms, animals, and human cadavers (202). It has been shown to accurately quantify AT content *in vivo,* showing good agreement with the values produced by dissection and chemical analysis (203, 204). In addition, MRI has shown to be reliable and reproducible. The MRI fat content quantification reproducibly coefficients ranging from 0.3 – 2.3% and approximately 2% for reliability (204).

1.4.2.3 MR liver and pancreas fat measurements

Generally, in tissues containing lipid and water, there will be oscillation in signal intensity as a function of echo time. At some echo times, the fat and water signals are in phase (higher signal), and at others they are out of phase (lower signal); this gives rise to the oscillations in MR signal decay curve. An organ (liver or pancreas) with very little fat infiltration will generate a very smooth decay curve (without obvious oscillations in the decay), whereas one containing a higher level of fat shows significant oscillations throughout the decay. From these data, ‘heat-maps’ were generated to visualise regional differences in the fat deposition as in **Figure 1.5,** which shows the heat maps from four individuals with varying levels of fat in their liver and pancreas. Each process of the detailed method for MR fat quantification will be discussed in detail in the following chapters’ method sections.

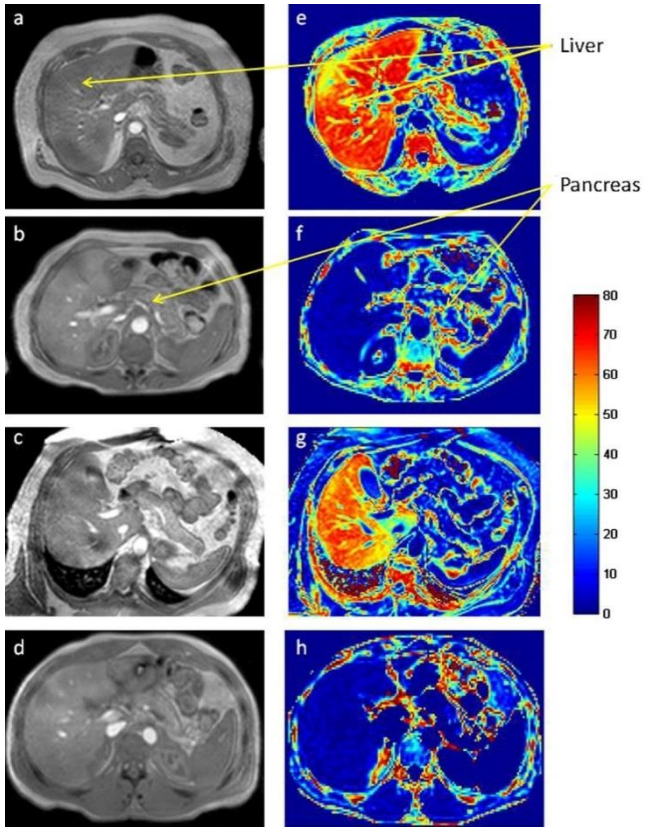

Figure 1.5 Multi-echo and heat-map images from the liver and pancreas. A series of multi-echo (**a–d**) and corresponding heat-map images (**e–f**) from four volunteers, with varying levels of ectopic fat in both the liver and pancreas. A scale reflecting fat content from blue (low) to red (high) is also shown. Images (**a**) + (**e**) show high liver and high pancreatic fat. Images (**b**) + (**f**) show low liver fat and with fat infiltrating into the pancreas. Images (**c**) + (**g**) show high liver and low pancreatic fat. Images (**d**) + (**h**) show low liver and low pancreatic fat. The heat-map values were localised to the liver, hence the lack of relationship between the levels of fat in the adipose tissue and its colour.

1.4.3 Quantifying obesity contributing factor: ethnicity

Ethnicity has a noticeable impact on both AT distribution with SA having higher abdominal AT mass and lower lean mass compared with Cau (205). This ethnic group are more susceptible to obesity-related cardiometabolic consequences, with higher incidence rates of T2D equivalent to those with a BMI of 30 kg/m^2, but occurring at much lesser obesity levels in SA, and BA (20, 206). The National Institute for Health and Clinical Excellence published in 2013 on BMI and preventing ill health and premature death in SA groups recommendation for using lower BMI thresholds (23 kg/m^2 to indicate increased risk and 27.5 kg/m^2 to indicate high risk) to trigger action to prevent

T2D among SA populations (207). A distinct phenomenon has been shown with increased abdominal adiposity at lower BMI in SA compared to Cau. Thus, the accurate assessment of regional AT distribution is an important predictor for assessing obesity-associated disorders in SA.

1.5 Ethnicity

Ethnicity is a complex and multifactorial concept which reflects the sharing of similar culture, religion and history (208). Although 99.9% of the human DNA is identical, natural selection and mutations have led to population differences (genetic drift) which are sufficiently characterised to identify an individual's ethnicity (209) (**Figure 1.6**).

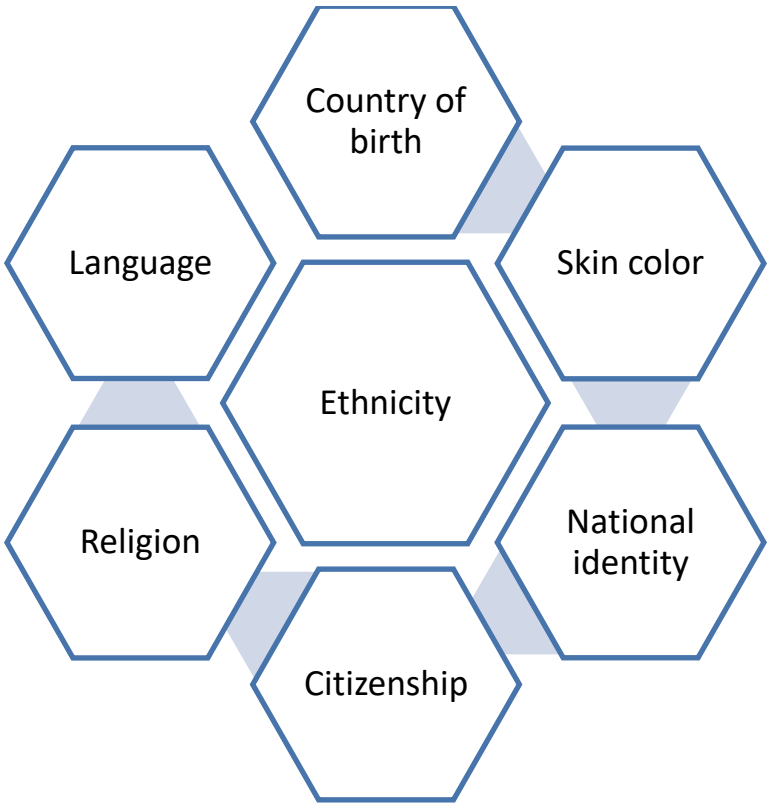

Figure 1.6 A representation of some of the complex factors that contribute to ethnicity definition.

In the topic of obesity measured via BMI in epidemiological studies, the ethnic disparities are clear (20, 210). For example, a meta-analysis review included data from National Health and Nutrition Examination Survey and the Behavioural Risk Factor Surveillance System looking at ethnic disparities in obesity prevalence in US between 1990 and 2001 and reported that obesity prevalence varied significantly by ethnicity, with Native American and Pacific Islanders at high risk, and Asian Americans (of Vietnamese, Korean, Japanese or other Asian ancestries) exhibiting remarkably low rates of obesity (**Figure 1.7**)(65). These differences may be influenced by a variety of cultural, lifestyle and socio-economic effects, although it has been suggested that these ethnic differences in propensity to obesity, persist after adjusting for these factors (211, 212).

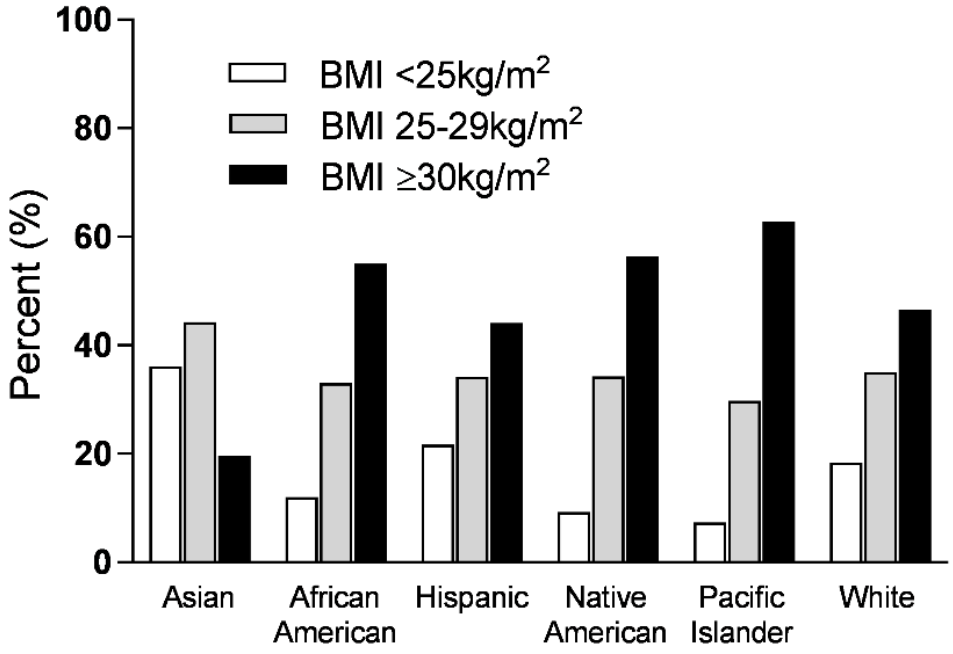

Figure 1.7 Adult obesity rates by ethnicity Percentage of normal weight, overweight, and obese adults (>30 years) within each ethnic group. Adapted from Wang et al. 2007 (65).

The UK is a cosmopolitan country with high ethnic diversity, between 2005 and 2009, 9.3% of all babies born in England were of SA origin, including individuals from Pakistan, India, Bangladesh, Nepal and Sri-Lanka. Further 5.3% were from Black British origins, including individuals from BA and African Caribbean ancestry (213). Table 1.2 shows the ethnic diversity distribution in the UK with 12.6% of the total UK population as non-Cau, and SA representing almost 50% of the non-Cau population in the UK (**Table 1.2**). Moreover, London was the most ethnically diverse area, with the highest proportion of non-white ethnic groups and the lowest proportion of white population, at 59.8% (213).

Table 1.2 Ethnic diversity in the UK according to the 2011 Census, the Office of National Statistics.

Ethnic group	Population	% of total population
Caucasian	55,010,359	87.1
Asian or Asian British	4,373,339	6.9
Black or Black British	1,904,684	3.0
Mixed or Multiple	1,250,229	2.0
Gypsy/Traveller/Irish Traveller	63,193	0.1
Other Ethnic Group	580,374	0.9
Total	**63,182,178**	**100**

In the UK, SA accounts for 14.0% of total subjects with T2D despite representing less than 6.9% of the overall population, according to the Association Of Public Health Observatories (214) (**Figure 1.8**).

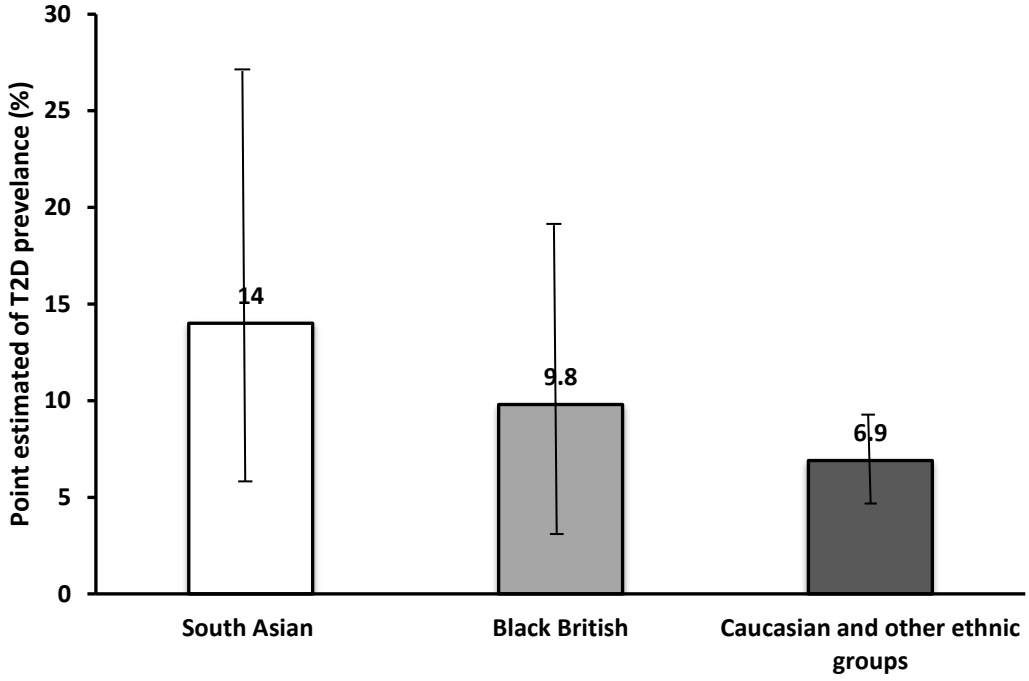

Figure 1.8 Estimates of type 2 diabetes prevalence by ethnicity for England in 2010 from The Association of Public Health Observatories. South Asian (14.0% UR 6.5–26.6%) and Black (9.8% UR 3.9–20.8%) ethnic groups have a higher prevalence than Caucasian and other ethnic groups (6.9% UR 5.2–9.4%).

A large cross-sectional study of 490,288 subjects in the UK investigated the association between the incidence of T2D and adiposity, and showed significant variations by ethnicity, with T2D developed at remarkably low BMI in SA (male 21.6 kg/m^2, female 22 kg/m^2) compared with Black British (male 26 kg/m^2, female 28 kg/m^2) and Cau (30 kg/m^2 for male and female) populations (20) (**Figure 1.9**).

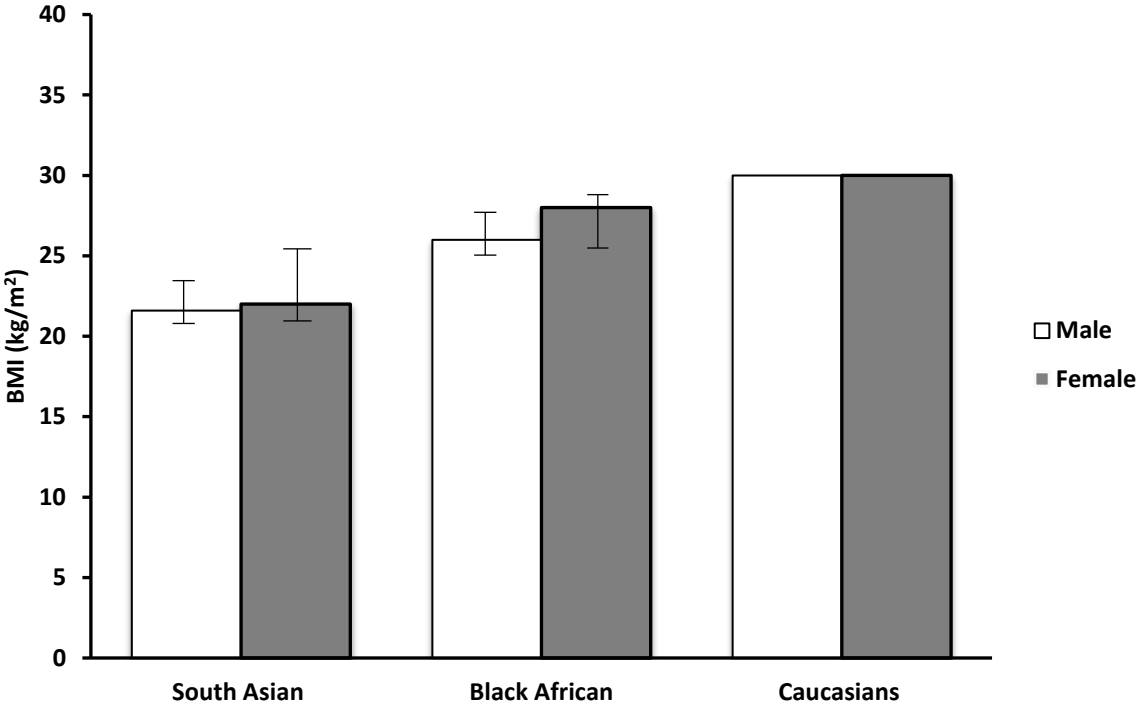

Figure 1.9 Age-adjusted associations between type 2 diabetes prevalence and adiposity by ethnicity; South Asian (male 21.6 kg/m^2, female 22 kg/m^2), Black British (male 26 kg/m^2, female 28 kg/m^2) and Caucasian (30 kg/m^2 for male and female). No variances are shown in the Caucasian data because it represents the defined reference cut-off value for the analysis. Data adapted from Ntuk *et al.* 2014 (20).

Studies looking at the incidence of obesity-related metabolic disorders such as insulin resistance, metabolic syndrome, T2D, HTN, CVD and stroke in different populations showed significant disparities among different ethnic groups (215-217). SA and Black individuals living in Europe have a remarkably higher prevalence of T2D compared to Cau (218-220), with the prevalence of T2D in SA adults shown to be between 6-10 times higher than Cau adults (221), developed at a younger age with a faster progression (222-224). In a study in Oslo, looking at T2D susceptibility among four ethnic groups, SA had 3-4 fold higher risk compared to other ethnic groups after

adjusting for age and socioeconomic factors, despite comparable BMI, WC, and waist to hip ratio (WHR) (225). Black individuals showed three times higher susceptibility to develop T2D compared to Cau (226, 227). Moreover, SA and Black individuals, tend to progress from impaired glucose tolerance (IGT> 100 mg/dL of plasma blood) to T2D at a faster rate (usually two times higher) than Cau (215, 228, 229).

SA males, in particular, may be genetically predisposed to premature CVD as it is widely reported that they have significantly higher CVD mortality rates compared to other ethnicities, along with increased CVD risk in childhood (230, 231). The predisposition of SA toward insulin resistance, together with raised TG and lower HDL cholesterol, are contributing factors to their elevated CVD risk (232-234). As such, SA populations are characterised as metabolically obese, even at normal-weight individuals (235). Furthermore, BA individuals showed increased susceptibility to HTN and insulin resistance, while TG levels are substantially lower compared to Cau (236-238). On the contrary, BA showed substantially lower CVD mortality rates when compared with Cau, whereas foreign-born BA populations have lower CHD mortality rates than USA-born BA who have increased rates compared with Cau (239-241). In addition, studies demonstrated that BA individuals have a higher risk of stroke compared to Cau (242). In the UK, a study conducted among people with T2D reported mortality from stroke was 3.5–4 times higher in BA than in Cau (243). Furthermore, in a 9-year follow-up study looking at the changes in plasma glucose, Black subjects developed HTN earlier than Cau or SA patients, but interestingly showed the most favourable lipid profiles (244, 245). This is in agreement with the high incidence and prevalence of HTN in Black subjects with and without T2D (244, 246).

1.6 Aims of the thesis

- To quantify visceral, abdominal subcutaneous, ectopic fat in the liver and pancreas in free-living general population, pre-diabetic and SA populations.
- To examine the association between MRI derived measurements and age, gender and anthropometric measurements in the general population, pre-diabetic and SA populations.
- To investigate the association between MRI derived measurements and metabolic parameters in high-risk populations for developing metabolic disorders, especially South Asians.
- To determine the ethnic differences in phenotypes of body fat in South Asian, Cau and BA populations.

1.6.1 Hypothesis

The hypothesis investigated in this thesis is that SA populations have increased VAT and ectopic fat deposition in the liver compared to Cau populations, which may help to explain the differences in obesity-related disease prevalence.

Personal contribution to the research presented in this thesis

All the work presented in this Thesis is the original work of the Thesis candidate unless otherwise stated.

Briefly, this research aims to provide an in-depth body fat phenotyping in SA, Cau and BA populations. This necessitates being a part of national and international collaborations, including Indian, UK, and European cohorts.

During the time as a PhD student, the author was involved on a day-to-day basis with the MR analysis of adipose tissue and ectopic fat quantification. My role involved:

- Arranging the raw data and metadata with a clean presentation and ensuring all subjects scored valuable inputs.
- Preparing the raw images for fat analysis and quantification.
- Assessing the quality control of the images in the Diabetes Research on Patient Stratification (DIRECT), and Pune Maternal Nutrition Study (PMNS).
- Optimizing the image tools to recover the non-analysable images, where possible.
- Quantify the amount of VAT, ASAT contents, liver fat, pancreas fat contents from MR images in PMNS and DIRECT.
- Co-ordinating the datasets for all the PMNS, DIRECT, The West London Observation study and UK Biobank including anthropometry, metabolic numbers, DXA, and MRI images.
- Planning and executing the proper statistical analyses in presenting the results.

The job entitled post-MR scanning operations. Therefore, the author was not involved in the subject recruitment or performing the scans because the breadth of the research required acquiring the data from multi-international collaborations. However, the author was closely monitoring the required detailed information on the recruitment and scanning from collaborators in the UK, Europe, and India.

During the time of my PhD, I performed **adipose tissue quantification for 2,001 subjects including the VAT, ASAT and liver fat contents** presented in this research. I also managed a large dataset of 12,272 subjects, including anthropometry, metabolic profile, DXA, and MRI scan outcomes.

Chapter 2

Phenotyping Body Fat Deposition and Ectopic Fat in Free-Living and Pre-diabetic Populations.

Chapter 2 Phenotyping body fat deposition and ectopic fat in free-living and pre-diabetic populations.

2.1 Introduction

As discussed in Chapter 1, different body fat compartments have different association with metabolic diseases, in particular, the development of T2D; with VAT, liver and pancreatic fat showing a positive association with metabolic risk, and in some cases independent of each other (247), whereas other fat compartments such as ASAT showed an apparent protective role with metabolic risk (108).

While various methodologies exist to measure overall body adiposity, available methods for measuring regional adiposity are more limited due to high technical demands or resource availability. Simple anthropometry, while cheap and easily obtainable, does not always provide a very accurate assessment for negative health outcomes. For example, despite its convenience, BMI fails to account for body composition (187). Furthermore, previous studies have confirmed that the relationship between BMI and regional body adiposity varies considerably by factors such as age (248), gender (249), and race/ethnicity (250). Similarly, WHR is also a poor marker of regional AT distribution since it is influenced by a number of factors such as frame size and skeletal muscle mass (251).

The accurate measurement of different abdominal fat depots, including VAT, ASAT, liver and pancreatic fat, is crucial. This is because the risks associated with excess adiposity have consistently been shown to be a function of regional fat distribution, rather than overall fatness; accumulation of VAT is linked to the development of metabolic syndrome features, including insulin resistance, T2D, dyslipidaemia, inflammation, HTN and CVD (83). As mentioned in Chapter 1 The introduction, ectopic fat build-up, particularly in the liver, is also associated with insulin resistance and other metabolic complications (148), independently of age, gender, and BMI (252). In addition, body fat compartments (VAT and liver fat) can be found in high

amounts in lean subjects and increase their susceptibility for metabolic diseases such as T2D (72, 137). Amongst anthropometric measures, WC represents the strongest correlation with abdominal adiposity and liver fat deposition (72). This is further complicated by the fact that abdominal, subcutaneous, VAT and liver fat may expand independently of each other (139, 253). Individual body fat compartments show different associations with all-cause mortality, relationships affected by age (254), gender (255), race/ethnicity (256) and socioeconomic and lifestyle factors such as diet and exercise (257, 258). In contrast to VAT, SAT may demonstrate a protective function against CVD development (108).

Moreover, there are established gender differences in body fat compartments with males tending to accumulate higher VAT and liver fat, while females tend to accumulate higher ASAT (249, 259). However, less is known about these gender differences during early metabolic alterations such as pre-diabetes status and the progression to T2D. Indeed, loss of VAT or liver fat is associated with an improvement in metabolic health without significant weight loss (257). Moreover, a recent study showed that both liver and pancreatic fat are key factors for the remission of T2D (171). Therefore, it is important to accurately measure VAT, ASAT, liver and pancreatic fat in order to determine metabolic health.

Large scale analysis of the compartmental distribution of AT is often limited by the expense and time required to employ requisite imaging techniques. In this chapter, I analyse the relationship between body fat distribution and anthropometry of free-living and pre-diabetic populations from two of the largest available UK and European population studies: the UK Biobank and the DIRECT study. The UK Biobank provides a comprehensive means of assessing the relationship between body composition and anthropometry in a large population-based cohort of adults aged 40-70 years old, recruited between 2007 and 2010 (260). The DIRECT study recruited 1,558 pre-diabetic subjects between the ages of 30-75 years, beginning in 2012 (261). Abdominal MR images were obtained for all participants allowing for the quantification of VAT, ASAT and ectopic fat content in the liver and pancreas.

The aim of this chapter is to examine the association between MRI derived abdominal adiposity measurements (ASAT, VAT, liver fat fraction, pancreatic fat) and age, gender, anthropometry and blood pressure in a free-living population. These results will be compared to those obtained in a cohort of pre-diabetic subjects.

2.1.1 Objectives

1. Investigate the association between MRI derived measurements (VAT, ASAT, liver fat) and age, gender and anthropometries in a free-living population (UK Biobank)
2. Quantify ectopic fat depots (liver, pancreas) in a pre-diabetic population (DIRECT).
3. Compare total body fat, VAT, ASAT and liver fat content between free-living and pre-diabetic populations.

2.2 Methods

2.2.1 Free-living population (UK Biobank)

2.2.1.1 UK Biobank participants

Written, informed consent was acquired from all participants as part of the large cross-sectional UK Biobank resource (260). The UK Biobank has approval from the North West Multi-Centre Research Ethics Committee (MREC). In total, data from 5,986 individuals (2,849M, 3,137F) from the UK Biobank, recruited between 2007 and 2010, were included. All participants were aged between 40 and 70 years old. The complete UK Biobank data set includes 502,656 UK adults (229,182 males and 273,474 females). Participant recruitment was conducted via centrally coordinated identification and invitation from population-based National Health Service patient registers of individuals living within a reasonable distance of an assessment centre. All participants from the general population were invited to participate. Exclusion criteria included participants who had metal in their bodies or devices such as pacemakers, were claustrophobic or anyone taking prescribed medication or females on the contraceptive pill. Participants underwent anthropometric

assessment, blood pressure, total body MRI scanning, MR liver proton density fat fraction (PDFF), detailed below in Section 2.2.1.4, acquired through UK Biobank Access Applications number 6569 and 9914 (**Appendix 1**).

2.2.1.2 UK Biobank anthropometric measurements

At the UK Biobank centres, a touchscreen questionnaire was used to collect information on demographic characteristics and lifestyle exposure, including day to day events (www.ukbiobank.ac.uk/resources). Height (cm) was measured from bare feet using a Seca 202 height measure (Seca, Hamburg, Germany). Weight and body fat percentage was measured with a Tanita BC418ma BEI device (Tanita, Tokyo, Japan). From these values, BMI was calculated. 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. The average of two blood pressure measurements, taken moments apart, was obtained using an automated device (Omron, UK) (260).

2.2.1.3 UK Biobank physical activity

Physical activity and inactivity were measured from the International Physical Activity Questionnaire (IPAQ) during the participant's essential visit to UK Biobank centre. The terms and the definition used for the physical activity and inactivity are summarized in the Appendix **(Appendix 2)**. Participants responded to questions on physical activity and inactivity including walking, moderate, vigorous physical activity and time spent watching TV or driving. Scores for each of these questions were then used to calculate a single score for "total physical activity" over the previous week, in line with IPAQ guidelines (described in details in **Appendix 2**, expressed as "metabolic equivalent minutes" (MET minutes) of activity per week.

2.2.1.4 Measures of body fat content

After the initial assessment, all eligible UK Biobank participants were invited for imaging assessment at UK Biobank Imaging centres. AT and ectopic fat content were acquired at the UK Biobank imaging Centre at Cheadle (UK) using a Siemens 1.5T Magnetom Aera. MR acquisitions were components of an extensive scanning protocol, including brain and cardiac imaging as well as DXA (GE-Lunar, Madison) to measure FM, fat percentage and lean mass.

Visceral fat and ASAT were measured via MR dual-echo Dixon Vibe protocol. The participants were scanned in a supine position with arms along the sides, scan area from the neck to knees. There was no localiser (pilot scan) used to position the anatomical landmarks for the scan. Instead the subjects' clavicles were used. The Dixon protocol covered a total of 1.1 m, divided over six overlapping slabs of axial 3D spoiled gradient dual-echo images. Images were acquired using the following parameters: TR=6.69 ms, TE=2.39/4.77 ms, and bandwidth 400 Hz. Integrated scanner software (AMRA profile) was used to reconstruct water-fat Dixon images (in phase and out of phase) (262). A multi-echo spoiled-gradient-echo acquisition was used to calculated T2* and PDFF maps for the liver. A single transverse slice of the liver was captured using the following parameters; field of view=40x40 cm, 160x160 acquisition matrix yielding a voxel size of 2.5 mm x 2.5 mm, 6 mm slice thickness, 20 flip angle, 27 ms TR, and 2 signal averages. Ten echo times were selected such that the signals from fat and water were in phase and out of phase at 1.5T. The acquisition of echoes needed for the liver PDFF images construction occurred during a single expiration breath-hold using the following echo times: TE: 2.38, 4.76, 9.52, 11.90, 14.28, 16.66, 19.04, 21.42, and 23.80 ms (263).

2.2.2 Pre-diabetic population (DIRECT)

2.2.2.1 DIRECT participants

The DIRECT (Diabetes Research on Patient Stratification) Study is an Innovative Medicine Initiative (IMI) and a part of the seventh European Union

Framework. IMI DIRECT is a joint undertaking between four industries and 21 academic partners throughout Europe (**Figure 2.1**). The DIRECT IMI was carried out under grant agreement number 115317 (DIRECT), resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013), and European Federation of Pharmaceutical Industries and Associations. The DIRECT IMI consortium had two phases; the first phase to identify pre-diabetic individuals, discover and validate biomarkers that predict the rate of glycaemic deterioration before and after T2D onset. The aim of the second phase was to predict the response to diabetes therapies and help stratify T2D into clearly definable subclasses that can be treated more effectively. Potential participants from four large Scandinavian studies were contacted if they were classified as pre-diabetic based on glycated hemoglobin (HbA1c) inclusion values ranging from 5.7 to 6.4% or 40-48 mmol/mol.

Inclusion criteria comprised 1) HbA1c values ranging from 5.7 to 6.4% or 40-48 mmol/mol, 2) Fasting blood glucose <10 mmol/l at recruitment, 3) Age 30-75 years. Exclusion criteria comprised; 1) Diagnosis of type 1 or T2D, HbA1c ≥6.5% (48 mmol/mol), fasting plasma glucose ≥7.0 mmol/L or 2 h plasma glucose >11.0 mmol/L; 2) Treatment with insulin-sensitising, glucose-lowering or other antidiabetic drugs; 3) Pregnancy, lactation or plans to conceive within the study period; 4) Use of a pacemaker.

Written, informed consent was acquired from all participants. Subjects with early stage of pre-diabetes were invited to take part in the study. Ethical approval was received from the National Research Ethics Service and Newcastle Hospital NHS Foundation Trust. All eligible volunteers were invited to a baseline assessment at one of six clinical research facilities (**Figure 2.1**) where they completed an MRI scan and a lifestyle questionnaire between November 2012 and November 2014 at seven different locations across northern Europe. In total, 1,558 participants (1,125 males, 433 females) were recruited and assessed at one of six centres (**Figure 2.1**).

Figure 2.1 The Diabetes Research on patients' stratification (DIRECT) project landscape. A map representing the co-ordination teams and partners for the DIRECT cohort. Scanning took place at one of the 6 following centres; from Copenhagen; Technical University of Denmark, University of Copenhagen, From Kuopio; University of Eastern Finland, From Newcastle; University of Newcastle upon Tyne, From Exeter; University of Exeter, From Amsterdam: VU University Medical Centre Amsterdam; From Dundee: University of Dundee. Figure adapted from DIRECT Diabetes Research on patient stratification website. Access date 25 May 2018.

For the purpose of the current research, subjects with Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) were grouped as pre-diabetes.

2.2.2.2DIRECT anthropometric, body fat percentage and blood pressure measurements

Examinations were carried out in the morning after a 10 h overnight fast. All measurement procedures were standardized across study sites and performed by trained nurses. Height was measured using calibrated wall-mounted stadiometers, weight using calibrated scales, and waist and hip circumferences using non-stretchable measuring tapes. 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. Average of two blood pressure measurements, taken moments apart, was obtained using an automated device (Omron, UK). Body fat percentage measured using DXA (GE-Lunar, Madison).

2.2.2.3 DIRECT Physical activity assessment

Habitual physical activity was assessed using a wrist-worn triaxial accelerometer (ActiGraph GT3X+; ActiGraph LLC, Pensacola, FL, USA). The monitor was fitted to the participant's non-dominant wrist using an adjustable strap (ActiGraph LLC). The participant was requested to wear the monitor continuously for 10 days to allow habitual uninterrupted measures of physical activity. Participants were asked to wear an additional monitor on their dominant hip. The participants were instructed to remove the monitor only when undertaking water-based activities (deeper than 1m and lasting longer than 30min), or if the monitor caused discomfort. Participants were given a prepaid, addressed, padded envelope in which to deposit the monitor and return it. The raw data from the monitor was assessed and translated into Euclidean Norm Minus One (ENMO) metric outcome. ENMO is a widely used metric indicator of physical activity from raw data in epidemiological studies (264).

2.2.2.4 DIRECT imaging protocol

MR scanning protocols were standardized across study centres to harmonise the scan methodology due to different equipment used by each centre. Scanners used were 1.5 Tesla (T) Philips Intera at the University of Exeter in the UK, Siemens Espree 1.5T at the University of Newcastle in the UK, Siemens Avanto 1.5T in University of Eastern Finland in Finland and VU University Medical Centre Amsterdam in the Netherlands. Siemens Trio 3T used in the University of Dundee in the UK and an Achieva 3T in Copenhagen University in Denmark. All participants were scanned in the prone position with arms extended above the head. T1-weighted images were acquired from the diaphragm to acetabulum using the maximum fields of view during free breathing with a slice thickness of 10 mm x 10 mm slice gap. VAT and ASAT

were quantified from transverse T1-weighted MR images using Slice-O-Matic software.

Liver and pancreas images were acquired using Multi-Echo sequence with a surface coil with the following parameters; TR = 1500 ms; field of view = 500 mm; slice thickness = 10 mm, TE varied between 8-20 ms depending on the scanner used (increases the scanner strength, reduces the echo time), and were chosen to represent in and out of phase signals.

2.2.2.5 DIRECT imaging analysis

Raw MRI data was converted into an analysable format using ImageJ (Image; National Institute of Health, Bethesda, MD). After assessing the quality control of the image by ensuring: 1. enough contrast for visualising the organs, 2. the image was free from artefacts such as breathing or motion artefacts. For each specific image, an entire organ was selected using continued dots placed to cover the entire organ (in this case the liver or the pancreas), avoiding vascular structures. Representative area covered for liver and pancreas are shown in **Figure 2.2**. The liver and pancreas organ extractions were performed separately.

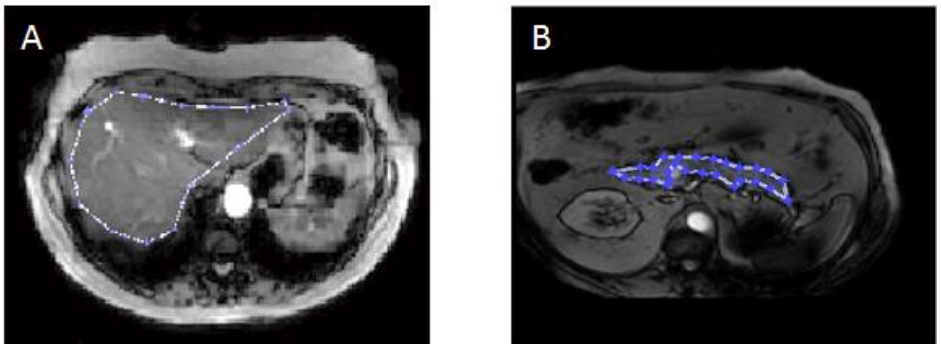

Figure 2.2 Magnetic resonance organ extraction for fat quantification in the liver (A) and the pancreas (B) in the Diabetes Research on patients' stratification (DIRECT) project.

The mean signal intensity was measured at each echo time. A curve-fitting algorithm using the exponential model was used to derive a fat fraction, as well as the component T2* decays for fat and water. An automated pixel by pixel analysis was performed to obtain colour-coded parametric heat maps of

the entire liver and pancreas using script code in Matlab version 2013 (Mathworks, Natick, MA, USA) (see the script code in **Appendix 3**). The relative portion of fat and water within each organ were then calculated from the T2* fitting curve of water and fat (**Figure 2.3**). The outcomes from each image were liver/pancreatic fat percentage, T2* (relaxation time), and R2* (relaxation rate).

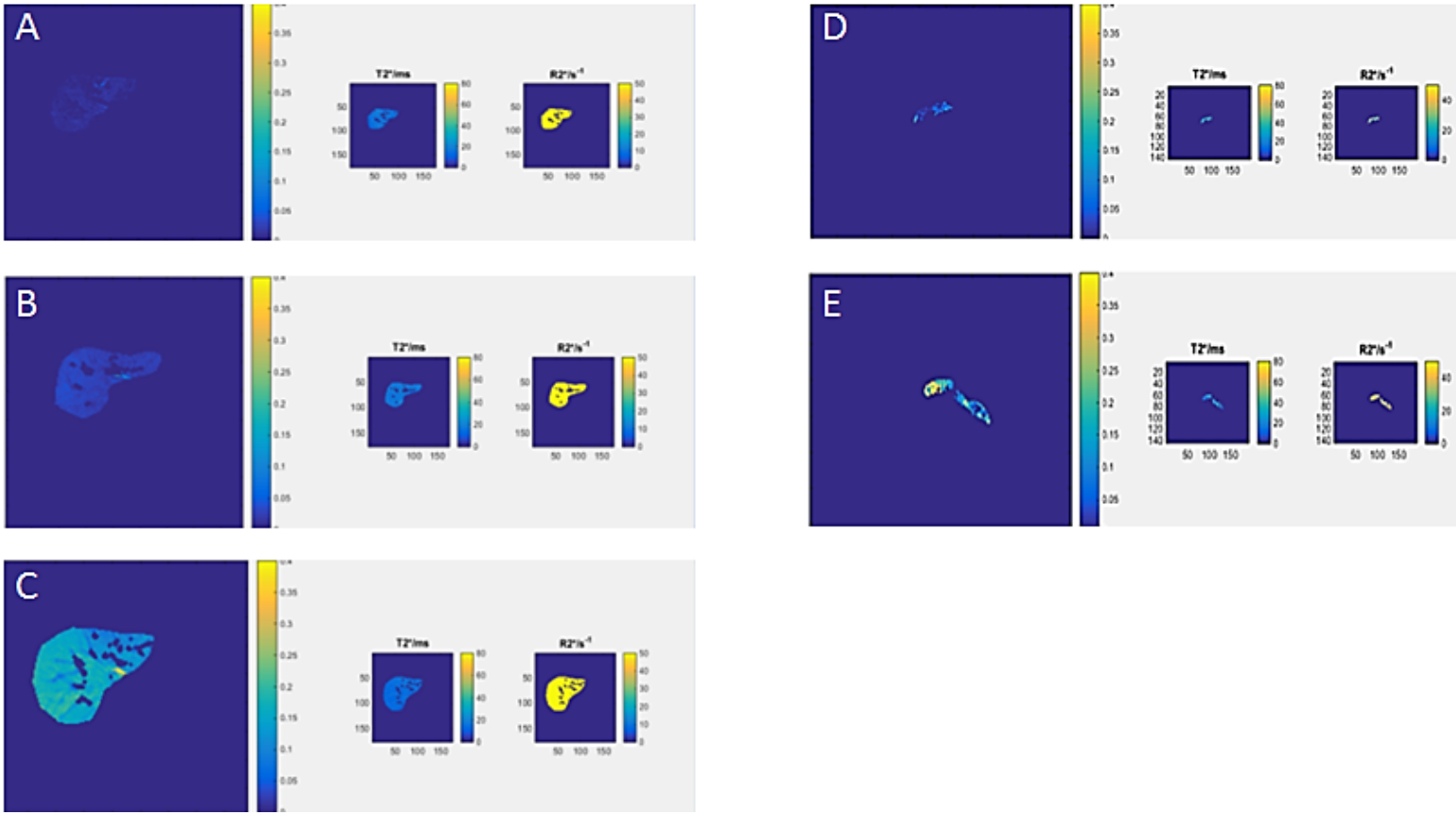

Figure 2.3 Magnetic resonance ectopic fat quantification in the liver and the pancreas of the Diabetes Research on patients' stratification (DIRECT) project. Examples of ectopic fat quantifications from 5 individuals in the liver (**A-C**) and pancreas (**D**, **E**). **A)** Low liver fat = 1.0%, **B)** Moderate liver fat content= 2.8%, **C)** High liver fat content= 15.1%. **D)** Low pancretic fat content= 4.7%, **E)** High pancretic fat content= 17.9%.

2.2.3 Statistical analysis

Descriptive statistics were obtained for anthropometric, blood pressure and AT measurements, and are reported as mean and standard deviation. The log of liver fat fraction data was performed prior to analysis due to the non-normally distributed nature of the liver data. Analyses were done in male and female subjects separately due to the established gender differences in regional adiposity. Gender differences were assessed using non-parametric Mann-Whitney U test.

The differences in body fat distribution by age and BMI were assessed using Kruskal-Wallis test with multiple comparisons. Correlation analysis was performed between anthropometric, MRI measures of visceral, ASAT and liver fat outcomes.

Gender-specific comparison of MR body fat measurements between free-living (UK Biobank) and pre-diabetic (DIRECT) populations was assessed using the non-parametric Mann-Whitney U test. All statistical analyses were performed using the Statistical Package for The Social Sciences (SPSS) version 23.0 (SPSS Inc. Chicago, USA) and graphs were generated using GraphPad Prism version 5.0 (GraphPad Software Inc. California, USA).

2.3 Results

2.3.1 Free-living population UK Biobank

2.3.1.1 UK Biobank population data

Population characteristics for data from 5,986 individuals (2,849M, 3,137F) in the free-living population are shown in **Table 2.1**, including indirect measures of body fat as measured by dual emission X-ray absorptiometry (DXA) and MR.

Table 2.1 Baseline characteristics of free-living population of the UK Biobank cohort. Outcome data from UK Biobank participants. VAT; Visceral adipose tissue; ASAT: Abdominal subcutaneous adipose tissue; DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure, VAT and ASAT: n=6021, liver fat =5971 and DXA outcomes: n=5170. Mean ± standard deviation calculated using SPSS 23.0.

	UK Biobank Cohort	Mean ± SD	Range
Anthropometry	Age (years)	61.7 ± 7.1	44 - 73
	Waist circumference (cm)	87.4 ± 12.1	55 - 150
	Hip (cm)	101.3 ± 8.6	73 - 152
	Height (cm)	169.5 ± 9.2	141 - 203
	Weight (kg)	75.8 ± 15.1	39 - 160
	BMI (kg /cm^2)	26.7 ± 4.4	14.2 - 49.2
Blood pressure	DBP (mmHg)	78.7 ± 10.0	36 - 120
	SBP (mmHg)	133.9 ± 17.7	75 - 221
DXA	Total Fat Mass (kg)	25.8 ± 9.1	2.7 – 76.1
	Total Fat Free Mass (kg)	49.8 ± 10.2	7.4 - 84.9
	Total Lean Mass (kg)	47.1 ± 9.7	6.8 - 80.3
	Total Tissue Fat (%)	34.9 ± 8.2	8.2 - 58.4
	VAT mass (kg)	1.2 ± 0.90	0 - 6.2
MR	VAT(litres)	3.7 ± 2.3	0.1 - 14.4
	ASAT (litres)	7.0 ± 3.2	0.7 – 23.5
	Liver fat PDFF (%)	4.1 ± 4.6	0.5 – 34.5

MR data were complete and available in VAT for 5,985 (M=2,849, F=3,136), ASAT for 5,985 (M=2,849, F=3,136) and in liver fat for 5,971 (M=2,839, F=3,132) in the free-living population from the UK Biobank study. There was no pancreas fat data available because while it was included in the imaging protocol of the UK Biobank, the data had not been released for analysis by the UK Biobank; **Figure 2.4** shows a flow chart demonstrating the MR images available for each body fat depots in the free-living population from the UK Biobank (**Figure 2.4**).

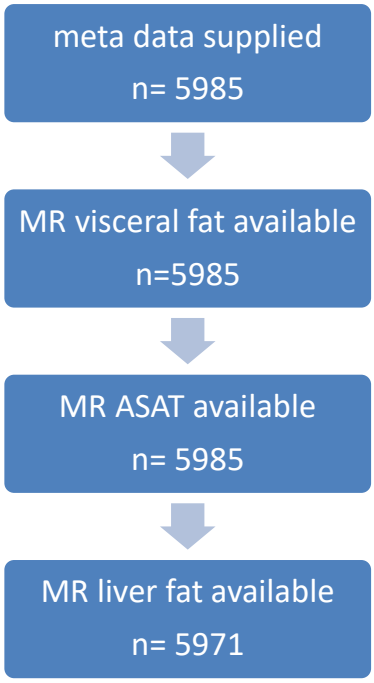

Figure 2.4 Flow chart demonstrating the MR images available for body fat depots of the free-living population from the UK Biobank

2.3.1.2 UK Biobank population data by gender

Table 2.2 presents the free-living population anthropometry, blood pressure, body composition via DAX and MRI for males and females with significant gender differences observed in all outcomes. In the free-living population from UK Biobank study, males were older, heavier with wider WC than females ($p<0.001$ for all) (**Table 2.2**). DBP and SBP were significantly higher in males than females in the free-living population ($p<0.001$). Total FM, total FFM and total lean mass were available from the DXA scan (**Table 2.2**), with females having a significantly higher total FM and tissue fat percentage than males in the free-living population ($p<0.001$ for all) (**Table 2.2**). Lean mass and FFM were significantly higher in males than females in the free-living population from UK Biobank ($p<0.001$ for all) (**Table 2.2**). There was good agreement in the measurements of VAT via DXA and MR with males having higher VAT than females in both modalities ($p<0.001$ for all) (**Table 2.2**).

There was a significant higher liver fat amount in males compared to females in the free-living population (p<0.001 for all) (**Table 2.2**).

Table 2.2 Gender specific characteristics of the free-living population of the UK Biobank cohort. Outcome data from UK Biobank participants. VAT; Visceral adipose tissue; ASAT: Abdominal subcutaneous adipose tissue; DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure, DXA: dual energy X-ray absorptiometry, n= M: 2,839, F: 3,132 except VAT and ASAT: M: 2,864, F: 3,157. A *U*-test for gender comparison was performed in SPSS 23.0. Mean ± standard deviation calculated using SPSS 23.0.

		Males		Females		*U*-test
		Mean ± SD	**Range**	**Mean ± SD**	**Range**	**p value**
Anthropometry	Age (years)	62.4 ± 7.1	44 to 73	61.1 ± 7.1	45 to 73	<0.001
	Waist circumference (cm)	93.4 ± 10.0	63 to 150	81.8 ± 11.2	55 to 137	<0.001
	Hip (cm)	101.4 ± 8.4	77 to 150	101.3 ± 8.7	73 to 152	0.028
	Height (cm)	176.4 ± 6.5	153 to 203	163.3 ± 6.3	141 to 193	<0.001
	Weight (kg)	83.6 ± 13.4	51 to 160	68.7 ± 12.9	39 to 154	<0.001
	BMI (kg\m^2)	27.1 ± 3.9	16 to 47	26.2 ± 4.7	14 to 49	<0.001
Blood pressure	DBP (mmHg)	80.3 ± 9.7	50 to 120	77.2 ± 11.1	36 to 118	<0.001
	SBP (mmHg)	137.5 ± 16.4	75 to 221	130.7 ± 18.1	82 to 202	<0.001
DXA	Total Fat Mass (kg)	24.8 ± 8.6	5.3 to 76.1	26.8 ± 9.4	2.7 to 73.0	<0.001
	Total Fat Free Mass (kg)	58.7 ± 6.9	38.4 to 84.2	41.9 ± 4.9	7.5 to 63.9	<0.001
	Total Lean Mass (kg)	55.5 ± 6.6	36.4 to 80.3	39.7 ± 4.7	6.8 to 60.5	<0.001
	Total Tissue Fat (%)	30.3 ± 6.4	8.2 to 50.7	39.2 ± 7.3	14 to 58.4	<0.001
	VAT mass (kg)	1.7 ± 1.0	0.01 to 3.2	0.8 ± 0.6	0 to 4.3	<0.001
	VAT volume (kg)	1.8 ± 1.0	0.01 to 6.6	0.8 ± 0.6	0 to 4.5	<0.001
MR	VAT(litres)	4.9 ± 2.3	0.4 to 14.4	2.6 ± 1.5	0 to 12.1	<0.001
	ASAT (litres)	5.9 ± 2.5	0.7 to 22.3	8.0 ± 3.4	0.8 to 23.5	<0.001
	Liver fat PDFF (%)	4.7 ± 4.7	0.7 to 34.0	3.6 ± 4.5	0.5 to 34.5	<0.001

Given that the sample size age in the free-living population ranged between 44 years (middle age) and 73 years old (old age), a breakdown of VAT, ASAT and liver fat by age and gender was performed (**Figure 2.5**). Overall, the effect of age and gender on VAT in free-living population was significant; with males showing higher VAT compared to females in all age groups, except in 70-73 years where VAT was similar between males and females in the free-living population (**Figure 2.5**). Males showed significantly lower VAT in the age group 70-73 years compared to younger males in the free-living population (p<0.0001) (**Figure 2.5**). However, the number of subjects in the 70-79 years group was limited (n = 5). Females also showed significant differences in VAT with age; the lowest amount of VAT (2.32 ± 1.41 litres)

observed in the youngest females (40-49 years), while the peak of VAT (2.83 ± 1.52 litres) was observed in 60-69 years group (p<0.0001) (**Figure 2.5**).

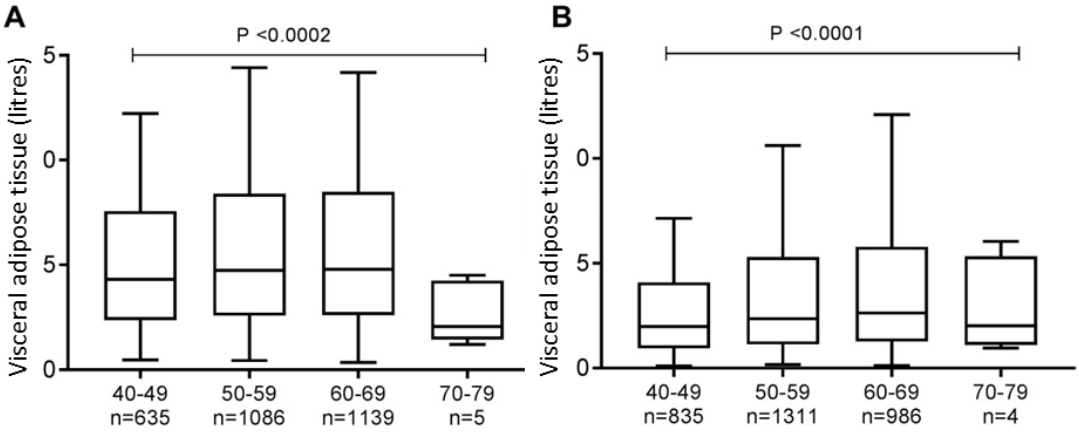

Figure 2.5 Gender specific visceral adipose tissue (VAT) distribution by age in the free-living population. VAT distribution by age in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. P values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23.0). Data obtained from UK Biobank. Graphs were done using Prism GraphPad version 5.0.

Investigating the age groups impact on ASAT and liver fat in free-living populations showed no significant differences in ASAT or liver fat with age in males (ASAT: p=0.1601, Liver fat: p=0.1595), while the amount of ASAT and liver fat was significantly higher with age in females (ASAT: p=0.0002, liver fat: p<0.0001) (**Figure 2.6**). Liver fat showed significantly higher accumulation with age in females while it showed the opposite in males in the free-living population, but not significant (**Figure 2.7**).

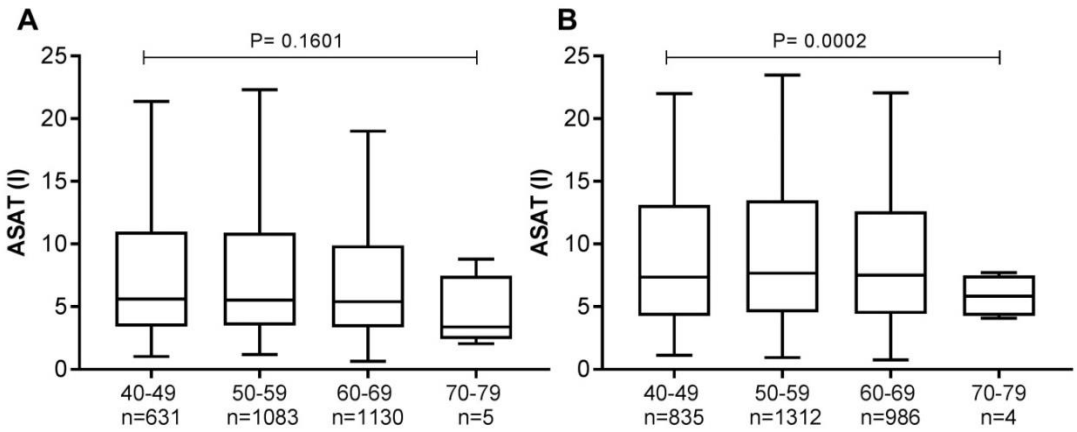

Figure 2.6 Gender specific abdominal subcutaneous adipose tissue (ASAT) distribution by age in the free-living population. ASAT distribution by age in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. P values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.24). Data obtained from UK Biobank. Graphs were done using Prism GraphPad Version 5.0.

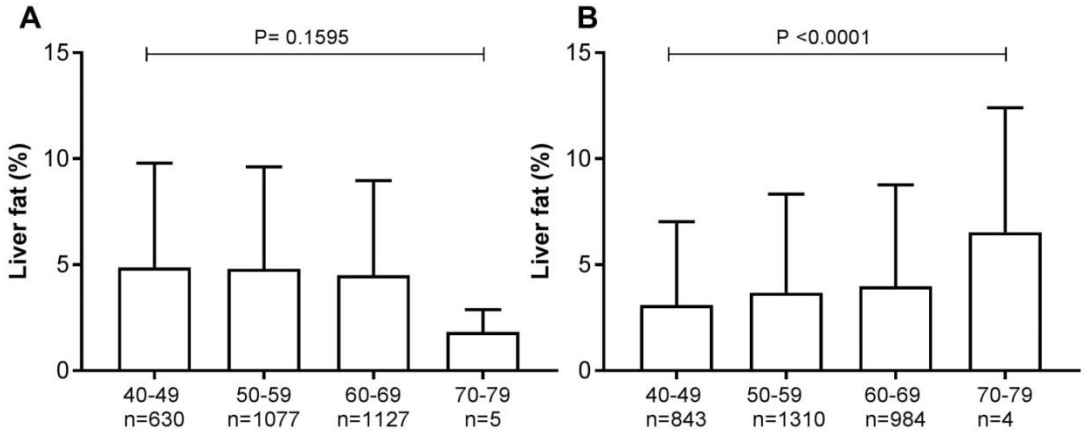

Figure 2.7 Gender specific distribution of liver fat distribution by age in the free-living population. Liver fat percentage distribution by age in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. P values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.24). Data obtained from UK Biobank. Graphs were done using Prism GraphPad version 5.0.

2.3.1.3 UK Biobank correlation analysis by gender

Gender specific correlation analysis between liver fat fraction, VAT and ASAT with anthropometric measures and blood pressure in the free-living population are shown in **Table 2.3**. Overall, there were significant gender differences between body fat depots and ectopic fat in the free-living population. All measures were found to positively correlate with liver fat fraction, except age in males from free-living population (r= -0.016, p=ns) (**Table 2.3**). VAT showed a weak association with age in females (r=0.137, p<0.001) but not in males (**Table 2.3, Figure 2.8**). There was no significant association in ASAT with age in males or females (**Table 2.3**) (**Figure 2.9**). The strongest association with liver fat fraction in the free-living population was WC in both males (r= 0.510, p< 0.001) and females (r= 0.606, p<0.001). WC was also the strongest correlate with VAT in males (r= 0.777, p<0.001) and females (r=0.834, p<0.001). For ASAT, WC was the strongest correlate in males (r= 0.847) whereas, in females, it was hip circumference (r= 0.880) (p<0.001 for all, **Table 2.3**).

In the free-living population, blood pressure and all anthropometry measures were significantly positively correlated with VAT, except height in females (**Table 2.3**). Amongst anthropometry, the strongest association with VAT was found with WC in both males (r=0.834, p<0.001) and females (WC r= 0.777, p<0.001). Similarly, anthropometric variables and blood pressure positively correlated with ASAT, except height in females; the strongest association with ASAT was WC in males (r=0.847, p<0.001) and HC in females (HC: r= 0.888, p<0.001) (**Table 2.3**). In general, correlations in the free-living population were strongest for ASAT, followed by VAT then liver fat fraction.

Table 2.3 Gender specific correlations between VAT, ASAT, liver fat and anthropometry, blood pressure in the free-living population. An R-value for non-parametric spearmen's test was performed. The strongest correlations for each fat depot are in bold font, n=M: 2,839, F: 3,132 except VAT and ASAT: M: 2,864, F: 3,157. Data obtained from UK Biobank. Statistics calculated using SPSS 23.0.

	Liver Fat Fraction				**VAT**				**Abdominal Subcutaneous AT**			
	Male		**Female**		**Male**		**Female**		**Male**		**Female**	
	R	**P value**	**r**	**P value**	**R**	**P value**	**r**	**P value**	**R**	**P value**	**r**	**P value**
Age (years)	-0.016	ns	0.129	0.001	0.080	ns	0.137	0.001	-0.055	ns	-0.038	ns
Weight (kg)	0.480	0.001	0.491	0.001	0.742	0.001	0.742	0.001	0.837	0.001	**0.897**	0.001
Height (cm)	0.388	0.001	-0.135	0.001	0.101	0.001	-0.011	ns	0.137	0.001	0.024	ns
BMI (kg/m^2)	0.449	0.001	0.485	0.001	0.755	0.001	0.696	0.001	0.792	0.001	0.839	0.001
Waist (cm)	0.510	0.001	0.606	0.001	**0.777**	0.001	**0.834**	0.001	**0.847**	0.001	0.861	0.001
Hip (cm)	0.388	0.001	0.441	0.001	0.604	0.001	0.667	0.001	0.793	0.001	0.880	0.001
IPAQ	-0.156	0.001	-0.122	0.001	-0.180	0.001	-0.187	0.001	-0.181	0.001	-0.193	0.001
SBP (mmHg)	0.189	0.001	0.239	0.001	0.166	0.001	0.228	0.001	0.108	0.001	0.162	0.001
DBP (mmHg)	0.209	0.001	0.230	0.001	0.206	0.001	0.250	0.001	0.176	0.001	0.261	0.001
Total fat mass (%)	0.432	0.001	0.467	0.001	0.670	0.001	0.690	0.001	0.714	0.001	0.804	0.001
VAT (litres)	**0.634**	0.001	**0.718**	0.001	-	-	-	-	0.684	0.001	0.761	0.001
ASAT (litres)	0.460	0.001	0.548	0.001	0.684	0.001	0.761	0.001	-	-	-	-
Liver fat (%)	-	-	-	-	0.530	0.001	0.605	0.001	0.389	0.001	0.433	0.001

There were gender differences in the association between abdominal AT distribution, liver fat and age; VAT and liver fat showed a weak correlation with age in females (VAT r=0.137, liver fat r=0.129, p<0.001), however in males, age did not show any significant correlation with VAT or liver fat (**Figure 2.8, 2.9**). On the other hand, ASAT did not show any gender differences with age in the free-living population (**Figure 2.10**).

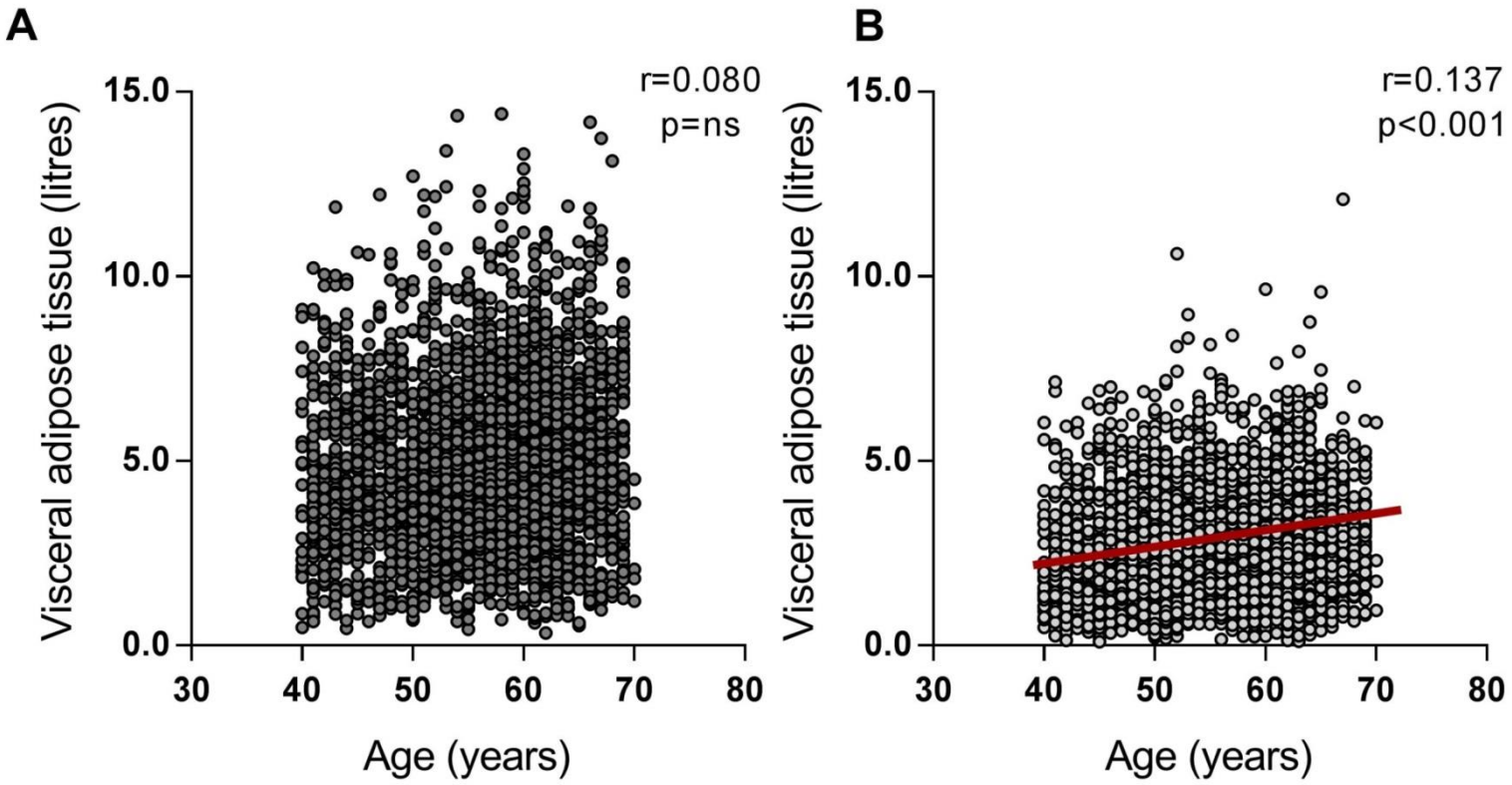

Figure 2.8 Gender-specific association between visceral adipose tissue distribution and age in the free-living population in (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. Data obtained from UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0.

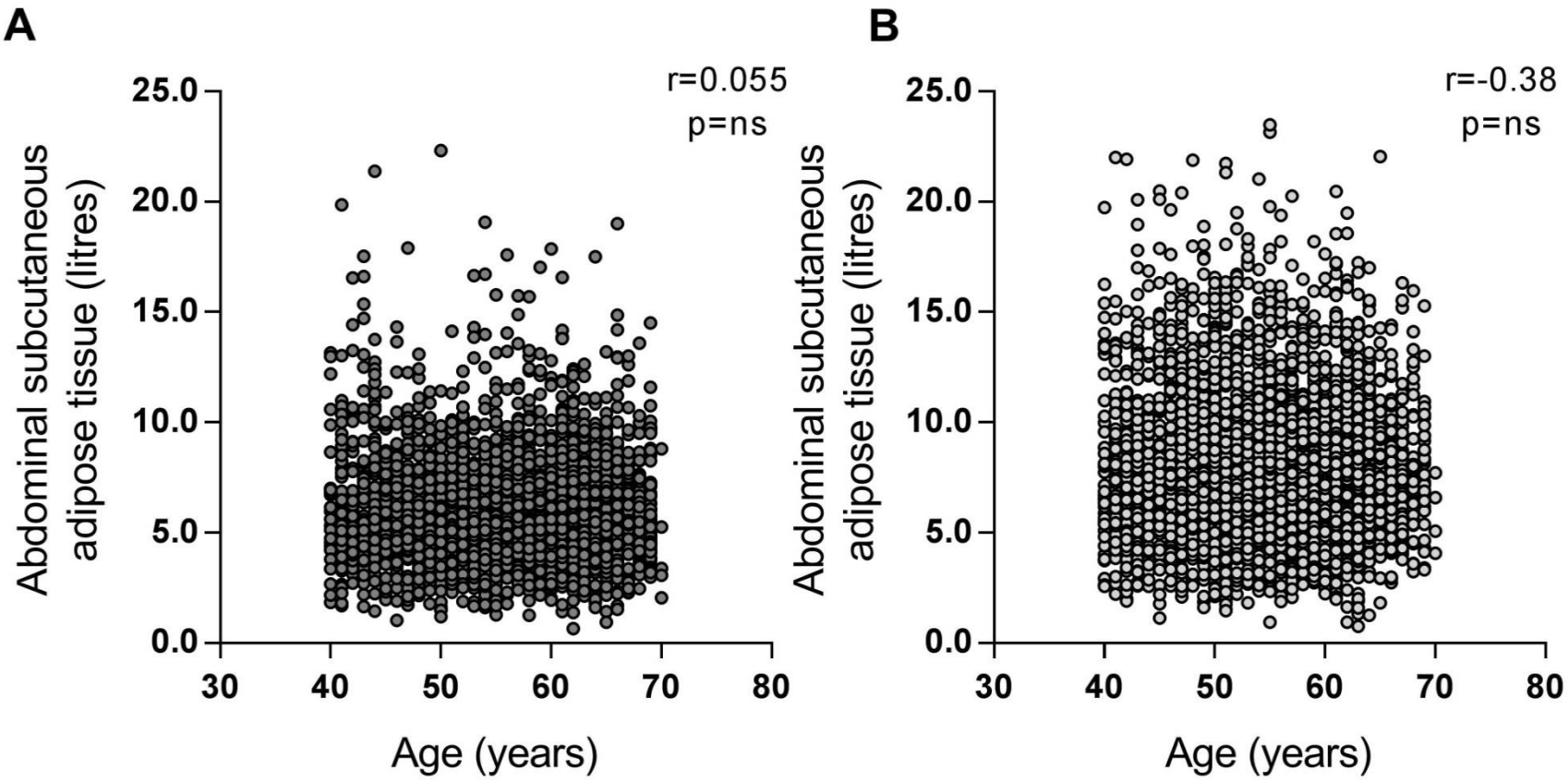

Figure 2.9 Gender-specific association between abdominal subcutaneous adipose tissue distribution and age in the free-living population in (**A**) males and (**B**) females. Non-parametric spearmen's test was performed. Data obtained from UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0.

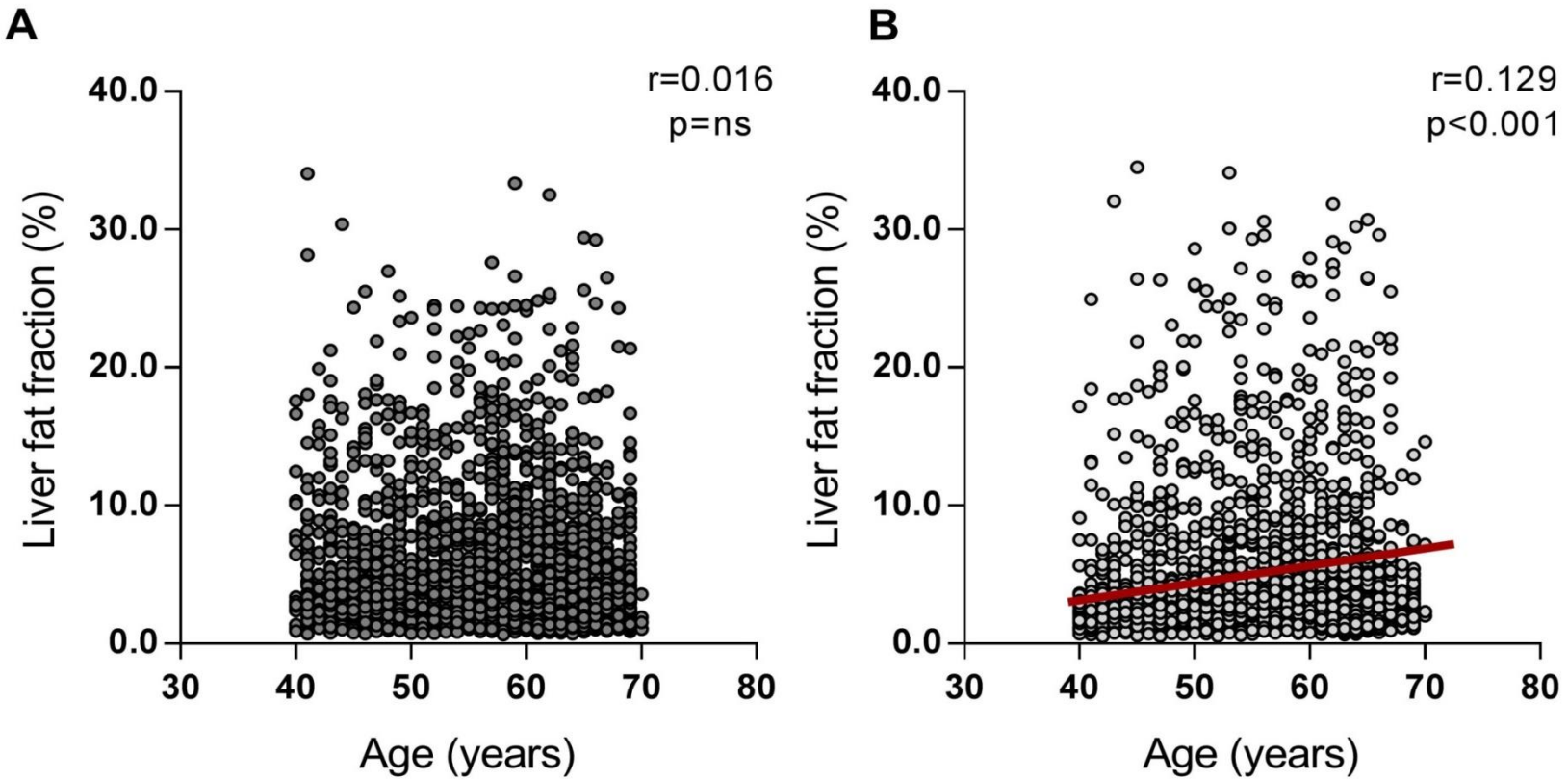

Figure 2.10 Gender-specific association between liver fat distribution and age in the free-living population in (**A**) males and (**B**) females. Non-parametric spearmen's test was performed. Data obtained from UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0.

There were gender specific differences in the association between VAT, ASAT and BMI. Overall, there was a significant association with BMI in VAT, ASAT and liver fat in the free-living population from the UK Biobank study (**Figure 2.11, 2.12, 2.13)**. In VAT, the association with BMI was stronger in males (r=0.775, p<0.001) than females (r=0.696, p<0.001) in the free-living population, whereas in ASAT it was the opposite gender association with BMI (M: r=0.792, F: r= 0.839, p<0.001) (**Figure 2.11, 2.12**). Liver fat content showed similar gender pattern (moderate correlation) with BMI in the free-living population (M: r=0.449, F: r=0.485, p<0.001) (**Figure 2.13**). From all body fat depots, the strongest association with BMI was observed with ASAT in both males and females in the free-living population, followed by VAT and then liver fat content (**Figure 2.11, 2.12, 2.13)**.

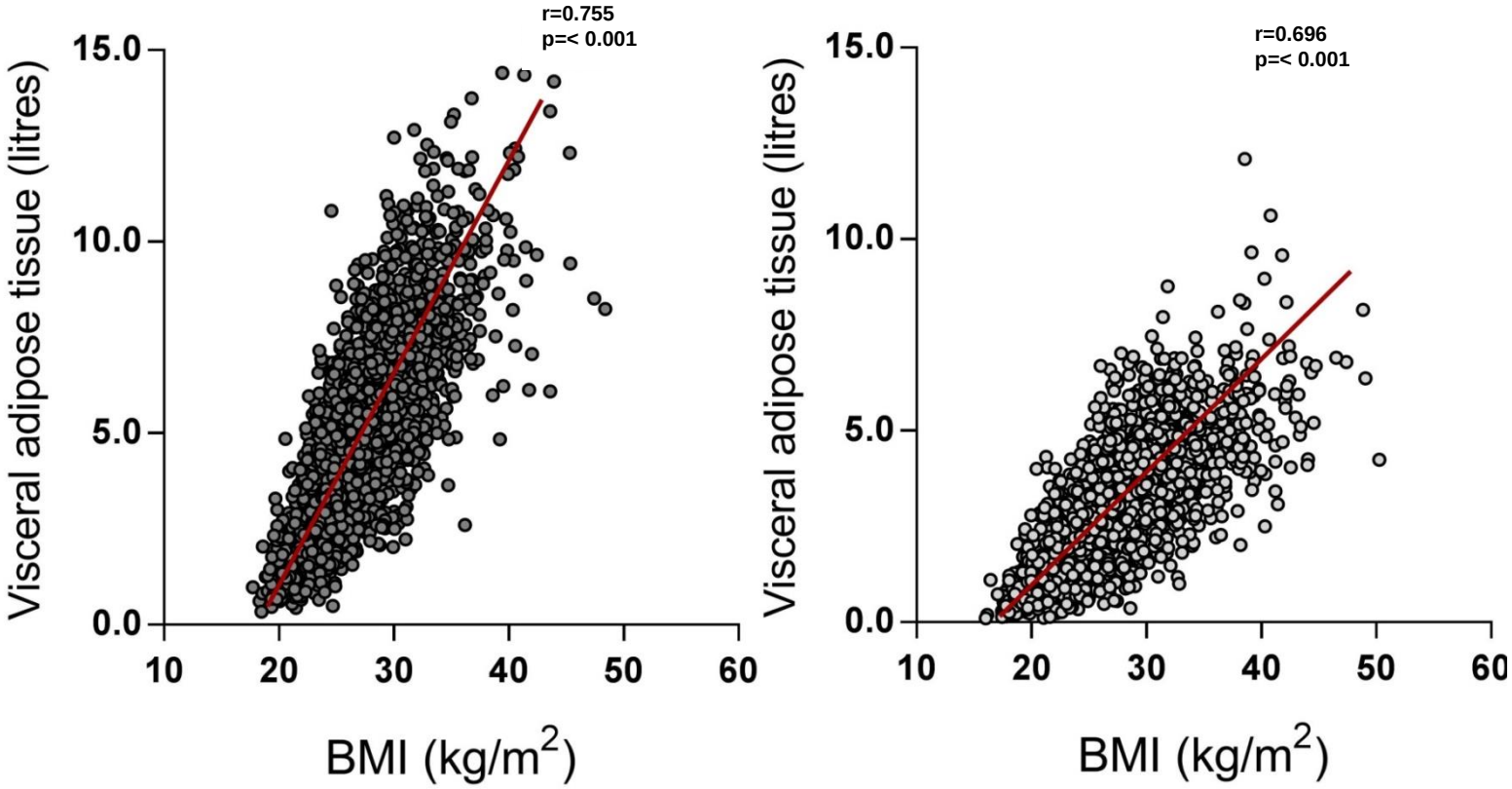

Figure 2.11 Gender-specific associations between visceral adipose tissue distribution and BMI in the free-living population in (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0.

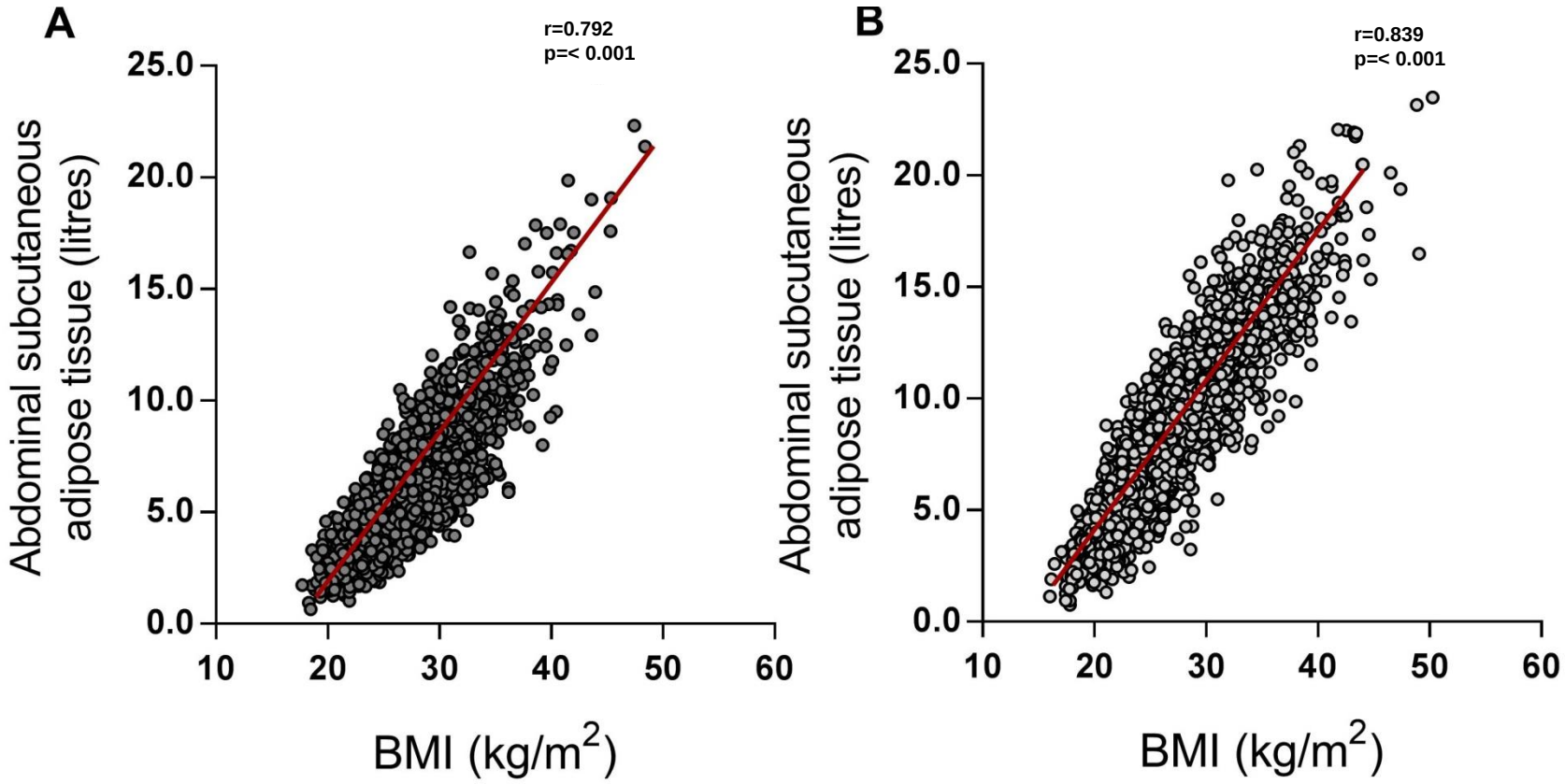

Figure 2.12 Gender-specific association between abdominal subcutaneous adipose tissue distribution and BMI in the free-living population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0.

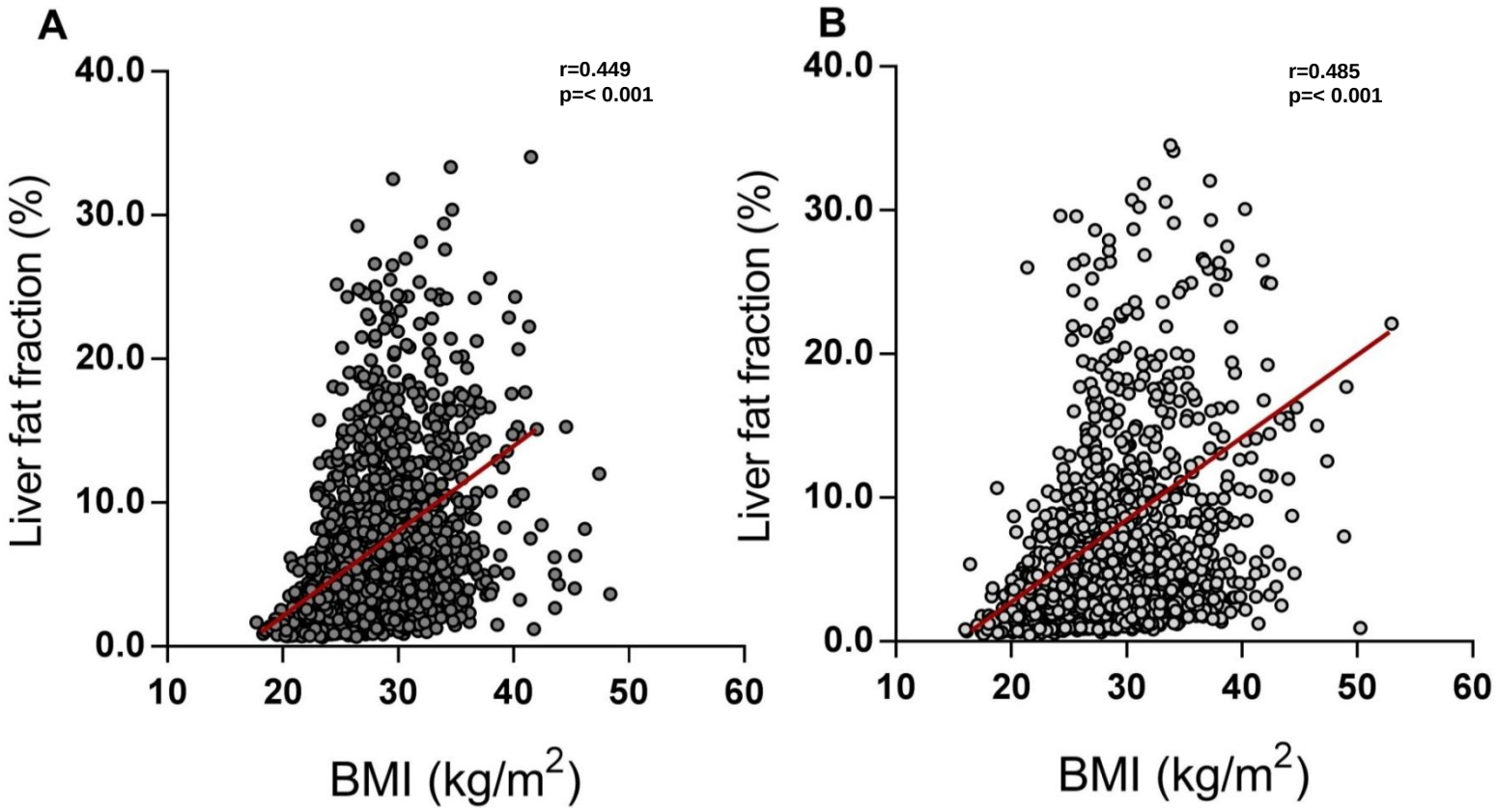

Figure 2.13 Gender-specific association between liver fat distribution and BMI in the free-living population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from the UK Biobank cohort. Graphs were done using GraphPad Prism version 5.0

Given the gender differences observed by age and BMI in VAT, ASAT, and liver fat in the free-living population, box and whisker plots were performed to illustrate the distribution of VAT and ASAT and liver fat fraction, by both gender and BMI group (**Figure 2.14, 2.15, 2.16).** The gender specific breakdown of VAT, ASAT and liver fat into BMI groups showed significantly higher fat depots as BMI groups increase in both genders ($p<0.05$), except liver fat in females in the free-living population (**Figure 2.14, 2.15, 2.16)**. Overall, in the free-living population, the amount of VAT was higher in males than females, whereas it was the opposite with ASAT in all BMI groups (**Figure 2.14, 2.15)**. The amount of liver fat was higher in males than females in all BMI groups in the free-living population. Further examination of gender differences in liver fat by BMI group revealed a similar amount of liver fat content in underweight males and females in the free-living population (<18.5 kg/m^2 median; M=0.91, F=1.01) (**Table 2.4**) (**Figure 2.15)**.

Table 2.4 Gender specific summary statistics of liver fat in the free-living population. Data obtained from UK biobank. Statistics perform on SPSS (v. 23.0).

		Female					Male					
BMI group	**n**	**Min**	**25th percentile**	**Median**	**75th percentile**	**Max**	**n**	**Min**	**25th percentile**	**Median**	**75th percentile**	**Max**
<18.5 kg/m²	4	0.52	0.83	1.01	1.29	6.31	36	0.65	0.74	0.91	1.05	2.86
18.5 < 25 kg/m²	869	0.25	1.00	1.31	1.91	32.7	1389	0	1.08	1.51	2.53	26.2
25 < 30 kg/m²	1442	0	1.42	2.22	4.28	31.9	1139	0	1.79	2.89	5.66	33.1
30 < 35 kg/m²	474	0.51	2.12	3.92	8.11	34.5	422	0	3.08	5.71	10.8	36.2
35+ kg/m²	111	0.62	3.21	6.22	11.4	31.4	177	0.99	5.97	9.09	14.3	36.2

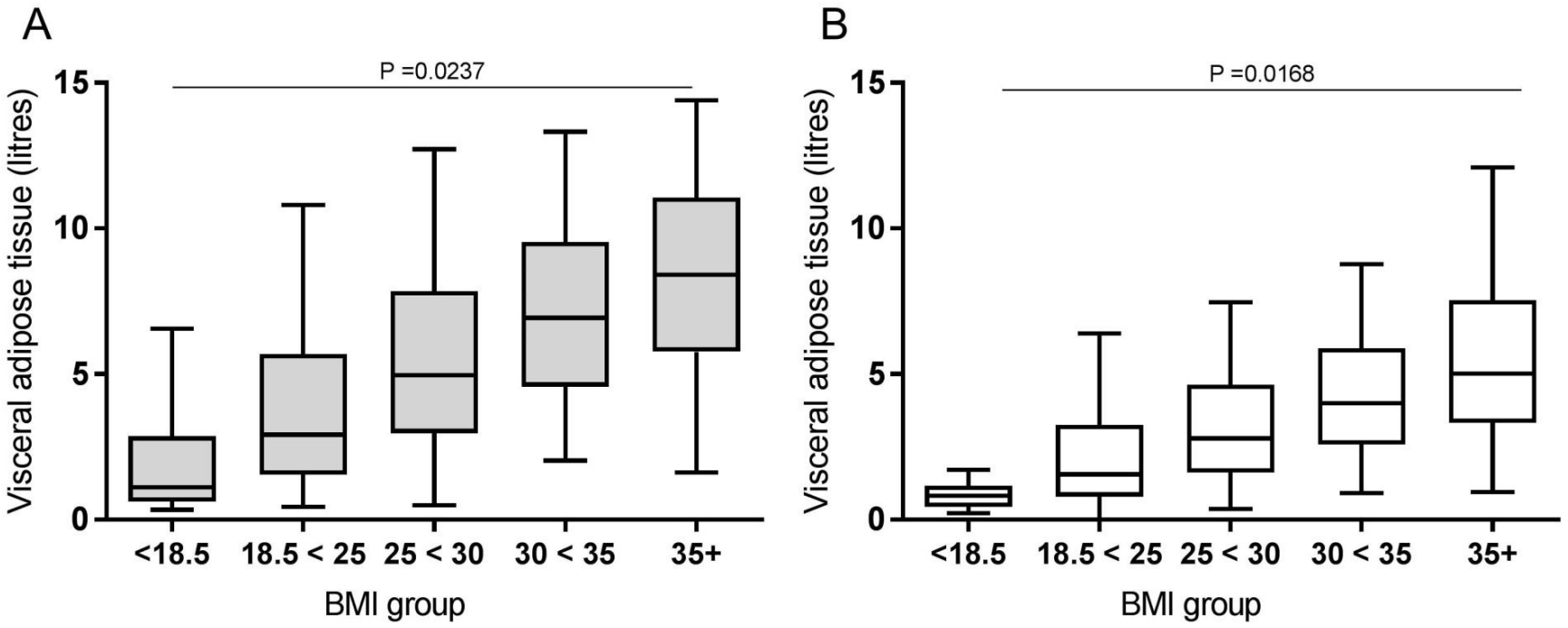

Figure 2.14 Gender specific distribution of visceral adipose tissue by BMI groups in the free-living population in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23.0). BMI: body mass index presented in kg/m^2. Data obtained from UK biobank. Graphs were done using GraphPad Prism version 5.0

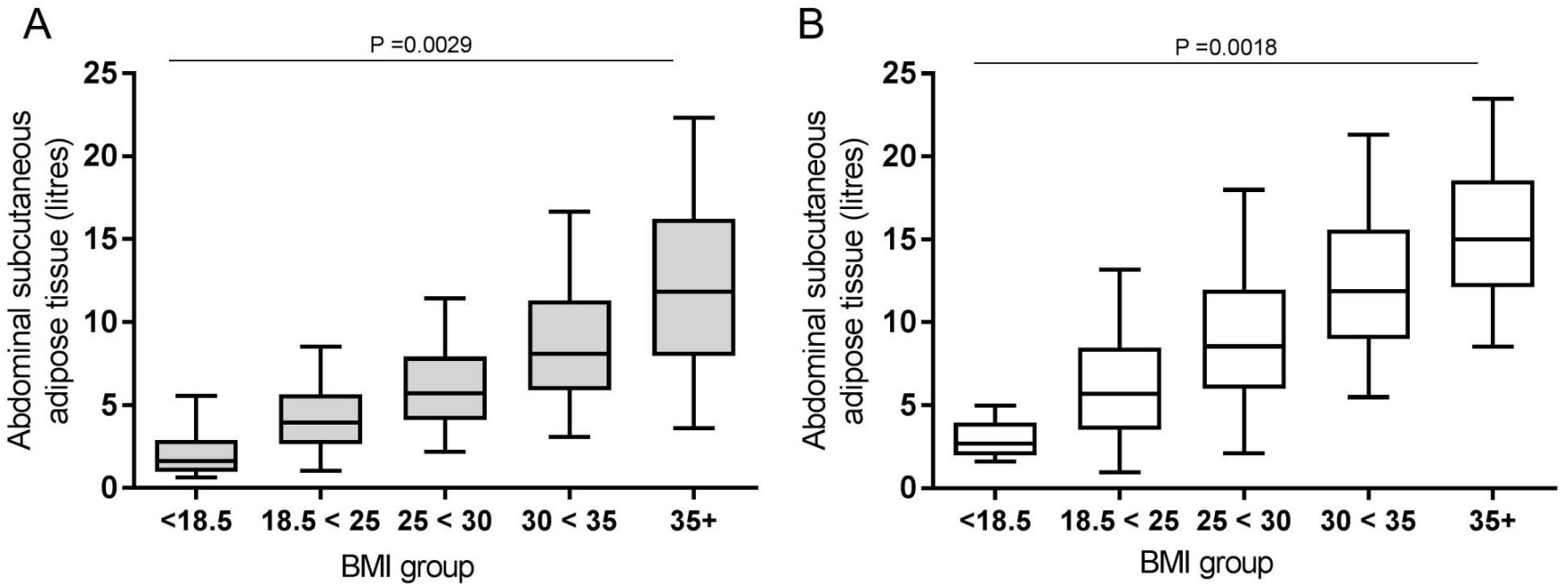

Figure 2.15 Gender specific distribution of abdominal adipose tissue distribution by BMI groups in the free-living population. In (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23.0). BMI: body mass index presented in kg/m^2. Data obtained from UK biobank. Graphs were done using GraphPad Prism version 5.0

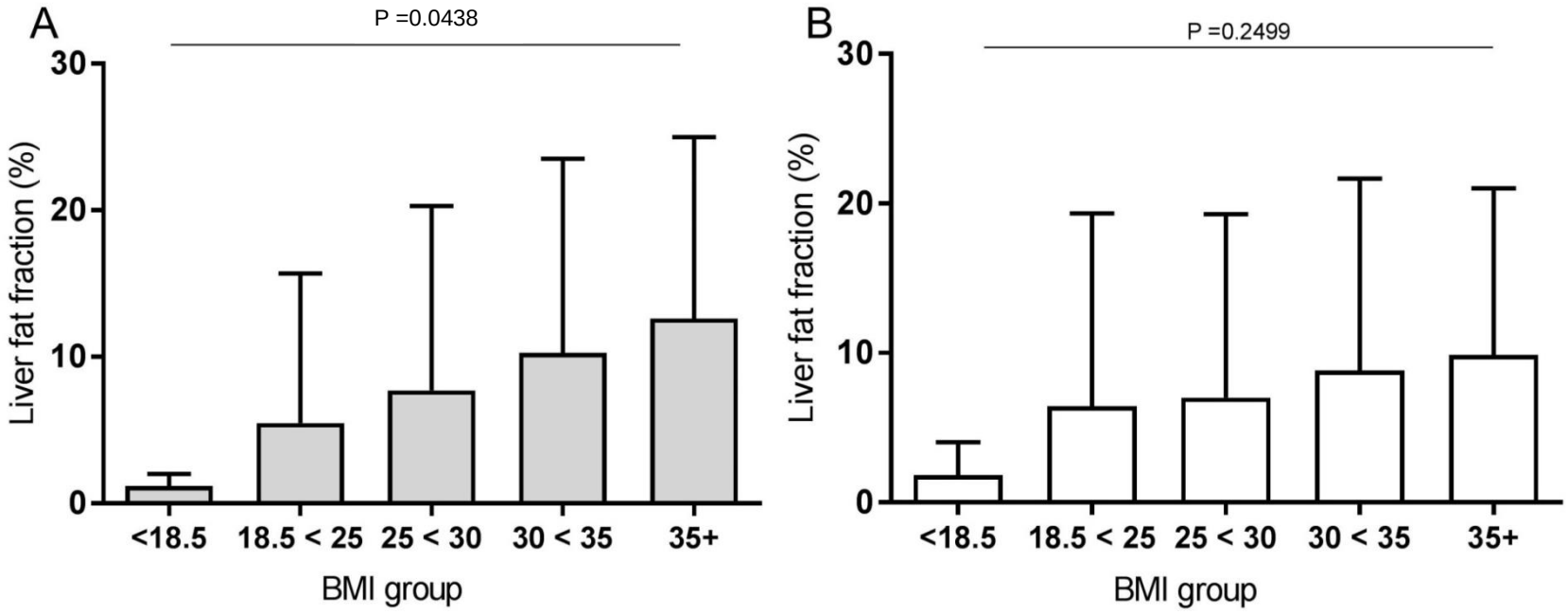

Figure 2.16 Gender specific distribution of liver fat by BMI groups in the free-living population in (**A**) males and (**B**) females. Data presented as mean and standard deviations. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23.0). BMI: body mass index presented in kg/m^2. Data obtained from UK biobank. Graphs were done using GraphPad Prism version 5.0.

2.3.1.4 Correlations between adiposity, liver fat fraction and physical activity in the UK Biobank

In order to investigate the impact of day-to-day events on phenotypes of body fat depots in the free-living population, a gender-specific correlation analyses between VAT, ASAT and liver fat content in the free-living population was performed. The gender specific correlation between abdominal body fat compartments (VAT, ASAT, and liver fat fraction) and measures of day to day events; daily physical activity and inactivity questionnaire are shown in males in (**Table 2.5**) and females in (**Table 2.6**). Overall, all types of day to day events that include movement showed a negative association with abdominal AT and ectopic fat in the liver in the free-living population, with interesting variations in the type of movement, and all day to day events that include no body movements showed a positive association with abdominal AT and ectopic fat. In males, amongst physical activity measures, ***usual walking pace*** provided the strongest correlation with liver fat fraction ($r = -0.263$), VAT ($r= -0.341$) and ASAT ($r= -0.355$, $p<0.001$ for all, **Table 2.5**). In females, ***usual walking pace*** provided the strongest correlation with both VAT ($r= -0.228$) and ASAT ($r= -0.230$) ($p<0.001$ for both, **Table 2.6**). ***Days per week performing vigorous physical activity*** (>10 min) provided the strongest correlation with liver fat fraction in females ($r= -0.181$, $p<0.001$, **Table 2.6**). Amongst physical inactivity measures, ***time spent watching television*** provided the strongest correlation in both genders for all depots; VAT ((r values) M: 0.243, F: 0.201), ASAT (M: 0.198, F: 0.161) and liver fat fraction (M: 0.209, F: 0.134) ($p<0.001$ for all, **Table 2.5** and **Table 2.6**).

Table 2.5 Correlation between abdominal body fat compartments (Liver fat fraction, VAT and ASAT) and measures of daily physical activity and inactivity in the free-living population males from UK Biobank. R values for the correlations between abdominal adiposity and liver fat outcomes with individual physical activity and inactivity outcomes. The strongest correlations are marked in bold font. AT: Adipose tissue; Significant results are highlighted by shading and in bold, with the strongest correlation highlighted in orange. n=2,839 except VAT and ASAT=2,864. Data obtained from UK Biobank. Statistics using SPSS 23.0.

	Correlations of physical activity and inactivity in Males	Liver Fat Fraction		Visceral AT (MRI)		Abdominal Subcutaneous AT	
		Correlation	P value	Correlation	P value	Correlation	P value
Physical Activity Measures	Days/weeks walked 10+ minutes	-0.105	<0.001	-0.159	<0.001	-0.172	<0.001
	Duration of Walks	-0.069	<0.001	-0.105	<0.001	-0.112	<0.001
	Days/wk moderate physical activity 10+ min	-0.077	<0.001	-0.135	<0.001	-0.172	<0.001
	Duration of moderate activity min	-0.028	0.145	-0.079	<0.001	-0.109	<0.001
	Days/weeks vigorous physical activity 10+ min	-0.154	<0.001	-0.210	<0.001	-0.191	<0.001
	Duration of vigorous activity	-0.058	0.005	-0.096	<0.001	-0.104	<0.001
	Usual walking pace	**-0.263**	**<0.001**	**-0.341**	**<0.001**	**-0.355**	**<0.001**
	Freq of stair climbing in last 4 weeks	-0.137	<0.001	-0.183	<0.001	-0.164	<0.001
	Freq of walking for pleasure in last 4 weeks	-0.041	0.031	-0.065	0.001	-0.057	0.003
	Duration of walking for pleasure	-0.089	<0.001	-0.102	<0.001	-0.110	<0.001
	Freq of strenuous sports in last 4 weeks	-0.062	0.171	-0.113	0.012	-0.088	0.049
	Duration of strenuous sports	0.015	0.743	0.014	0.757	0.039	0.39
	Freq of light DIY in last 4 weeks	-0.029	0.163	-0.021	0.293	-0.014	0.478
	Duration of light DIY	-0.044	0.033	-0.060	0.003	-0.056	0.006
	Freq of heavy DIY in last 4 weeks	0.015	0.534	-0.007	0.774	-0.004	0.864
	Duration of heavy DIY	-0.044	0.072	-0.049	0.047	-0.007	0.763
	Freq of other exercises in last 4 weeks	-0.114	<0.001	-0.162	<0.001	-0.149	<0.001
	Duration of other exercises	-0.053	0.013	-0.070	0.001	-0.100	<0.001
	Types of physical activity in past 4 weeks	-0.064	<0.001	-0.066	<0.001	-0.043	0.017
	Job involves heavy lifting	0.021	0.334	0.007	0.75	0.009	0.668
	Time spent doing vigorous physical activity	-0.050	0.034	-0.069	0.003	-0.080	0.001
	Time spent doing moderate physical activity	-0.069	0.004	-0.091	<0.001	-0.119	<0.001
	Time spent doing light physical activity	-0.021	0.373	-0.074	0.002	-0.072	0.002
Physical Inactivity Measures	Time spent watching television	**0.209**	**<0.001**	**0.243**	**<0.001**	**0.198**	**<0.001**
	Time spent using computer	0.028	0.119	0.045	0.012	0.069	<0.001
	Time spent driving	-0.050	0.034	-0.069	0.003	-0.080	0.001

Table 2.6 Pearson correlation between abdominal body fat compartments in UKBB (Liver fat fraction, VAT and ASAT) and measures of daily physical activity and inactivity in the free-living population females from UK Biobank. R values for the correlations between abdominal adiposity and liver fat outcomes with individual physical activity and inactivity outcomes. AT: Adipose tissue; Significant results are highlighted by shading and in bold, with the strongest correlation highlighted in orange. The strongest correlations for each fat depot are in bold font, n= 3132 except VAT and ASAT=3157. Data obtained from UK Biobank. Statistics using SPSS 23.0.

Correlations of physical activity and inactivity in Females		Liver Fat Fraction		Visceral AT (MRI)		Abdominal Subcutaneous AT	
		Correlation	P value	Correlation	P value	Correlation	P value
Physical Activity Measures	Days/weeks walked 10+ minutes	-0.062	<0.001	-0.069	<0.001	-0.084**	<0.001
	Duration of Walks	-0.065	<0.001	-0.092	<0.001	-0.086	<0.001
	Days/wk moderate physical activity 10+ min	-0.121	<0.001	-0.117	<0.001	-0.158	<0.001
	Duration of moderate activity min	-0.037	0.06	-0.036	0.068	-0.048*	0.013
	Days/weeks vigorous physical activity 10+ min	-0.181	<0.001	-0.210	<0.001	-0.183	<0.001
	Duration of vigorous activity	-0.083	<0.001	-0.085	<0.001	-0.076	<0.001
	Usual walking pace	-0.134	<0.001	-0.228	<0.001	-0.230	<0.001
	Freq of stair climbing in last 4 weeks	-0.080	<0.001	-0.111	<0.001	-0.117	<0.001
	Freq of walking for pleasure in last 4 weeks	-0.018	0.376	-0.014	0.476	-0.042*	0.036
	Duration of walking for pleasure	-0.049*	0.014	-0.084	<0.001	-0.102	<0.001
	Freq of strenuous sports in last 4 weeks	-0.059	0.115	-0.094*	0.012	-0.084*	0.026
	Duration of strenuous sports	0.023	0.543	0.03	0.429	-0.001	0.976
	Freq of light DIY in last 4 weeks	-0.018	0.409	-0.033	0.121	-0.048*	0.024
	Duration of light DIY	-0.003	0.906	-0.03	0.156	-0.03	0.163
	Freq of heavy DIY in last 4 weeks	-0.018	0.414	-0.03	0.157	-0.025	0.25
	Duration of heavy DIY	-0.002	0.942	0.005	0.81	0.03	0.161
	Freq of other exercises in last 4 weeks	-0.121**	<0.001	-0.097**	<0.001	-0.111**	<0.001
	Duration of other exercises	-0.035	0.123	-0.039	0.088	-0.033	0.153
	Types of physical activity in past 4 weeks	0.019	0.325	0.017	0.352	0.025	0.182
	Job involves heavy lifting	-0.002	0.937	0.029	0.198	0.019	0.407
	Time spent doing vigorous physical activity	-0.064*	0.012	-0.058*	0.022	-0.087	0.001
	Time spent doing moderate physical activity	-0.080	0.002	-0.041	0.101	-0.043	0.088
	Time spent doing light physical activity	-0.022	0.376	-0.023	0.356	-0.032	0.203
Physical Inactivity Measures	Time spent watching television	0.134	<0.001	0.201	<0.001	0.161	<0.001
	Time spent using computer	0.056	0.003	0.053	0.005	0.053	0.005
	Time spent driving	-0.062	<0.001	-0.069	<0.001	-0.084	<0.001

2.3.2 Pre-diabetic population (the Diabetes Research on Patient Stratification)

2.3.2.1 DIRECT Descriptive Statistics

The characteristics of the 1,558 (1,125 males and 433 females) individuals in the pre-diabetic population from the DIRECT study are shown in **Table 2.7**. Participants' average age in the pre-diabetic population was 61.0 ± 7.2 years, with a BMI of 28.8 ± 4.5 kg/m^2 (**Table 2.7**).

Table 2.7 Baseline characteristics of pre-diabetic participants from the DIRECT cohort. Outcomes data from DIRECT participants. ENMO: Euclidean Norm minus One; MRI: Magnetic resonance imaging; VAT: visceral adipose tissue; ASAT: Abdominal Subcutaneous Adipose Tissue; Trunk AT: Trunk Adipose Tissue; DXA: dual energy X-ray absorptiometry. Data presented as mean ± standard deviation calculated using SPSS 23.0.

		N	Mean ± SD	Range
Anthropometry	Age(years)	1558	61.0 ± 7.2	30.0 - 75.0
	Weight (kg)	1558	86.4 ± 14.4	43.0 - 142.5
	Height (cm)	1558	173.2 ± 8.7	145.0 - 204.0
	BMI (kg/m^2)	1558	28.8 ± 4.5	16.9 – 54.3
	Waist Circumference (cm)	1552	100.9 ± 11.7	65.0 – 145
	Hip Circumference (cm)	1552	103.7 ± 8.8	85.0 – 152
Blood pressure	Diastolic blood pressure (mmHg)	1558	95.8 ± 12.2	51.3 – 135.3
	Systolic blood pressure (mmHg)	1558	129.5 ± 18.1	62.3 – 186.7
MRI	Liver fat (%)	1551	6.3 ± 5.9	0.3 - 37.6
	Pancreas fat (%)	1454	12.7 ± 8.6	0.2 – 39.3
	VAT (litres)	1408	5.5 ± 2.4	0.2 - 14.5
	ASAT (litres)	1405	6.6 ± 3.2	0.9 - 21.9
	VAT/ASAT	1405	1 ± 0.46	0.1 - 4.2
DXA	Body fat %	1034	28.2 ± 7.8	3.9 – 49.9
Physical activity	ENMO	1215	22.7 ± 7.2	0.6 – 54.6

Data were available and complete for all variables for 1,405 out of 1,558 pre-diabetic subjects due either missing data or missing variables, except for body fat (n=1034), and ENMO (n=1215) (**Figure 2.17).**

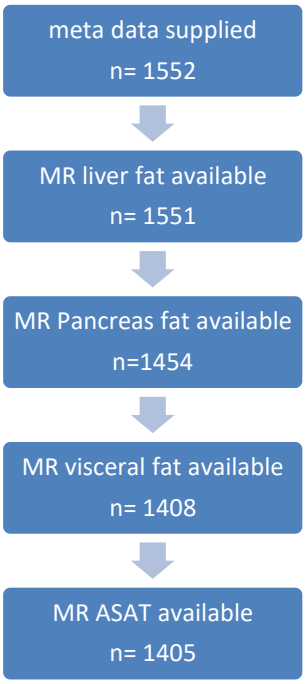

Figure 2.17 Flow chart demonstrating the MR images available for body fat depots in the pre-diabetic population.

Gender specific differences in study outcomes of the pre-diabetic population are shown in **Table 2.8**. Significant differences between pre-diabetic male and females were found for the majority of measures, except age (p=0.478) (**Table 2.8**). Pre-diabetic females had a significantly higher BMI than pre-diabetic male (F: BMI: 29.6 ± 5.5 kg/m^2, M: 28.4 ± 3.9 kg/m^2, p<0.001) (**Table 2.8**).

Pre-diabetic males were taller (p<0.001), and weighed more (p<0.001), with wider WC (p<0.001) but smaller hip circumference (p<0.001) compared to pre-diabetic females. Pre-diabetic males were more hypertensive than pre-diabetic females (p<0.001 for DBP and SBP) (**Table 2.8**). Furthermore, pre-diabetic males had significantly higher VAT than pre-diabetic females, whereas pre-diabetic females had significantly higher ASAT content (p<0.001 for all) (**Table 2.8**). The ratio of VAT to ASAT observed to be significantly higher in pre-diabetic males compared to pre-diabetic females (p<0.001) (**Table 2.8**). There were significant gender differences in ectopic fat of pre-

diabetic population. Pre-diabetic males had significantly less liver fat than pre-diabetic females (p<0.001) but had significantly more pancreas fat (p<0.001) (**Table 2.8**). The assessment of day to day events in the pre-diabetic population, using an objective physical activity assessment tool (wristband), showed to be effective in the pre-diabetic population. Pre-diabetic males showed significantly higher physical activity status than pre-diabetic female (p=0.013). (**Table 2.8**).

Table 2.8 Gender specific characteristics of pre-diabetic participants from the DIRECT cohort. Outcomes data from DIRECT participants. MRI: Magnetic resonance imaging; VAT: visceral adipose tissue; ASAT: Abdominal Subcutaneous Adipose Tissue; SBP: Systolic Blood Pressure: DBP: Diastolic Blood Pressure: DXA: dual energy X-ray absorptiometry, ENMO: Euclidean Norm minus One. Data presented as mean ± standard deviation. Gender differences were assessed using *t*-test and performed using SPSS 23.0.

		Male (N=1,045)	Female (N=362)	*p* value
Anthropometry	Age (years)	60.8 ± 6.9	61.2 ± 8.0	0.478
	Weight (kg)	88.9 ± 13.5	79.9 ± 14.7	<0.001
	Height (cm)	176.7 ± 6.6	164.0 ± 6.6	<0.001
	BMI (kg/m^2)	28.4 ± 3.9	29.6 ± 5.5	<0.001
	Waist Circumference (cm)	101.9 ± 10.6	98.1 ± 13.7	<0.001
	Hip Circumference (cm)	102.2 ± 7.2	107.8 ± 11.0	<0.001
	Waist-to-Hip Ratio (WHR)	0.997 ± 0.06	1.0 ± 0.1	<0.001
Blood pressure	Systolic blood pressure (mmHg)	130.4 ± 16.1	127.6 ± 21.6	0.006
	Diastolic blood pressure (mmHg)	97.1 ± 10.7	92.7 ± 21.6	<0.001
MRI	Liver fat (%)	5.9 ± 5.4	7.3 ± 7.0	<0.001
	Pancreas fat (%)	13.9 ± 8.5	9.3 ± 8.0	<0.001
	VAT (litres)	6.0 ± 2.3	4.2 ± 1.9	<0.001
	ASAT (litres)	5.8 ± 2.4	9.3 ± 3.5	<0.001
	VAT/ASAT	1.1 ± 0.4	0.4 ± 0.1	<0.001
DXA	Body fat %	26.0 ± 6.5	36.8 ± 5.8	<0.001
Physical activity	ENMO	23.0 ± 7.3	21.9 ± 7.1	0.013

Participant characteristics and summary abdominal body composition compartments by gender and BMI group (lean versus overweight/obese) are shown in **Table 2.9**. Despite that, liver fat content was significantly higher in pre-diabetic females than pre-diabetic males, after dividing the subjects into lean and overweight/obese group, the amount of liver fat content was similar between lean pre-diabetic male and female (M: 3.2 ± 3.5, F: 3.6 ± 4.0) showing that the gender differences in pre-diabetic subjects in liver fat might be mediated by weight (**Table 2.9**). The gender differences in VAT and ASAT in the whole cohort showed the same pattern after dividing the groups into

lean and overweight/obese subjects; VAT was higher in pre-diabetic males compared to pre-diabetic females (among all BMI groups), and ASAT was higher in pre-diabetic females than pre-diabetic males (among all BMI groups) ($p<0.001$ for all) (**Table 2.9).**

Table 2.9 Gender specific characteristics in lean versus overweight pre-diabetic participants. Outcomes data from DIRECT participants. Data presented as mean ± standard deviation. Data obtained from DIRECT study in males and females split by (lean group represents BMI ≤ 25 kgm^{-2}; the overweight/obese group represents individuals with a BMI above 25 kgm^{-2}). MRI: Magnetic resonance imaging; VAT: visceral adipose tissue; ASAT: Abdominal Subcutaneous Adipose Tissue; SBP: Systolic Blood Pressure: DBP: Diastolic Blood Pressure, DXA: Dual energy X-ray absorptiometry, ENMO: Euclidean Norm minus One. Data presented as mean ± standard deviation. *T-test* was performed for gender differences calculated using SPSS 23.0.

		Male			Female		
		< 25 kg/m^2	>25 kg/m^2	p value	< 25 kg/m^2	>25 kg/m^2	p value
Anthropometry	Age (years)	61.4 ± 6.8	60.7 ± 6.9	0.22	62.8 ± 7.0	60.7 ± 9.4	0.61
	Weight (kg)	73.8 ± 6.5	92.0 ± 12.3	<0.001	62.4 ± 7.0	80.1 ± 17.3	<0.001
	Height (cm)	177 ± 6.1	176 ± 6.71	0.22	164 ± 5.8	163 ± 14.9	<0.001
	BMI (kg/m^2)	23.4 ± 1.2	29.4 ± 3.4	<0.001	23.1 ± 1.8	29.6 ± 6.0	<0.001
	Waist Circumference (cm)	90.0 ± 6.3	104 ± 9.5	<0.001	82.3 ± 8.6	97.9 ± 16.3	<0.001
	Hip Circumference (cm)	94.9 ± 4.0	103 ± 6.7	<0.001	95.2 ± 5.0	107 ± 14.3	<0.001
	Waist-to-Hip Ratio (WHR)	0.9 ± 0.1	1.0 ± 0.1	<0.001	0.9 ± 0.1	0.9 ± 0.1	<0.001
Blood pressure	SBP (mmHg)	127 ± 16.0	131 ± 15.9	0.001	123 ± 26.1	126 ± 24.5	0.004
	DBP (mmHg)	94.4 ± 10.3	97.6 ± 10.6	<0.001	88.0 ± 15.8	92.0 ± 16.5	<0.001
MRI	Liver fat MR (%)	3.2 ± 3.5	6.5 ± 5.6	<0.001	3.6 ± 4	7.4 ± 7.1	<0.001
	Pancreas fat MR (%)	11.4 ± 7.9	14.3 ± 8.6	<0.001	8.2 ± 7.4	9.5 ± 8.2	<0.001
	Visceral adipose tissue (litres)	4.1 ± 1.8	6.4 ± 2.2	<0.001	2.8 ± 1.4	4.2 ± 2.0	<0.001
	ASAT (litres)	3.6 ± 1.4	6.3 ± 2.4	<0.001	5.6 ± 2.0	9.3 ± 3.7	<0.001
	Trunk adipose tissue (litres)	7.7 ± 2.8	12.6 ± 3.8	<0.001	8.4 ± 2.8	13.5 ± 4.9	<0.001
Physical activity	ENMO	24.9 ± 8.4	22.6 ± 6.9	<0.001	23.8 ± 7.4	21.6 ± 7.6	0.014
DXA	Body Fat (%)	21.8 ± 6.2	27.0 ± 6.1	<0.001	30.9 ± 5.2	36.2 ± 7.3	<0.001

Gender specific breakdown of VAT, ASAT, liver and pancreatic fat by age was performed in the pre-diabetic population (**Figure 2.18, 2.19, 2.20. 2.21**). Overall, the effect of age and gender on VAT was minimal in the pre-diabetic population. The impact of age on the amount of VAT was less in pre-diabetic females compared to pre-diabetic males, but not significantly (M: p=0.412, F: p= 0.209) (**Figure 2.18)**. The only major gender difference in VAT was observed in 30-39 years; with pre-diabetic females showed significantly more VAT than pre-diabetic males (VAT differences = 1.9 litres; median: M; 2.2, F; 4.0, p<0.001) (**Figure 2.18**) interestingly, the correlation between VAT and age was not significant in either pre-diabetic males or females (M: r=0.015, F: r= 0.040, p=NS).

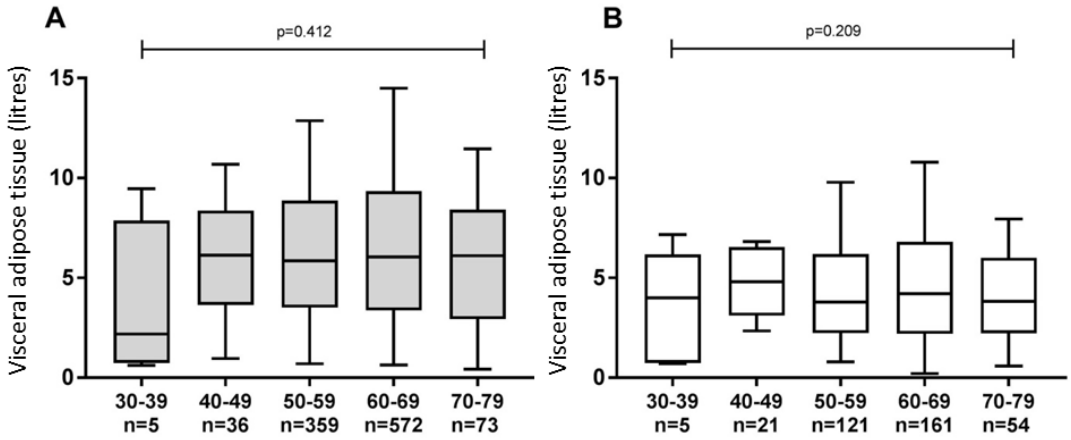

Figure 2.18 Gender specific distribution of visceral adipose tissue (VAT) by age groups in the pre-diabetic population. In (**A**,) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23). Data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0

The gender-specific breakdown of ASAT by age showed a consistent pattern with higher ASAT in pre-diabetic females compared to pre-diabetic males among all age groups (M: p=0.0002, F: p<0.0001), (**Figure 2.19**).

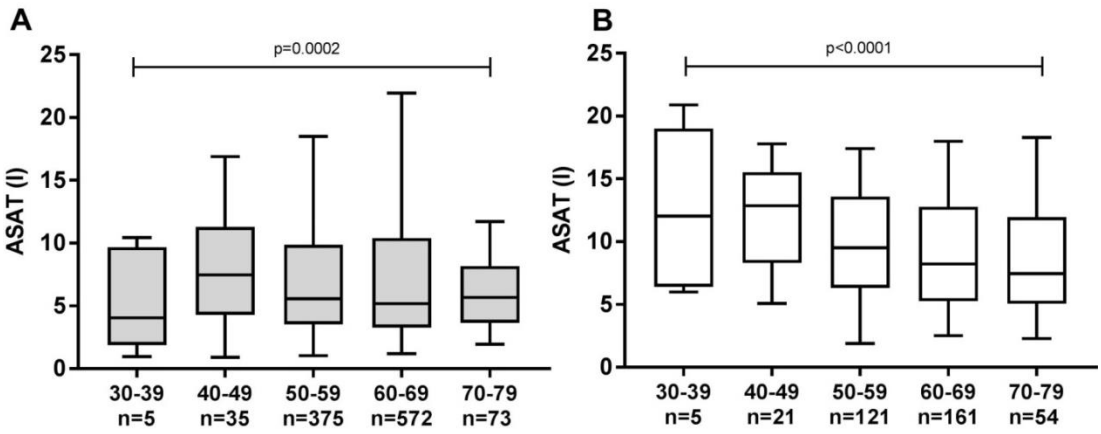

Figure 2.19 Gender specific distribution of abdominal subcutaneous adipose tissue (ASAT) by age groups in the pre-diabetic population in (**A**,) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23). Data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0

Among pre-diabetic females, the highest ASAT was observed in 40-49 years compared to other pre-diabetic females, but compared to pre-diabetic males, the youngest pre-diabetic females had the highest ASAT content (ASAT M= 12.0, F=4.1 litre) (**Figure 2.19**). The association of ASAT and age was significantly negative and stronger in pre-diabetic females than pre-diabetic males (M: r= -0.095, F: r=-0.252, p>0.001) (**Figure 2.19**).

The gender specific breakdown of ectopic fat by age showed a distinct pattern in the youngest pre-diabetic males and females. Pre-diabetic females in the 30-39 age group had almost 3-fold liver fat content than pre-diabetic males (median; M: 3.38% F: 9.68%, p<0.001) (**Figure 2.20**).

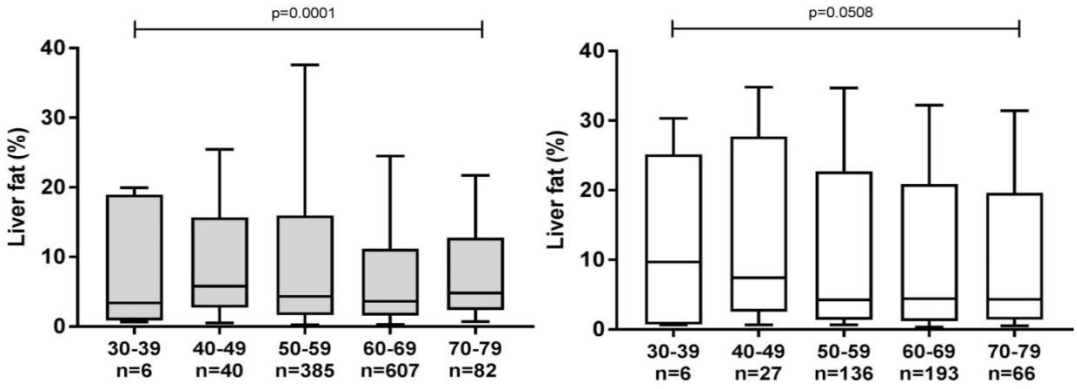

Figure 2.20 Gender specific distribution of liver fat by age group groups in the pre-diabetic population. In (**A**,) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23). Data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0

On the contrary, pancreatic fat showed an opposite pattern; with pre-diabetic males had significantly more pancreas fat than pre-diabetic females in all age groups, except for 30-39 years (**Figure 2.21)**.

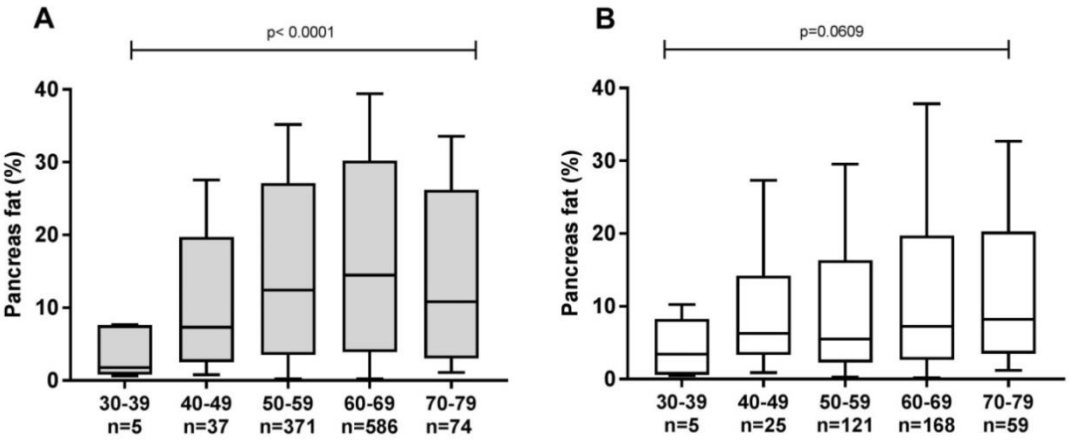

Figure 2.21 Gender specific distribution of pancreas fat content by age group in pre-diabetic cohort. In (**A**,) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles and line median. *P* values are calculated from Kruskal-Wallis test with multiple comparison corrections in SPSS (v.23). Data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0

2.3.1.2 DIRECT Correlation analysis

Gender specific correlations between anthropometry, blood pressure, VAT and ASAT, liver and pancreas fat in the pre-diabetic population were done shown in Table 2.9. In pre-diabetic males and females, WC provided the strongest correlate with VAT (M r= 0.607, F r= 0.445, p<0.001) (**Table 2.10**). Looking at ASAT, BMI was the strongest correlate in pre-diabetic males (r= 0.774, p<0.001), while hip circumference was the strongest correlate in pre-diabetic females (r= 0.832, p<0.001) (**Table 2.10**). The correlation of ectopic fat in the pre-diabetic population showed that liver fat most strongly correlates with BMI in pre-diabetic males (r=0.41, p<0.001), and with WC in pre-diabetic females (r= 0.438, p<0.001) (**Table 2.10**). The strongest correlation with pancreatic fat was observed with VAT in males (r=0.376, p<0.001) and females (r= 0.208 p<0.001) (**Table 2.10**).

Table 2.10 Gender specific correlations between VAT, ASAT and liver fat fraction, anthropometry, blood pressure, physical activity in the pre-diabetic population. R values for correlations. The significant correlations for each fat depot are highlighted as **= *p*<0.001. Correlation done in SPSS (v.23.0). Data obtained from DIRECT IMI. BMI; body mass index: WHR; waist-to-hip ration: ENMO; Euclidean Norm minus One: SBP; systolic blood pressure: DSP; diastolic blood pressure: VAT; visceral adipose tissue: ASAT; abdominal subcutaneous adipose tissue: DXA: dual energy X-ray absorptiometry: MR; magnetic resonance. ** indicates correlation is significant at the 0.01 and * indicates correlation is significant at the 0.05.

		VAT		ASAT		Liver Fat		Pancreatic fat	
		Male	Female	Male	Female	Male	Female	Male	Female
Anthropometry	Age	0.015	0.040	-0.095**	-0.252**	-0.083**	-0.151**	0.09**	0.13**
	Weight	0.514**	0.386**	0.758**	0.808**	0.337**	0.393**	0.093**	0.125*
	Height	0.018	-0.078	0.126**	0.034	-0.059*	-0.051	-0.051	-0.061
	BMI	0.567**	0.437**	0.774**	0.828**	0.412**	0.407**	0.133**	0.157**
	Waist	0.607**	0.445**	0.757**	0.779**	0.379**	0.438**	0.149**	0.028
	Hip	0.437**	0.310**	0.755**	0.832**	0.292**	0.350**	0.038	0.071
	WHR	0.530**	0.382**	0.430**	0.305**	0.301**	0.307**	0.128**	0.197**
Physical Activity	ENMO	-0.269**	-0.262**	-0.257**	-0.181**	-0.138**	-0.204**	-.114**	-0.051
Blood pressure	SBP	0.170**	0.082	0.038	0.069	0.092**	0.000	0.018	-0.084
	DBP	0.219**	0.054	0.051	0.125*	0.103**	0.008	0.061*	-0.116*
DXA	Body fat (%)	0.392**	0.281**	0.502**	0.710**	0.222**	0.317**	-0.006	-0.307**
MR	VAT			0.498**	0.377**	0.395**	0.419**	0.376**	0.208**
	ASAT	0.498**	0.377**			0.297**	0.405**	0.102**	0.084
	Liver fat	0.395**	0.419**	0.297**	0.405**			0.037	0.142**
	Pancreatic Fat	0.376**	0.208**	0.102**	0.084	0.037	0.142**		

The association between abdominal adiposity and age in the pre-diabetic population revealed a consistent gender pattern in VAT (**Figure 2.22**) ASAT (**Figure 2.23**) and liver fat (**Figure 2.24**), with negative correlations between age and ASAT and liver fat (**Figure 2.23, 2.24**) but not VAT in pre-diabetic males and females. The correlations between ASAT, liver fat, pancreatic fat and age were stronger in pre-diabetic females than pre-diabetic males (ASAT: M: r=-0.095, F: r=- 0.252, p< 0.001, Liver fat: M: -0.083, F: r= -0.151, p< 0.001, Pancreatic fat: M: r=0.09, p<0.002, F: r=0.13, p<0.008) (**Table 2.9**) (**Figure 2.23, 2.24, 2.25**).

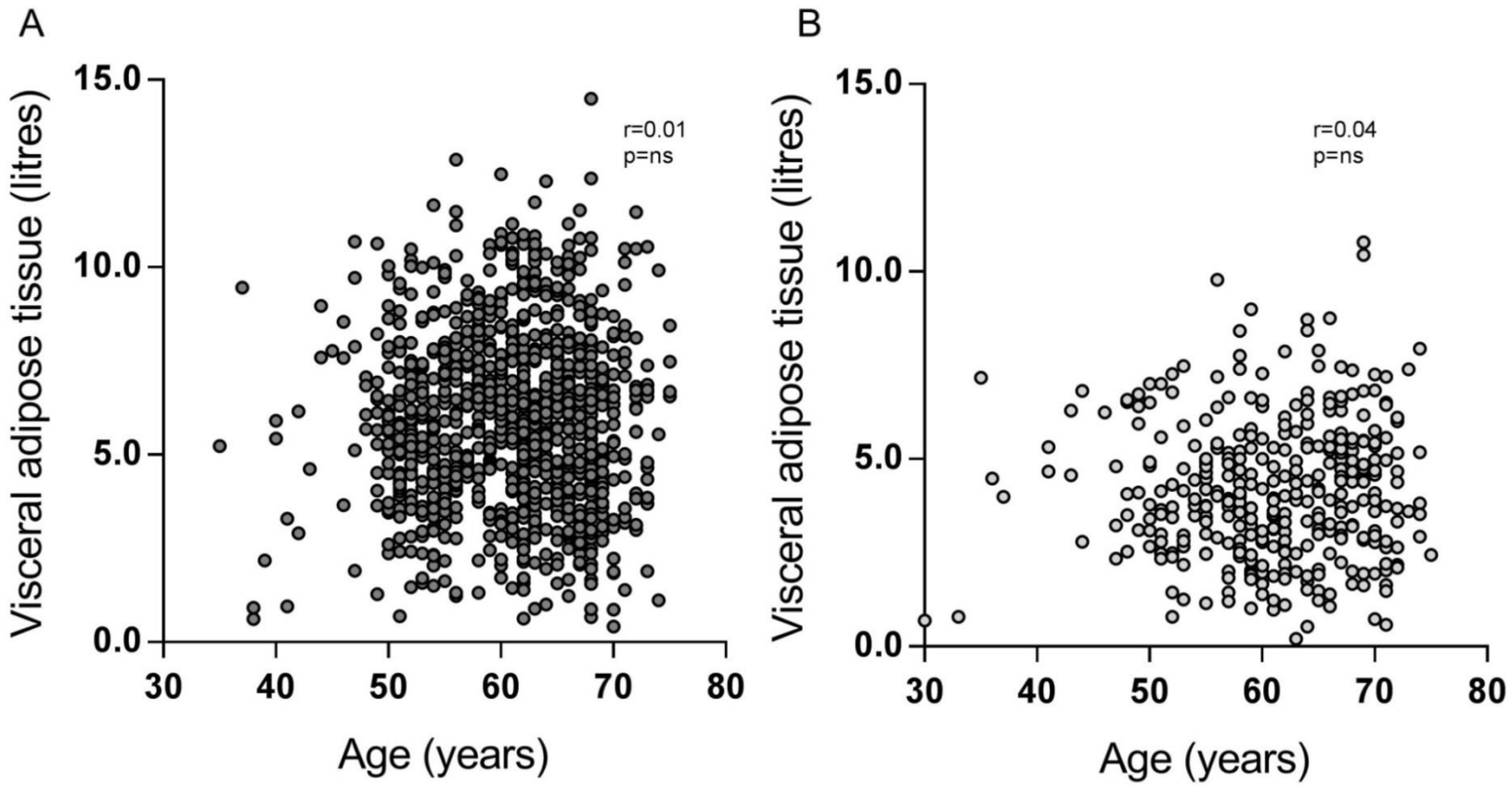

Figure 2.22 Gender specific distribution of visceral adipose tissue content by age in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

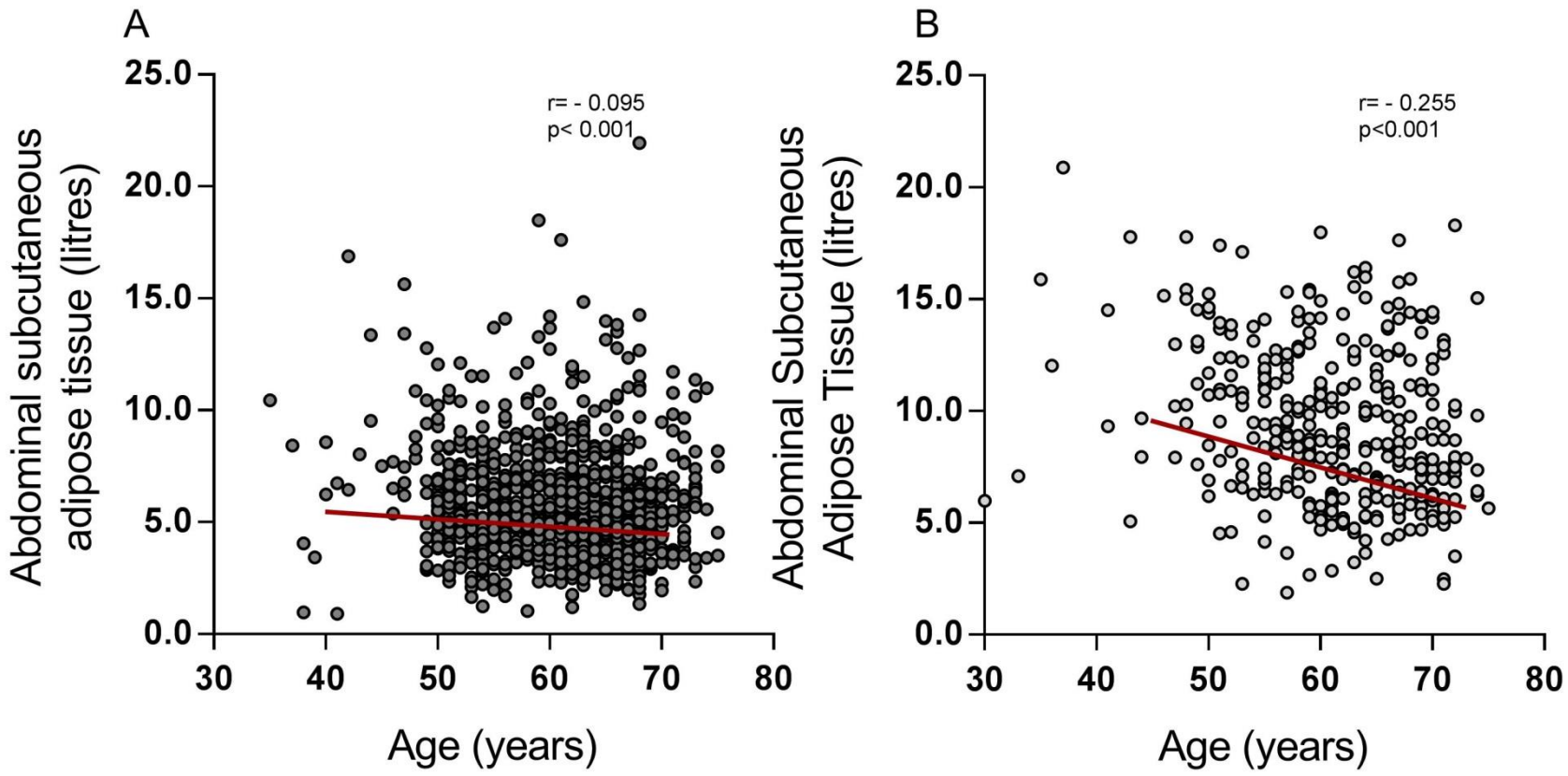

Figure 2.23 Gender specific distribution of abdominal subcutaneous adipose tissue by age in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

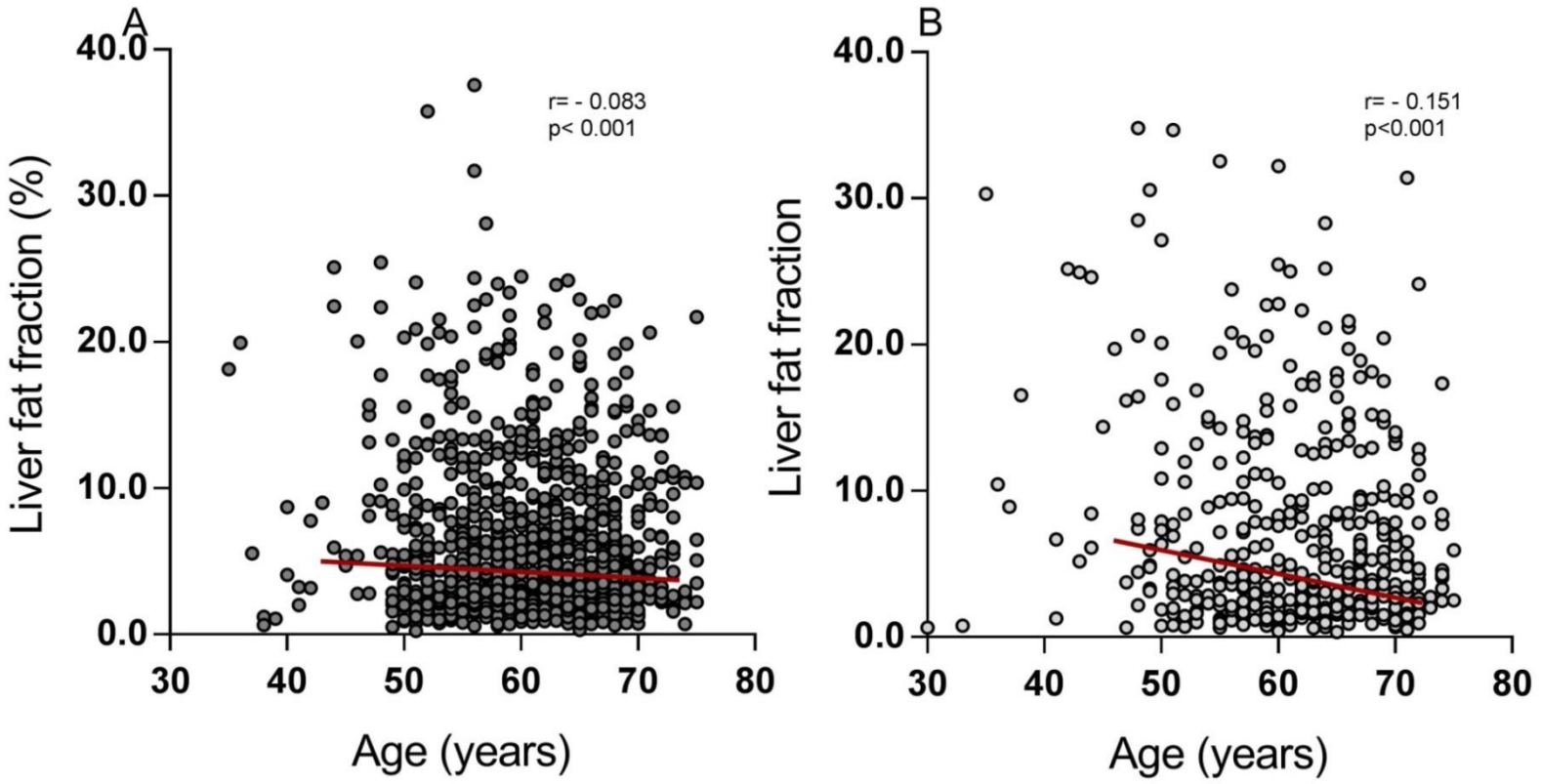

Figure 2.24 Gender specific distribution of liver fat fraction by age in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

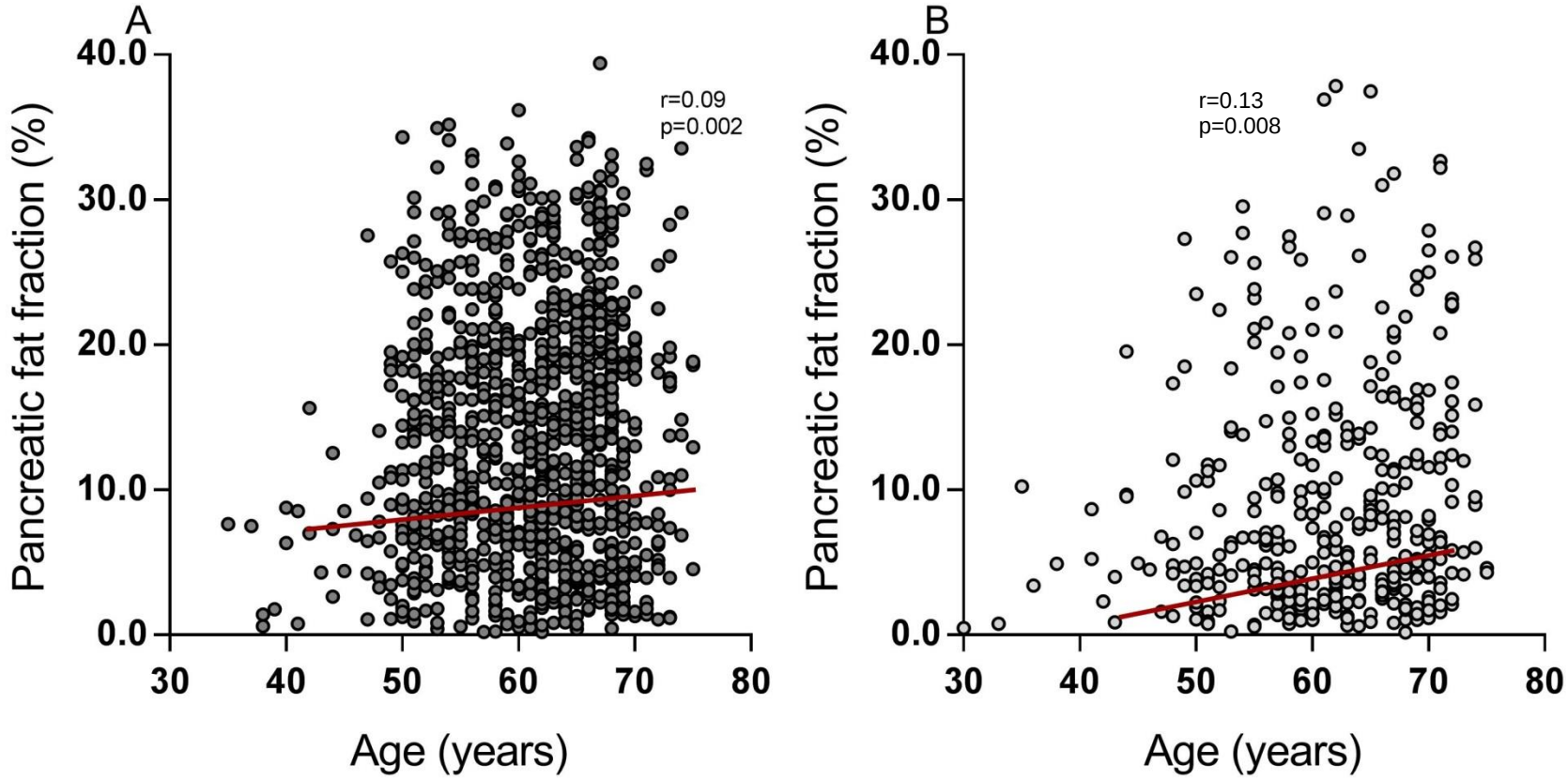

Figure 2.25 Gender specific distribution of pancreas fat fraction distribution by age in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

There were gender specific differences in the association between VAT (**Figure 2.26**), ASAT (**Figure 2.27**), ectopic fat (**Figure 2.28, 2.29**) and VAT, with pre-diabetic males showing stronger association with BMI ($r=0.567$, $p<0.001$) than pre-diabetic females ($r=0.437$, $p<0.001$), whereas in ASAT, pre-diabetic females had a stronger association with BMI ($r=0.828$, $p<0.001$) than pre-diabetic males ($r=0.774$, $p<0.001$) (**Figure 2.26, 2.27**).

The association between ectopic fat and BMI showed gender differences in the pre-diabetic population. In terms of liver fat, there was a similar moderate association with BMI in pre-diabetic males ($r=0.412$, $p<0.001$) and females ($r=0.407$, $p<0.001$) (**Figure 2.28)**. However, there was no significant association observed in pancreatic fat and BMI in pre-diabetic males, while pre-diabetic females showed a weak significant association with BMI ($r=0.130$, $p<0.001$) (**Figure 2.29)**

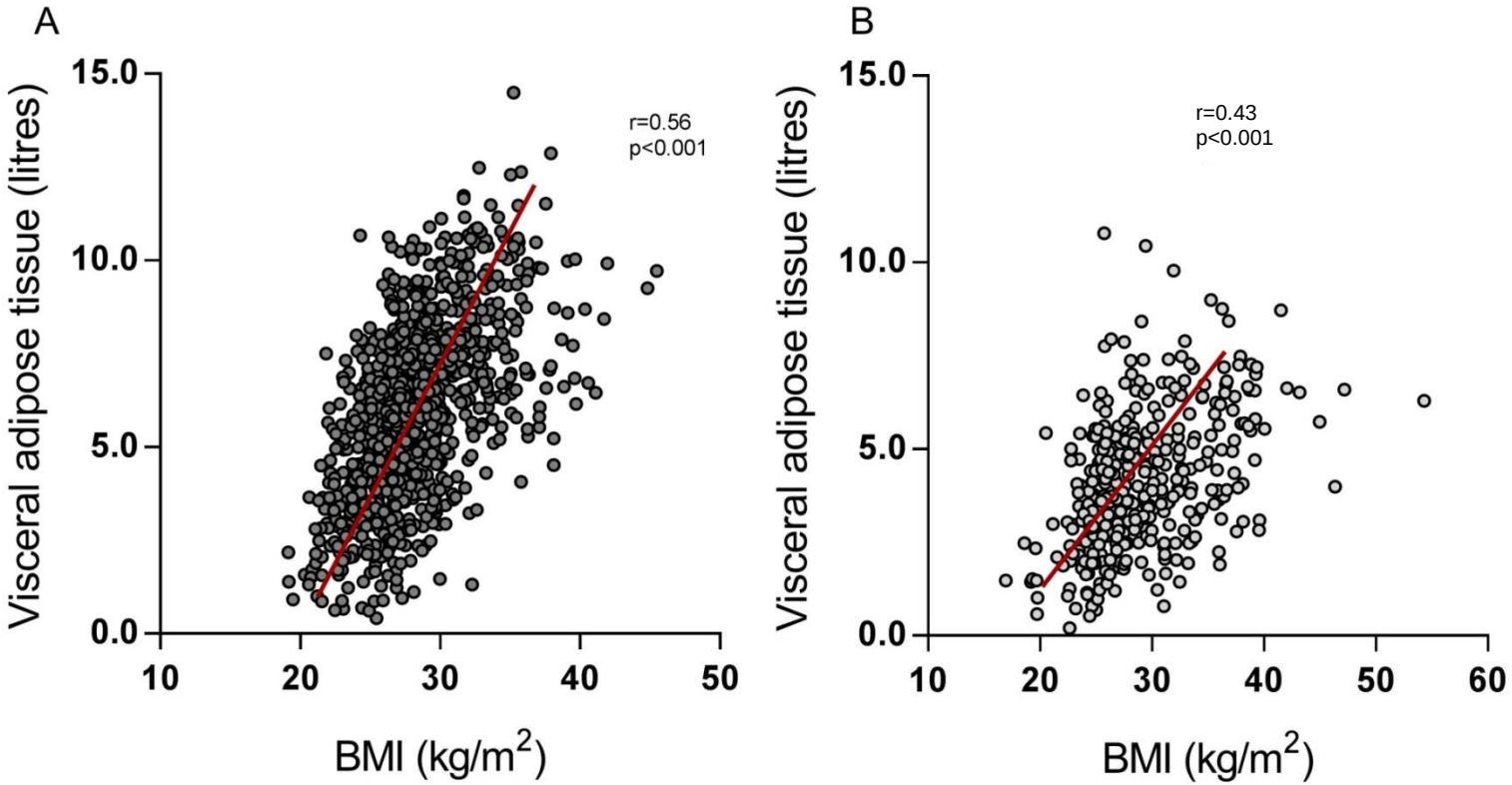

Figure 2.26 Gender specific distribution of visceral adipose tissue content with BMI in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

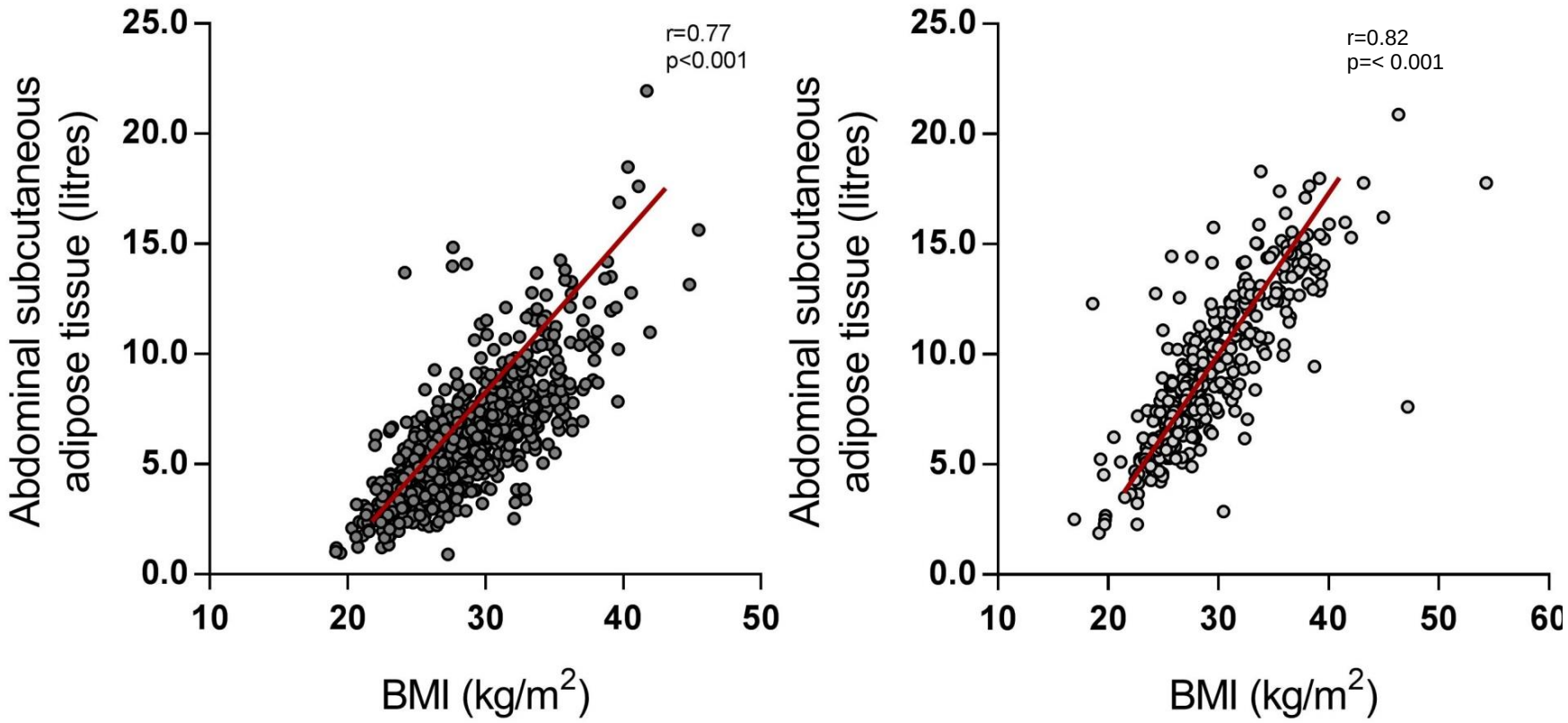

Figure 2.27 Gender specific distribution of abdominal subcutaneous adipose tissue by BMI in the pre-diabetic population in (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

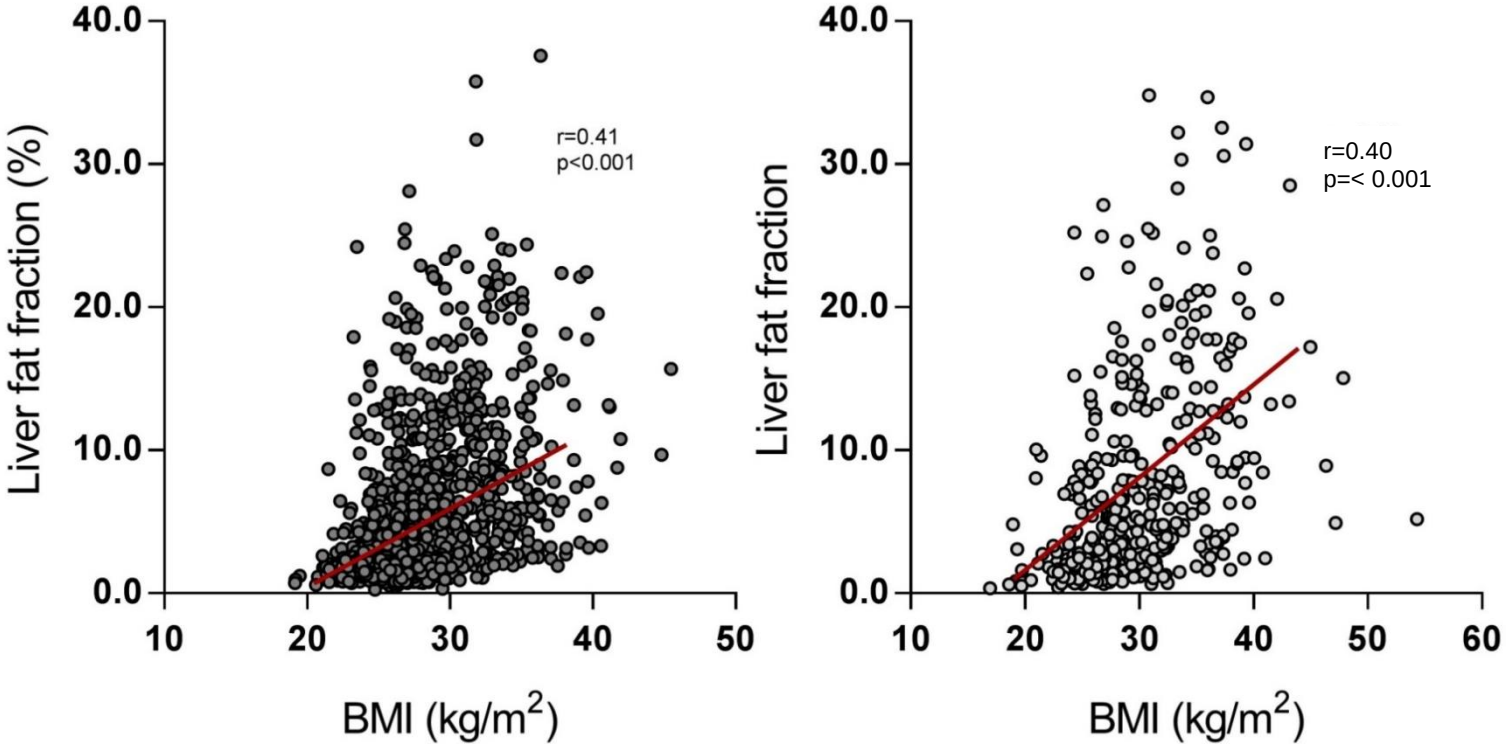

Figure 2.28 Gender specific distribution of liver fat by BMI in the pre-diabetic population. In (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

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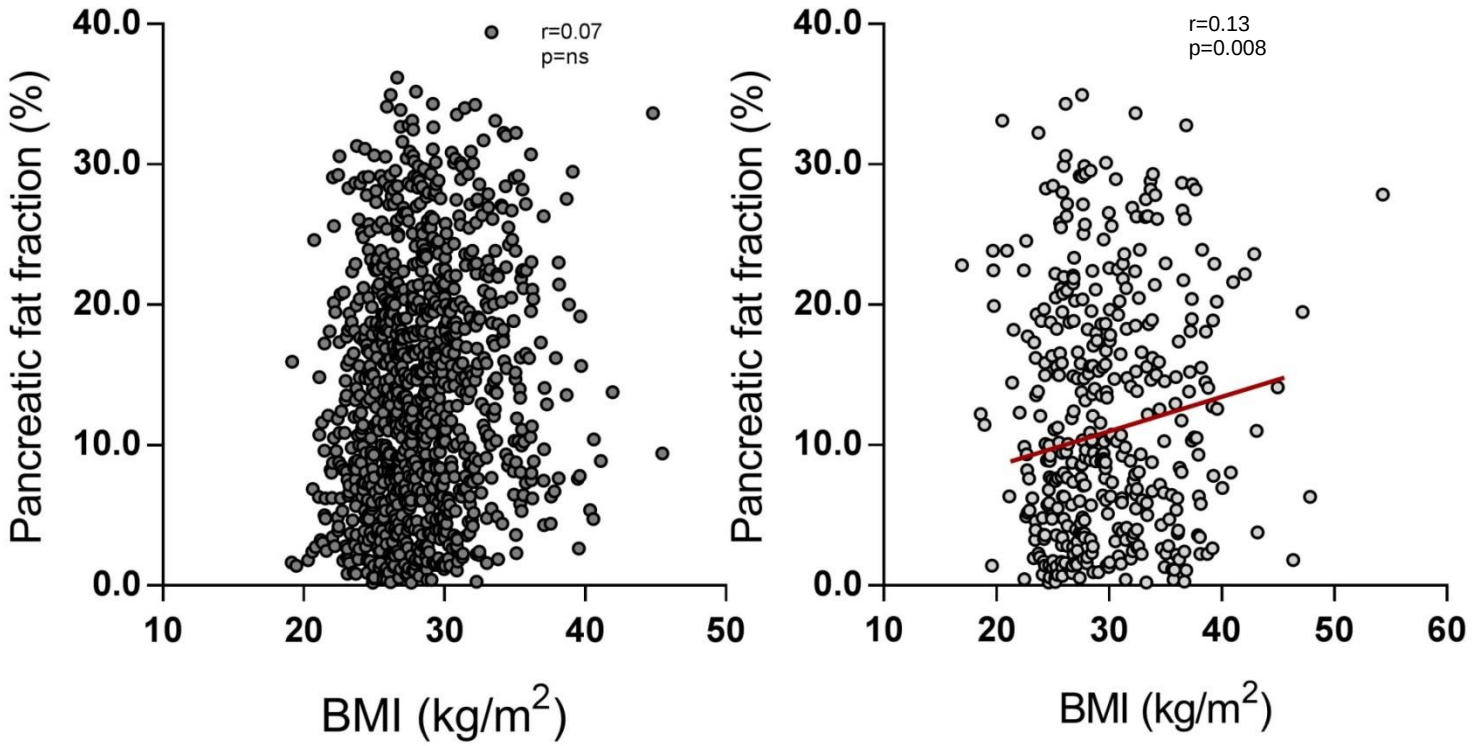


Figure 2.29 Gender specific distribution of pancreas fat by BMI in the pre-diabetic population in (**A**) males and (**B**) females. Non-parametric Spearmen's test was performed. BMI: body mass index. Data obtained from DIRECT cohort. Graphs were done using GraphPad Prism version 5.0

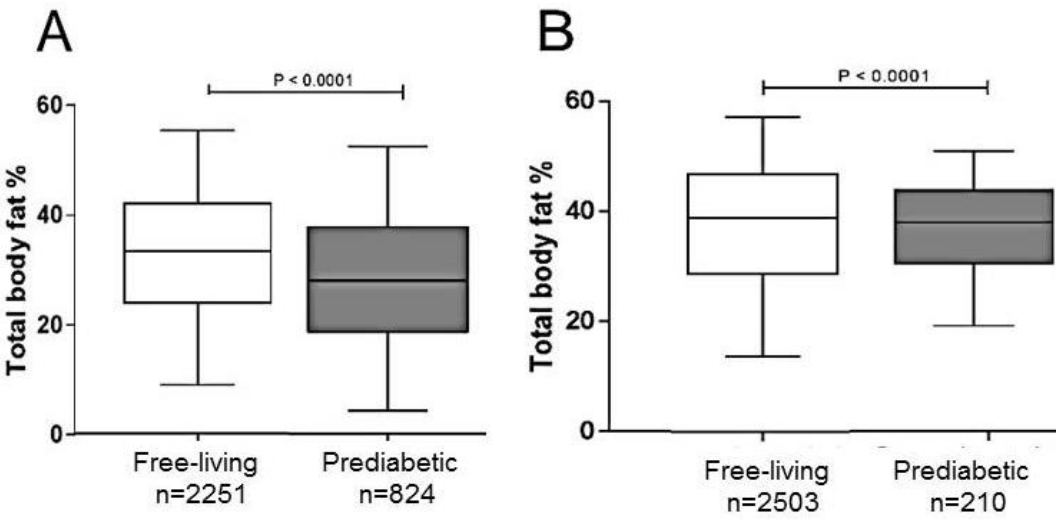
2.3.3 Comparison of total, regional and liver fat between free-living and pre-diabetic population

The comparable age between the free-living and pre-diabetic population (61.7 ± 7.1 years in the free-living population versus 61.0 ± 7.2 years in the pre-diabetic population), enables a homogenous comparison in body composition, fat distribution and ectopic fat between free-living and pre-diabetic populations. With age as an exception, body composition and blood pressure in free-living versus pre-diabetic populations showed significant distinct patterns. Overall, pre-diabetic subjects were taller (173.2 ± 8.7 cm in pre-diabetic versus 169.5 ± 9.2 cm in the free-living populations, $p< 0.0001$), heavier (28.8 ± 4.50 kg/m^2 in pre-diabetic versus 26.7 ± 4.40 kg/m^2 in the free-living populations, $p<0.0001$) with widest WHR (0.95 ± 0.07 in pre-diabetic versus 0.86 ± 1.37 in the free-living population, $p=0.0009$). Furthermore, the pre-diabetic population were more pre-hypertensive whereas the free-living population were borderline pre-hypertension (DBP 95.8 ± 12.2, SBP 129.5 ± 18.1 mmHg in pre-diabetic versus DBP 78.7 ± 10.0, SBP 133.9 ± 17.7 mmHg in the free-living population, $p<0.0001$ for both) (**Table 2.11**).

Table 2.11 Comparison in baseline characteristics and blood pressure between free-living and pre-diabetic populations. Data presented as mean ± Standard deviations. Significance was calculated from nonparametric Mann-Whitney with multiple corrections in SPSS (v.23). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. VAT; visceral adipose tissue, ASAT; abdominal subcutaneous adipose tissue.

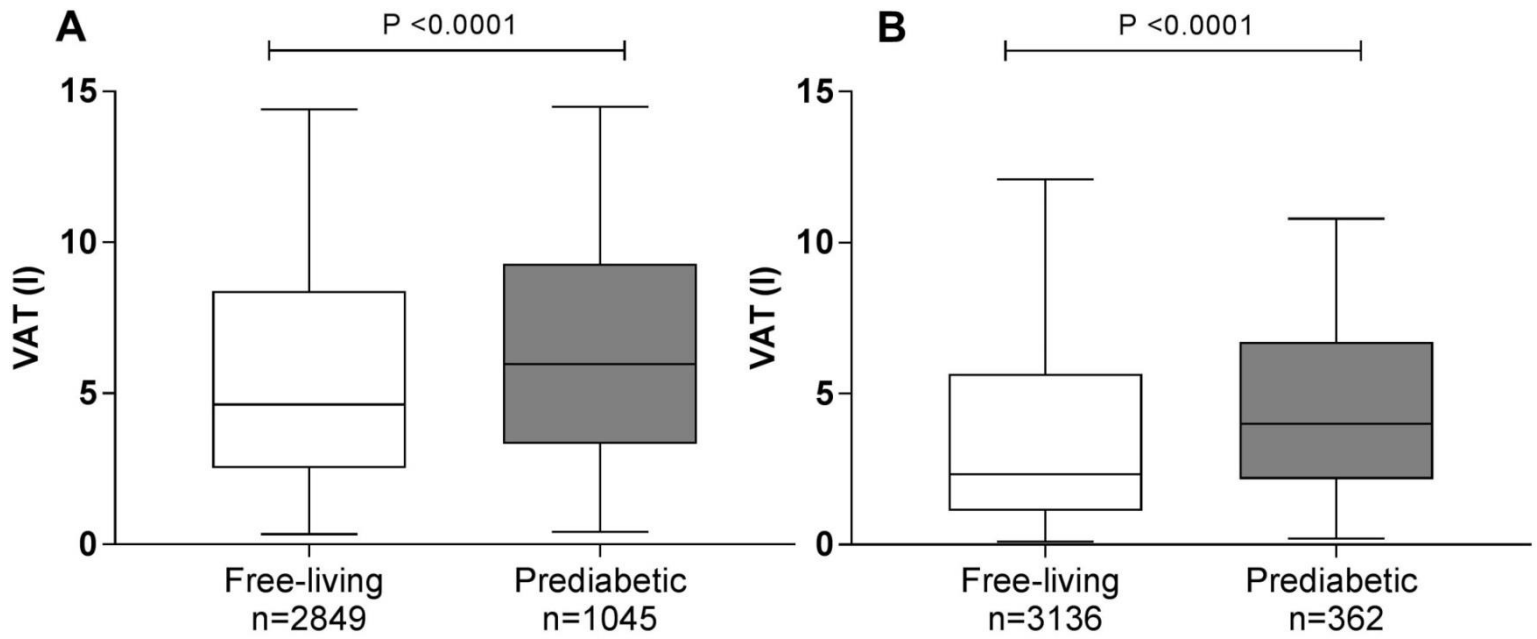
		Male		*U*-test	Female		*U-test*
		Free-living	**Pre-diabetic**	***p* value**	**Free-living**	**Pre-diabetic**	***p* value**
Anthropometry	Age (years)	62.4 ± 7.1	60.8 ± 6.9	<0.001	61.1 ± 7.1	61.2 ± 8.0	0.478
	Waist (cm)	93.4 ± 10.0	101.9 ± 10.6	<0.001	81.8 ± 11.2	98.1 ± 13.7	<0.001
	Hip (cm)	101.4 ± 8.4	102.2 ± 7.2	0.028	101.3 ± 8.7	107.8 ± 11.0	<0.001
	Height (cm)	176.4 ± 6.5	176.7 ± 6.6	0.026	163.3 ± 6.3	164.0 ± 6.6	<0.001
	Weight (kg)	83.6 ± 13.4	88.9 ± 13.5	<0.001	68.7 ± 12.9	79.9 ± 14.7	<0.001
	BMI (kg\m^2)	27.1 ± 3.9	28.4 ± 3.9	<0.001	26.2 ± 4.7	29.6 ± 5.5	<0.001
Blood pressure	DBP (mmHg)	80.3 ± 9.7	97.1 ± 10.7	<0.001	77.2 ± 11.1	92.7 ± 21.6	<0.001
	SBP (mmHg)	137.5 ± 16.4	130.4 ± 16.1	<0.001	130.7 ± 18.1	127.6 ± 21.6	0.006
DXA	Total Tissue Fat (%)	30.3 ± 6.4	26.0 ± 6.45	<0.001	39.2 ± 7.3	36.8 ± 5.80	<0.001
MR	VAT(litres)	4.9 ± 2.3	6.0 ± 2.3	<0.001	2.6 ± 1.5	4.2 ± 1.9	<0.001
	ASAT (litres)	5.9 ± 2.5	5.8 ± 2.4	<0.045	8.0 ± 3.4	9.3 ± 3.5	<0.001
	Liver fat (%)	4.7 ± 4.7	5.9 ± 5.4	<0.001	3.6 ± 4.5	7.3 ± 7.0	<0.001
	VAT/ASAT	0.9 ± 0.3	1.1 ± 0.4	<0.001	0.3 ± 0.1	0.5 ± 0.2	<0.001

In term of body composition in free-living versus pre-diabetic populations, percentage total body fat, measured via DXA scan, revealed unexpectedly significant higher adiposity in the free-living population (UK Biobank) males and females compared to their pre-diabetic counterparts (DIRECT) (males: p<0.0001, females: p<0.0001) (**Figure 2.30**).

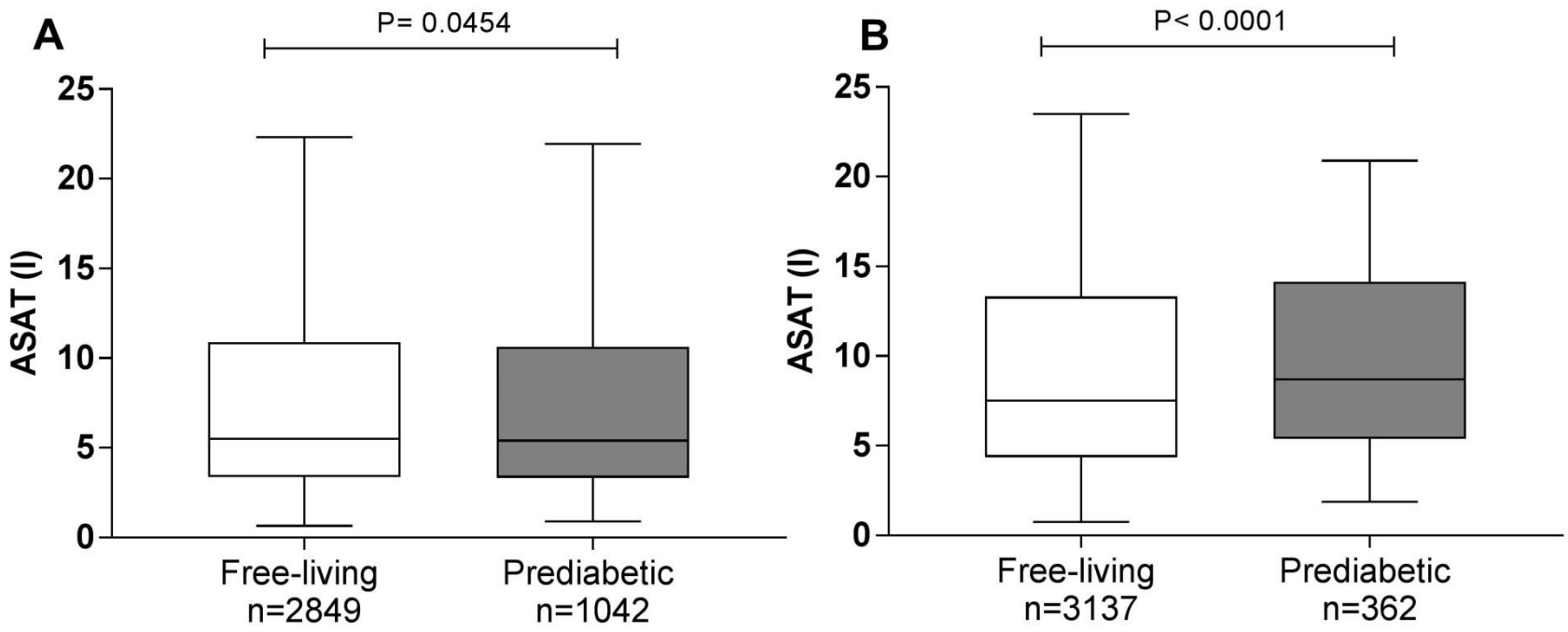


2.30 Gender specific phenotyping of total body fat percentage between free-living and pre-diabetic population in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles. *P* values are calculated from nonparametric Mann-Whitney in SPSS (v.23). Free-living population data obtained from UK Biobank and pre-diabetic data obtained from DIRECT. Graphs were performed using GraphPad Prism version 5.0

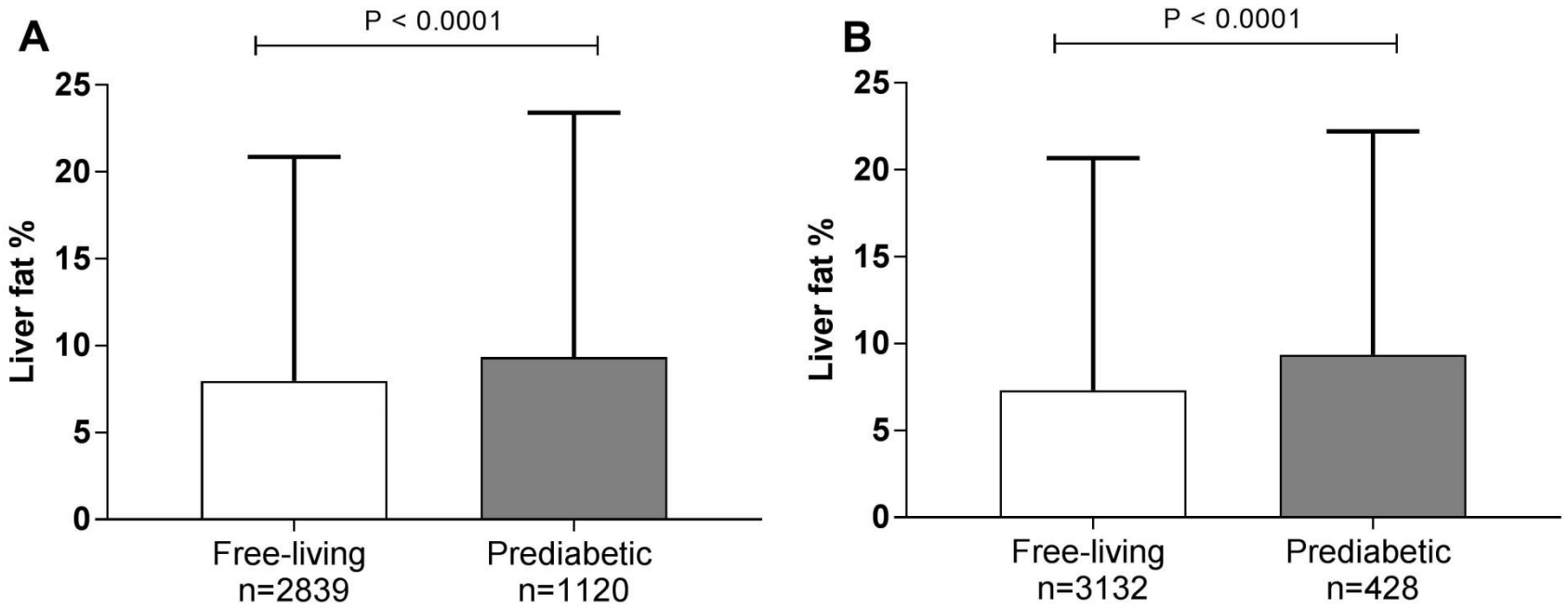
The opposite pattern was observed in regional body fat distribution, with males and females from the former population revealing significantly less VAT (total mean difference= -1.35 litre in VAT, 7.6%), more ASAT (total mean difference = 0.05 litre in ASAT, 0.2%) and less liver fat (total mean difference = -2.45 % in liver fat, 11.4%) compared to their pre-diabetic counterparts (**Figure 2.31, 2.32, 2.33** and **Appendix 4** for Detailed Gender specific phenotyping of VAT, ASAT and liver fat between free-living and pre-diabetic population). The findings of higher total fat but lower VAT, ASAT and liver fat in the free-living population compared to the pre-diabetic population is intriguing. Clearly, the differences in subcutaneous fat, which may make up the differences in overall adiposity as measured by DXA, must be in the non-abdominal area in the general population. Unfortunately, there was no data available to assess total subcutaneous AT in either population due to the MRI protocol used, which focused principally on the abdominal area of the participants. Regardless, and notwithstanding the fact that the free-living population was not fully tested for their metabolic status, these results appear to confirm the importance of fat distribution, especially abdominal obesity (visceral and liver fat), as being the key factor in the development of metabolic dysfunction. In addition, the ratio of VAT to ASAT found to be higher in pre-diabetic than free-living population (p>0.0001).



2.31 Gender specific phenotyping of visceral adipose tissue (VAT) between free-living and pre-diabetic population in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles. *P* values are calculated from nonparametric Mann-Whitney in SPSS (v.24). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0**.**



2.32 Gender specific phenotyping of abdominal subcutaneous adipose tissue (ASAT) between free-living and pre-diabetic population in (**A**) males and (**B**) females. Data presented as box and whisker plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles. *P* values are calculated from nonparametric Mann-Whitney in SPSS (v.24). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.0.



2.33 Gender specific phenotyping of liver fat percentage between free-living and pre-diabetic population in (**A**) males and (**B**) females. Data presented as mean ± standard deviations. P values are calculated from nonparametric Mann-Whitney in SPSS (v.24). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. Graphs were performed using GraphPad Prism version 5.

The greatest fat difference between pre-diabetics and subjects from the free-living population was observed in liver fat in males (percentage difference = 22.6%), and in females (percentage difference = 67.9%). This suggests liver fat as a potential candidate biomarker in determining metabolic risk. Smaller differences between cohorts were observed for ASAT, suggesting it has less of a contribution to differences arising from pre-diabetes (**Table 2.11**).

Significant differences between VAT and liver fat in free-living and pre-diabetic population were observed in both males and females, these tended to be greater in females (percentage difference: VAT=47.1%, liver fat = 67.9%) than in males (percentage difference: VAT=20.1%, liver fat = 22.6%) (**Table 2.11**).

2.4 Discussion

Large-scale imaging studies are a way to investigate physiological variation, disease development, and identify novel biomarkers of risk. Recent examples include the Rotterdam study (265), the Framingham Heart Study (266), the German National Cohort (267) and the Multi-Ethnic Study of Atherosclerosis (268). These studies have provided significant insights into complex disease processes, as well as identifying novel imaging biomarkers as a precursor for disease states. In this chapter, MRI data is analysed in order to explore the relationship(s) between anthropometry and adiposity in two large, distinct, cohorts.

It is recognised that the increased health risks of obesity and associated features of the metabolic syndrome are more strongly associated with central rather than total adiposity (99), with increased VAT and ectopic fat in the liver is the key determinants (72, 148, 247). BMI is the current standard for obesity classification, but as with all anthropometric measurements, only offers a surrogate measure of body adiposity (187). WC is widely used as a surrogate of central fat distribution, but while easily obtainable, it is unable to distinguish between VAT and ASAT deposition (269). MRI, as described in Chapter 1, is a non-invasive technique that allows accurate measurement of whole-body fat and specific internal stores of AT and ectopic fat (89). MRI studies have demonstrated significant variation among individual AT compartments that

are not fully predicted by total body or trunk fat or standard anthropomorphic characteristics; such as skin-fold measurements, BMI, and WHR (200, 254). Ectopic fat in organs has also been linked to obesity, insulin resistance, T2D, in particular, liver and pancreas fat (137, 162). Moreover, a recent cross-sectional study of 7,464 Chinese subjects demonstrated that the association between NAFLD and fatty pancreas with T2D is independent of age, gender, adiposity, and other cardio-metabolic risk factors (270).

2.4.1 Genders differences in adiposity

Gender differences in body adiposity, as previously described in Chapter 1, are well established, with females having a higher percentage of total body fat and SAT in the glutofemoral region (72, 249). These differences were confirmed in both the free-living (UK Biobank) and the pre-diabetic (DIRECT) cohorts. Nonetheless, there is some conflicting data in the literature regarding gender differences in VAT. While the great majority of studies show that males have greater VAT than females (254), a few showed no difference (271) .

Here, was found a significant increase in VAT in males compared to females in both the free-living (UK Biobank) and pre-diabetic (DIRECT) populations, representing a combined total of around 7,500 males and females. While these differences only apply to an age range of 30-70 years, this data would suggest that there is a clear gender difference in VAT. Whether these gender differences in VAT are altered by age or not is fully understood. While previous papers have found ectopic and visceral fat increase as a person ages (72, 272, 273), here, only a relatively weak correlations between VAT and liver fat fraction with age in both free-living and pre-diabetic populations was observed. Given the lower age limit of these cohorts is around 30-40 years of age, this data suggest that there is little effect of age on VAT and liver fat in middle age in free-living and pre-diabetic populations. Previous studies have indicated that VAT and ectopic fat increases in females post-menopause, an effect due to alterations in sex hormone regulation (249). Furthermore, male to female transsexuals showed a proportional effect of sex hormone with VAT after sex hormonal therapy (274). Very little evidence was

found of increased VAT or liver fat fraction in females as they enter their 50s, suggesting further detailed analysis including precise information on an individual's pre- and post-menopausal timeframe is necessary to fully resolve the impact of the menopause on the deposition of VAT and liver fat fraction.

Gender differences in liver fat were observed between free-living and pre-diabetic populations. As expected from the gender specific association seen in this Chapter and with others (249), in the free-living population, males had higher liver fat content than females, while unexpectedly, pre-diabetic females had higher liver fat content than pre-diabetic males. The gender difference in liver fat content pattern between free-living and pre-diabetic populations is interesting because it was the only fat depot that showed different gender body fat pattern. This might be because the trajectory threshold for liver fat content in order to develop pre-diabetes in females is higher than in males. In other words, it requires a higher accumulation of liver fat in females than in males to allow for the development of pre-diabetes. In addition, pre-diabetic females in my analysis had higher BMI than pre-diabetic males which were not seen in the free-living population and therefore might contribute to their higher liver fat. Increased BMI is associated with increased adverse psychological symptoms such as depression and social stigmatization, an association that was found to be stronger in females compared to males (275). Studies have shown that psychological factors such as depression and stress have a greater impact on T2D females than males, whilst females appeared to be more vulnerable to the adverse effects of the metabolic impact of such psychological factors, as well as unhealthy behaviours (276). Furthermore, mice studies showed that female mice who were exposed to psychological stress via electrical floor shock had higher liver fat content than females control despite significant weight loss in both groups (277). The mechanism by how psychological stress can affect liver fat accumulation with possible larger magnitude in females than males remains unclear and certainly complex to measure. Indeed, several mechanisms have been suggested including markers related to increased inflammation, cytokine production, and oxidative stress species (276). However, these plausible mechanisms are less likely to be liver fat specific (as it is also observed with VAT).

2.4.2 Relationship between anthropometry, adiposity, and ectopic fat depots

In agreement with previous data (72), anthropometric variables were more closely related to individual adiposity and ectopic fat stores. Similar to previous publications (72, 254, 269), it was found that WC provided the strongest correlate with liver fat fraction and VAT in both genders and in both cohorts (except in males for liver fat). Hip circumference was the strongest correlate for ASAT in females, as confirmed in most of the current literature (72, 254). However, in this Chapter, these associations are confirmed in two metabolically separate cohorts; free-living (UK Biobank) and pre-diabetic (DIRECT) populations. Hence, if MRI, or other imaging modalities are not available to measure VAT, ASAT or liver fat, the data here suggests that these markers are the most accurate ones to estimate internal adiposity in both free-living and pre-diabetic populations. It should be noted that in both cohorts there was a large amount of variation in all abdominal fat depots by BMI group. Increased BMI showed great association with CVD and T2D in epidemiological studies (278), but it remains largely insensitive to detect changes at an individual level (187).

There is evidence that VAT and ASAT play contrasting roles in the development of metabolic syndrome associated disorders. A recent review of 2,515 T2D subjects, demonstrated that liver fat and VAT (measured with CT) were associated with T2D (measured by glucose intolerance compared to normal glucose tolerance (NGT) individuals), whereas abdominal subcutaneous adiposity showed an inverse relationship with T2D (279). The International Study of Prediction of Intra-abdominal Adiposity and its Relationships with Cardio-metabolic risk of 4,144 individuals showed that VAT, but not ASAT, was strongly related to cardio-metabolic risk factors in patients regardless of T2D status (280). In the analysis, the results showed that VAT, ASAT and liver fat were higher in pre-diabetic males and females compared to free living-population ($p>0.001$ for all). This observation suggests that VAT, ASAT, and liver fat can be used for stratifying at-risk phenotypes of developing T2D in particular liver fat since it showed the

highest mean difference in body fat depots between free-living and pre-diabetes populations. In agreement with my results, Stefan *et al*. in an analysis of 1,003 subjects (405 pre-diabetic) suggested that liver fat and insulin resistance were independent determents of pre-diabetes and predict the progression from NGT to pre-diabetes status (165). A limitation of my analysis in order to draw fat depot-specific threshold with metabolic deleterious is the lack of available metabolic outcomes, such as fasting glucose and insulin. For the pre-diabetic population (DIRECT), β-cell function and insulin sensitivity were assessed using validated modelling methods based on an oral glucose tolerance test (OGTT), however, unfortunately at present these data were not available. My ability to compare the metabolic roles of VAT and ASAT is therefore limited.

2.4.3 Pancreatic fat and insulin resistance

Insulin resistance and pancreatic β-cells play an important role in pre-diabetes and in the progression to T2D since insulin resistant tissues do not normally respond to the hormone insulin (to allow normal glucose uptake) which in turn induce β-cells to produce insulin further leading to β-cell failure and eventually developing chronic hyperglycaemia and pre-diabetes (159, 281). Furthermore, insulin resistance has a close association with fat metabolism – subjects with insulin resistance showed impaired fat metabolism which might be due to insulin hormone which is a known factor for lipolysis inhibition (282). Today, there is an ongoing debate about whether β-cell failure actually results from chronic insulin resistance due to β-cell exhaustion or from increased pancreas fat due to increased lipid toxicity, which is due to inflammation and cytokine production in subjects with fatty pancreas (282).

This debate is further complicated by the conflict in the current literature as to whether increased pancreatic fat is associated with pre-diabetes and T2D development or not. Yamazaki *et al.* in a longitudinal study of five years on 813 subjects without T2D at baseline showed no association with CT derived pancreas fat and the development of T2D (283). Despite that the Yamazaki *et al.* study has the power of a longitudinal study rather than a cross-sectional one, the progression rate of T2D in the study was only 7%, and it is further limited by the usage of CT as the modality of choice for the assessment of pancreas fat. CT assessment of pancreas fat based on the ratio of the pancreas to the spleen Hounsfield's units attenuation which makes it sensitive to changes in the spleen as a cofounder rather than an accurate assessment of pancreas fat as when using MR (283).

Further conflicts in the literature arise where few MR studies showed no association between increased pancreatic fat and pre-diabetes. Kuhn *et al*. in a study of 431 pre-diabetic subjects demonstrated no association between increased MR-derived pancreatic fat and pre-diabetes as measured by OGTT between 5.6 and 9.6 mmol/L (284). However, these findings and others (285)

who showed no association between increased pancreatic fat and pre-diabetes have a technical limitation in mostly using three regions of interests (as ±1.0 cm circular dotted area) as the employed method for assessing pancreatic fat content instead of a whole organ extraction as presented in this Chapter. Indeed, there are noticeable variations in pancreas fat content between different anatomical locations and therefore whole organ extraction, which is employed in this chapter, is the superior method for an accurate pancreas fat assessment (285).

In this chapter, was presented an analysis of pancreas fat using MR quantification of whole organ extraction and showed increased pancreatic fat in pre-diabetic males and females. In agreement with the results of my analysis, a recent meta-analysis and a systemic review of the association between pancreas fat and T2D from CT and MR studies on a total of 3,403 subjects (33.4% with T2D) showed increased pancreas fat content in patients with T2D compared to non-diabetic individuals (285). The strength of my analysis of pancreas fat is using MR whole organ extraction for the assessment of pancreas fat as well as a large sample size of homogenous pre-diabetic subjects. However, a major limitation of my pancreas fat analysis is that there was no data regard β-cell function in order to allow for a better understanding of the mechanism of altered pancreas function and fat deposition in the manifestation of T2D, which may allow for new insights in T2D prevention, diagnosis and treatment. Further research including comprehensive metabolic data is recommended to evaluate the effect of increased pancreas fat on the development of T2D. A limitation of my pancreas analysis is that the pancreas fat was only available in the pre-diabetic population and therefore, it was not possible to make a comparison between free-living and pre-diabetic population.

2.4.4 Impact of physical activity on adiposity

It is well-established that increased physical activity is linked with abdominal body fat depots reduction (85). However, setting a dose-response relationship is challenging as part because assessing physical activity is predisposed to certain measurement challenges. IPAQ has been designed to

assess physical activity indirectly by engaging participants in answering questions on their everyday live physical activity. IPAQ is the most frequently used method to assess general physical activity in population studies such as in the UK Biobank because it is practical and comes with low cost (286). However, since IPAQ is largely dependent on the participants reporting their own level of physical activity, it has the capacity to over or underestimate true physical activity levels because of issues associated with memory, the participants ability and motivation to report accurately (286). In addition, IPAQ requires translation in particular in population studies such as UK Biobank aiming to capture an actual representation of the whole population and ensure the inclusion of ethnic minorities and vulnerable subjects who may not speak English (286). Furthermore, IPAQ responses were shown to be largely affected by participants' sociodemographic and health status, for example, participants with higher education and better self-reported health status are likely to overestimate their physical activity using self-reported measures (287). On the other hand, objective direct methods for physical activity measurements such as wearable fitness accelerometers are commonly used for precise physical activity assessment and to overcome some of the self-reported data issues related to memory, response bias and language barriers (288). Objective direct physical activity assessment, despite its advantages, remains costly, requires time and particular training and in some wearable devices, such as triaxial which is used in the pre-diabetic cohort in this study (DIRECT), and unable to measure physical water activities (288). Therefore, objective measures are often used as validation or a complement for the indirect physical activity assessment (288).

In this chapter, was also presented physical activity assessments using both methods; the objective direct accelerometer used in the pre-diabetic cohort (DIRECT), and the self-reported physical activity assessment using IPAQ in the free-living population (UK Biobank). The two physical activity measurement outcomes were not comparable for various reasons including the technical differences in acquiring the data (direct versus indirect physical activity assessments), and their availability from two different cohorts (IPAQ from UK Biobank and ENMO from DIRECT). However, VAT, ASAT and liver fat negatively correlated with physical activity to a similar degree in both

DIRECT (ENMO score) and UK Biobank (IPAQ). Correlation with individual physical activity parameters in the UK Biobank also showed similar *r* values for both VAT and ASAT. **Table 2.12** demonstrates the correlation between VAT, ASAT, and liver fat using IPAQ and ENMO. Overall, the objective physical activity assessment using ENMO showed stronger correlations with fat depots than the IPAQ. It is noteworthy to mention that the ENMO covered a period of 10 days while the IPAQ covered two weeks.

Table 2.12 The correlations between MR measurements and physical activity assessment using objective physical activity assessment (IPAQ) and subjective physical activity assessment (ENMO). IPAQ and ENMO. Values representing r from Pearson's correlation. IPAQ: International Physical Activity Questionnaire, ENMO: Euclidean Norm Minus One. Statistics performed using SPSS v. 23.

	IPAQ (UK Biobank)		ENMO (DIRECT)	
MR measurements	**Male**	**Female**	**Male**	**Female**
VAT	-0.180	-0.187	-0.269	-0.262
ASAT	-0.181	-0.193	-0.257	0.181
Liver fat	-0.156	-0.122	-0.138	-0.204

Further work needs to be done to combine measurements of physical activity (IPAQ with wearable devices) for accurate assessment of physical activity, full inclusion of the population studied and ability to determine dose-response relationships between day to day lifestyle and phenotyping of body fat depots.

Previous data from the UK Biobank has shown low levels of physical activity, high television viewing and poor sleep duration cluster together in overweight and obese individuals (289). Additional work showed an inverse relationship between physical activity and both BMI and body fat percentage (290). Here, was found higher liver fat deposition was associated with specific sedentary lifestyle variables, including time spent watching TV, time spent using a computer, and the presence of long-standing illness or disability. TV viewing is also linked with other unhealthy behaviours like snacking and is consistently associated with higher liver fat deposition (291). A recent large meta-analysis showed watching TV for more than 3 hours was strongly linked to all-cause mortality (292). The strongest correlation was observed between an individual physical activity outcome with liver fat fraction and VAT was the subjects usual walking pace. Conversely, lower liver fat was associated with

physical activity variables, including brisk walking pace and frequency of walking (as means of transport), performing moderate, vigorous or strenuous physical activity, and stair climbing.

Previous studies have demonstrated that daily walking and active commuting is linked to reductions in VAT and improvements in insulin resistance (293, 294). A recent systematic review and meta-analysis of 24 studies examined the effects of physical activity on visceral fat and liver fat in subjects with T2D (295). The authors demonstrated that aerobic exercise, but not resistance training, effectively reduced both (295). Furthermore, even low levels of physical activity have been associated with reduced mortality in individuals with the metabolic syndrome (296). These data, together with the correlational analyses presented, suggest that even low-intensity aerobic exercise, such as walking or stair climbing, has a beneficial effect on lowering levels of VAT and ectopic fat. The lack of association between the duration of physical activity and liver fat as opposed to the frequency and reduced liver fat is interesting, suggesting that it is not the exercise duration for that important, rather performing it repeatedly. Overall, these relationships observed in this data represent a promising opportunity for effective intervention to reduce abdominal fat, especially in an elderly group of individuals for whom strenuous vigorous activity may not be feasible. Indeed, a number of interventional studies have linked an increase in physical activity (aerobic and resistance) with a reduction in liver fat (297-299).

2.4.5 Strength and weakness of this study (phenotyping body fat deposition and ectopic fat in free-living and pre-diabetic populations)

As mentioned in the introduction, there are various methods for body fat assessment. In this chapter, the gold standard modality, which is MR was used for the quantification of visceral, ASAT and ectopic fat in the liver and pancreas (89). Unfortunately, both cohorts did not include full body MR data to allow the quantification of total body fat. Therefore, DXA scan data (which was available for both cohorts) was used for the assessment of total body fat and its association with MR modality measurements. The correlation of total

fat percentage measured by DXA with MR fat depots was similar in pre-diabetic and the general population. ASAT showed the strongest correlation with body fat percentage in pre-diabetic and general population males and females. UK Biobank cohort provided DXA quantifications of FFM and lean mass in addition to body fat percentage, and as expected, FFM and lean mass revealed significant gender differences with males having higher FFM and lean mass which might be due to physical variations between males and females (249). Despite the overall agreement in the correlation *r* values between DXA and MR measurements in both cohorts, DXA as a radiation source remains a concern (300). Therefore, further studies using MR to quantify full body fat with comprehensive physical activity assessment and metabolic data is required in order to fully understand the biological association of body fat phenotypes in metabolic disease developments.

The major strengths of my data are the numbers of individuals included with MR data for body fat depots quantification. While the cross-sectional nature of the data-set is limiting in its inability to imply causality, it is the largest study of its kind to incorporate MRI-acquired measures of VAT, ASAT and liver fat, with detailed measures of physical activity. In addition, there are inherent issues regarding the self-reported physical activity propensity to measurement error, an effect more prevalent in aged populations where cognitive regression can impact on accuracy. The lack of age-related increases in VAT and liver fat fraction observed in males are especially of note, suggesting that levels of these depots are established by the time individuals reach 40 years of age. Secondly, the MR fat quantification included in this chapter did not include young adults (>30 years old) where an early manifestation of metabolic disease may occur, because both cohorts recruitment fallen between 30-70 years. Finally, this analysis did not include information on the ethnicity background of the subjects studied because the pre-diabetic cohort was mainly homogenous Cau cohort and the free-living population cohort, which had ethnicity data but unfortunately was not available at the time of this analysis. Ethnicity, as mentioned extensively in Chapter 1 Section 1.5, is a major factor altering the deposition of VAT and

ASAT, and a determining factor of regional AT deposition and its association with insulin resistance and the development of T2D.

Chapter 3

Phenotyping body fat deposition in South Asian

Chapter 3 Phenotyping body fat deposition in South Asians

3.1 Introduction

SA, who makes one-quarter of the world population including individuals of India, Pakistan, Bangladesh and Nepal, have a higher prevalence of the metabolic syndrome compared with Cau populations (178, 206, 231). In India, the largest SA country, it is estimated that 65 million people are affected by T2D, with the number predicted to reach 109 million by 2035 (301, 302). As mentioned in Chapter 1 Section 1.5 Ethnicity, at a similar or even lower BMI, SA adults present a higher percentage body fat, lower lean mass and more visceral fat compared to Cau (20, 221, 225, 303). The 'thin-fat phenotype' in South Asians reflects a body composition comprised of reduced muscle mass but increased adiposity (235, 304, 305). It is evident that neonatal SAs are characterised with smaller anthropometric measurements but increased adiposity with relative preservation of body fat (306, 307). The compartmental distribution of AT is a key factor for metabolic deterioration, with VAT linked to increased CVD risk (308). This tendency towards increased central obesity, together with reduced HDL cholesterol levels and elevated circulating TG and cholesterol, are thought to contribute to SA increased susceptibility to develop metabolic syndrome associated morbidities (230, 233, 234).

The foetal origins hypothesis, as previously mentioned in Chapter 1, Section 1.2.1 Obesity: the epigenetic causes, has also been proposed as a further effect to explain the vulnerability of SA individuals to metabolic disease. This hypothesis posits that challenges *in utero*, such as malnutrition, lead to adaptive changes in metabolic-endocrine pathways necessary for the foetus to survive (44, 309). These changes persist into adult life, subsequently triggering degenerative conditions, including metabolic syndrome, CVD and T2D (310, 311). Such alterations in metabolic–endocrine pathways are reflected in reduced foetal growth and small size at birth; SA babies are among the smallest in the world, given that half of the world's low birth weight babies are born in SA (low birth weight <2500 g) (312).

In order to better understand how this phenotype manifests, attempts have been made to compare the body composition of individuals born in India against White Cau counterparts. One such investigation is the PMNS (305), which was established in 1993. The PMNS monitored over 800 pregnant females recruited from 6 rural villages near Pune, one of the largest urban cities in West India. Mothers were comprehensively observed during pregnancy for anthropometric changes, nutritional intake, physical activity, and circulating nutrient levels. Once infants were born, growth measurements were performed every 6 months and individuals followed up every 6 years, investigating risk factors for T2D and CVD.

In this Chapter, the amount of abdominal fat (VAT and ASAT) in the offspring of the PMNS was quantified from the MR imaging data collected at their 18-year follow up. The relationships between anthropometry, blood biochemistry and abdominal adiposity was examined in this cohort. Furthermore, the relevance of ethnicity specific BMI cut-offs and the prevalence of sub-phenotypes, including the thin-fat phenotype within this population was also investigated. For further exploration on how ethnicity impacts of body fat deposition, an attempt was made to compare this young SA cohort with a comparable Cau cohort.

3.1.1 Aims

1. Quantify the amount of VAT and ASAT in young SA adults in the PMNS.
2. Investigate the associations between MRI derived measurements (ASAT and VAT) and age, gender, anthropometry and metabolic profile in the Pune Maternal Nutrition Study.
3. Determine the prevalence of South Asian sub-phenotype; thin-fat phenotype in 18 years SA in India.

3.2 Methods

3.2.1 Pune Maternal Nutritional Study (PMNS) participants

PMNS was established in 1993 to study the Indian adiposity phenotype and has monitored a birth cohort raised in rural villages around Pune in Southern India. It provides measurements collected at various time points from conception up to 18 years of age. Written, informed consent was acquired from all volunteers. Ethical approval permission for this study was given by the village authorities in King Edward Memorial Hospital in Pune, India. The volunteers were recruited from six villages approximately 50 km from Pune, including Dhamari, Karandi, Kendur, Pabal, Pimpale-Jagtap, and Shikrapur. Ethnicity was self-reported, and all parents and grandparents were required to be of SA descent.

All married females of reproductive age living in the six villages were approached and 2675 recruited (between June 1994 and April 1996). Of these, 1102 became pregnant and 762 delivered live babies. A flow diagram describing data collection and exclusions from the PMNS is presented in **Figure 3.1**. The data in this Chapter corresponds to 423 offspring (261 M, 162 F, mean age 18.0 ± 0.60 yrs.) who returned for a follow-up assessment between 2008 and 2010 in King Edward Hospital, Pune, India.

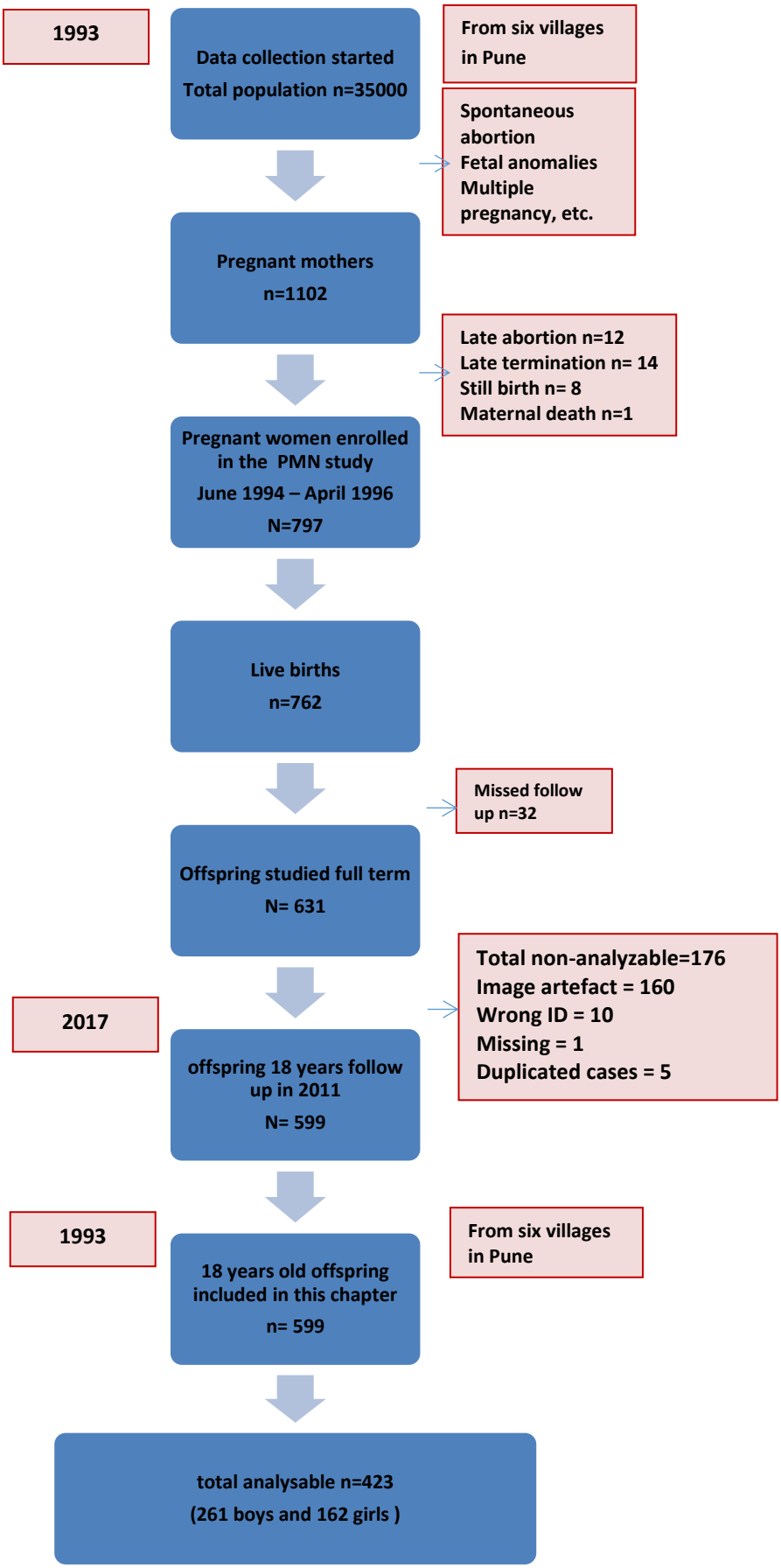


Figure 3.1. A flow diagram describing data collection and exclusions in the Pune Maternal Nutrition Study from six villages in rural India; adapted from (313).

3.2.1.2 Study measurements

Height (stadiometer, ATCO Healthcare Ltd, Mumbai, India) and weight (calibrated scale, CMS Instruments Ltd, London, UK) were measured by trained research nurses, and BMI calculated as weight divided by height squared. Callipers (CMS Instruments Ltd, London) was used to measure the sum of skin-fold thicknesses (biceps, triceps, subscapular and suprailiac), an index of subcutaneous adiposity. Blood pressure was taken by a semi-automated sphygmomanometer (Welch-Allyn, Beaverton, OR, USA) and the mean of the 2nd and 3rd of three recordings used in the analysis.

3.2.1.3 Metabolic markers

Plasma glucose, cholesterol, HDL-cholesterol and triacylglycerol concentrations were measured using standard enzymatic methods (Roche Diagnostics, Mannheim, Germany). Between-batch coefficient of variation (CV) for all these assays were <3% in the normal range. Plasma insulin, proinsulin and 32–33 split proinsulin were measured using a two-site immunoenzymometric assay (Medgenix, Fleurus, Belgium); between-batch CV for insulin measurements were <6%. HOMA-IR, which is an index used to gauge insulin sensitivity calculated from fasting glucose and insulin levels, was calculated using the currently accepted standard (online Oxford HOMA calculator: available from www.dtu.ox.ac.uk) (314). OGTT was carried out according to the WHO protocol, using 75g glucose. Blood samples were collected for measurement of glucose and insulin at 0, 30 and 120 min. Individuals were classified as NGT if their fasting blood glucose between 4.4 and 5.5 mmol/L (between 72 and 100 mg/dL) (**Table 3.1**). Elevated blood glucose or pre-diabetes status was classified as IFG between 5.6 and 6.9 mmol/L (100-125 mg/dL)) or impaired glucose tolerance (> 7.0 mmol/L or >126 mg/dL). In this chapter, IFG threshold was implemented for the diagnosis of pre-diabetes as it is recommended for appropriate diagnosis of pre-diabetes in the general population with no observed disorders of glucose metabolism (i.e. hypertensive) (**Table 3.1**) (315).

Table 3.1 Recommended criteria for normal glucose and pre-diabetes. Updated from the American Diabetic Association 2013.

Test	Normal blood glucose	Pre-diabetes
Fasting plasma glucose (mmol/L)	4.5 - 5.5	5.6 – 6.9 (Impaired fasting Glucose)
Oral Glucose Tolerance Test (mmol/L)	Below 6.9	7.0 – 9.0 (Impaired glucose tolerance)

3.2.1.4 MRI scanning protocol

T1-weighted MR images were acquired at the Anushka Scanning Centre at Kem Hospital, Rasta Peth, Pune using a 1.0T Siemens Magnetom Harmony scanner (Siemens, Munich, Germany). Three 10 mm thick transverse slices located in the abdomen were acquired with subjects lying in a supine position using the following parameters; TR: 100 ms, echo time: 7.49 ms, 512x384 matrix size and an 11 mm gap between slices.

3.2.1.5 Technical quality assurance protocol for abdominal images prior to quantification of visceral and abdominal subcutaneous adiposity

Before images were analysed, technical quality assurance was performed in order to evaluate the prevalence of image artefacts and to investigate factors affecting image analysability (**Figure 3.2**). Images were considered not analysable if any part of the anatomical region was missing in the dataset or constrained significant, any respiratory artefacts motion artefact over the abdominal region, and incomplete abdominal coverage.

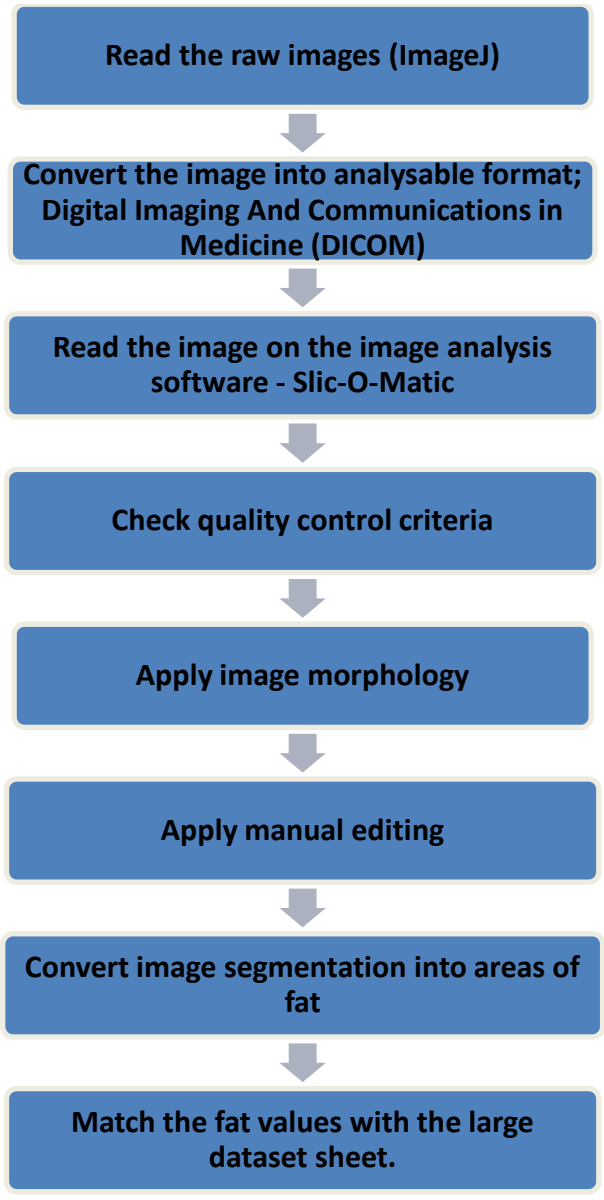


Figure 3.2. A flow chart describing the process of generating the dataset for Pune Maternal Nutrition Study from data handling, quality control and creating the large dataset.

3.2.1.6 MRI Image analysis.

All dataset was analysed using Slice-O-Matic (Tomovision, Montreal, Canada) for abdominal subcutaneous and visceral adipose tissue quantification (316) with the area of adipose tissue recorded in cm^2. The ASAT and VAT segmentation method based on two main tools; image morphology and manual editing that was conducted to label regions as ASAT or VAT.

With the scanning parameters employed, fat appears as a high signal against a muted background of other tissues and noise. The images were segmented and analysed by labelling voxels as fat and non-fat components (316). The analysis procedure employed a contour-following algorithm to isolate individual structures from binary images produced by thresholding. The threshold needed to identify fat-component associated voxels was computed automatically from grey intensity histogram analysis and background-noise computation (317). Separated regions were then manually filled with appropriate tags for visceral fat (red) and subcutaneous fat (green) (**Figure 3.3**). To verify segmentation precisely and make corrections, the tag coloured images (red or green) were superimposed on a greyscale image in transparency mode during analysis. After finishing the morphology phase, a manual edit was done to detect fine details, includes all the missing pixels from the semi-automated phase and remove any bowel content from the adiposity segmentation. The outcome was an adipose tissue area (cm^2) for each compartment, which was calculated by summing the relevant voxel counts. Note that this analysis provides a direct measurement of the area of adipose tissue rather than of the quantity of triglyceride contained within the adipose tissue.

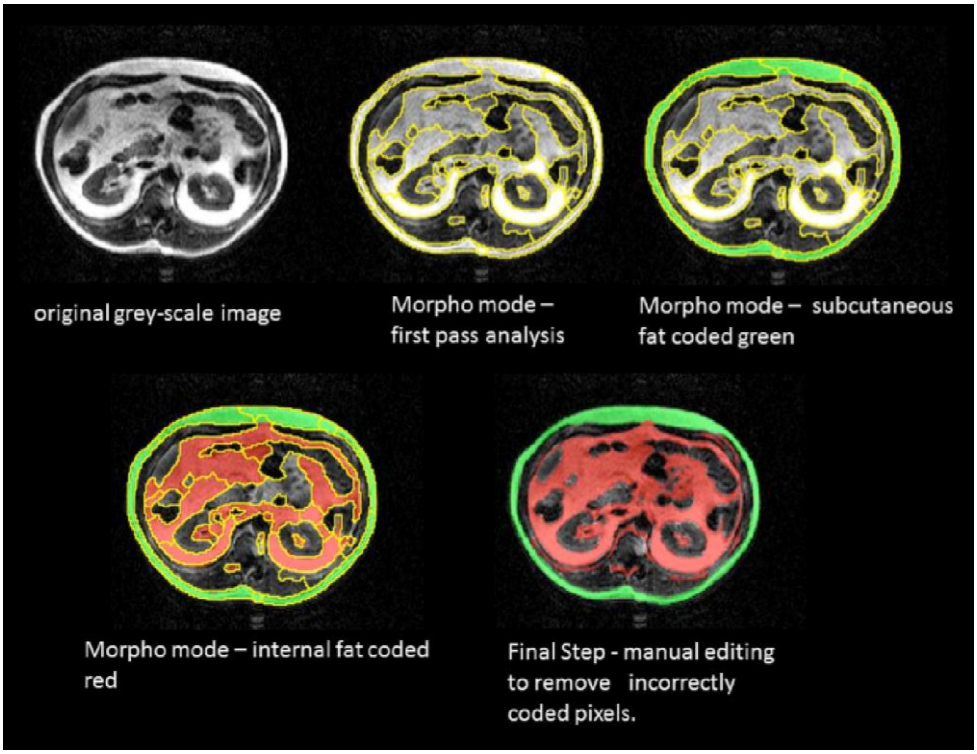


Figure 3.3 Quantification of visceral and abdominal adipose tissue in South Asian population using Slice-O-Matic software. The greyscale image was initially processed using mathematical morphology to segment the subcutaneous and internal fat. These were labelled (Tagged) with specific colour codes for each depot. In these studies, subcutaneous fat was coded green and internal fat (visceral fat) coded red.

3.2.2 Statistical analysis

Descriptive measures are reported as means ± standard deviation (s.d.). Data were checked for normality using the Shapiro-Wilk's test. In the case of a normal distribution, means were compared using Student's t-test; otherwise, the non-parametric Mann-Whitney U-test was used. Comparison of VAT and ASAT between different BMI cut-offs (WHO general population recommendations and proposed WHO SA recommendations) was examined using t-test for males and females separately. The relationship between quantitative variables (age, weight, height, BMI, VAT and ASAT) was analysed using Spearman's rank correlation coefficient for non-normal distributed data in SPSS (version 23.0). Individuals were assessed for sub-phenotypes including thin-fat and TOFI phenotype, which represents a ratio of VAT/ASAT. Cut-offs for the phenotype were defined as individuals with a VAT/ASAT ratio of greater than 1.04 in males and 0.45 in females (72). Circulating levels of glucose were used to define individuals as NGT (blood

glucose <5.5mmol/L) or pre-diabetic (blood glucose >5.6mmol/L) based on Diabetes UK guidelines (https://www.diabetes.co.uk/pre-diabetes.html). Linear regression analysis was used to model the relationship between VAT and ASAT with glycaemic status (as defined by a fasting blood glucose >5.6mmol/L). In Model 1, the contribution of glycaemic status was assessed for VAT and ASAT, in Model 2: gender and BMI were adjusted for.

3.3 Results

3.3.1 Pune Maternal Nutritional Study (PMNS) participants baseline characteristics

MRI data was available from 599 subjects, 176 subjects were excluded from the analysis due to poor outcome after technical quality assurance protocol (**Table 3.2**), resulting in a total of 423 subjects included in the final analysis.

Table 3.2 Exclusion criteria for PMNS magnetic resonance imaging data. Table listing the reasons for excluding MRI data of the PMNS. MRI; magnetic resonance imaging, PMNS; Pune Maternal Nutritional Study

	n
False ID number on the image	10
ID number not on the list	1
Not analysable due to image artefact	160
IDs for not available images	5
Total	**176**

The results for the 423 individuals included in the cohort with study characteristics are shown in **Table 3.3** The mean age of the sample was 18.0 ± 0.60 years (range: 16.6 - 19.5 years) and BMI ranged from 13.0 - 37.6 kg/m^2.

Table 3.3 Baseline characteristics of anthropometry, body composition, and metabolic profiling in adolescent South Asian in the PMNS cohort. Data presented as mean ± standard deviation. PMNS; Pune Maternal Nutritional Study, MR: Magnetic resonance imaging, DXA: Dual-energy X-ray, BMI: Body mass index, HOMA-IR: Homeostatic model assessment insulin resistance. Statistics done using SPSS version 23.0.

		Mean ± SD	Range
Anthropometry	Age (yrs.)	18.0 ± 0.60	16.6 - 19.5
	Weight (kg)	53.1 ± 11.0	23.6 - 97.8
	Height (cm)	164 ± 9.2	134 - 186
	BMI (kg/m^2)	19.5 ± 3.3	13.0 - 37.6
	Waist circumference (cm)	71.6 ± 8.7	51.5 - 104
	Hip circumference (cm)	87.5 ± 8.5	19 - 117
	Waist-to-Hip Ratio	0.8 ± 0.2	0.7 - 3.8
	Skinfold Thickness (cm)	51.9 ± 28.9	14.3 - 177.3
Blood pressure	Systolic Blood Pressure (mmHg)	108 ± 9.2	45 - 140
	Diastolic Blood Pressure (mmHg)	59.8 ± 7.6	34 - 82
MRI	Visceral Fat Area (cm^2)	186 ± 63.6	34.9 - 400
	Abdominal Subcutaneous Area (cm^2)	344 ± 216	34.9 - 1521
	VAT / ASAT ratio	0.6 ± 0.2	0.1 – 2.6
DXA	Total Fat Mass (kg)	11.2 ± 6.8	2.1 - 40.0
	Total Lean Mass (kg)	39.1 ± 8.4	16.1 - 64.8
	Total Trunk Fat (kg)	5.4 ± 3.3	0.8 – 19.2
Metabolic profile	Fasting Glucose (mmol/L)	8.0 ± 1.0	6.6 – 25.9
	Fasting Insulin (mU/L)	10.7 ± 5.6	2.0 - 55.6
	Cholesterol (mmol/L)	3.3 ± 0.6	1.6 - 6.1
	HDL Cholesterol (mmol/L)	1.0 ± 0.3	0.5- 3.9
	Triglycerides (mmol/L)	0.7 ± 0.3	0.2 - 3.1
	HOMA IR	1.4 ± 0.7	0.3 - 6.7

3.3.1.2 Pune Maternal Nutritional Study characteristics by gender

Gender specific characteristics of the Pune Maternal Nutritional Study are shown in **Table 3.4.** Significant gender differences were recorded for the majority of parameters in the PMNS cohort. Overall, males were significantly taller ($p<0.001$) and heavier ($p<0.001$) with larger WC and waist to hip ratio than females in the PMNS cohort ($p< 0.001$ for all) (**Table 3.4**). There were gender differences in all body composition outcomes in the PMNS cohort; females had a large skinfold-thickness (difference=42.6%, 23.3 cm^2 in skinfold thickness, $p<0.001$) and more total FM (34.8%, 4.1 kg differences in FM, $p<0.001$) but with less lean mass (difference= 18.8%, 14 kg in lean mass: F: 30.4 ± 3.6, M: 44.5 ± 5.5, $p<0.001$) than males (**Table 3.4**). Contrarily,

males presented significantly more VAT (differences= 20.1%, 37 cm^2 in VAT, p< 0.001) than females in the PMNS cohort, despite having less total FM (**Table 3.4**), whereas females had significantly more ASAT (difference=23.3%, 80 cm^2 in ASAT, p<0.001) than male counterparts in the PMNS study (**Table 3.4**).

With few exceptions seen in insulin parameters, there were intriguing gender differences in all metabolic profile outcomes in reflection with body composition outcomes (**Table 3.4**). Males were more hyperglycaemic (difference= 2.5%, 0.2 mmol in blood glucose, p>0.052), than females, which is interesting given that the latter had less lean mass (p<0.001), and therefore it is expected to observe lower glucose uptake with less muscle mass, particularly that both genders had similar age and BMI (**Table 3.4**). Furthermore, males had higher plasma triglyceride (differences= 15.4%, 0.1 mmol in triglyceride, p>0.005), while having significantly less FM than females (**Table 3.4**).

Table 3.4 Gender specific baseline characteristics, blood pressure, body composition and metabolic phenotyping in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT; Visceral adipose tissue (cm2), ASAT; Abdominal subcutaneous adipose tissue (cm2), PMNS; Pune Maternal Nutritional Study. MR: Magnetic resonance imaging, DXA: Dual-energy X-ray, BMI: Body mass index, HOMA-IR: Homeostatic model assessment insulin resistance Data presented as mean ± standard deviation. Gender comparison performed by independent t-test in SPSS, version 24.0. Significance was taken at $p<0.05$ level.

		Male (n=261)		Female (n=162)		
		Mean ± SD	**Range**	**Mean ± SD**	**Range**	**p**
Anthropometry	Age (yrs.)	18.2 ± 0.5	16.9 - 19.5	17.7 ± 0.61	16.6 - 19.2	0.504
	Weight (kg)	56.9 ± 10.6	36.0 - 97.8	46.8 ± 8.6	23.6 - 82.2	<0.001
	Height (cm)	169 ± 7.0	141 - 186	156 ± 6.2	134 - 185	<0.001
	BMI (kg/m^2)	19.8 ± 3.2	13.7 - 31.2	19.1 ± 3.5	13.0 - 37.6	0.043
	Waist circumference (cm)	73.2 ± 9	53 - 104	68.9 ± 7.5	51.5 - 98	<0.001
	Hip circumference (cm)	87.7 ± 9.2	19 - 114	87.1 ± 7.3	61.4 - 117	0.451
	Waist-to-Hip Ratio	0.8 ± 0.2	0.7 - 3.8	0.8 ± 0.1	0.7 - 1	0.001
	Skinfold Thickness (cm)	43.0 ± 25.6	14.3 - 161	66.3 ± 28.2	20.4 - 177	<0.001
Blood Pressure	Systolic Blood Pressure (mmHg)	111 ± 10.1	45 – 146	105 ± 8.3	85 - 137	<0.001
	Diastolic Blood Pressure (mmHg)	58 ± 8.3	34 -78	61.5 ± 6.8	43 – 82	<0.001
MRI	VAT Area (cm^2)	203 ± 64.8	72.5 - 400	166 ± 62.3	34.9 - 344	<0.001
	ASAT Area (cm^2)	304 ± 215	34.9 - 1372	384 ± 218	116 - 1521	<0.001
	VAT/ASAT ratio	0.9 ± 0.4	0.1 – 2.6	0.5 ± 0.2	0.1 – 1.4	<0.001
DXA	Total Fat Mass (kg)	9.7 ± 6.8	2.1 - 35.0	13.7 ± 5.9	4.2 - 40.0	0.026
	Total Lean Mass (kg)	44.5 ± 5.5	28.8 - 64.8	30.4 ± 3.6	16.1 - 40.4	<0.001
	Total Trunk Fat (kg)	4.6 ± 3.6	0.8 - 19.2	6.1 ± 2.9	1.6 - 16.9	<0.001
Metabolic Profile	Fasting Glucose (mmol/L)	8.1 ± 0.5	6.7 - 10	7.9 ± 1.5	6.6 - 25.9	0.052
	Fasting Insulin (mU/L)	10.3 ± 6	2.0 - 55.6	11.3 ± 4.7	2.6 - 30.4	0.075
	Cholesterol (mmol/L)	3.3 ± 0.6	1.6 - 5.1	3.5 ± 0.6	1.9 - 6.1	0.001
	HDL Cholesterol (mmol/L)	1.0 ± 0.3	0.6 - 3.9	1.1 ± 0.2	0.5 - 1.7	<0.001
	Triglycerides (mmol/L)	0.7 ± 0.3	0.3 - 3.1	0.6 ± 0.3	0.2 - 2.1	0.005
	HOMA IR	1.4 ± 0.8	0.0 - 6.7	1.5 ± 0.6	0.3 - 3.8	0.127

3.3.2 Pune Maternal Nutritional Study correlation analysis

Given the interesting gender differences in body composition and metabolic profile phenotyping in SA in PMNS cohort, the gender specific association between MR central adiposity compartmentalisation (VAT, ASAT), anthropometry, body composition (FM, lean mass) and metabolic profile phenotyping were further investigated (**Table 3.5**).

Correlation analysis between VAT and ASAT (cm^2) compartments with anthropometry, body composition, metabolic profile and additional outcomes, by gender, are shown in **Table 3.5**. In both genders, VAT was significantly associated with a number of outcomes including WC (M: r=0.547; F: r=0.561, p<0.001) and hip circumference (M: r=0.556, F: r=0.469 p<0.001). Fasting glucose (r=0.148) and cholesterol (r=0.282) were only significantly correlated with VAT in males (p<0.001 for both). ASAT was not associated with fasting glucose (M: r=0.089, F: r=-0.073), but showed a strong correlation with BMI (M: r=0.869, F: r=0.888), skinfold thickness (M: r=0.923, F: r=0.897) and fasting insulin (M: r=0.481, F: r=0.263) (p<0.001 for all **Table 3.5**).

Table 3.5 Gender specific correlation of VAT and ASAT compartments with anthropometry, body composition and metabolic profile phenotyping in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT: Visceral adipose tissue (cm2), ASAT: Abdominal subcutaneous adipose tissue (cm2), HDL: High-density lipoproteins. PMNS; Pune Maternal Nutritional Study. MR: Magnetic resonance imaging, DXA: Dual-energy X-ray, BMI: Body mass index, HOMA-IR: Homeostatic model assessment insulin resistance Data presented as mean ± standard deviation Spearman's correlation carried out in SPSS (v. 24.0); ** indicates correlation is significant at the 0.01 and * indicates correlation is significant at the 0.05 level.

		Male (n=261)		Female (n=162)	
		VAT	**ASAT**	**VAT**	**ASAT**
	Age (years)	0.264**	0.083	0.445**	0.124
Anthropometry	Weight (kg)	0.548**	0.831**	0.511**	0.765**
	Height (cm)	0.198**	0.181**	0.008	-0.074
	BMI (kg/m^2)	0.519**	0.869**	0.537**	0.888**
	Waist circumference (cm)	0.547**	0.896**	0.561**	0.850**
	Hip Circumference (cm)	0.556**	0.851**	0.469**	0.801**
	Waist-to-Hip Ratio	0.346**	0.592**	0.317**	0.401**
	Sum of Skinfolds	0.504**	0.923**	0.458**	0.897**
Blood Pressure	Systolic blood pressure (mmHg)	0.284**	0.321**	0.218**	0.257**
	Diastolic blood pressure (mmHg)	0.153*	0.290**	0.137	0.317**
DXA	Total fat mass (kg)	0.531**	0.961**	0.516**	0.936**
	Total lean mass (kg)	0.377**	0.422**	0.398**	0.349**
	Trunk fat (kg)	0.551**	0.959**	0.544**	0.942**
Metabolic Profile	Fasting glucose (mmol/L)	0.148*	0.089	0.061	-0.073
	Fasting insulin (mu/L)	0.321**	0.481**	0.160*	0.263**
	Cholesterol (mmol/L)	0.272**	0.381**	0.054	0.189*
	HDL (mmol/L)	-0.039	-0.125*	-0.174*	-0.106
	Triglycerides (mmol/L)	0.219**	0.373**	0.159*	0.163*
	HOMA IR	0.316**	0.463**	0.119	0.193*

3.3.2.1 Pune Maternal Nutritional Study distribution characteristics

Abdominal compartment of VAT and ASAT showed a weak but significant association with height in male (VAT: M; r=0.198, p<0.001, ASAT: M; r=0.181, p<0.001) (**Figure 3.3**). However, there was no observed association between VAT and ASAT with height in females, despite that the latter was shorter (**Table 3.5**) **(Figure 3.4).** The absence of an association between VAT and ASAT with height seen in females might indicate that height is less likely to contribute to VAT and ASAT content in adolescent SA females.

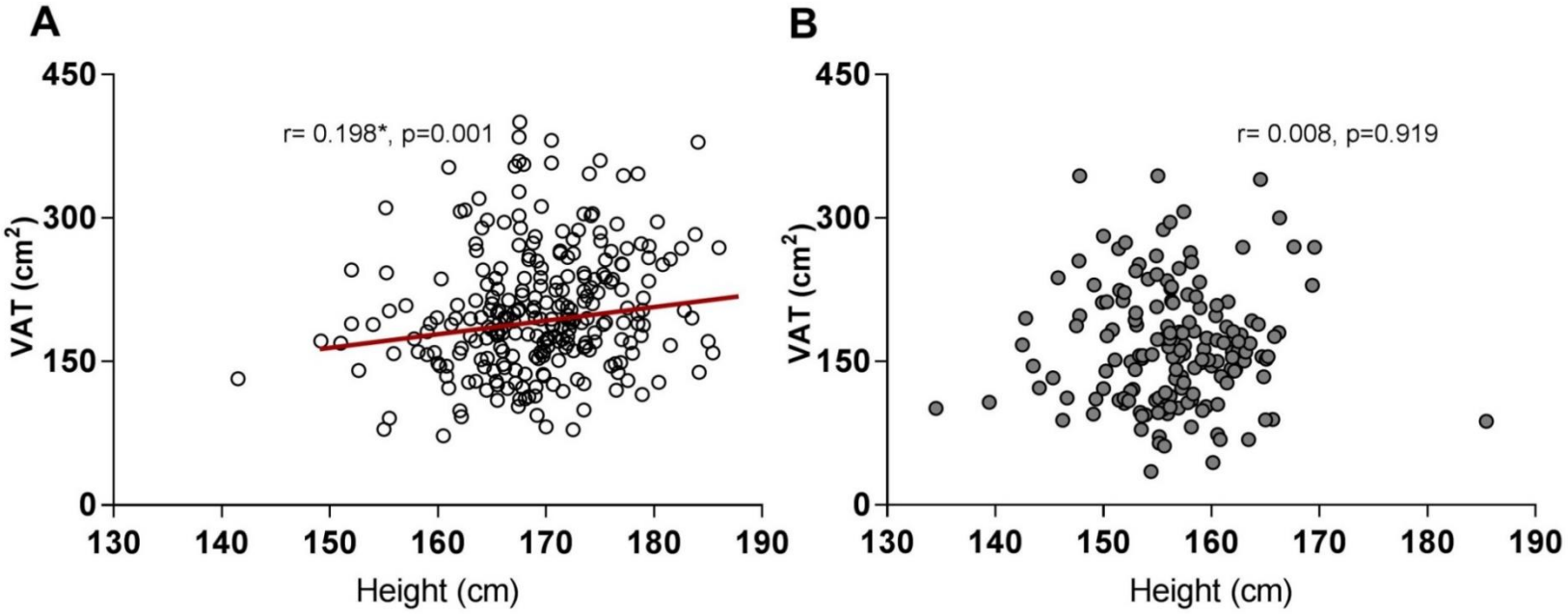


Figure 3.4 Gender specific distribution of visceral adipose tissue (VAT) by height in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT: Visceral adipose tissue (cm^2. PMNS; Pune Maternal Nutritional Study in (A**)** n= 261 male, (B) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as $p<0.05$. Graphs done using GraphPad Prism version 5.0

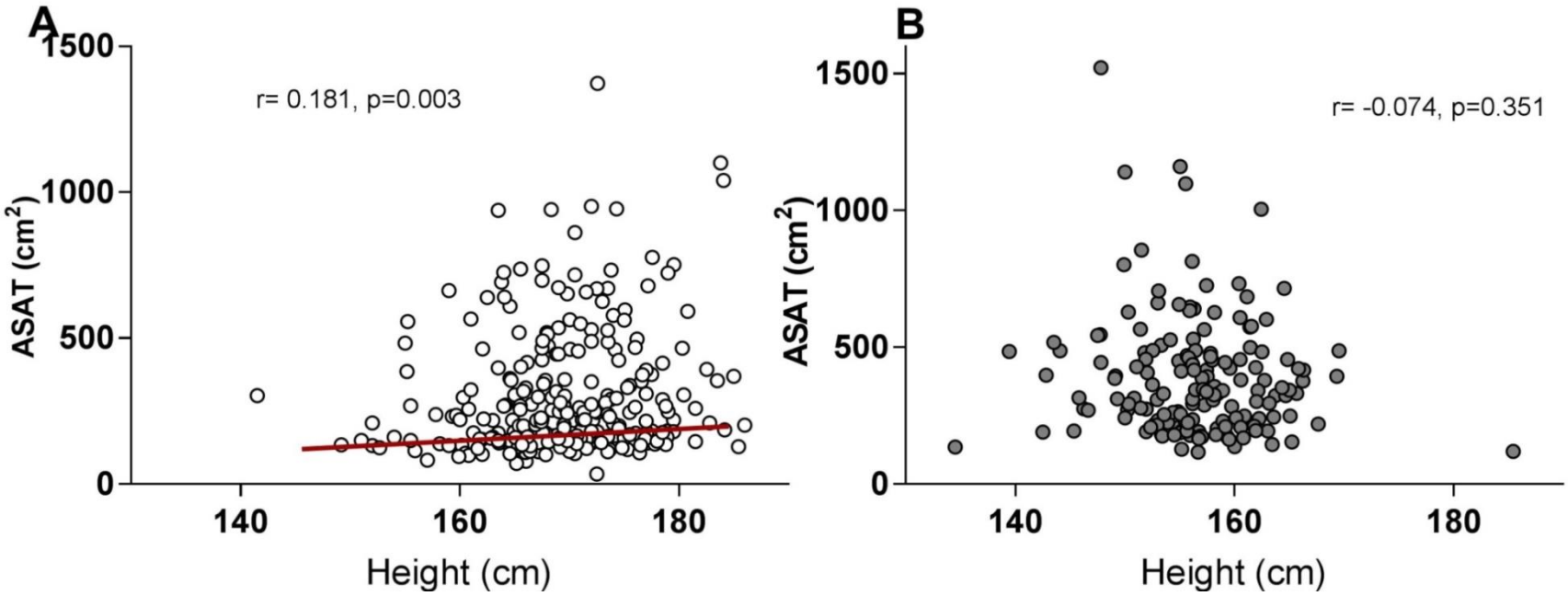


Figure 3.5 Gender specific distribution of abdominal subcutaneous adipose tissue (ASAT) by height in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. ASAT: abdominal subcutaneous adipose tissue ($cm^{2)}$. PMNS; Pune Maternal Nutritional Study in (**A)** n= 261 male, (**B**) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as $p<0.05$. Graphs done using GraphPad Prism version 5.0

There was no observed gender differences in the association between VAT and weight; both adolescent SA males and females showed a significant association between VAT and weight (M: r=0.548, F: r=0.511, p<0.001 for both) **(Figure 3.6)**. The significant association with weight was stronger for ASAT in both adolescent SA males and females (M: r=0.831, F: r=0.765, p<0.001 for both) (**Figure 3.7**).

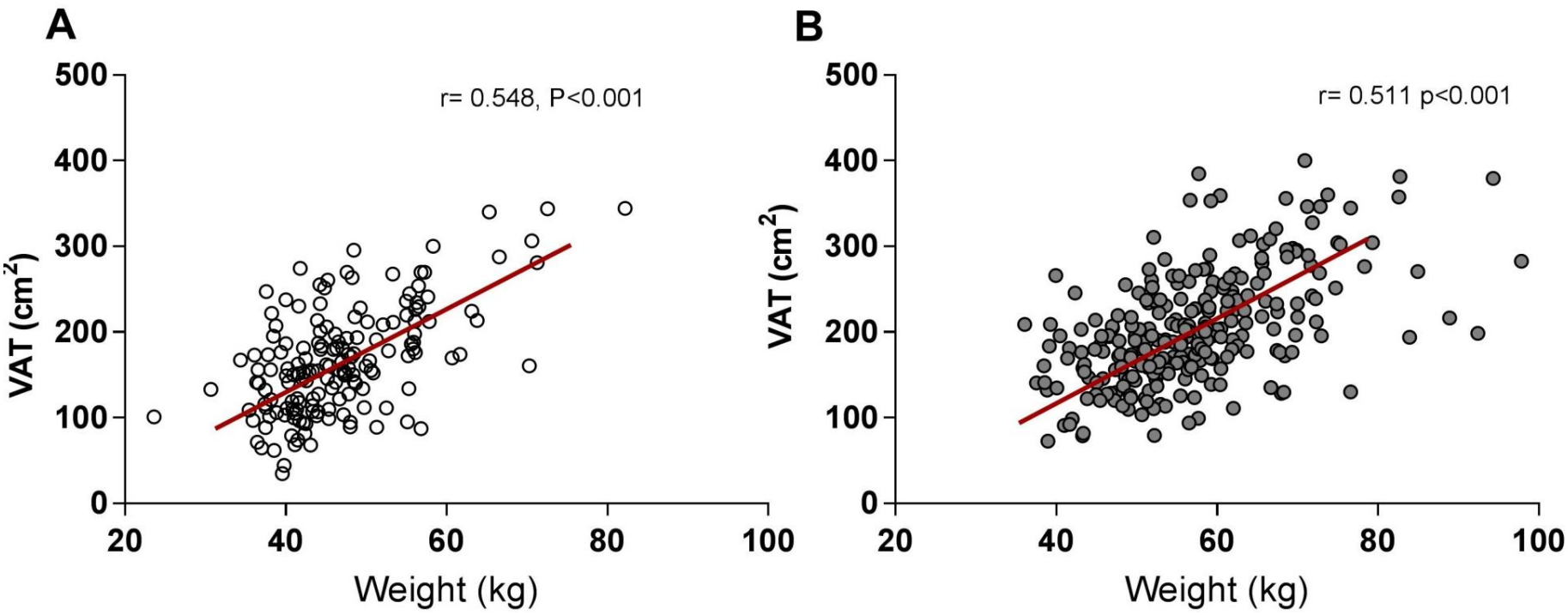


Figure 3.6 Gender specific distribution of visceral adipose tissue (VAT) by weight in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT: Visceral adipose tissue ($cm^{2)}$. PMNS; Pune Maternal Nutritional Study in (**A)** n= 261 male, (**B**) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as p<0.05. Graphs done using GraphPad Prism version 5.0

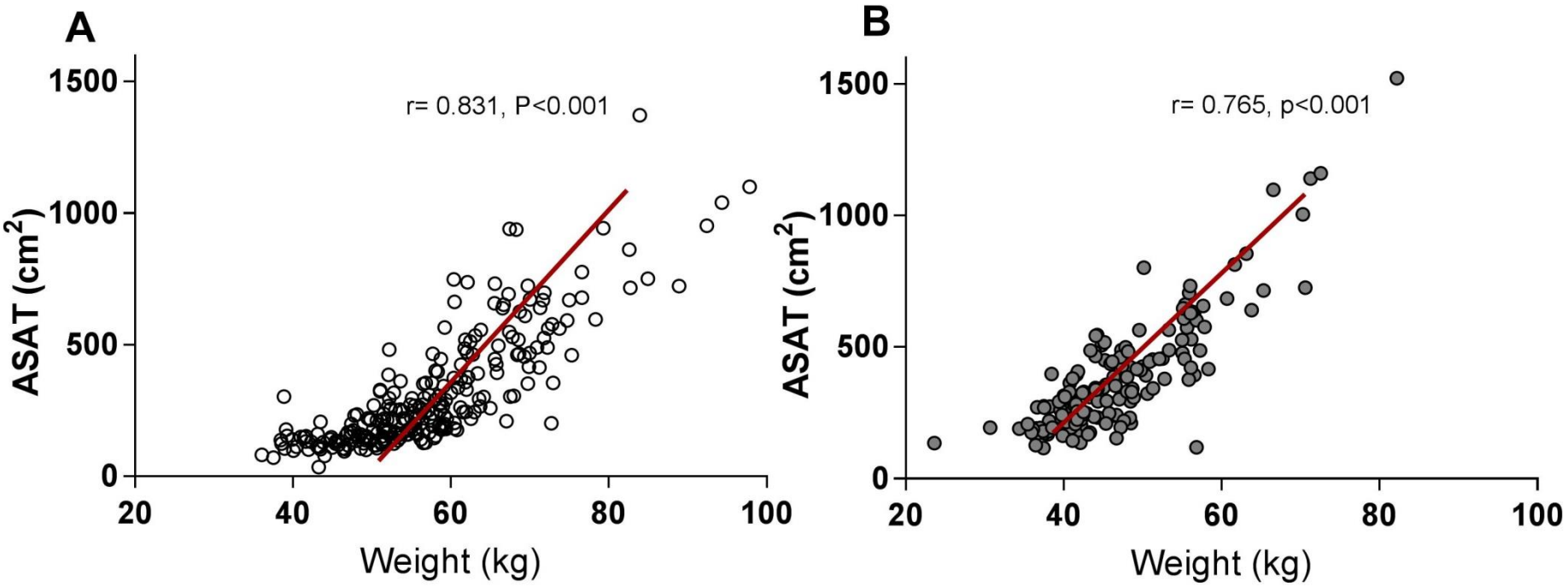


Figure 3.7 Gender specific distribution of abdominal adipose tissue (ASAT) by weight in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT: Visceral adipose tissue ($cm^{2)}$. PMNS; Pune Maternal Nutritional Study in (**A)** n= 261 male, (**B**) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as p<0.05. Graphs done using GraphPad Prism version 5.0

As expected from the abdominal adiposity compartments association with weight in both genders, there was an observed strong association between BMI and VAT, the association was stronger with ASAT than VAT in both male and female (VAT; M: r=519, F: r= 0.537, ASAT; M: r=0.869, F: r=0.888, $p<0.001$ for all) (**Figure 3.8, 3.9)**.

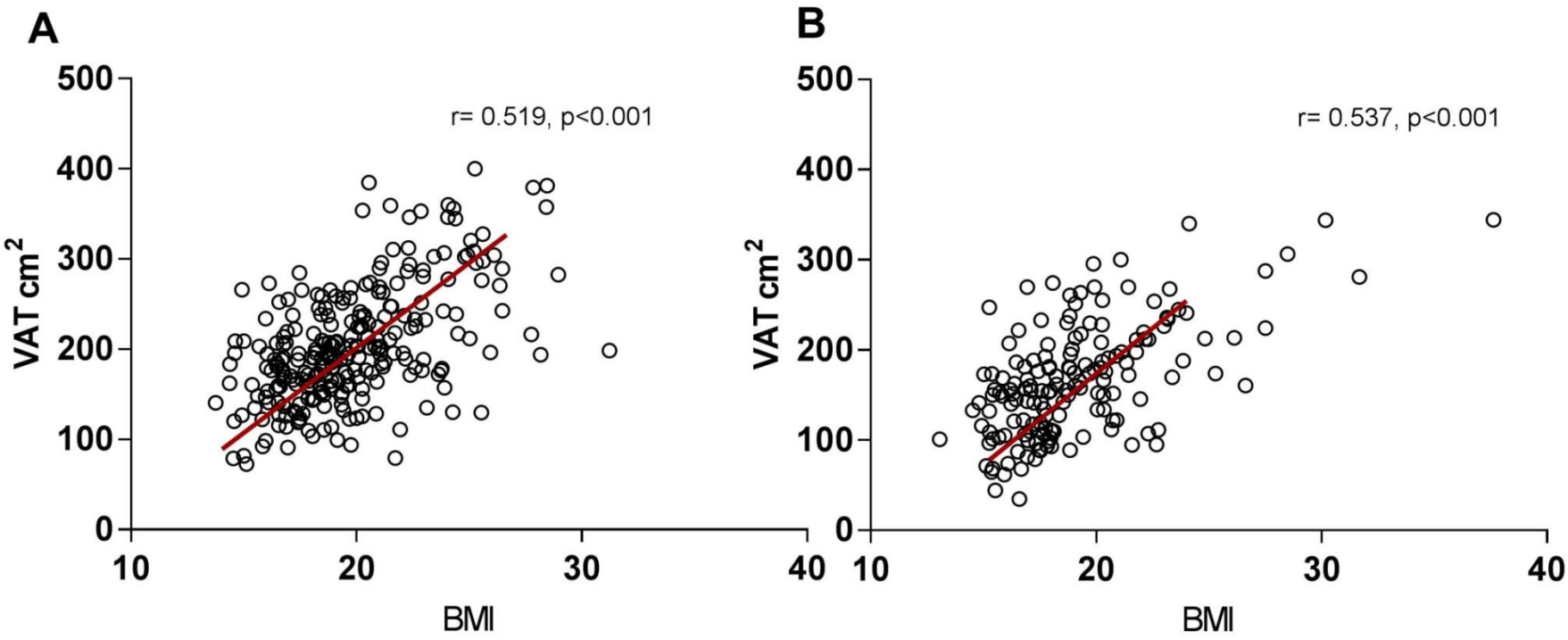


Figure 3.8 Gender specific distribution of visceral adipose tissue (VAT) by BMI in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. VAT: Visceral adipose tissue ($cm^{2)}$. PMNS; Pune Maternal Nutritional Study in (A**)** n= 261 male, (B) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as $p<0.05$. Graphs done using GraphPad Prism version 5.0.

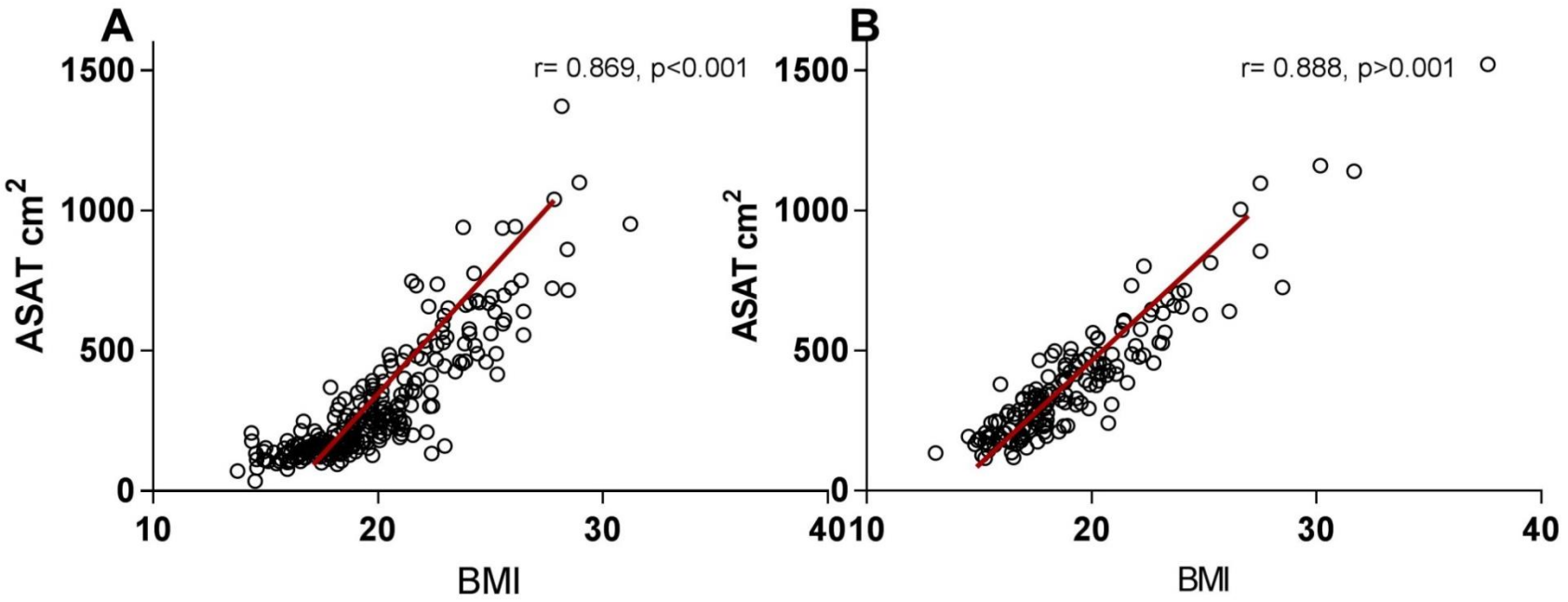


Figure 3.9 Gender specific distribution of abdominal subcutaneous adipose tissue (ASAT) by BMI in adolescent South Asian in the PMNS cohort. Data obtained from the PMNS cohort. ASAT: abdominal subcutaneous adipose tissue ($cm^{2)}$. PMNS; Pune Maternal Nutritional Study in (A**)** n= 261 male, (B) n=162 female; r values represent Spearman's test. Spearman's correlation carried out in SPSS (v. 24.0); * significance was taken as $p<0.05$. Graphs done using GraphPad Prism version 5.0.

3.3.2.2 BMI cut-offs

The gender specific distribution of abdominal compartments (VAT and ASAT) by BMI grouping (underweight, normal, overweight, and obese; based on WHO general guidelines) in adolescent SA in the PMNS cohort is shown for VAT in **Figure 3.10** and ASAT in **Figure 3.11**. There were significant differences in both depots between BMI groups in males and females ($p<0.001$ for all groups) (**Figure 3.10, 3.11**). There was an increase in ASAT as BMI group number increased for male and female subjects ($p<0.001$) (**Figure 3.11**). At any BMI point, ASAT was higher in female compared to male in the PMNS cohort ($p<0.001$) (**Figure 3.10, 3.11**).

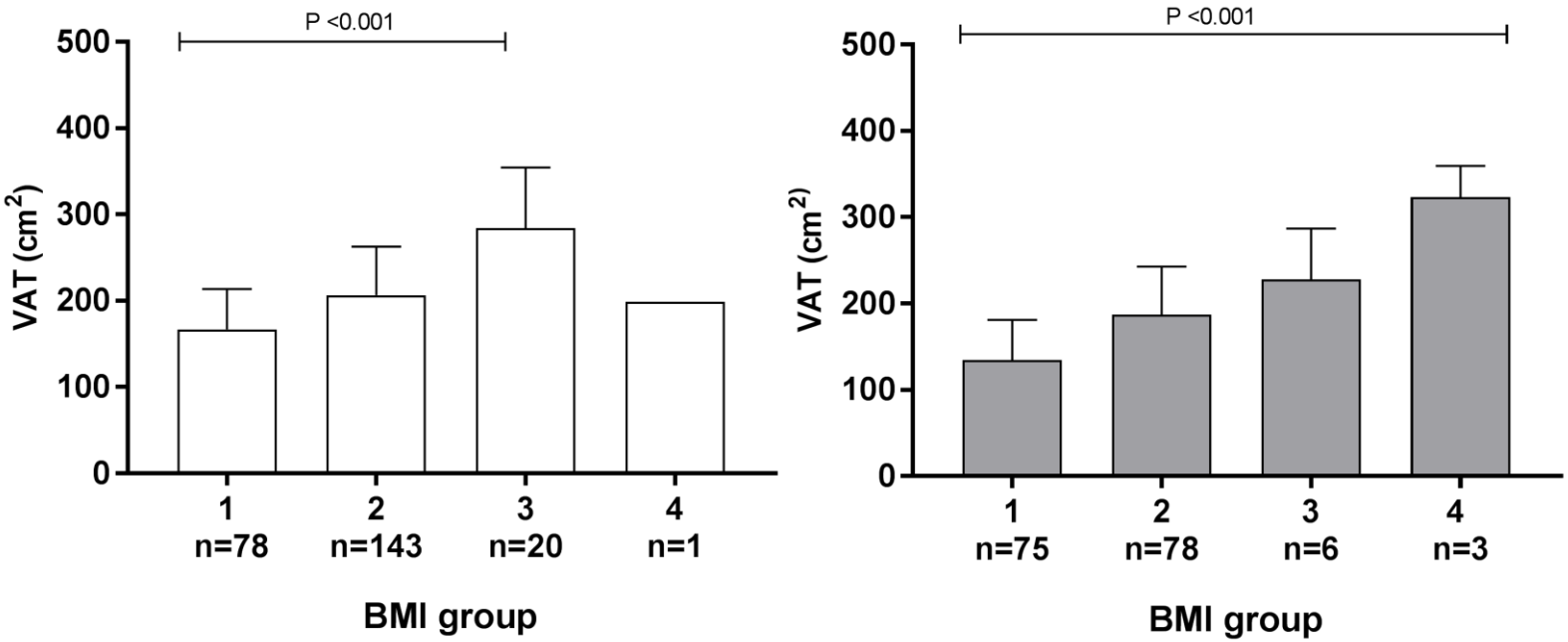


Figure 3.10. Gender specific volume of visceral adipose tissue (VAT) by BMI groups in adolescent South Asian in PMNS cohort in (**A**) n= 261 male, (**B**) n=162 female BMI groups 1: Underweight: (<18kg/m^2); 2: Normal (18<23 kg/m^2), 3. Overweight (23.0<25.0 kg/m^2), 4 Obese (>25 kg/m^2), One-way Anova test was used to assess differences between all BMI groups except in males where the test was run excluding Obese BMI category (4) due to insufficient subject number;n=1. PMNS**:** Pune Maternal Nutritional Study, BMI: body mass index. Data presented as mean ± standard deviation using SPSS (v. 24.0); * significance was taken as p<0.05. Graphs done using GraphPad Prism version 5.0.

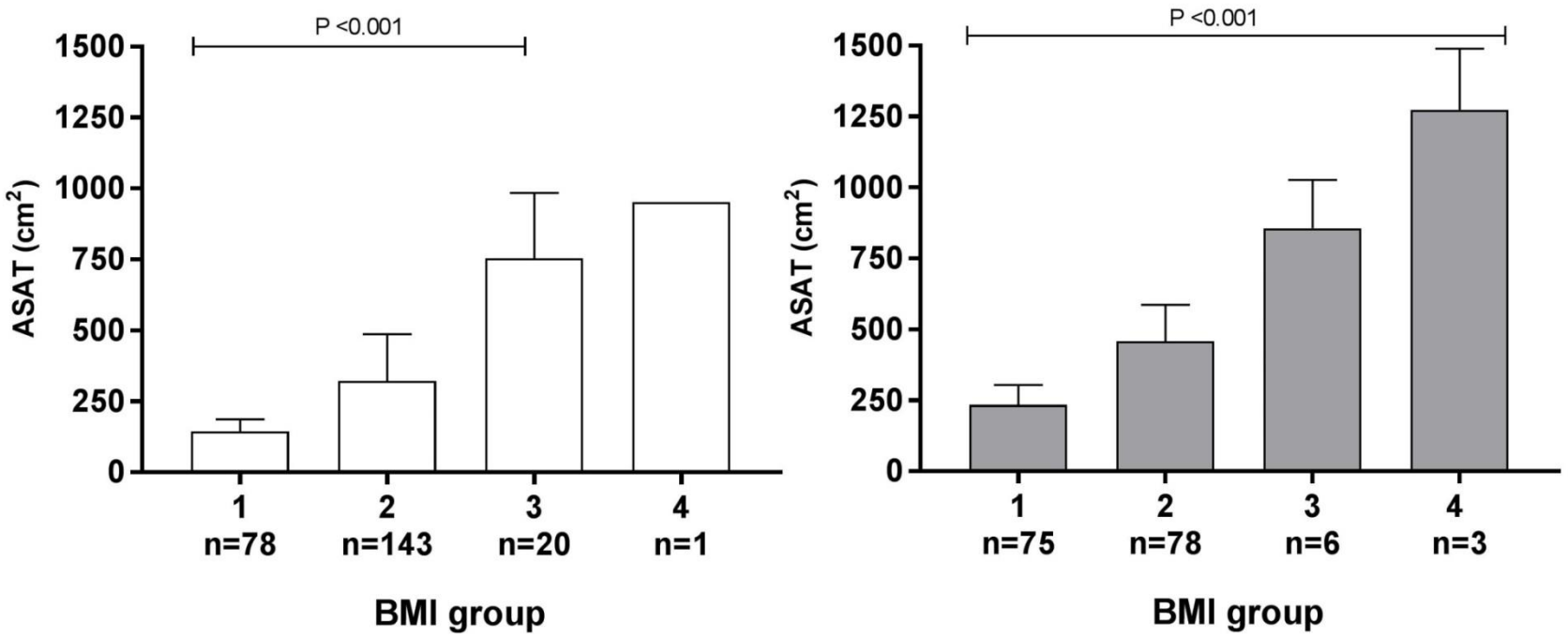


Figure 3.11. Gender specific volume of abdominal subcutaneous adipose tissue (ASAT) by BMI groups in adolescent South Asian in PMNS cohort. In (A**)** n= 261 male, (B) n=162 female BMI groups 1: Underweight: (<18kg/m^2); 2: Normal (18<23 kg/m^2), 3. Overweight (23.0<25.0 kg/m^2), 4 Obese (>25 kg/m^2), One-way Anova test was used to assess differences between all BMI groups except in males where the test was run excluding Obese BMI category (4) due to insufficient subject number;n=1. PMNS**:** Pune Maternal Nutritional Study, BMI: body mass index. Data presented as mean ± standard deviation using SPSS (v. 24.0); *significance was taken as p value (P)<0.05. Graphs done using GraphPad Prism version 5.0.

The distribution of VAT and ASAT was subsequently re-assessed using SA specific BMI cut-off (overweight >23 kg/m^2, obese >27 kg/m^2) and compared to standard WHO cut-off (overweight >25kg/m^2, obese >30kg/m^2) (**Figure 3.12**). No significant differences between ranges were observed in VAT or ASAT area, in either gender between employing SA specific BMI cut-off or WHO cut-off. The comparison was not possible in obese males due to the limited number in WHO cut-offs (BMI >30 kg/m^2, M: n=1). Since there were no significant differences observed between WHO and SA specific BMI cut-offs, all the data presented from this point will be using WHO BMI cut-offs.

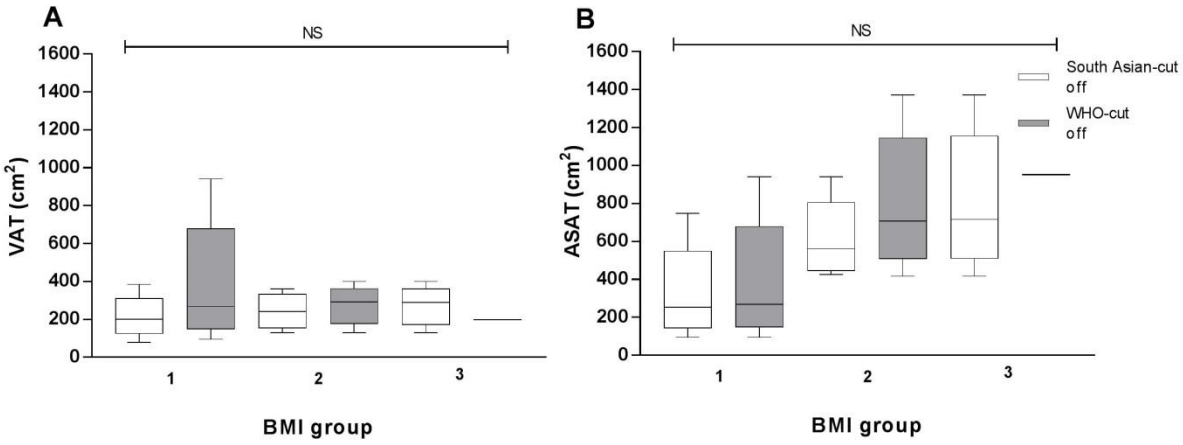


Figure 3.12 Abdominal adiposity area distribution in adolescent South Asian male by BMI cut-offs in the PMNS cohort. The distribution of VAT (**A**) and ASAT (**B**). BMI cut-off; South Asians specific BMI cut-offs (1= 18<23 kg/m^2, 2= 23<25 kg/m^2, 3= <25 kg/m^2, white columns) versus WHO BMI cut-offs (1 =18<25 kg/m^2, 2= 25<30 kg/m^2, 3= >30 kg/m^2, grey columns). **A , B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t test with no significant differences between groups. Graphs done using GraphPad Prism version 5.0.

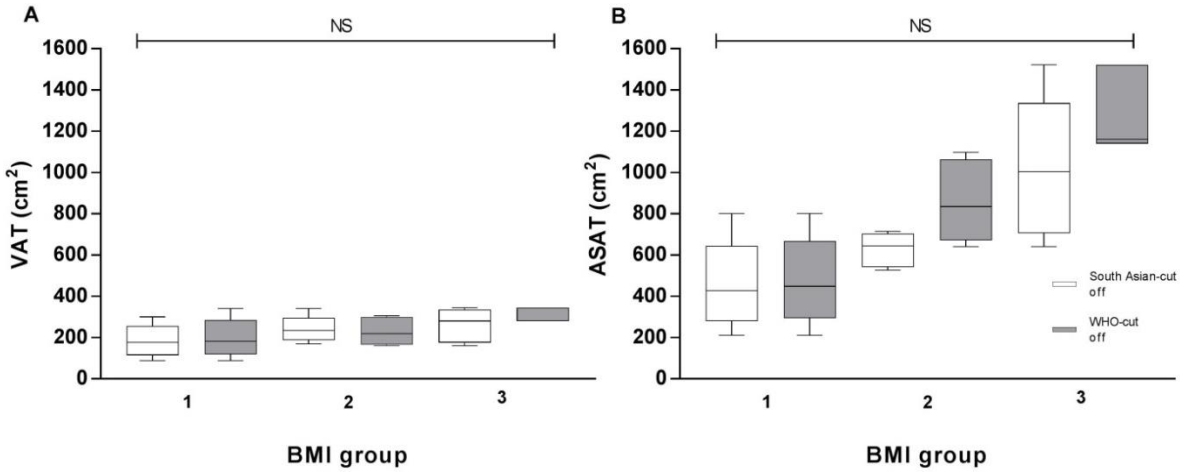


Figure 3.13 Abdominal adiposity area distribution in adolescent South Asian female by BMI cut-offs in the PMNS cohort. The distribution of VAT (A) and ASAT (B). BMI cut-off; South Asians specific BMI cut-offs (1= 18<23 kg/m^2, 2= 23<25 kg/m^2, 3= <25 kg/m^2, white columns) versus WHO BMI cut-offs (1 =18<25 kg/m^2, 2= 25<30 kg/m^2, 3= >30 kg/m^2, grey columns). **A , B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t test with no significant differences between groups. Graphs done using GraphPad Prism version 5.0.

3.3.3 South Asian sub-phenotypes of body fat

3.3.3.1 The thin-fat phenotype:

The thin fat phenotype is a SA specific phenotype in individuals with low or normal BMI but characterized by increased total body adiposity with less muscle mass and noticeable high insulin resistance compared to Cau. The thin fat phenotype has been reported in SA PMNS cohort compared to Cau since infancy and up to the age of 6 years (318, 319) but no information if this adverse phenotype persisted in adolescent SA compared to Cau. Since the study in this chapter (PMNS) did not include a control group, it was inaccessible to compare the cohort to Cau, however, an attempt was made to find a Cau cohort that is similar in anthropometry, and body composition measurements technique with PMNS and its comparison is discussed in more detail in the Discussion. A full statistical comparison of the two cohorts was unfortunately not possible as only mean ± standard deviation values were provided for general comparison, and no further analysis was allowed by the research group who provided these limited data. Overall, thin fat phenotype was evident in 18 years old SA compared to Cau with SA presented with lower BMI (BMI: PMNS M: 19.8 ± 3.2 kg/m^2, F: 19.1 ± 3.46

kg/m^2; versus Cau M: 22.5 ± 2.2 kg/m^2, F: 21.7 ± 2.7 kg/m^2), higher glucose levels (Glucose: PMNS: M: 8.11 ± 0.5 mmol/L, F;7.9 ± 1.55 mmol/L, Cau: M: 5.2 ± 0.35 mmol/L, F: 5.1 ± 0.45 mmol/L) and higher insulin (Insulin: M: 10.3 ± 6.0 mU/L, F: 11.3 ± 4.7 mU/L, Cau: M: 3.9 ± 2.2 mU/L, F: 5.9 ± 3.2 mU/L) than Cau (Cau data obtained from unpublished work of Dr James Parkinson).

3.3.3.2 The thin outside fat inside phenotype:

TOFI phenotype is a characterized by lean/ normal-weight individuals with an increased amount of VAT to ASAT and increased susceptibility for high risk of adverse metabolic profile, and it has been reported in Cau. Because there was a stronger correlation between BMI and ASAT in females, despite similarities in BMI, between both genders, an attempt was made to assess the incidence of TOFI phenotype in Pune cohort in males and females. A gender comparison was performed by assessing the number of PMNS individuals classified as TOFI within the “normal” weight range. TOFI was calculated as from a previous publication (72) as the mean of the ratio of VAT/ASAT for healthy individuals was reported to be 0.59 (male) and 0.25 (female), this was calculated as two standard deviations above the measured (mean VAT/ASAT) in healthy individuals (+2 s.d. male: 1.04, female: 0.45). Applying this published TOFI cut-off to my study of SA, I identified 35 males and 29 females presented with TOFI phenotype.

Table 3.6 shows the number and percentage of male and females identified as TOFI for 18-25kg/m^2. In females, the percentage of TOFI was higher than in males (TOFI %: M: 21.6%, F: 37.1%) despite that females had smaller WC than males ($p<0.001$) (**Table 3.6**). Comparing to TOFI prevalence in Cau (TOFI in Cau; M=14%, F=12%, reported previously in (72)), SA had almost 2 fold higher TOFI in lean males, and 3 fold higher TOFI in lean females. These ethnic differences in TOFI phenotype indicate that almost a quarter of lean SA males and half of lean SA females have a phenotype associated with an adverse metabolic profile.

Table 3.6 Gender specific epidemiology of TOFI phenotype in adolescent South Asian in the PMNS. The number of male and female presenting as TOFI in the PUNE cohort WHO recommended 18-25 kg/m^2 ranges. Individuals were defined as TOFI if their VAT/ASAT ratio was >1.04 in males, and >0.45 in females (from (72)). TOFI; thin outside fat inside. PMNS; Pune Maternal Nutritional Study.

	Male	Female
	18-25 kg/m^2	**18-25 kg/m^2**
TOFI number	35/162	29/78
TOFI (%)	21.6	37.1

Since the published TOFI cut off was derived from Cau population, it may not be useful in the SA population (one size does not fit all); therefore an attempt was made to create a TOFI cut-off suitable for a SA population. To do so, a healthy subgroup was identified from the PMNS based on a BMI normal rage. The mean ratio of VAT/ASAT in healthy SA population was 0.88 (males) and 0.50 females. Two standard deviations were added to the mean (SD: M: 0.43, F: 0.23) for the ratio of VAT/ASAT in order to create a TOFI threshold derived from SA population. The TOFI threshold for SA was set to 1.75 for males and 0.97 for females. Using this threshold, 13 males (4.9 %) and 8 females (10.2 %) were identified as TOFI-South Asian. Compared to Cau TOFI, SA specific TOFI estimated a lower percentage of TOFI in lean SA males (16.7% lower than TOFI-Caucasian cut-offs), and lean South Asian females (3.7% lower than TOFI-Caucasian cut-offs) than Cau male and female. Furthermore, lean SA used in this statistical comparison were 21 years younger than the Cau, and age showed a significant positive association with VAT ($p<0.001$) in adolescent SA males and females. Hence it is estimated that the prevalence of TOFI in older SA may be higher.

The TOFI and thin-fat phenotypes are related to each other in term of lean characteristics but unfavourable adiposity and adverse metabolic risk. The main difference between the two phenotypes is that the TOFI phenotype was originally derived from individuals with a BMI range of 20 – 25 kg/m^2 and it is unclear how applicable it is to subjects outside this range.

3.3.4 Pune Maternal Nutritional Study gender specific characteristics by Impaired Fasting Glucose status

As described in Section 3.2.1.1, pre-diabetes diagnosis falls into two categories; IFG: when the fasting plasma glucose result is between 5.6

mmol/L and 6.9 mmol/L, or Impaired Glucose Tolerance: when the OGTT is between 7.8 and 11.0 mmol/L. IFG for the measurements of pre-diabetes is recommended in the general population with no observed glucose metabolism disorders, including HTN. In this study of adolescent SA there was no observed HTN and therefore circulating levels of fasting glucose were used to define individuals as “normal fasting glucose” (NG: <5.5mmol/L) or pre-diabetic (PD) as IFG (PD: >5.6mmol/L, <6.9 mmol/L) (**Table 3.7**).

Table 3.7 Distribution of normal blood glucose and pre-diabetes in adolescent South Asian from PMNS cohort. PMNS; Pune maternal nutritional study.

Test	Normal blood glucose (NGT)		Pre-diabetic (PD)	
	Value	N	Value	N
Fasting plasm glucose (mmol/L)	Below 5.5	281	5.6 – 6.9 (Impaired fasting Glucose)	142

Gender specific presentation of anthropometry, body composition and blood biochemistry based on NGT and pre-diabetes classification are shown in **Table 3.8**. In males, significant differences between NGT and pre-diabetes groups were observed for several parameters, with pre-diabetes showing increased WHR (NG: 0.8 ± 0.1, pre-diabetes: 0.9 ± 0.3, p<=0.004), sum of skinfold thickness (NG: 40.8 ± 23.5 cm^2, pre-diabetes: 46.0 ± 28.1 cm^2, p=0.006) (**Table 3.8**). Interestingly, there were no observed differences in total FM or lean mass measured via DXA between NGT and pre-diabetes males, whereas VAT was more (15 cm^2 higher VAT) in pre-diabetes than NGT adolescent SA males but not females. It appeared from the lack of differences in FM and lean mass with the observed increased in VAT in pre-diabetes than NGT that VAT might be an early biomarker for T2D in adolescent SA males but not females.

Apart from blood glucose levels (p<0.001), there were no observed significant differences in anthropometry, body composition, and metabolic profile between NGT and pre-diabetes adolescent female, although the latter showed a trend toward lower BMI (**Table 3.8**).

Table 3.8 Gender specific characteristics by fasting glucose status in adolescent South Asian from the PMNS. VAT; Visceral adipose tissue, ASAT; Abdominal subcutaneous adipose tissue. NG: Normal glucose tolerance; PD: Pre-diabetic; Statistical analysis comparing NG and PD groups was carried out separately in males and females by Student's t-test; significance was taken as $p<0.05$ and indicated by *. Statistics carried out in SPSS version 23.0.

		Male		Female	
		NG (n=150)	**PD (n=111)**	**NG (n=131)**	**PD (n=31)**
Anthropometry	Weight (kg)	56.6 ± 10.7	57.3 ± 10.5	47.2 ± 8.1	45.0 ± 10.4
	Height (cm)	170 ± 7.2	169 ± 6.8	156 ± 6.0	155 ± 6.8
	BMI (kg/m^2)	19.6 ± 3.1	20.0 ± 3.25	19.2 ± 3.2	18.7 ± 4.5
	Waist circumference (cm)	72.6 ± 8.6	74.1 ± 9.4	69.3 ± 7.3	67.4 ± 8.4
	Hip circumference (cm)	87.9 ± 7.2	87.5 ± 11.4	87.5 ± 6.7	85.2 ± 9.3
	Waist-to-Hip Ratio	0.8 ± 0.1	0.9 ± 0.3*	0.8 ± 0.1	0.8 ± 0.1
	Skinfold Thickness (cm)	40.8 ± 23.5	46.0 ± 28.1*	67.3 ± 27.7	62.1 ± 30
Blood Pressure	Systolic Blood Pressure	111 ± 10.5	111 ± 9.6	105 ± 7.8	108 ± 9.7
	Diastolic Blood Pressure	58.7 ± 8.3	57.1 ± 8.1	61.2 ± 6.5	62.4 ± 7.8
MRI	VAT Area (cm^2)	197 ± 64.3	212 ± 64.9*	166 ± 61.3	165 ± 67.6
	ASAT Area (cm^2)	291 ± 200	321 ± 233	393 ± 205	348 ± 267
	VAT/ASAT	0.9 ± 0.4	0.9 ± 0.4	0.5 ± 0.5	0.5 ± 0.2
DXA	Total Fat Mass (kg)	9.2 ± 6.4	10.3 ± 7.2	14.0 ± 5.6	12.7 ± 7.0
	Total Lean Mass (kg)	44.7 ± 5.9	44.2 ± 4.9	30.5 ± 3.4	30.0 ± 4.6
	Total Trunk Fat (kg)	4.4 ± 3.4	5.0 ± 3.9	6.2 ± 2.9	5.5 ± 3.2
Metabolic Profile	Fasting Glucose (mmol/L)	5.2 ± 0.2	5.7 ± 0.2*	5.1 ± 0.2	6.1 ± 2.1*
	Fasting Insulin (mmol/L)	9.5 ± 5.2	11.5 ± 6.8*	11.3 ± 4.8	11.8 ± 4.7
	Cholesterol (mmol/L)	3.3 ± 0.6	3.3 ± 0.6	3.4 ± 0.6	3.6 ± 0.8
	HDL Cholesterol (mmol/L)	1 ± 0.2	1.1 ± 0.34	1.1 ± 0.2	1.1 ± 0.2
	Triglycerides (mmol/L)	0.8 ± 0.4	0.8 ± 0.3	0.7 ± 0.3	0.7 ± 0.4
	HOMA IR	1.2 ± 0.7	1.5 ± 0.9*	1.4 ± 0.7	1.5 ± 0.7

Gender specific distribution of metabolic profile in NG and pre-diabetes is shown in (**Figure 3.14**), and as reported pre-diabetes females showed a trend toward lower BMI yet more insulin resistant than pre-diabetes males (**Figure 3.14**).

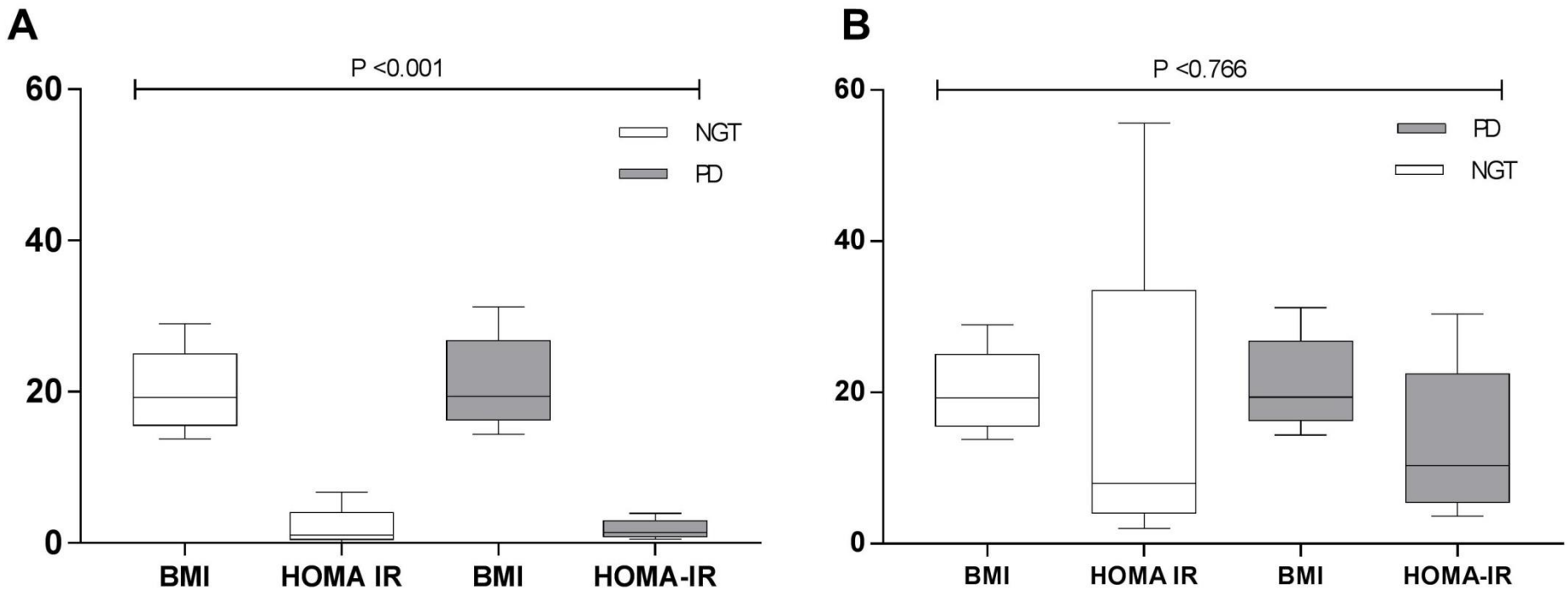


Figure 3.14 Illustration of gender specific metabolic profile distribution in NGT and PD adolescent South Asian in the PMNS cohort. BMI: body mass index, HOMA IR: homeostasis model assessment of insulin resistance, NGT: normal glucose tolerance (<5.5mmol/l), PD: pre-diabetic (>5.5mmo/L, >6.9mmol/L). One-way ANOVA carried out in SPSS (v.24.0**).(A**) male,(**B)**: female **A & B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t with no significant differences between groups. Graphs done using GraphPad Prism version5.0.

The results of the OGTT as defined by normal fasting glucose tolerance or pre-diabetes classification is shown in **Figure 3.15**. A significant increase in circulating glucose profile was observed in males and females termed pre-diabetic ($p<0.001$) compared to those defined as having NGT, with similarities in insulin secretion (**Figure 3.15**).

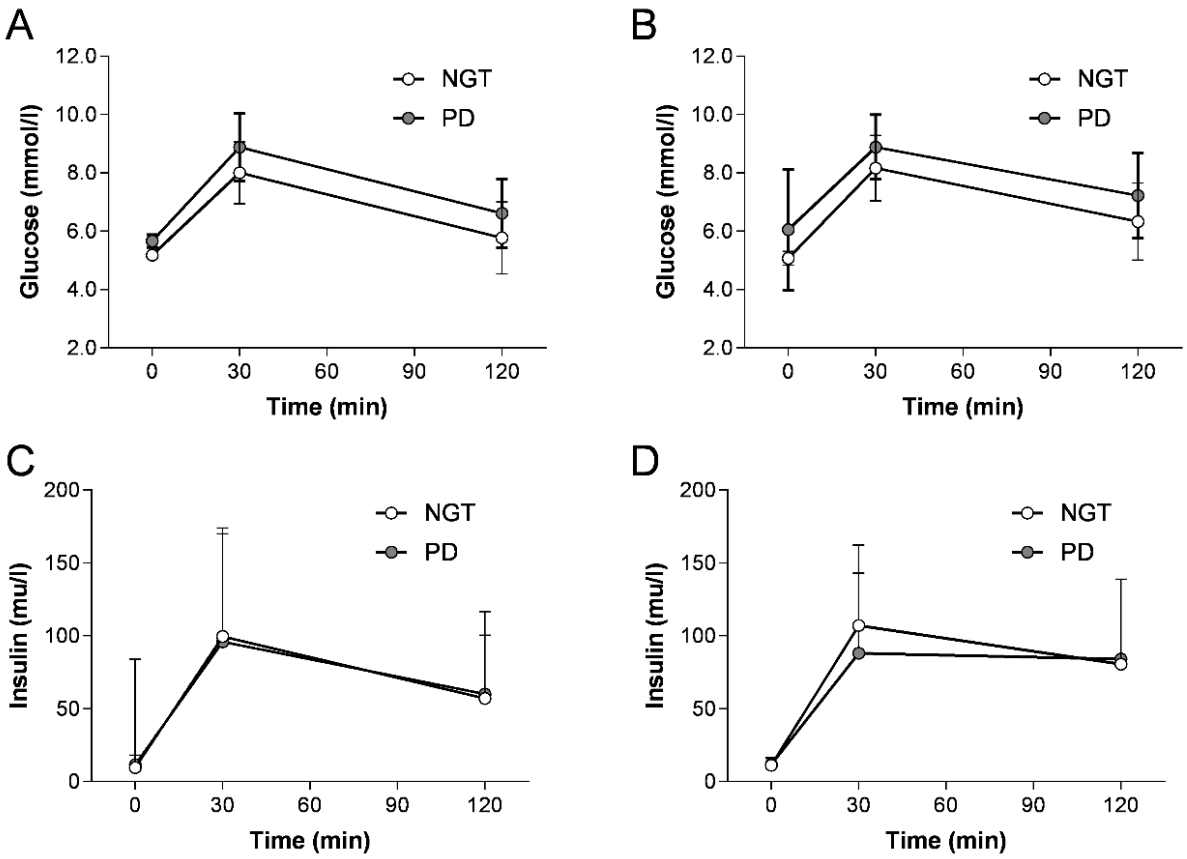


Figure 3.15 Oral glucose tolerance test (OGTT) in the PMNS. NGT: normal glucose tolerance (<5.5mmol/l), PD: pre-diabetic (>5.5mmo/ml). Glucose (**A**, **B**) and insulin (**C**, **D**) levels following a 75g oral dose of glucose in fasted individuals from the PUNE cohort in males (**A**, **C**) and females (**B** , **D**). Data presented as mean ± s.d. Graphs done using GraphPad Prism version 5.0.

VAT was significantly higher in pre-diabetes than NG males ($p=0.03$), while there were no significant differences in VAT between NG and pre-diabetes females (**Figure 3.16**). ASAT showed a trend to be higher in pre-diabetes compared to NG males and females, but this was not significant ($p=0.9$) (**Figure 3.17**). The ratio between VAT and ASAT was not different between NGT and pre-diabetes males and females (**Figure 3.18**).

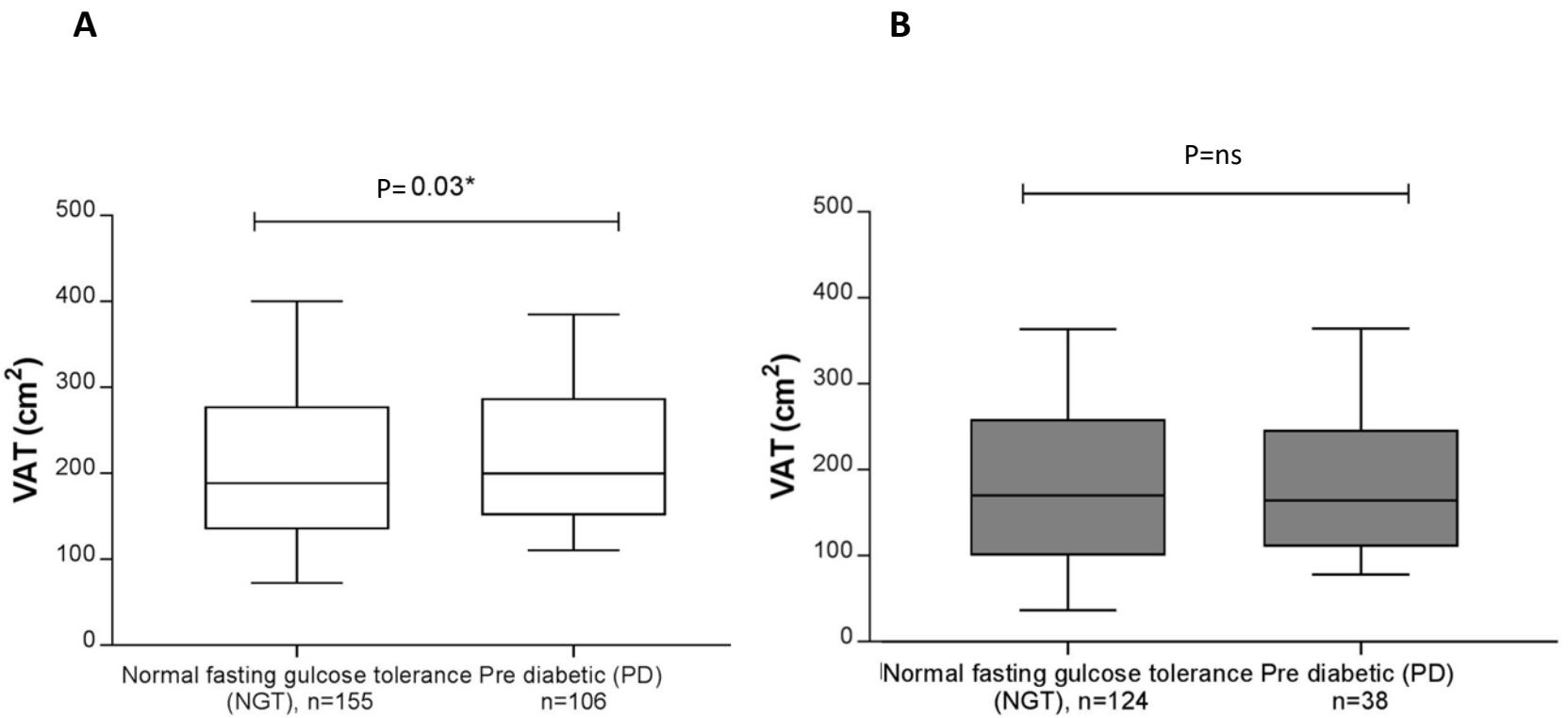


Figure 3.16 Gender specific visceral adipose tissue (VAT) distribution in the PMNS in NG and PD adolescent South Asian from PMNS. VAT: Visceral adipose tissue, ASAT: Abdominal subcutaneous adipose tissue, NGT: normal glucose tolerance (<5.5mmol/l), PD: pre-diabetics (>5.5mmo/ml). Mann-Whitney carried out in SPSS (v.24.0). **A, B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t test. While columns in male, grey columns in female.

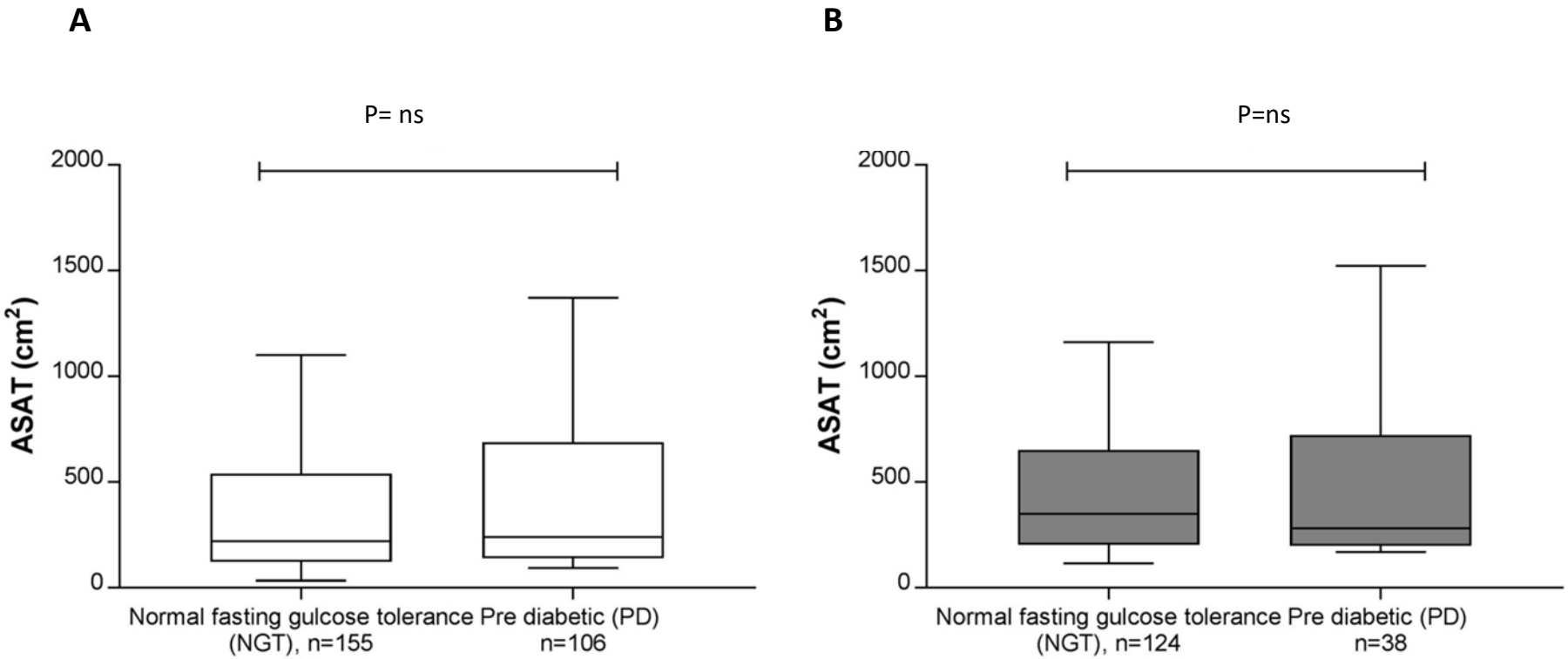


Figure 3.17 Gender specific abdominal subcutaneous adipose tissue (ASAT) distribution in the PMNS in NG and PD adolescent South Asian from PMNS. ASAT: Abdominal subcutaneous adipose tissue, NGT: normal glucose tolerance (<5.5mmol/l), PD: pre diabetics (>5.5mmo/ml). Mann-Whitney carried out in SPSS (v.24.0). **A, B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t test with no significant differences between groups. While columns in male, grey columns in female.

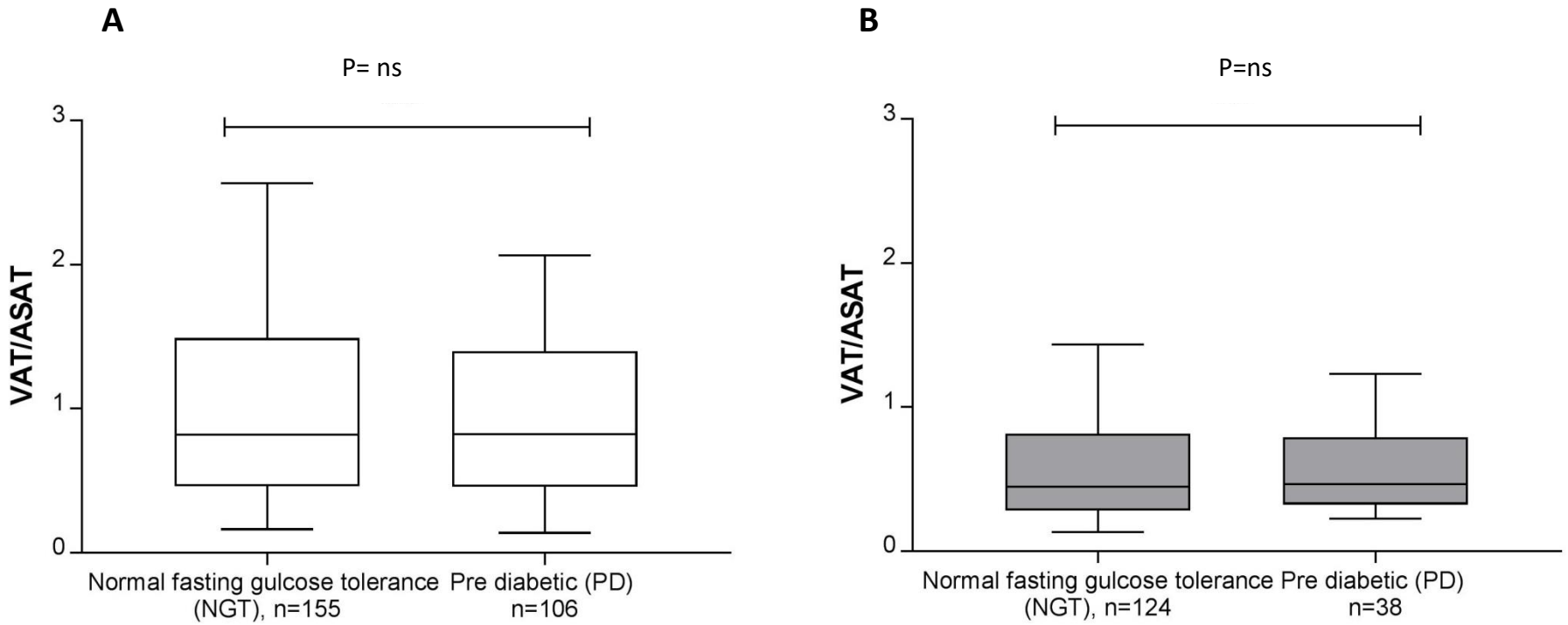


Figure 3.18 Gender specific adipose tissue distribution in the PMNS in NG and PD adolescent South Asian from PMNS. VAT: Visceral adipose tissue, ASAT: Abdominal subcutaneous adipose tissue, NGT: normal glucose tolerance (<5.5mmol/l), PD: pre diabetics (>5.5mmo/ml). Mann-Whitney carried out in SPSS (v.24.0). **A, B** are box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data analysed by t-test with no significant differences between groups. While columns in male, grey columns in female.

Linear regression analysis was used to model the relationship between VAT (**Table 3.7**) and ASAT (**Table 3.8**) with glycaemic status.

Table 3.9 Modelling of VAT in adolescent South Asian of PMNS participants via linear regression. Data showing the results of linear regression of VAT comprising two models; M1 is Model 1: Glycaemic status; M2 is Model 2: Glycaemic status adjusted for gender and BMI.

		Standardized Coefficients Beta	P value
M1	Glycaemic Status	0.13	0.007
M2	Glycaemic Status adjusted for gender and age	0.06	0.104
	Gender	-0.21	<0.001
	BMI	0.54	<0.001

Table 3.10 Modelling of ASAT in adolescent South Asian of PMNS participants via linear regression. Data showing the results of linear regression of VAT comprising two models; M1 is Model 1: Glycaemic status; M2 is Model 2: Glycaemic status adjusted for gender and BMI.

		Standardized Coefficients Beta	P value
M1	Glycaemic Status	0.03	0.570
M2	Glycaemic Status adjusted for gender and age	0.02	0.421
	Gender	0.27	<0.001
	BMI	0.88	<0.001

Results indicated a significant contribution of glycaemic status in VAT (p=0.007) but not ASAT (p=0.570), suggesting a more important role of VAT in the development of insulin resistance. Models for both VAT (p<0.001) and ASAT (p<0.001) showed more significant effect after including gender and BMI in the model.

3.4 Discussion

The prevalence of T2D is increasing worldwide, with 659 million people predicted to present with the condition by 2045 (301). Many elements contribute to its development including age, gender, diet, exercise and socioeconomic factors (320). Ethnicity is also a factor, with data from numerous countries indicating that SA populations present much higher rates of T2D compared to Cau (218, 245, 321-323). Studies have shown that SA populations have a higher body fat percentage compared to Cau at any given BMI (up to 5% higher) (250, 303, 324). SA populations also have a higher susceptibility to develop features of the metabolic syndrome at any given WC or WHR compared with Cau (225, 325). It has been proposed that this increased risk may result from increased central adiposity observed in SA (325); fat deposition strongly linked to insulin resistance and CVD (93, 326).

This chapter presents the quantitative analysis of MRI acquired abdominal images from the 18-year-old participants of the PMNS. The relationships between VAT, ASAT and additional outcomes; including anthropometry, blood biochemistry and body composition were examined. Notable gender specific correlations between VAT and markers of insulin resistance were found, not observed with ASAT. In addition, the study data based on pre-diabetic status and the generally accepted BMI cut-offs versus proposed BMI cut-offs specific for SA population were assessed. Lastly, given the young age of the SA population included in this study (18.0 ± 0.6 years), an unexpectedly high number of individuals of both genders in the PMNS who present with the TOFI phenotype were identified, suggesting the development of metabolic difficulties will be a feature of adult life for many of these individuals.

The PMNS is a large ongoing study that provides in-depth mother-infant data from a homogenous SA population located in rural Southern India (327). Females in these communities usually work farming cash crops, with few educated beyond the primary school level (327). The PMNS was designed to examine the relationship between a mother's size, body composition, diet and micronutrient status on foetal development and subsequent infant growth (327). In addition to the wealth of data from India, PMNS investigations have

also utilised data from a mother-infant cohort for use as a Cau comparator group. This study, the Southampton's Women's Survey (SWS), represents the only study in Europe of females and their children to obtain information directly from mothers before conception in order to learn more about the dietary and lifestyle factors that influence infant health (328).

Previous publications from the PMNS and SWS have demonstrated that, compared to their Cau counterparts, SA mothers were younger, lighter and shorter with a lower BMI (328). SA mothers gave birth to infants that were smaller in all anthropometric measurements at birth and became relatively even smaller up to 2 years of age, before a degree of catch-up growth at 3 years (328). At 6 years, PMNS children were slimmer, thinner and had a higher body fat percentage (thin-fat phenotype) compared to their UK based counterparts (318, 328). Furthermore, SA boys had increased body fat deposition, with SA children of both genders demonstrating a metabolically unhealthy profile that was not explained by their adiposity (319). In addition to these data, a study by Krishnaveni *et al.* examined the anthropometry of urban SA children at three time points up to the age of 4 years and showed that the thin-fat phenotype persists in childhood and may explain SA 'diabetogenic' phenotype in adulthood (329). All these studies have provided clear evidence of the "thin-fat phenotype" manifesting in SA children.

The PMNS 18-years cohort is a reflection of the 'Predictive Adaptive Response' hypothesis mentioned in Chapter 1 Section 1.2.1, which is that foetal adaptations to scarcity become maladaptive only when affected individuals are later exposed to an environment of plenty as from rural to urban areas (39). PMNS adolescents were for malnourished mothers and so experienced scarce environment in utero while later in life they moved from rural to urban areas so experienced an environment of plenty and therefore this disparities in both environments might lead to the observed adverse metabolic dysregulation in PMNS offspring (39).

In this Chapter, was found significant differences between males and females in the PMNS cohort; these includes established gender differences, such as

reductions in height, weight, VAT and increased ASAT, skinfold thickness in females compared to males, which have also been established in other ethnicities at a comparable age (330, 331). Height was weakly associated with VAT and ASAT in males but not females, despite those females being shorter, with less lean mass but a similar BMI to the males and therefore it expected that they would develop a stronger association between height and VAT or/and ASAT. Moreover, females were more insulin resistant compared to males, which might be explainable by their lower lean mass. Such an association demonstrates further gender differences in the pathophysiology of insulin resistance in SA.

As mentioned previously in Chapter 2, pre-diabetes is a condition of glucose abnormalities; an intermediate status above-normal glucose (lower than 5.6 mmol/L) and T2D, and it is estimated to affect more than 457 million individuals worldwide by 2045 (301). Its prevalence in the UK increased from 11.6% to 35.3% from 2003 to 2011 and expected to affect 50% of all overweight and obese population in the UK (332). Pre-diabetes defined as elevated blood glucose above the normal level but below T2D threshold (301). Pre-diabetes can be diagnosed as IFG using fasting plasma glucose of 5.6 to 6.9 mmol/L, or as impaired glucose tolerance using OGTT of 2 hours plasma glucose of 7.8 – 11.0 mmol/L (after ingestion of 75 mg oral glucose), and glycaemic haemoglobin A1C, which indicates average blood glucose in the last 2-3 months of between 5.7% and 6.4% (333). Other health institutions have published different thresholds for the diagnosis of pre-diabetes with slightly higher cut-offs of IFG (6.1 - 6.9 mmol/L) (334). HbA1c considered a superior method for diagnosing pre-diabetes due to its practicality (only one blood test) and temporal capturing but unfortunately, HbA1c was not measured in the PMNS protocol. Therefore, and given the young age (18.0 ± 0.6 years) and low average BMI (19.5 ± 3.3 kg/m^2) of PMNS cohort, the pre-diabetes definition that I used was using the lower threshold of IFG of 5.6 to 6.9 mmol/L to ensure proper sensitivity.

However, there is a growing scientific criticism of pre-diabetes; firstly, the label ‘pre-diabetes’ suggests that the condition has a linear link to T2D (the word ‘*pre*’ is a Latin word means before) (335). However, a Cochrane review of a total of 103 prospect cohort studies that investigated the prognosis from pre-diabetes (defined by FPG: <5.6 mmol/L >6.9 mmol/L, IFG: <6.1 mmol/L >6.9 mmol/L, IGT: plasma glucose after 2 hours of 75 g glucose ingestion < 7.8 mmol/L > 11.1 mmol/L, IFG+IGT or HbA1C: < 5.7% > 6.4%) to T2D demonstrated no clear linear association (335). Additional studies have shown that the prognosis from pre-diabetes to T2D could be as low as 1.3 % of the studied population (336). While the emphasis on pre-diabetes is mainly from a prevention perspective, there are high rates of pre-diabetes patients who revert to normal glucose status at any time, even after more than one decade of being pre-diabetic (335). As an additional criticism, the way pre-diabetes is currently defined allows the pharmaceutical industry to target a relatively large population without enough clinical evidence supporting either the necessity of a drug treatment nor the long–term Hazard Ratio for the prognosis to T2D (335). For example, when the American Diabetes Association reduced the threshold of pre-diabetes from IFG of 6.1 mmol/L to 5.6 mmol/L, this dramatically increased the number of people diagnosed with pre-diabetes worldwide (337). Revisiting the label of pre-diabetes as an independent condition; for example, labelling it as an abnormality in glucose signalling or impaired glycaemia, rather than implying a direct link to T2D, would provide much needed clarity for researchers, physicians and most importantly patients (337).

In this Chapter, the term pre-diabetes used to categorize young individuals based on their elevated glucose status because they had similar main cofounders such as age, BMI, WC, total FM, total lean mass and ethnicity in order to provide in-depth understating of AT role in a well-characterised population. This characterization revealed interesting results with pre-diabetes females represent a normal adipose tissue profile more than their normoglycaemic peers and therefore indicating the need for further studies

to understand T2D development in Indian focusing on AT content and distribution.

Increased fasting blood glucose levels are an early indicator of glucose insensitivity, which may progress to insulin resistance and ultimately, T2D (338, 339). Comparison of individuals when categorised as NGT or pre-diabetic revealed a similar increase in VAT, but not ASAT, in pre-diabetic males. Pre-diabetic males also presented increased fasting insulin, WHR, skinfold thickness and HOMA index. Linear regression analysis revealed a significant contribution of glycaemic status when modelling VAT, effects not observed when modelling ASAT. It is important to identify outcomes that indicate greater susceptibility to metabolic disease in later life. While these data are cross-sectional, meaning causality cannot be implied, the associations observed, notably in males, indicate that VAT appears to be an important marker for these effects. It should be noted that cholesterol, triglycerides and the HOMA-IR are found to be more strongly correlated with ASAT than VAT in both males and females. Surprisingly, when the cohort was divided into normal glucose and pre-diabetic, I found an increase in ASAT in pre-diabetic males. This is surprising given several factors: first, previous evidence has demonstrated that VAT, not ASAT, has a positive association with insulin resistance (83, 93, 326). Secondly, some evidence indicated that ASAT usually outweighs VAT negative metabolic outcomes (93). Thirdly, epidemiological studies demonstrated that VAT is associated with all-cause mortality, while the SAT is associated with decreased all-cause mortality (188). Finally, according to the adipose tissue expandability hypothesis, the adverse effect of excess fat is linked to SAT ability to expand to accumulate excess lipid (the protective effect), and therefore prevent visceral and ectopic adiposity (91). In this cohort the contrary was seen, with pre-diabetic SA having a higher SAT than VAT. All these factors together with the findings described here, indicate a possible different metabolic pathophysiology in adipose tissue metabolism in SA. Longitudinal follow-up of these individuals will be required to clearly ascertain the role VAT and ASAT may play in the development of metabolic syndrome associated morbidities. It is also possible that SA in this

cohort had elevated deep ASAT which as mentioned in Chapter 1 Section 1.3.2, has a similar adverse metabolic effect to VAT. However, during my analysis for the PMNS, I was not able to differentiate between deep and superficial ASAT due to low image resolution. The facia layer that separate superficial ASAT from deep ASAT is a thin collagen layer that requires high image resolution for visualization.

BMI is strongly associated with mortality and morbidity in epidemiological studies, as mentioned in Chapter 1, and has a clear linear relationship with anthropometry and fat depots (14). However, additional data have shown a significant variation in an individual's body fat within established BMI and WC cut-offs groups (72, 330, 340). The use of standardised BMI ranges for clinical classification of obesity across ethnic boundaries is also limited by the increased susceptibility to develop T2D at lower BMI and age in SA compared to Cau (20); SA, develop metabolically adverse outcomes at a lower BMI (approximately 6 kg/m^2 lower) (20, 193, 341). As such, conventional clinical thresholds for obesity that were originally derived from populations of white Cau descent may not be appropriate for an ethnically diverse population (192).

SA populations exhibit elevated adiposity at a lower body weight compared to Cau (248). As such, the number of SA who are classified as obese (and subsequently at higher risk of related comorbidities) is substantially underestimated when using unadjusted BMI classifications. This has led to revised BMI cut-offs for SA being published in order to more accurately reflect the increased metabolic risk in these populations (342, 343). In my analysis, I have compared the distribution of VAT and ASAT in the PMNS cohort based on the established WHO guidelines for obesity classification (189), and the SA specific BMI cut-offs (343). I found no significant differences in abdominal body fat distribution in either males or females between WHO and SA BMI cut-offs. Extensive work has been done to change these guidelines from overweight >25 kg/m^2, obese >30 kg/m^2 to overweight > 23 kg/m^2 and obese 27> kg/m^2 (193, 343), however, it appears from this analysis that the scientific community may need to focus on implementing accurate obesity assessment tools such as MR (89) before calling for new guidelines. Moreover, the significant differences I observed between fat depots between all BMI groups

using WHO BMI cut-off guidelines indicate that using WHO BMI guidelines can be accepted with caution as a proxy measure to separate groups when MR is not available, although this may be limited by the very small number of individuals presenting higher BMI classifications of overweight and obese in PMNS.

Different BMI ranges were compared by assessing the number of individuals in each BMI group that presented with the TOFI phenotype. The TOFI phenotype is calculated from a ratio of VAT and ASAT, with cut-offs originally derived from the range of abdominal adiposity stores in a defined healthy subset of volunteers (72). It is a mean of identifying those with an adverse fat distribution that may be at increased metabolic risk. It is appropriate for use in individuals with a BMI within the normal range (18-23kg/m^2 specific for SA or 18-25kg/m^2 general WHO). Anyone classified as overweight or obese cannot be classified as "thin"; therefore, high levels of VAT in these individuals represent a separate body composition type of disproportionately large amounts of visceral adiposity. The TOFI index is a useful quantitative tool to assess SA given the prevalence of the thin-fat phenotype in this population. While no differences in the number or percentage of individuals presenting with the TOFI phenotype was found between normal BMI group by WHO and SA specific cut-offs, what is striking is the exceptionally high numbers of young adults in the PMNS, of both genders, classified as TOFI as 21.6% in lean SA males and 37.9% in lean SA females. The original TOFI cut-offs were defined using a Cau population with a mean age of 38 years (72). In that population of substantially older individuals, only 14% of males and 12% of females presented as TOFI, compared to the 21.6% of males and 37.9% of females of 18 years of age. Adiposity, hyperinsulinemia and the thin-fat phenotype in this population of SA have been reported at birth (318, 328) and the data here indicate this phenotype is maintained into young adulthood. Taking the large difference in age and ethnicity between the published TOFI phenotype (72) and my SA cohort, a South Asian-specific TOFI was created (applying the published method (72) on my cohort), which results in a lower TOFI prevalence in SA specific than was found when using the generic cut-off. This indicated significant racial differences in the TOFI

threshold between Cau and SA proposed TOFI threshold. These differences, together with no observed differences in BMI SA specific cut-offs showed that central adiposity (VAT and ASAT) has significant differences in SA than Cau and an accurate tool for abdominal adiposity is required to determine the obesity risk in SA.

One limitation of my work is the lack of liver fat data available for this cohort. In addition to SA neonates have been reported with increased all abdominal AT compartments (344) and therefore, it would have been beneficial to measure liver fat given its association with metabolic disease (247). Interestingly, Prof Yanjik (PMNS lead researcher) reported orally that PMNS cohort was scanned for liver fat by ultrasound and only very few (~3% of the total cohort) had fatty liver (Yajnik 2018 personal communication). However, ultrasound remains a method of limited accuracy for determining liver fat content especially with increased SAT (as seen in the PMNS cohort), which may cause large scatter during the ultrasound scan (345). Therefore, and as this cohort reaches 24 years of age and will be going through additional follow-up scans, it is propose here that the MR imaging protocol includes liver and pancreas, as these two ectopic fat depots are important determinants of metabolic health. It was demonstrated in Chapter 2 that liver and pancreatic fat were two-fold higher in pre-diabetic versus the free-living population in Europe and therefore it would be beneficial to examine if the same pattern exist in pre-diabetic versus normal SA population. This further detail in liver fat as well as VAT and ASAT will open up rich resources of allowing robust diagnosis, treatment and prevention of T2D in SA.

As previously discussed in Chapter 1, the overflow hypothesis suggests that immature SAT will lead to an overflow of excess fat and subsequently increased VAT, and liver fat accumulation (256, 346). The analysis of SAT and VAT appears to partly contradict this hypothesis, as SA who were more insulin resistant showed higher levels of SAT. The reason for this difference, compared to what is normally observed in Cau subjects, may relate to differences in “deep ASAT” (347). Deep ASAT has been shown to have a similar association with metabolic health as in VAT and therefore ASAT may need more in-depth quantification than simply assessing its overall volume

(348). Unfortunately, it was not possible to further quantify the ASAT into deep and superficial ASAT due to the limited resolution of the MRI images. The quantification of deep and superficial ASAT requires very high resolution images to be able to detect the fascia layer which builds up of thin collagen (348).

Another limitation of this study is the lack of infant and maternal longitudinal data available for analysis. According to the thrifty phenotype hypothesis (43), reduced foetal growth is strongly associated with the development of chronic conditions in later life (44, 349). Neonatal outcomes such as birth weight, growth rate, catch-up growth and nutrition, as well as maternal factors such as age, diet and gestational diabetes status have all been implicated in the development of metabolic disease (37, 310, 350-353) and having such longitudinal data would have provided interesting correlates for the adiposity data generated here. Unfortunately, the longitudinal data of infant and mothers in the PUNE analysis was not available by the time of this analysis. Hopefully future work with our collaborators in India will allow these important relationships to be assessed, which will facilitate new horizons in the prevention of obesity associated metabolic disorders.

The largest limitation in the analyses here is the lack of a Cau control group with a similar age range, with which to compare the PMNS cohort. The most recent follow-up of the University of Southampton SWS was carried out when children were 11 years of age and remain unpublished (https://www.mrc.soton.ac.uk/sws/the-survey/childrens-follow-up/). There is, therefore, a need for comparative imaging data. In order to try and address this issue, some unpublished data was obtained from a study of Cau of white European descent, aged 19 -27 years of age. These individuals were recruited based on their gestational age (37 to 42 weeks) and were defined as healthy, free from chronic disease and neuro-disability. Whole body imaging by MRI, anthropometry and blood biochemistry data were all obtained. While a direct statistical comparison of these two data sets would not be appropriate due to differences in image acquisition (PMNS covered three MR slices of the abdomen while Cau dataset covered all body), as well

as issues regarding limited use of the data**, Table 3.11** shows study data from this population next to that of the PMNS.

Table 3.11 Anthropometry, blood biochemistry and body composition in South Asian and Caucasian young adults. Study data from age comparable South Asian and Caucasian young adults. *Unpublished data courtesy of Dr J Parkinson*. PMNS= Pune Maternal Nutritional Study, TOFI= Thin outside fat inside, HOME-IR = homeostatic model assessment of insulin resistance. Data presented as mean ± SD.

		PMNS – South Asians		Caucasians of European descent	
		Male (n=261)	**Female (n=162)**	**Male (n=27)**	**Female (n=25)**
Anthropometry	Age (yrs.)	18.2 ± 0.5	17.7 ± 0.6	22.0 ± 2.3	23.5 ± 2.9
	Weight (kg)	56.9 ± 10.6	46.8 ± 8.6	71.7 ± 9.4	59.7 ± 8.7
	Height (cm)	169 ± 7.0	156 ± 6.2	178 ± 9.2	165 ± 6.5
	BMI (kg/m)	19.8 ± 3.2	19.1 ± 3.5	22.5 ± 2.2	21.7 ± 2.7
Blood pressure	Systolic Blood Pressure (mmHg)	111 ± 10.1	105 ± 8.3	123 ± 7.4	119 ± 7.9
	Diastolic Blood Pressure (mmHg)	58.0 ± 8.3	61.5 ± 6.8	73.9 ± 7.1	72.8 ± 7.4
Metabolic profile	Fasting Glucose (mmol/L)	8.1 ± 0.5	7.9 ± 1.5	5.2 ± 0.3	5.1 ± 0.4
	Fasting Insulin (mU/L)	10.3 ± 6	11.3 ± 4.7	3.9 ± 2.2	5.9 ± 3.2
	Fasting Cholesterol (mmol/L)	3.3 ± 0.6	3.5 ± 0.6	4.0 ± 0.6	4.4 ± 0.8
	Fasting HDL Cholesterol (mmol/L)	1.0 ± 0.3	1.1 ± 0.2	1.5 ± 0.5	1.1 ± 0.2
	Fasting Triglycerides (mmol/L)	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.3	0.6 ± 0.3
	HOMA IR	1.4 ± 0.8	1.5 ± 0.6	0.9 ± 0.5	1.1 ± 0.5
MRI derived measurements	TOFI score (VAT/ASAT)	1 ± 2.1	0.5 ± 0.2	0.5 ± 0.2	0.3 ± 0.1
	TOFI percentage (%)	21.6	37.1	3.7	0.0

The reductions in height, weight and BMI observed in individuals from the PMNS represent established ethnic differences in anthropometry. Furthermore, the large increase in fasting blood glucose, insulin (double in SA than Cau) and HOMA-IR in PMNS participants of both genders suggest a marked difference in glucose metabolism and insulin sensitivity (**Table 3.11**). The number of individuals presenting as TOFI is also markedly higher (10 times) in the PMNS than Cau (**Table 3.11**). These observed racial differences might be due to reduced muscle mass in SA, which is associated with increased glucose circulation (354). In addition, PMNS children were born to malnourished mothers, which may indicate that the offspring (PMNS children) may have received inadequate substrate for skeletal and/or lean

mass growth as mentioned in Chapter 1, Sections 1.2.1 (328, 355). Furthermore, changes in the environment from scarce (intrauterine; as the mothers were malnourished) into plenty (Indian mothers moved from rural to urban areas, which considered obesogenic environment), might reprogram SA children to survival adaption by preserving higher level on insulin and glucose to maintain sufficient sustainable energy (310). Finally, epigenetic environmental factors, which are highly complex, such as dietary intake and urban infrastructure, might be a prominent indicator of increased central adiposity in SA (356). Therefore, further studies that combine all aspects that contribute to metabolic health in SA is recommended to capture the full magnitude of the epigenetic contribution. Furthermore, comprehensive multidiscipline efforts to reduce metabolic diseases from primary care (monitoring mothers nutritional status), research community (for accurate assessment of adiposity) and government (physical activity promoting infrastructure) are urgently recommended to be put in place in order to tackle the increased burden of metabolic illness in South Asia.

Increased blood pressure is a defining feature of the metabolic syndrome (357) and might have been expected to be increased in PMNS individuals. However, the large reduction observed here between the two cohorts suggests there may be separate pathophysiology to metabolic disease in SA. Published data on blood pressure is inconclusive, with a high degree of heterogeneity in blood pressure within different SA groups (Indians, Pakistanis and Bangladeshis) complicating matters (358). However, published work examining additional markers for CVD seems to indicate ethnic differences; muscle TG is reported to be associated with insulin sensitivity in Cau but not Asians (172). Furthermore, it has been suggested that SA have larger abdominal subcutaneous adipocytes size than Cau (339). Large adipocytes are dysfunctional, predicting insulin resistance and T2D independently of obesity, indicating genetic factors influencing adipocyte size may play a role in the pathogenesis insulin resistance (359).

The MRI acquisition for the Cau individuals presented in **Table 3.11** was a whole body MRI whereas a more limited acquisition of only three abdominal

slices was acquired in SA cohort. Therefore, the measurements of VAT + ASAT in the two studies are not directly comparable. Hence, the TOFI index based on a ratio of VAT and ASAT was calculated as a means to compare these data. While whole body MRI is considered the gold standard, due to time and cost, it is common to obtain more limited data sets with fewer slices, such as that in the presented study. Indeed many MRI studies only report a single slice through the abdomen (360). While this approach has drawbacks, a strong correlation has been reported between VAT measured from a single slice and whole volume measurements of VAT (360). The main criticism of single slice studies is that they often do not reflect the heterogeneous distribution of visceral fat within the entire depot (361). Here, the data from three MR abdominal slices were combined, which minimised time and cost while increasing sampling. This approach provides reasonable coverage through the abdomen and enables a shorter acquisition and analysis times compared to whole abdomen approach (200).

The strengths of my study are the comprehensive phenotyping of a relatively large cohort of adolescent SA and the use of precise MR imaging techniques to characterise abdominal adiposity. The homogenous nature of the population, limited to a small rural region, is another factor worth considering. While the overall incidence of T2D in South Asia is high there is considerable heterogeneity in prevalence across SA countries (224, 225). Some of this variation can be attributed to differences in lifestyle factors, and socioeconomic development, which showed to be quite homogeneous for this rural farming population who moved into urban areas, and the accuracy of undiagnosed versus diagnosed diabetes statistics (224, 229). A high prevalence of T2D is also observed in SA migrant populations compared with other ethnic groups in the host country (245, 321, 322). Furthermore, studies investigating the incidence of T2D in migrant SA populations found a strong association with an increased duration of residence in Western countries (362). This effect, observed in both North America Western and Middle Eastern countries, has been attributed to exposure to a greater abundance of calorie-dense foods and reductions in physical activity (363, 364). As such,

the homogenous nature of the PMNS cohort is much less likely to be affected by the additional confounders of ethnic diversity within a SA population in Western countries, such as migration or the length of exposure to the potentially negative effects of a more Westernised lifestyle.

The thin-fat phenotype whereby smaller SA babies have reduced muscle mass, but preserved body fat during their intrauterine development is hypothesised to underpin the increased susceptibility of these individuals to developing CVD. My analyses indicate that this phenotype, previously observed in infants and children of the PMNS cohort persists into young adulthood. Furthermore, my analysis of abdominal fat indicates that there is an increased proportion of VAT compared to ASAT, especially notable in SA males, a body composition which predisposes to an insulin-resistant state. Future work on this cohort should focus upon trying to obtain the extensive longitudinal data of both infants and mothers, in order to try and determine causal markers of increased visceral and abdominal subcutaneous fat. Determining the physiological pathways which lead to an adverse metabolic state will help clinicians in identifying individuals at increased risk and inform interventional studies aimed at reducing the burden of CVD and associated morbidities in SA.

Chapter 4

Phenotyping Ethnic Differences in Body Fat Distribution and Ectopic Fat

Chapter 4. Phenotyping ethnic differences in body fat distribution and ectopic fat

4.1 Introduction

As was demonstrated in Chapter 3 and supported by literature studies, ethnic differences in anthropometry and body composition may be partly responsible for the variation in metabolic risk observed between different racial groups (215, 216). Compared to Cau, SA population have a higher WC and WHR (365, 366), while at any given BMI value, SA has 5% higher body fat (303, 324). It was also shown in Chapter 3 that SA have a higher prevalence of TOFI phenotype compared to Cau which manifest from early adulthood. Furthermore, it has been proposed that an increased susceptibility to VAT accumulation underpins the higher rates of T2D, insulin resistance and CVD observed in SA (256, 367, 368), which contrast with my findings in Chapter 3 where it was showed that higher SAT to be associated with insulin resistance in SA living in India. This is surprising and contrary to most of the literature on SA AT metabolism; where SAT showed either no or negative association with insulin resistance (108). Therefore, the aim is to examine whether this association was specific to the Pune population or whether it could also be found in SA living in Western Countries.

A further conundrum in ethnic differences in body fat deposition and metabolic health outcomes have also been reported in BA populations, as described in Chapter 1. Individuals of BA descent present differences in anthropometry compared to Cau; with BA being heavier, with higher muscle mass, and increased prevalence for developing HTN and T2D compared to other ethnic groups. (216, 369, 370).

Previous studies assessing body fat percentage in different ethnic groups including SA, BA and Cau have often relied on indirect anthropometric measurements, with limited data available on direct imaging for accurate mapping of adiposity. Furthermore, there is a lack of ethnic data regarding specific body fat distribution and ectopic fat in the liver. MR represents the gold standard for the measurement of body fat and ectopic fat deposition and distribution (139). In the previous Chapter, the body composition was

examined in an adolescent SA cohort, and an increased prevalence of an adverse fat distribution such as elevated VAT and ASAT was observed. While there was no Cau group to perform statistical analysis for comparison, the data was suggestive of strong differences between SA and Cau. This Chapter (4) describes access to ethnically diverse population allowing detailed examination of anthropometry, regional fat deposition, ectopic fat content and other metabolic markers in Cau, SA and BA from two different UK-based populations.

4.1.1 Aims

- Phenotype body fat depots (TAT, VAT, ASAT, liver fat) in SA, BA and Cau in two UK based studies of adults:
  1. The West London Observation Study (TWLO)
  2. The UK Biobank study
- Compare the ethnic differences in phenotypes of body fat (VAT, ASAT and liver fat) and anthropometry in the two UK based studies.
- Examine the SA paradox (which observed in the previous Chapter) in older SA living in the UK.

4.2 Methods

4.2.1 The West London Observation Study (TWLO)

4.2.1.1 TWLO study subjects

The ethnic groups included in the TWLO study were; Cau, SA and BA (BA includes the Black Caribbean and Black African).

The TWLO study consists of 747 healthy volunteers recruited from the UK general population in West London between 2000 and 2014. All volunteers provided written informed consent with ethics obtained from the Hammersmith and Queen Charlotte's and Chelsea Hospital Research Ethics Committee, London (REC: 07Q04011/19). Volunteers were recruited via advertisements in websites, newspapers, and academic newsletters. Participants from all ethnicities were invited to take apart. Exclusion criteria included any individual with chronic disease, diabetes, CVD or liver disease, anyone taking prescribed medication, metal implants, claustrophobic subjects, pregnancy or females on the contraceptive pill. The mean age of all subjects was 41 years (range 17-75 years) with 59.7% male and 40.3% female.

4.2.1.2 TWLO study anthropometry and body fat assessment

Anthropometric measurements including age, weight, height, waist and hip were measured in each subject in the morning following an overnight fast. Subjects wore light scrubs to be weighed in kg by a Seca scale and height in m using a wall-mounted stadiometer. The Tanita Body Composition analyser -418MA (Tanita, Tokyo, Japan) was used to assess bioimpedance measures including body fat percentage, and FFM.

4.2.1.3 TWLO study Magnetic Resonance Imaging (MRI)

MRI of total and regional AT content was measured using a 1.5 T Philips Achieva scanner (Philips, Best, Netherlands) as described in Chapter 3 (200). Briefly, subjects lay in a prone position with arms straight above the head and were scanned from fingertips to toes for up to 15 minutes. Images were acquired with a whole body axial T1-weighted fast-spin-echo sequence using Q-body coil, without respiratory gating. Imaging parameters: TR 560 ms; TE

18 ms; slice thickness 10 mm; inter-slice gap 10 mm; flip angle 90 degrees; number of excitation 1. Transverse images were acquired as nine equal stacks of 12 or 13 slices at the iso-centre of the magnet to avoid image distortion.

Images were analysed using Slice-O-Matic (Tomovision, Montreal, Quebec, Canada) as described in Chapter 3. There were approximately 120-140 images per subject. The area of AT depots in (cm^2) was calculated as the product of pixel number and pixel area. The AT volumes (cm^3) of each compartment were calculated by multiplying the AT depot area by the sum of the slice thickness (10 mm) and slice gap (10 mm). Total and regional volumes were recorded in litres (L). The abdominal region was delineated as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/bottom of the lungs; therefore, the measurement of VAT contains a mixture of visceral, perineal, and retroperitoneal AT. Total adipose tissue (TAT) was calculated from the sum of SAT and internal adipose stores: TAT = SAT + Internal.

4.2.1.4 TWLO study Magnetic Resonance Spectroscopy (MRS)

Magnetic Resonance Spectroscopy (MRS) was performed during the same scanning session as MRI to determine Intra hepatocellular lipid (IHCL) (139, 371). Liver fat content measured via MRS commonly known as IHCL while liver fat content measured via MRI is commonly known as liver fat fraction (345). Participants were positioned supine with arms resting by their side. Transverse images of the liver were used to ensure an accurate position of a (2 x 2 x 2 cm) voxel in the liver, avoiding blood vessels, fatty tissue and gallbladder. ^{1}H MR spectra were obtained from the right lobe of the liver using an MRS localisation technique known as the Point-Resolved-Spectroscopy (PRESS) sequence (TR 1500 ms, TE 135 ms, 128 signal averages) without water suppression (372). ^{1}H MR spectra were analysed to determine the levels of IHCL or liver fat content using jMRUI analysis package (AMRARES) (373, 374). IHCL or liver far content was quantified in the spectrum as a percentage ratio of the -CH_2- (part of a chain of CH_2 groups lipid resonances

with references to water resonance, after correcting for T1 and T2 (**Figure 4.1**).

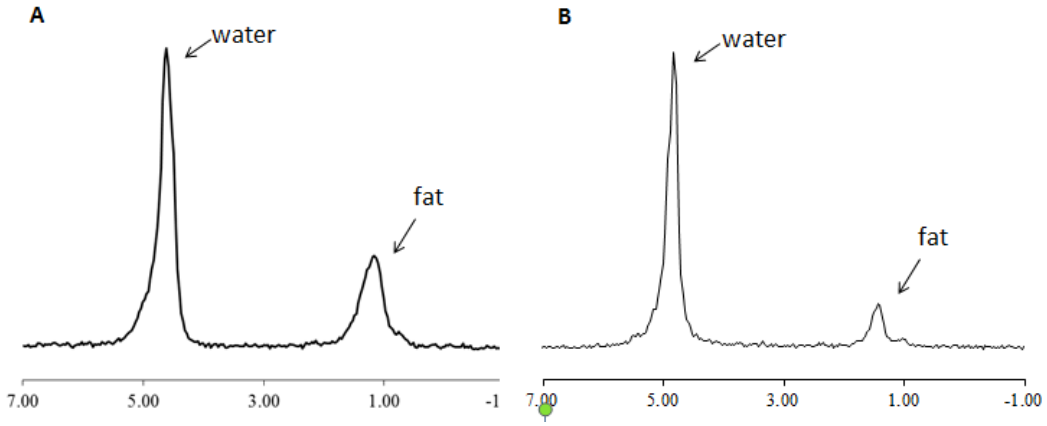


Figure 4.1: Representative ^{1}H MR spectra from the liver of (**A**) South Asian female subject with an IHCL of 13.7% and (**B**) from a Caucasian female subject with an IHCL = 5.5 %. Values refer to the peak area of the IHCL peak with reference to the water peak after correcting for T1 and T2. Results are expressed as percentage ratio of the CH_2 lipid peak area relative to the water peak area. The X axis of the spectrum represents the frequency of the resonance and expressed in parts per millions. The Y axis represented the intensity of the resonance. IHCL, intrahepatocellular lipids. Original data obtained from (139).

4.2.1.5 TWLO study ethnicity assessment

All participants ethnicity in the TWLO study was determined from a self-reported questionnaire.

4.2.2 UK Biobank

4.2.2.1 UK Biobank participants

This study represents a cross-sectional assessment of a subset of the UK Biobank, consisting of 9389 individuals from the multimodal-imaging cohort (375). As seen in Chapter 2 in the UK Biobank cohort, the age range for inclusion was 44-73 years, with individuals excluded if they had metal or electric implants, medical conditions that prohibited scanning or planned surgery within six weeks before scanning date. The 9389 subjects were scanned between August 2014 and September 2016. MR measurements for body fat compartments, percentage density liver fat fraction and patient meta-data were acquired through UK Biobank Access Application number 9914 and 6569. The ethical approval and the consent forms details were the same as detailed in Chapter 2. The ethnicity of UK Biobank participants was

defined genetically through the projection of UK Biobank individuals into the principal component space of the 1000 Genomes Project samples (PMID: 26432245) and supplied through research collaborator (see **Appendix 5**).

4.2.2.2 UK Biobank anthropometry

Anthropometry measurements were collected at UK Biobank assessment centres; height was measured using the Seca 202 height measure (Seca, Hamburg, Germany). The average of two blood pressure measurements, taken moments apart, was obtained using an automated device (Omron, UK).

4.2.2.3 UK Biobank Body fat assessment

After the initial assessment stage, all eligible participants were invited to the imaging centre. Images were acquired at the UK Biobank imaging Centre at Cheadle (UK) using a Siemens 1.5T Magnetom Aera. VAT and ASAT were measured using the dual-echo Dixon Vibe protocol as previously described in Chapter 2, and elsewhere (262). A multi-echo spoiled-gradient-echo acquisition was used to determine liver fat fraction (263). DXA scan (GE-Lunar, Madison) was used to assess body fat percentage, total body FM, total FFM.

4.2.2.4 UK Biobank physical activity

The assessment of physical activity in the UK Biobank using the IPAQ was described in Chapter 2 the Method of UK Biobank physical activity.

4.2.3 Statistical analysis

Descriptive statistics were obtained for anthropometric and volume measurements and presented as mean ± standard deviation. Data were checked for normality using Shapiro-Wilk's test. Analyses were carried out in male and female subjects separately given the established gender differences in body fat distribution. The log of liver fat fraction data was performed prior to analysis due to the non-normally distributed nature of data. The overall effect of ethnicity on study outcomes was assessed using one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons, employed to assess pairwise comparisons. ANCOVA was used to examine the relationship between ethnicity and body composition

outcomes, adjusting for age, BMI and gender. If the initial model was significant, pairwise Bonferroni post-hoc tests were carried out in order to compare individual ethnic groups. In the TWLO study, IHCL values were log transformed as (log (IHCL+1)) prior to analysis in order to address the non-normally distributed nature of the data, and an individual's FFM was calculated by converting total AT (litres) into kg by multiplying by 0.9 and subtracting from the overall weight. In the UK Biobank, liver fat PDFF was log transformed prior to the analysis to address the non-normally distributed nature of the data. Significance was taken as $p<0.05$. All data were presented as mean ± s.d. Statistical analysis was performed using SPSS version 23. All statistical graphs were done using GraphPad Prism version 5.0.

4.3 Results

4.3.1 The West London Observation Study (TWLO)

4.3.1.1 The West London Observation (TWLO) study descriptive statistics

747 volunteers participated in the study. The mean age of all subjects was 41 years (range 17-75 years) with 60% male and 40% female. Ethnic specific characteristics are shown for males in **Table 4.1** and females in **Table 4.2**.

Table 4.1 Ethnic specific baseline characteristics of anthropometry and body composition in Caucasian (Cau), South Asian (SA) and Black African (BA) males of TWOL study. WC: waist circumference; WHR, waist-to-hip ratio. IHCL: intra-hepatocellular lipid; TAT: total adipose tissue; ASAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; IHCL log transformed as IHCL+1. MRS: Magnetic resonance spectroscopy, MRI: Magnetic resonance imaging. TOFI; Thin outside fat inside, BEI; bio-electrical impedance. Data obtained from The West London Observation (TWLO) study. All data presented as mean ± SD; data analysed by one-way ANOVA with Bonferroni correction for pairwise comparisons (SPSS v 23.0). A significant was taken at < 0.05 and marked with bold font.

		Male		
		Cau (n=374)	**SA (n=68)**	**BA (n=14)**
Anthropometry	Age (years)	45.4 ± 14.5	41.5 ± 18	42.0 ± 15.9
	Weight (kg)	**89.3 ± 16.8**	79.2 ± 12.3	**89.6 ± 15.6**
	Height (cm)	**173 ± 7.0**	168 ± 6.0	168 ± 7.0
	BMI (kg/m^2)	28.2 ± 4.6	26.9 ± 3.8	28.8 ± 4.0
	Waist (cm)	98.2 ± 13.7	95.2 ± 12.7	96.6 ± 12.3
	Hip (cm)	**104.6 ± 8.4**	100. 6 ± 6.2	103.5 ± 11.2
	WHR	0.9 ± 1.6	1.0 ± 2.0	0.9 ± 1.1
MRS	IHCL	8.8 ± 16.0	6.0 ± 9.8	**2.9 ± 6.1**
MRI	TAT (litres)	27.6 ± 11.4	26.7 ± 8.9	25.9 ± 9.6
	VAT (litres)	4.1 ± 2.4	3.6 ± 1.9	**2.6 ± 1.8**
	ASAT (litres)	6.0 ± 3.2	6.2 ± 2.8	6.3 ± 3.1
	TOFI score (VAT/ASAT)	0.6 ± 0.3	0.5 ± 0.3	0.6 ± 0.7
	TOFI percentage	5.2 %	0 %	0 %
BEI	Fat Free Mass (kg)	**64.5 ± 9.8**	55.2 ± 7.3	**66.3 ± 12.7**
	Body Fat (%)	**27.1 ± 7.5**	29.9 ± 6.7	25.8 ± 8.1

In the TWOL study, SA males were shorter ($p<0.001$) and weighed less ($p<0.001$) with a smaller hip circumference ($p<0.001$) compared with Cau males (**Table 4.1**). SA males presented a higher total body fat percentage (2.8% differences in body fat) compared to Cau ($p=0.014$), and lower fat free mass (8.3 kg differences in fat free mass in SA males to other ethnic groups) compared to both Cau and BA males ($p<0.001$ for both, **Table 4.1**).

SA females were shorter than Cau counterparts ($p<0.001$) but had similar height with BA females (**Table 4.2**).

Table 4.2 Ethnic specific baseline characteristics of anthropometry and body composition in Caucasian (Cau), South Asian (SA) and Black African (BA) females of TWOL study. WC: waist circumference; WHR, waist-to-hip ratio. IHCL: intra-hepatocellular lipid; TAT: total adipose tissue; ASAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; IHCL log transformed as IHCL+1. MRS: Magnetic resonance spectroscopy, MRI: Magnetic resonance imaging. TOFI; Thin outside fat inside, BEI; bio-electrical impedance. Data obtained from The West London Observation (TWLO) study. All data presented as mean ± SD; data analysed by one-way ANOVA with Bonferroni correction for pairwise comparisons (SPSS v 23.0). A significant was taken at < 0.05 and marked with bold font.

		Female		
		Cau (n=240)	**SA (n=22)**	**BA (n=29)**
Anthropometry	Age (years)	39.3 ± 14.5	37.5 ± 13.2	41.1 ± 10.7
	Weight (kg)	75.0 ± 18.2	72.1 ± 17.6	86.0 ± 16.7
	Height (cm)	**170 ± 10**	160 ± 10	160 ± 40
	BMI (kg/m^2)	27.3 ± 6.7	28.2 ± 6.8	31.8 ± 6.3
	Waist (cm)	87.4 ± 17.5	90.9 ± 14.1	94.2 ± 15.8
	Hip (cm)	105.2 ± 12.8	104.6 ± 12.4	113.9 ± 12.2
	WHR	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
MRS	IHCL	4.1 ± 11.1	6.7 ± 12.4	1.2 ± 1.5
MRI	TAT (litres)	32.9 ± 15.7	35.2 ± 14.6	**41.0 ± 14.5**
	VAT (litres)	2.5 ± 1.7	2.4 ± 1.2	**1.7 ± 0.9**
	ASAT (litres)	8.2 ± 4.9	8.9 ± 4.6	**11.1 ± 4.8**
	TOFI score (VAT/ASAT)	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0
	TOFI prevalence	5.4 %	0%	0%
BEI	Fat Free Mass (kg)	**45.5 ± 7.6**	**40.4 ± 5.7**	**49.2 ± 5.7**
	Body Fat (%)	37.9 ± 10.2	42.5 ± 7.2	41.6 ± 7.5

In addition, ethnicity was found to have an effect on VAT distribution; with less VAT (1.5 litre difference in VAT) in BA males compared to Cau males (VAT: Cau: 4.1 ± 2.4 litres; BA: 2.6 ± 1.8 litres, p=0.043, **Table 4.1**) and females (0.7 litres differences in VAT) (VAT: Cau: 2.5 ± 1.7 litres; BA: 1.7 ± 0.90 litres, p=0.044, **Table 4.2)**. Furthermore, BA females had less VAT (0.8 litre difference) than SA females but it was not statistically significant. FFM was also less in SA females (10.3 kg differences in FFM in SA females compared to other ethnic groups) compared to females from other ethnic groups (p<0.001 for both, **Table 4.2**). Given the high percentage of SA TOFI phenotype reported in Chapter 3, no TOFI phenotype in lean SA males or females in the TWLO study were observed. Here, only the observation of the TOFI phenotype in Cau males (5.2%) and female (5.4%) but not in UK-based SA who lives in the UK.

Significant increase in TAT (7.6% differences, 5.8 litres in TAT, p=0.028, **Figure 4.2**) and ASAT (15%, 2.9 litres difference in ASAT, p=0.007, **Figure 4.4**) were observed in BA females compared with Cau counterparts (**Table 4.2**). Gender-specific distribution of total, visceral, ASAT and IHCL by ethnic group are shown in **Figure 4.2**. There was no observed increase VAT or liver fat in SA compared to other ethnic groups in both males and females (**Figure 4.3** for VAT, **Figure 4.5** for liver fat). The only observed ethnic variation was seen in BA with more TAT, SAT, but less VAT. No significant ethnic differences in IHCL were observed in either males (M: p=0.126) or females (F: p=0.180) (**Figure 4.5)**.

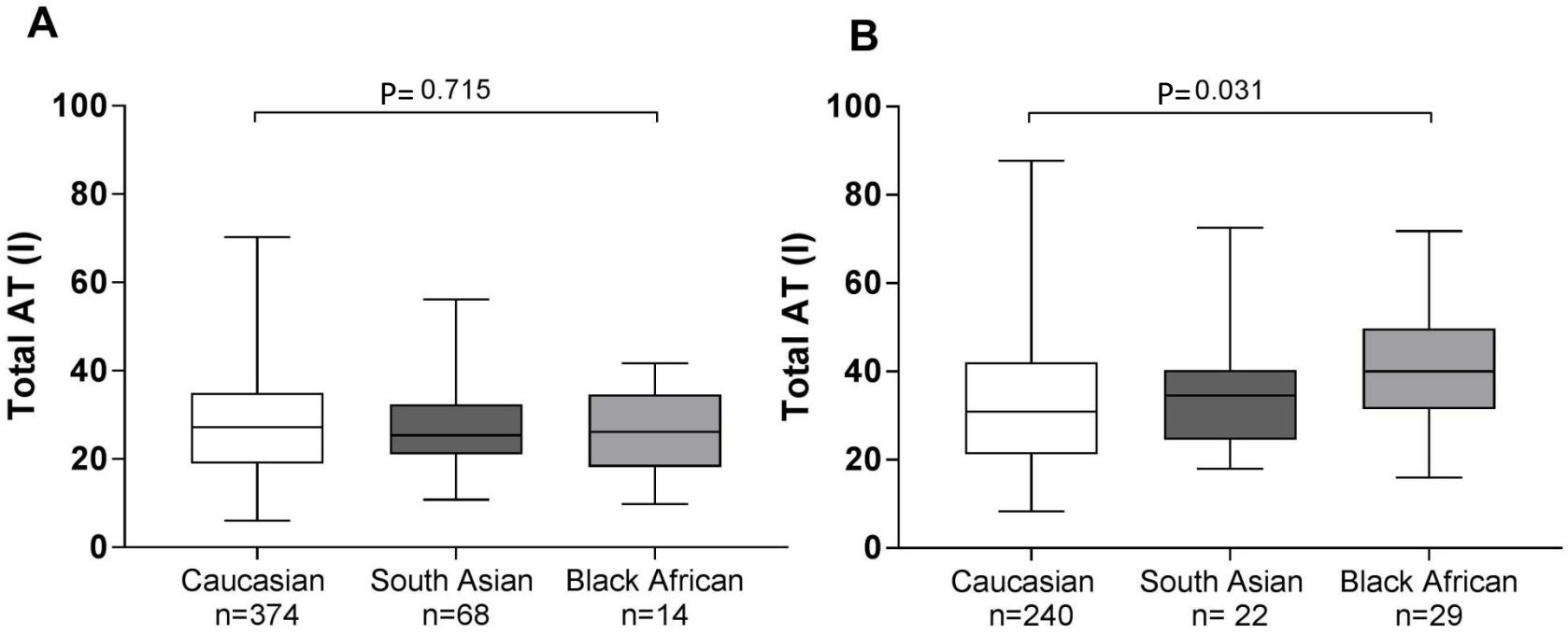


Figure 4.2 Ethnicity and Gender specific distribution of total adipose tissue (TAT) in Caucasians, South Asians and Black African adults from TWLO study. In males (**A**) in females (**B**); Data obtained from The West London Observation (TWLO) study. Data presented as box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. P values calculated from one-way ANOVA analysis (SPSS (v. 23)). Graphs were done using GraphPad Prism version 5.0

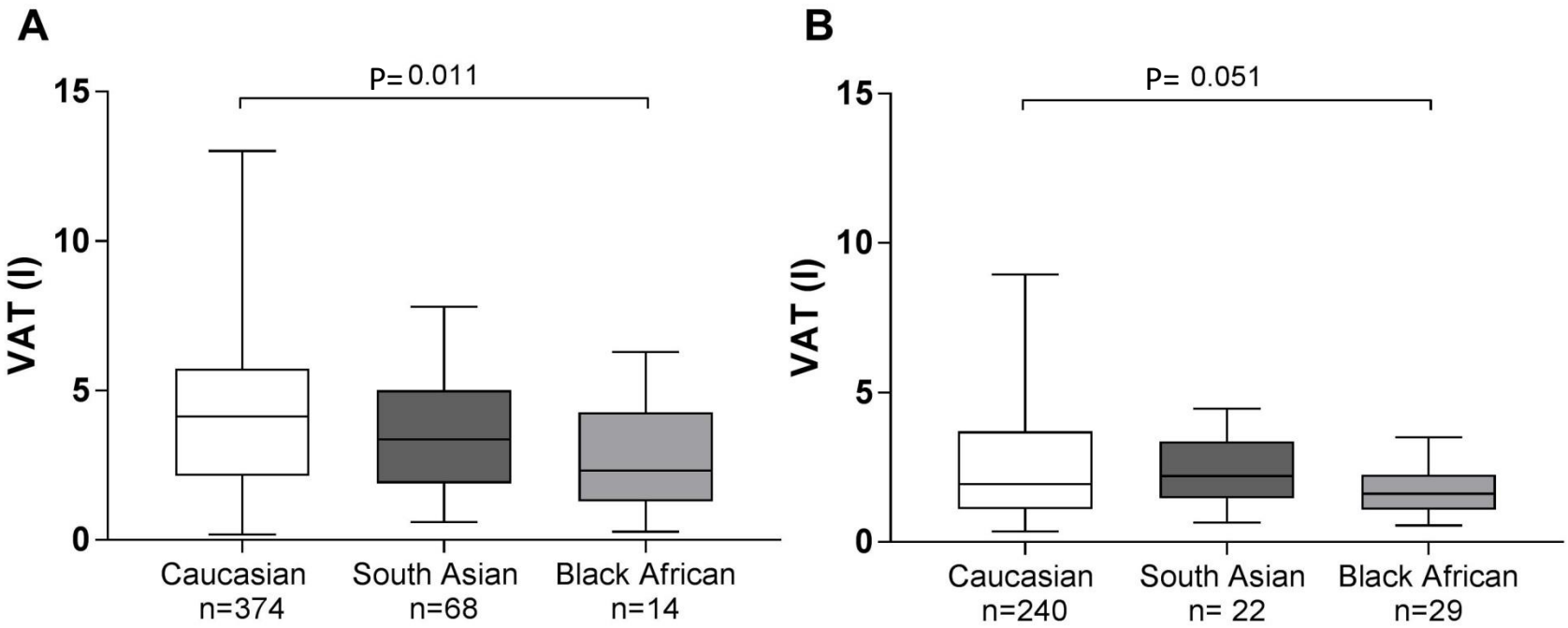


Figure 4.3 Ethnicity and Gender specific distribution of visceral adipose (VAT) in Caucasians, South Asians and Black African adults from TWLO study. In males (**A**) in females (**B**); Data obtained from The West London Observation (TWLO) study. Data presented as box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. P values calculated from one-way ANOVA analysis (SPSS (v. 23)). Graphs were done using GraphPad Prism version 5.0

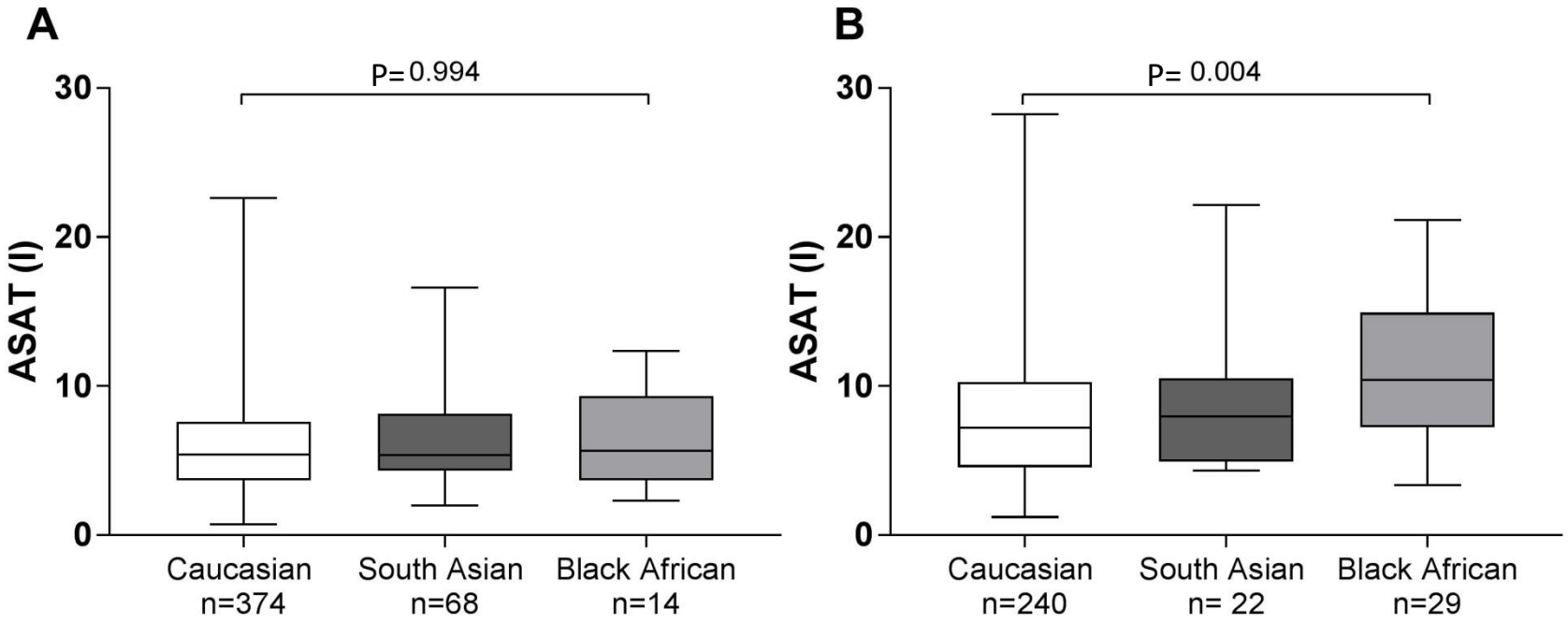


Figure 4.4 Ethnicity and Gender specific distribution of abdominal adipose tissue (ASAT) in Caucasians, South Asians and Black African adults from TWLO study. In males (**A**) in females (**B**); Data presented as box and whisker plots; where error bars are min/max range, upper and lower box edges are 25th and 75th percentiles and line median. Data obtained from The West London Observation (TWLO) study. P values calculated from one-way ANOVA analysis (SPSS (v. 23)). Graphs were done using GraphPad Prism version 5.0

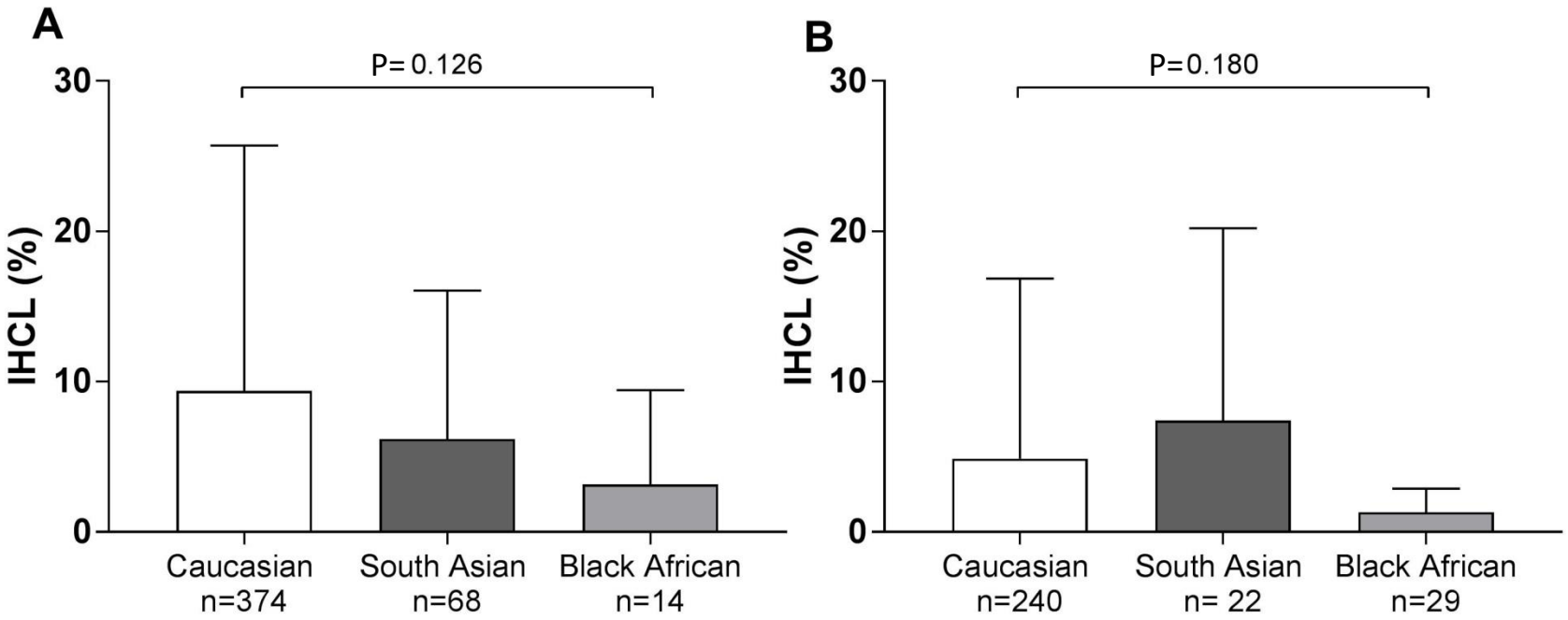


Figure 4.5 Ethnicity and Gender specific distribution of intrahepatocellular lipid (IHCL) in Caucasians, South Asians and Black African adults from TWLO study. In males (**A**) in females (**B**); Data obtained from The West London Observation (TWLO) study. Data presented as mean and standards deviations. P values calculated from one-way ANOVA analysis (SPSS (v. 23)). Data plotted in this graph using IHCL but p values determined from log data. Graphs were done using GraphPad Prism version 5.0

Given the variation in TAT, VAT, ASAT, and IHCL, a model blend of ANOVA and regression (known as ANCOVA) was performed for modelling the impact of ethnicity on each body fat compartment and ectopic fat, while adjusting for covariates such age, gender and BMI (**Table 4.3**). The results modelling the effect of ethnicity on TAT, ASAT, VAT and IHCL using an ANCOVA analysis, revealed a significant influence of all components (ethnicity, age, gender, BMI) in the model ($p<0.001$ for all, **Table 4.3**). The F statistic, which implies which of the factors has the lowest or the highest effect on the model, indicates that BMI contributes the most to TAT, VAT, ASAT, IHCL models, with ethnicity the least. These models show that in the TWLO study, ethnicity has a statistically significant effect on TAT, VAT, ASAT, and IHCL in the TWLO study, however, the impact is low compared to the effect of age, BMI and gender (**Table 4.3**).

Table 4.3 Modelling the ethnicity impact on body composition outcomes in TWLO study. The models were performed using analysis of covariance (ANCOVA). The results of ANCOVA analyses modelling the effects of ethnicity group on TAT, ASAT, VAT and IHCL, showing the overall corrected model, the F statistics, ethnicity and additional covariates: BMI, age and gender. This model is used to show the impact of ethnicity among other contributors on TAT, ASAT, VAT and IHCL. The F statistics indicates the degree of impact, significance taken as $p< 0.05$, TAT: total adipose tissue, ASAT: abdominal subcutaneous adipose tissue, VAT visceral adipose tissue, IHCL, intrahepatocellular lipid, BMI; Body mass index. Data obtained from The West London Observation (TWLO) study. Data analysed in SPSS (v. 23.0).

	TAT		ASAT		VAT		IHCL	
	F	p-value	F	p-value	F	p-value	F	p-value
The model	758.7	<0.001	682.1	<0.001	264.5	<0.001	32.323	<0.001
Age	13.5	<0.001	21.6	<0.001	237.9	<0.001	9.910	0.002
BMI	2976	<0.001	2796	<0.001	468.9	<0.001	100.839	<0.001
Gender	313.7	<0.001	366.3	<0.001	121.9	<0.001	9.712	0.002
Ethnicity	6.7	<0.001	8.44	<0.001	30.9	<0.001	6.203	0.002

Because all the contributors (age, gender, BMI and ethnicity) showed a significant impact on TAT, ASAT, VAT and IHCL in the previous models (**Table 4.3**), additional post-hoc pairwise comparisons between individual ethnic groups were performed to identify the differences observed within each ethnic group (**Table 4.4**).

Table 4.4 Ethnicity specific models for the analysis of covariance (ANCOVA) with pairwise comparison in TAT, VAT, ASAT, and IHCL in TWLO study. Ethnic comparison of TAT, ASAT, VAT and IHCL, following adjustment for gender, age and BMI. Data presented as mean differences ± standard error. IHCL log transformed as IHCL+1 prior to analysis. This model is used to identify the difference between ethnicities in TAT, ASAT, VAT, and IHCL. Significance taken as p<0.001. TAT: total adipose tissue, ASAT: abdominal subcutaneous adipose tissue, VAT visceral adipose tissue, IHCL, intrahepatocellular lipids, Cau: Caucasians, SA: South Asian, BA: Black African. Data obtained from The West London Observation (TWLO). Data analysed in SPSS 24 using Bonferroni post-hoc test for multiple comparisons.

		ANCOVA		
		Mean differences ± Standard error	**95% confidence interval**	**p value**
MRI	TAT (litre)			
	Cau versus SA	-1.7 ± 0.6	-3.2 to -0.2	0.015
	Cau versus BA	1.8 ± 0.9	-0.2 to 3.9	0.103
	SA versus BA	3.6 ± 1.0	1.1 to 6.0	0.002
	ASAT (litre)			
	Cau versus SA	-0.8 ± 0.2	-1.3 to -0.3	<0.001
	Cau versus BA	0 ± 0.3	-0.7 to 0.6	1.000
	SA versus BA	0.8 ± 0.3	0 to 1.6	0.052
	VAT (litre)			
	Cau versus SA	0.1 ± 0.2	-0.2 to 0.5	1.000
	Cau versus BA	1.7 ± 0.2	1.2 to 2.2	<0.001
	SA versus BA	1.6 ± 0.3	0 to 2.2	<0.001
MRS	IHCL (%)			
	Cau versus SA	0.3 ± 1.5	-3.3 to 3.9	1.000
	Cau versus BA	7.6 ± 2.2	2.4 to 12.8	0.001
	SA versus BA	7.4 ± 2.5	1.3 to 13.4	0.012

Significantly lower levels of VAT and IHCL were observed in BA compared to other ethnic groups (p<0.001), while no differences were observed between SA and Cau groups for either outcome (**Table 4.4**). After adjusting for BMI, age and gender, significantly higher levels of ASAT were observed in BA compared with SA and Cau, while TAT was greater in SA compared with both other ethnic groups (**Table 4.4**).

4.3.2 UK Biobank ethnicity project

The participants included in this chapter are a subset of the UK Biobank who completed MR scans for VAT, ASAT and liver fat fraction with available ethnicity information (n=9533). Gender and ethnicity were determined by Genome Wide Association Study (GWAS) analysis to describe the ancestry and for precise characterization of individuals' biological ancestry in order to

reduce bias from self-reported data and ensure accuracy. The ethnic distributions of the imaging cohort subset compared to the entire UK Biobank data set are shown in **Table 4.5**. **Table 4.5 Ethnicity distribution in the UK Biobank.** The number (n) and percentage of each ethnic group within the total UK Biobank cohort and the imaging cohort.

	Total Participants (n=533,726)	Imaging cohort (n=9533)
Caucasians (Cau)	503,837 (94.4)	9356 (95.6)
South Asian (SA)	10,141 (1.9)	123 (1.2)
Black African (BA)	8006 (1.5)	54 (0.6)
Other	11,742 (2.2)	230 (2.6)

A similar ethnic distribution was observed in both sets of data (**Table 4.5)**. Gender specific characteristics for the 9533 individuals are shown in **Table 4.6**. Overall, males, in the UK Biobank study, were older, heavier and pre-hypertensive than females in the UK Biobank study, whereas the latter had a more total body fat percentage (**Table 4.6**).

Table 4.6 Gender specific baseline characteristics, blood pressure and body composition by DXA scan in the UK Biobank study. BMI: body mass index, DXA: dual x-ray absorptiometry. Data presented as mean ± standard deviations using SPSS v 24.0.

		Male (n=4595)		**Female (n=4938)**	
		Mean ± SD	**Range**	**Mean ± SD**	**Range**
Anthropometry	Age (yrs.)	56.3 ± 7.7	40 - 70	54.8 ± 7.4	40 - 70
	Waist circumference (cm)	93.5 ± 10	63 - 150	81.9 ± 11.3	55 - 138
	Hip circumference (cm)	101.6 ± 7.2	78 - 150	101.2 ± 9.7	73 - 156
	Height (cm)	176 ± 6.0	152 - 201	163 ± 6.0	141 - 195
	Weight (kg)	83.7 ± 13.4	50 - 160	68.7 ± 12.8	39 - 154
	BMI (kg/m^2)	27.2 ± 3.5	16.8 - 51.6	25.9 ± 4.7	14.4 - 64.9
Blood pressure	Diastolic Blood Pressure (mmHg)	80.3 ± 9.8	47 - 120	77.2 ± 10.0	36 - 118
	Systolic Blood Pressure (mmHg)	137.6 ± 16.7	75 - 221	130.6 ± 18.0	82 - 202
DXA	Percentage body fat DXA (%)	30.3 ± 6.4	8.2 - 50.7	39.1 ± 7.3	14.0 - 58.4
	Total fat mass DXA (kg) -	24.9 ± 8.7	5.2 - 76.1	26.6 ± 9.3	2.7 - 73.0
	Total fat free mass DXA (kg) -	58.5 ± 6.8	38.4 - 84.2	41.9 ± 4.9	7.4 - 62.6

The breakdown of VAT, ASAT and liver fat by gender and ethnicity is shown in **Figure 4.6, 4.7, 4.8**. There were no significant differences in VAT, ASAT, and liver fat fraction in SA males and females than other ethnic groups

(**Figure 4.6, 4.7, 4.8**). The only observed ethnic difference in ASAT, VAT, liver fat fraction was in BA females with less VAT than other ethnic groups ($p>0.001$) (**Figure 4.6).** These differences show that BA females in the UK Biobank have more favourable adiposity than other ethnic groups.

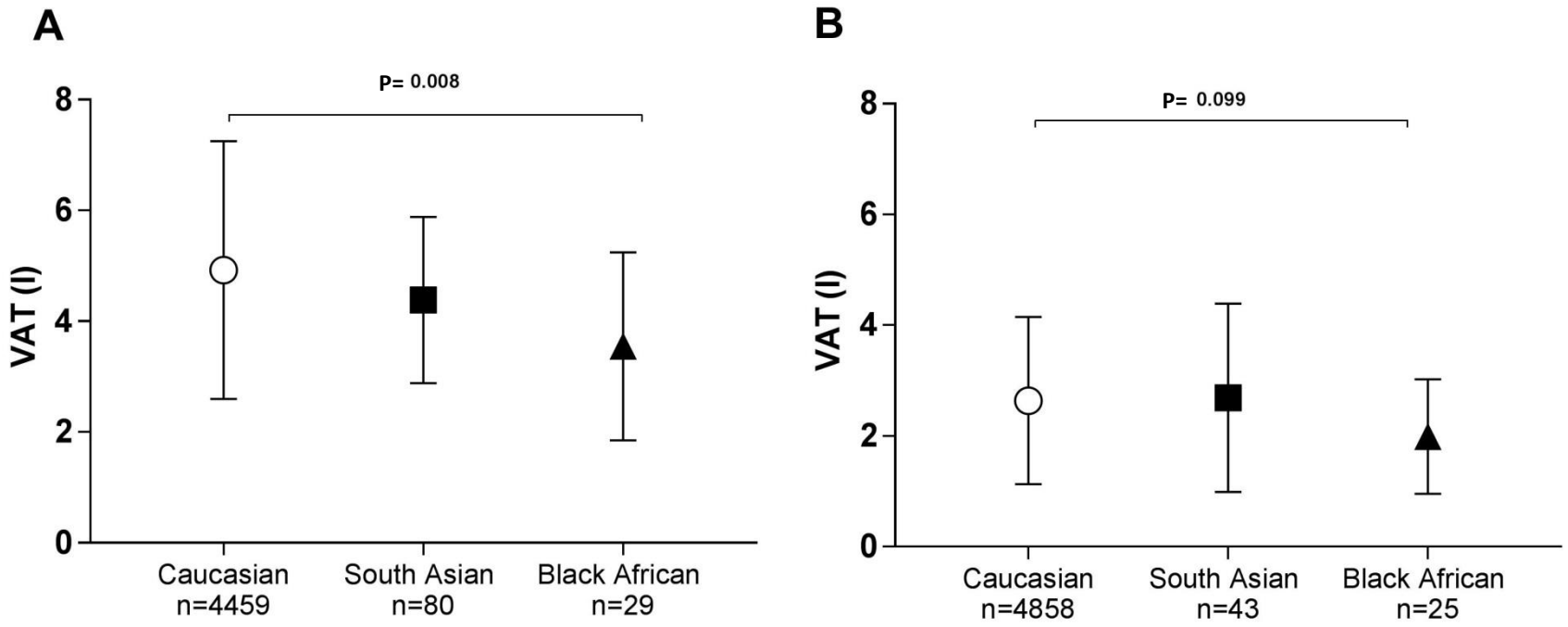


Figure 4.6 Ethnicity and gender specific distribution of visceral adipose tissue (VAT) in Caucasians, South Asians and Black African in the UK Biobank study in males (**A**) females (**B**). Data are presented as mean ± 2 standard deviation, p values calculated from ANOVA test, significance taken as $p<0.05$. Graphs were done using GraphPad Prism version 5.0.

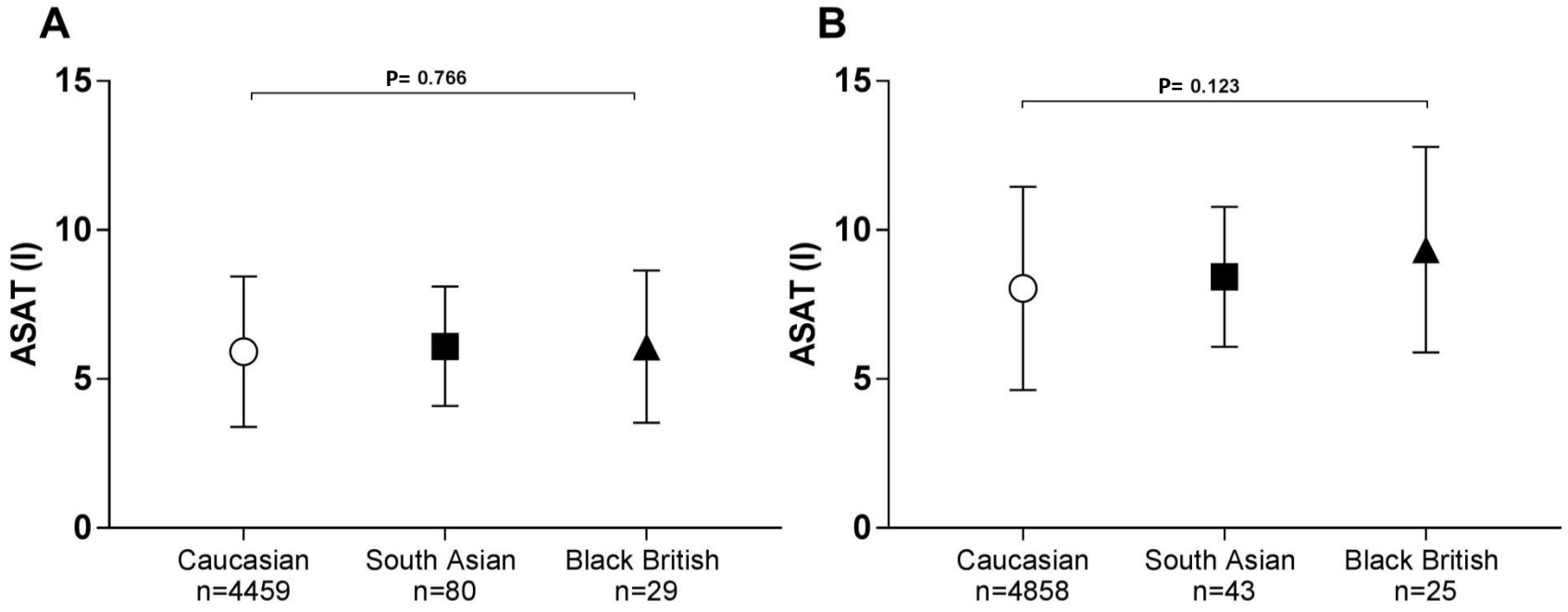


Figure 4.7 Ethnicity and gender specific distribution of abdominal subcutaneous adipose tissue (ASAT) in Caucasians, South Asians and Black African in the UK Biobank study in males (**A**) females (**B**). Data are presented as mean ± 2 standard deviation, p values calculated from ANOVA test, significance taken as $p<0.05$. Graphs were done using GraphPad Prism version 5.0.

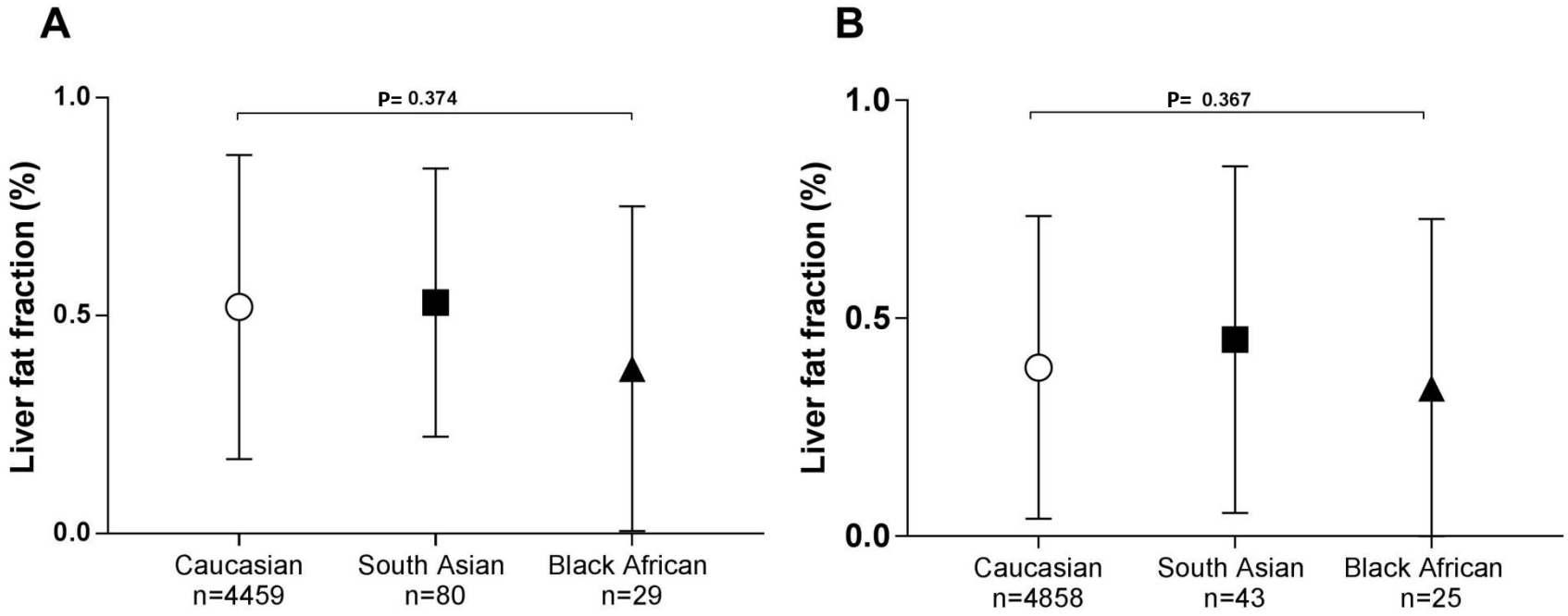


Figure 4.8 Ethnicity and gender specific distribution of abdominal subcutaneous adipose tissue (ASAT) in Caucasians, South Asians and Black African in the UK Biobank study in males (A) females (B). Data are presented as mean ± 2 standard deviation, p values calculated from ANOVA test, significance taken as $p<0.05$. Graphs were done using GraphPad Prism version 5.0.

Ethnic specific baseline characteristics of anthropometry, blood pressure and body composition are shown in **Table 4.7** for males and **Table 4.8** for females.

SA males were younger, lighter and shorter than both Cau (p<0.001 for all) and BA (p<0.001 for all) counterparts (**Table 4.7**). A significant ethnic difference in VAT is seen in BA males (30.6% differences in VAT) compared to Cau males (p=0.004) (**Table 4.**7). Total FFM was significantly lower in SA males compared to Cau and BA counterparts (p<0.001 for all).

Table 4.7 Ethnic specific baseline characteristics of anthropometry, blood pressure, and body composition in Caucasian (Cau), South Asian (SA) and Black African (BA) males in the UK biobank study. Data for UK biobank participants by ethnicity; Caucasian (Cau), South Asian (SA) and Black African (BA). ASAT: abdominal subcutaneous adipose tissue; MR: Magnetic resonance, DXA: dual x-ray absorptiometry; VAT: visceral adipose tissue, N/A: not applicable. Statistical analysis by one-way ANOVA to detect the differences between ethnic groups in SPSS (version 23.0). A significant was taken at < 0.05 and marked with bold font.

	Males	**Caucasian (n=4486)**	**South Asian (n=80)**	**Black African (n=29)**
Anthropometry	Age (yrs.)	56.3 ± 7.6	**53.5 ± 8.7**	48.7 ± 7.0
	Weight (kg)	83.8 ± 13.4	**76.6 ± 9.4**	87.6 ± 12.9
	Height (cm)	176 ± 6	**170 ± 5**	175 ± 5
	BMI (kg/m^2)	26.9 ± 3.9	26.2 ± 3.0	28.5 ± 3.6
	Waist circumference (cm)	93.5 ± 10.0	91.4 ± 8.0	92.0 ± 8.8
	Hip circumference (cm)	101.7 ± 7.1	99.2 ± 6.9	102.6 ± 6.3
	Waist to Hip Ratio	1.2 ± 0	1.3 ± 0.1	1.2 ± 0
Blood pressure	Diastolic Blood Pressure (mmHg)	80.3 ± 9.8	80.3 ± 9.4	81.1 ± 10.1
	Systolic Blood Pressure (mmHg)	137.0 ± 16.7	133.8 ± 17.5	136.2 ± 14.4
MR	VAT (litres)	4.9 ± 2.3	4.4 ± 1.5	**3.6 ± 1.7**
	ASAT (litres)	5.9 ± 2.5	6.1 ± 2.0	6.0 ± 2.6
	Liver fat fraction (%)	4.7 ± 4.7	4.4 ± 3.5	3.6 ± 4.0
	TOFI score (VAT/ASAT)	0.7 ± 0.3	0.7 ± 0.3	0.6 ± 0.3
	TOFI prevalence	4.5 %	0 %	0 %
DXA	Percentage body fat DXA (%)	30.3 ± 6.4	32.8 ± 5.7	28.8 ± 7.2
	Total fat mass DXA (kg)	24.9 ± 8.7	23.9 ± 6.3	23.9 ± 8.1
	Total fat free mass DXA (kg)	56.3 ± 7.6	**48.7 ± 7.0**	53.5 ± 8.7

Ethnic differences were observed in weight; BA females were significantly heavier than Cau (p=0.005) and SA (p=0.003) females. BA also presented significantly greater BMI, hip circumference, and total FFM compared to SA and Cau females (p<0.05 for all, **Table 4.8**).

Table 4.8 Ethnic specific baseline characteristics of anthropometry, blood pressure, and body composition in Caucasian (Cau), South Asian (SA) and Black African (BA) females in the UK biobank study. Data for UK biobank participants by ethnicity; Caucasian (Cau), South Asian (SA) and Black African (BA). ASAT: abdominal subcutaneous adipose tissue; MR: Magnetic resonance, DXA: dual x-ray absorptiometry; VAT: visceral adipose tissue, N/A: not applicable. Statistical analysis by one-way ANOVA to detect the differences between ethnic groups in SPSS (version 23.0). A significant was taken at < 0.05 and marked with bold font.

	Females	Caucasian (n=4870)	South Asian (n=43)	Black African (n=25)
Anthropometry	Age (yrs.)	54.8 ± 7.3	50.9 ± 8.3	51.0 ± 6.9
	Weight (kg)	68.6 ± 12.8	66.3 ± 12.0	**76.9 ± 11.9**
	Height (m)	1.75 ± 0.1	1.70 ± 0.1	1.78 ± 0.1
	BMI (kg/m^2)	25.9 ± 4.7	26.7 ± 4.4	**29.8 ± 4.3**
	Waist circumference (cm)	81.8 ± 11.3	84.1 ± 12.2	**88.3 ± 9.9**
	Hip circumference (cm)	101 ± 9.7	100.0 ± 9.0	**106.2 ± 8.5**
	Waist to Hip Ratio	1.5 ± 0.2	1.6 ± 0.1	**1.4 ± 0.1**
Blood pressure	Diastolic Blood Pressure (mmHg)	77.1 ± 10.0	79.6 ± 12.6	**83.9 ± 8.4**
	Systolic Blood Pressure (mmHg)	130.6 ± 18.1	126.0 ± 20.6	136.0 ± 13.7
MR	VAT (litres)	2.6 ± 1.5	2.7 ± 1.7	2.0 ± 1.0
	ASAT (litres)	8.0 ± 3.4	8.6 ± 2.6	9.3 ± 3.5
	Liver fat fraction (%)	3.6 ± 4.5	4.8 ± 5.7	3.3 ± 3.2
	TOFI score (VAT/ASAT)	0.3 ± 0.1	0.2 ± 0	0.1 ± 0
	TOFI prevalence	4.6 %	8.1 %	0%
DXA	Percentage body fat DXA (%)	39.1 ± 7.3	42.0 ± 6.5	37.2 ± 7.4
	Total fat mass DXA (kg)	26.7 ± 9.4	27.5 ± 10.4	26.3 ± 8.5
	Total fat free mass DXA (kg)	42.0 ± 5.0	38.5 ± 6.4	**45.6 ± 3.7**

BA presented increased DBP compared to Cau (p=0.002) (**Table 4.8**). BMI, waist, hip and WHR were all significantly increased in BA compared to Cau and SA females (p<0.05 for all, **Table 4.8**).

ANOVA analysis revealed significant ethnic differences in age; Cau individuals were older than SA and BA in both males and females. SA females were significantly shorter than Cau counterparts in both females ($p<0.001$ for both) and males ($p<0.001$). Significant ethnic differences in total FFM and body fat percentage (DXA) were observed in both males (**Table 4.7**) and females (**Table 4.8**), with lower values observed in SA compared to other ethnic groups.

As seen in The West London Observatory study in **Table 4.3,** and given the prior results of ethnicity variation in total body fat percentage, VAT, ASAT, and liver fat fraction, a model blends of ANOVA and regression (ANCOVA) was performed for modelling the impact of ethnicity on each body fat compartment and ectopic fat, while adjusting for covariates such as age, gender and BMI (**Table 4.9**).

Table 4.9 modelling the ethnicity impact on body composition outcomes in the UK Biobank. Analysis of covariance (ANCOVA) modelling of ethnicity on body composition outcomes. The results of ANCOVA analyses modelling the effects of ethnicity group on TAT, ASAT, VAT and liver fat fraction, showing the overall corrected model, the F statistics (F), ethnicity and additional covariates: BMI, age and gender. This model is used to show the impact of ethnicity among other contributors on TAT, ASAT, VAT and liver fat fraction. The F statistics indicates the degree of impact, significance taken as $p< 0.001$. TAT: total adipose tissue, ASAT: abdominal subcutaneous adipose tissue, VAT visceral adipose tissue, BMI; body mass index, Data analysed in SPSS (v. 24.0).

	Total fat mass (DXA)		ASAT		VAT		Liver fat fraction	
	F	p-value	F	p-value	F	p-value	F	p-value
The model	4040	<0.001	15394	<0.001	3771	<0.001	924	<0.001
Age	4.5	<0.001	17.1	<0.001	403	<0.001	43.1	<0.001
BMI	10560	<0.001	65987	<0.001	19264	<0.001	345.7	<0.001
Gender	19952	<0.001	17541	<0.001	8243	<0.001	177	<0.001
Ethnicity	10.1	<0.001	36.0	<0.001	84.0	<0.001	14.4	<0.001

The results modelling the effect of ethnicity on total body FM, ASAT, VAT and liver fat using the ANCOVA analysis, revealed a significant influence of all components (ethnicity, age, gender, BMI) in the model ($p<0.001$ for all, **Table 4.9**). The F statistic, which implies which of the factors that has the lowest or the highest effect on the model, indicates that BMI contributes the most to ASAT, VAT and liver fat models, with ethnicity the least. These models show

that in the UK Biobank cohort, ethnicity has a statistically significant effect on TAT, VAT, ASAT, and IHCL in the UK Biobank study, however, this impact is small compared to the effects of age, BMI and gender. The findings of UK biobank and the West London Observation studies in modelling of ethnicity on body fat measurements are similar, which implies the homogeneity between the two studies and confirms the findings of the TWLO in a larger UK based study on 9533 subjects.

Because all the contributes (age, gender, BMI and ethnicity) showed a significant impact on total body FM, ASAT, VAT and liver fat fraction in the previous models (**Table 4.9**), additional post-hoc pairwise comparisons between individual ethnic groups ANCOVA were performed to identify the differences observed within the ethnic groups on body FM, ASAT, VAT, liver fat fraction (**Table 4.10).**

Table 4.10 Ethnicity specific models for the analysis of covariance (ANCOVA) with pairwise comparison in total body fat mass, VAT, ASAT, and liver fat fraction in the UK Biobank study. Comparison of total body fat mass, VAT, ASAT and liver fat fraction by ethnic group, following adjustment for gender, age and BMI. This model is used to identify the difference between ethnicities in total fat mass, ASAT, VAT and liver fat fraction. Significance taken as p<0.001. ASAT: abdominal subcutaneous adipose tissue, VAT visceral adipose tissue, MR: Magnetic resonance, DXA: Dual x-ray absorptiometry, Cau: Caucasians, SA: South Asian, BA: Black African. Data presented as mean difference ± standard error. Data analysed in SPSS 23 using Bonferroni post-hoc test for multiple comparisons.

		ANCOVA		
		Mean differences ± Standard error	**95% confidence interval**	**p value**
DXA	Total body fat mass (kg)			
	Cau versus SA	-0.6 ± 0.6	-1.8 to 0.7	0.863
	Cau versus BA	3.1 ± 0.8	1.4 to 4.8	<0.001
	SA versus BA	-3.6 ± 0.9	1.5 to 5.7	<0.001
MR	VAT (litre)			
	Cau versus SA	-0.2 ± 0.1	-0.9 to 0.5	0.326
	Cau versus BA	1.8 ± 0.2	-2.2 to -1.3	<0.001
	SA versus BA	-1.6 ± 0.2	-2.1 to 1.1	<0.001
	ASAT (litre)			
	Cau versus SA	-0.4 ± 0.1	-0.7 to -0.1	0.005
	Cau versus BA	1 ± 0.2	0.5 to 1.4	<0.001
	SA versus BA	1.4 ± 0.2	0.8 to 2	<0.001
	Liver fat fraction (%)			
	Cau versus SA	0.5 ± 0	-0.1 to 0	0.200
	Cau versus BA	0.2 ± 0.0	0.1 to 0.3	<0.001
	SA versus BA	0.3 ± 0.1	0.1 to 0.4	<0.001

After adjusting for age, BMI and gender, significant differences were observed between SA and Cau in ASAT (-0.4 ± 0.1 litre, p=0.005 ASAT, data

expressed as mean difference). Significant differences between BA, SA and Cau were observed in total body FM (as measured by DXA), VAT, ASAT and liver fat fraction ($p<0.001$ for all) (**Table 4.10**). BA had a higher body FM than either (Cau 3.1 ± 0.8 kg, or SA -3.6 ± 0.9 kg, $p<0.001$) Cau and SA in the UK Biobank. Furthermore, BA had more favourable adiposity with less VAT (Cau 1.8 ± 0.2 litre, SA -1.6 ± 0.2 litre , $p<0.001$) and less liver fat (Cau 0.2 ± 0 %, SA 0.3 ± 0.1 %, $p<0.001$) than other ethnic groups in the UK Biobank after adjusting for age, gender, BMI (**Table 4.10**).

4.3.3 UK Biobank physical activity by ethnicity and gender

As presented in Chapter 2 (body fat depots in free-living and pre-diabetic populations), day to day events as in physical activity and inactivity have significant associations with total, internal body fat deposition and ectopic fat content, therefore, the ethnic differences in day to day activity with body fat depots was explored further. The differences in physical activity between Cau, SA and BA by gender by comparing mean IPAQ outcomes using ANOVA test were examined (**Table 4.11, 4.12**). In male subjects, gender and ethnic differences in physical activity were found, whereas in contrast there were no ethnic differences in physical activity as measured by the IPAQ between Cau, SA and BA females (**Table 4.11)**.

Figure 4.11 Overall ethnic specific differences in physical activity between Caucasian, South Asian, Black African females in the UK Biobank. IPAQ: International Activity Questionnaire. All data presented as mean ± SD and calculated using SPSS 23.0. Data analysed by ANOVA test.

	Caucasians	South Asian	Black African	P value
IPAQ	2016.8 ± 995.9	1970.3 ± 1177.3	1912.9 ± 1140	0.89

However, in males, SA were significantly less physically active (measured via IPAQ) than males from other ethnic groups in the UK Biobank (**Table 4.12**). This shows gender and ethnic differences in physical activity among individuals who share the same geographical environment and indicating that such differences might have an effect on increased susceptibility to metabolic diseases in SA males but not females in the UK Biobank. Furthermore, the gender differences observed in physical activity with no ethnic differences

among females in the UK Biobank might indicate that the adiposity variations are less likely to be explained by physical activity factors in females, while perhaps it is likely in males.

Figure 4.12 Overall ethnic specific differences in physical activity between Caucasian, South Asian, Black African males in the UK Biobank. IPAQ: International Activity Questionnaire, All data presented as mean ± SD and calculated using SPSS 23.0. Data analysed by ANOVA test.

	Caucasians	South Asian	Black African	P value
IPAQ	2020.6 ± 1009.3	1565 ± 1027	1803.9 ± 904	**0.005**

4.4 Discussion

In this Chapter, ethnic variations in anthropometry and body fat distribution in two UK-based studies of Cau, SA, and BA adults was investigated. Significant differences between the ethnic groups that were consistent in both studies from the UK was found. These characteristics may contribute to the variation in susceptibility to developing metabolic syndrome associated features.

While the frequency of global obesity continues to rise, there is a remarkable difference in its incidence between different ethnic groups. In the UK, obesity prevalence varies considerably between ethnic groups, with estimates differing according to the measurement used; for example, using BMI, obesity prevalence is higher among BA (32%) and SA (28%) females and lowest among Chinese (8%) females compared to females in the general population (21%). In males, obesity rates are lower in BA (17%) and SA (14%) populations and most markedly in Chinese individuals (6%) compared to males in the general population (22%) (376). There are therefore, gender and methodological considerations to accurately comparing obesity rates by ethnicity.

Different ethnicities are associated with a variety of separate body shapes and distinct physiological responses to fat storage (377-379). As a result, there has been an ongoing debate regarding the validity of using current definitions of obesity for non-Cau ethnic groups. Indeed, revised BMI

thresholds and WC measures have been proposed for SA, who are at greater risk of developing chronic diseases at lower BMI levels than Cau populations (234, 343, 380, 381). One study, which tracked weight gain and T2D development in a large cohort of females over 20 years, found that SA population were twice as likely to develop T2D as Cau counterparts (228). Furthermore, this study demonstrated weight gain in SA confers considerably greater risk than comparable increases in Cau.

One potential mechanism underlying this increased risk is increased body fat. Whilst the study here showed no differences in overall adiposity in UK-based Asians, previous studies have reported 3-5% increased body fat in Asians compared to Cau (324), with SA particularly susceptible to increased body fat, abdominal obesity and a predisposition to a high risk of T2D and CVD (20, 214, 233, 382). As mentioned previously, BMI does not distinguish between elevations in body weight from fat tissue or muscle mass, therefore, the estimates of central fat tissue have been proposed as a more accurate reflection of disease risk. Central adiposity may produce these effects via increased inflammatory cytokines release and decreased the release of factors, such as adiponectin, which are linked to increased insulin sensitivity, as discussed in Chapter 1 section 1.3.1 (93).

In this Chapter, the ethnic differences in both anthropometry and body composition in two separate studies of middle-aged, UK-based individuals was assessed. **Table 4.13** presents the percentage of Cau, SA and BA subjects within TWLO and UK Biobank studies imaging cohort, and how these percentages contrast with UK averages, as defined by data from the British Census 2011 (383). A close alignment would suggest that TWLO study and UK Biobank are representative of the normal UK population. However, in light of the census data, the UK Biobank cohort had a lower percentage of SA and BA individuals, while TWLO study had a higher proportion.

Table 4.13 Percentage distribution of Caucasians, South Asians and Black African in the UK general population, and the two included studies, TWLO, and UK Biobank. TWLO; the West London Observation study, UK Biobank (both overall and imaging cohorts) and British Census 2011 (384).

%	British Census 2011 (n=23,146,612)	UK Biobank Imaging cohort (n=9533)	TWLO study (n=747)
Caucasian	91.3	95.6	82.1
South Asian	3.2	1.2	12.0
Black African	2.4	0.6	5.9

Growing concerns about poorly represented ethnic minority groups in the UK Biobank led to the establishment of an Ethnicity Recruitment Sub-Group, tasked with ensuring that the recruitment drive took the ethnic diversity of the UK into consideration. The sub-group set targets to match these numbers and although a perfect match was not achieved, it successfully ensured that a significant number of ethnic minority individuals were included in the project. Recruiting ethnic minorities is challenging as there is evidence to suggest that ethnic minorities are less likely to enrol in clinical research compared to Cau (385). This is thought to relate to worries regarding the researchers motivation behind carrying out the clinical research, as well as worries about confidentiality of the information, especially in subjects with limited English proficiency (385). At the end of recruitment, the overall UK Biobank's Cau recruits were at just over 94%, with ethnic minorities represented by the remaining 6%. The UK Biobank imaging cohort analysed here is a slightly less diverse cross-section. As a result, the smaller number of SA and BA individuals, notably in the UK Biobank imaging cohort, clearly affects the power of the analysis to detect significant changes in study outcomes between groups (386).

Despite these differences in the distribution of ethnicities, the similarity between the two studies regarding ethnic differences in anthropometry and body composition is remarkably strong. In both TWLO and UK Biobank studies, SA males were significantly shorter and lighter than their BA and Cau counterparts as shown previously (20), with additional reductions in hip circumference and FFM. In females, a similar pattern was observed across both cohorts, with SA females being significantly shorter with reduced FFM compared to other ethnic groups in both TWLO and UK Biobank studies. As

mentioned in the introduction, WHR is strongly associated with abdominal adiposity and cardio-metabolic disease (387). In agreement with previous studies (366, 388), increased WHR in SA was found compared to Cau females in the UK Biobank cohort, and a non-significant trend towards an increased WHR in TWLO study (p=0.088). However, greater VAT or liver fat in either SA males or females, compared to Cau counterparts in TWLO and UK Biobank, did not accompany this. Previous studies have indicated that SA presents increased TAT and VAT compared to Cau matched for age and BMI (339, 368). SA neonates are also reported to have elevated liver fat compared to Cau babies within the first two weeks of life (307). Anand and colleagues have published several papers comparing the metabolic health of SA and Cau individuals matched by age gender, and BMI group. They show increased TAT, VAT, liver fat and insulin resistance in SA, with their analysis of adipocyte size indicating SA have a lower capacity to store fat in subcutaneous depots leading to overflow into ectopic fat depots (389, 390).

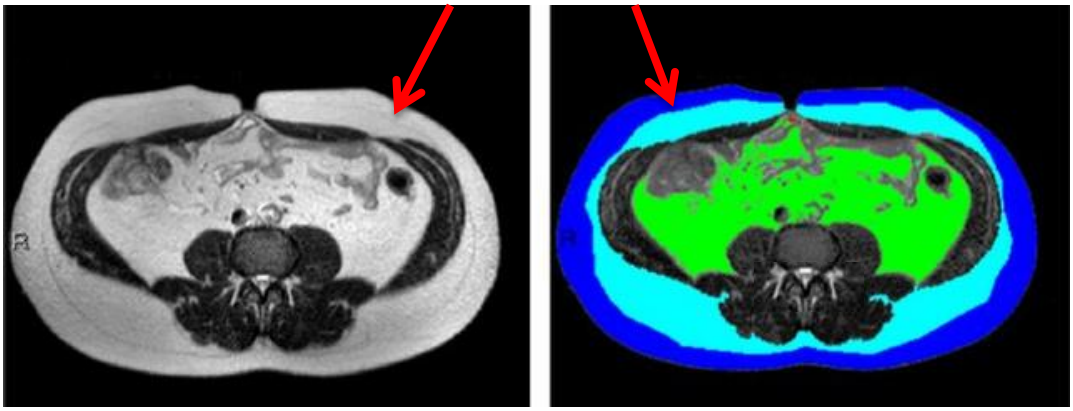
There are conflicting data regarding gender difference in VAT accumulation in SA; Park and colleagues reported an increase in VAT in SA females but not in SA males (238), whereas Lear *et al.* found increased VAT in both SA males and females after adjusting for age, BMI and total FM (250). However, neither of these findings were confirmed in the analyses here of TWLO or UK Biobank cohorts. No increased VAT or liver fat in SA compared to Cau was found, even after adjustment for age, gender and BMI. Given the increased average age of the two cohorts here compared to these studies, it is possible that by the mid- to late-forties Cau have “caught up” with regard to the increased VAT and liver fat deposition (353). Longitudinal studies in large populations will be needed to determine how ethnic specific differences in body fat accumulation manifest over time.

In this Chapter, a significant increase in ASAT in SA compared to Cau in both TWLO and UK Biobank studies was found, even after adjustment for age, BMI and gender. ASAT has been linked to a “protective” role against the development of CVD and is not associated with a linear increase in the prevalence of obesity related risk factors (391). Furthermore, it has been

proposed that SA have a less developed ASAT compartment (characterised by larger SAT adipocyte size, which is more insulin resistant), leading to greater accumulation of adipose in visceral compartments in situations of energy excess (348, 392). This model hypothesises that ASAT is the first adipose tissue compartment to develop and mature, and any restriction on its capacity to store triglyceride would lead to the expansion of VAT and accumulation of lipid in ectopic organs such as the liver (256). It should be noted that some studies have suggested that in SA, ASAT may play a more prominent role in the pathogenesis of metabolic syndrome related symptoms (256). The data here is contrary to the prevailing idea that SA have greater VAT and less ASAT, however, it should be noted that the significant differences in ASAT that were observed only manifest in ANCOVA modelling analysis following adjustment for gender, age and BMI, which means that the amount of variations seen in SA ASAT can only be explained by ethnicity when removing the effect of age, gender and BMI. Furthermore, it indicates that the effect of age, gender and BMI might outweigh the differences in ASAT in SA compared to Cau in the UK. Clearly, defining and comparing the metabolic activity of individual adipose compartments in different ethnicities will be required to determine the potential contribution of each depot in disease progression.

As described in Chapter 3, further compartmentalisation of ASAT into deep and superficial ASAT might be important; as deep ASAT showed a similar link with metabolic dysfunction as VAT (393). Furthermore, it has been shown that deep ASAT is associated with lower adiponectin levels in SA compared to Cau; this association was eliminated when the authors adjusted for adipocyte size (389). From the data available, it was impossible to ascertain details on adipocyte size nor the separation of deep and superficial ASAT. The separation between deep and superficial ASAT is a thin collagen layer (**Figure 4.4**) and therefore, further in-depth analysis of body fat compartments in different ethnic groups is needed.

Figure 4.9 MRI of abdominal fat tissues compartments. The red arrow is pointing to where the two ASAT compartments are separated, the superficial ASAT is segmented and coloured in dark blue, the deep subcutaneous adipose tissue is segmented and coloured in light blue, and visceral adipose tissue in green. MR: Magnetic Resonance. Image obtained from Golan et al 2012 (394).



One aspect of the analysis is that it is reproduced in both the TWLO study and UK Biobank cohorts is the reduced FFM, which includes lean mass that observed in both male and female SA compared to Cau. Increased lean mass, consisting mainly of skeletal muscle, is associated with improved insulin sensitivity while the adverse impact of sarcopenia, or low muscle mass, on insulin resistance and T2D is well-recognized (395, 396). FFM in the TWLO study was calculated by converting adiposity into kg and subtracting this figure from overall body weight (72), with bone mass not factored into the calculation. It therefore represents a different measure compared to DXA measurements of FFM available for UK Biobank individuals.

BA individuals also demonstrated consistent ethnic differences compared with Cau and SA individuals of the same gender, in the analysis. Participants of BA descent were generally heavier and taller than the other ethnicities, and presented significantly greater FFM and reduced percentage body fat. These findings are in agreement with previous reports showing BA individuals have increased weight and lean mass compared to Cau (216, 397). Hull *et al.* also reported similar results with BA females having higher FFM and SA females having lower FFM compared to Cau (398). In agreement with this, the study here found BA males to have significantly less VAT than Cau and SA males in both TWLO and UK Biobank studies. These data would appear to be at

odds with the increased prevalence (almost double) of T2D in BA compared to Cau for a given BMI (399).

In terms of metabolic syndrome susceptibility, BA presents a greater prevalence of HTN than Cau (369, 370). This is reflected in the UK Biobank data, with significantly increased DBP in BA compared with other ethnic groups in the UK Biobank, although this was only found in females. The lack of difference in blood pressure observed in males may be linked to the very small numbers of BA individuals with only 29 BA males included (compared to 4486 Cau males) in the UK Biobank. Previous studies have reported no difference in levels of obesity (400) and reduced abdominal visceral fat in Black American groups compared to Cau (401, 402). These studies, together with the data presented here, would indicate pathophysiology for T2D and CVD in subjects of Black race is perhaps in response to a different mechanism unrelated to increased abdominal and ectopic fat. The rates of HTN in BA are extremely high, with increased salt sensitivity and alterations in the renin-angiotensin system representing a possible underlying mechanism for the increased susceptibility to developing metabolic syndrome associated morbidities (403).

With regards to body fat distribution, one possible reason for the lack of ethnic differences is that both populations are subject to “healthy volunteer” selection bias (404). Neither cohort is fully representative of the general population, having excluded individuals with metabolic diseases that would predispose to higher abdominal fat. In order to substantiate the role of VAT and liver fat, or lack thereof, was limited to indirectly characterising groups as “normal”, based upon replication of previously published ethnic and sex differences in anthropometry (324, 365, 366).

From a statistical point of view, the approach employed to assess the ethnic differences in anthropometry and body composition is also worth consideration. By adjusting for BMI here, it was attempted to remove its potential confounding influence on ethnic differences in body fat. However, within this statistical adjustment is the tacit acceptance that BMI behaves similarly in different ethnicities. Accumulated evidence suggests that males

and females of SA origin have a greater risk for developing CVD at lower BMI levels than other ethnicities (250). Ethnic differences may, therefore, exist in the strength of the relationships between body size and metabolic and cardiovascular risk factors, and has prompted calls for lower BMI cut-offs for SA (343). However, directly excluding this confounder for my analyses by matching individuals on BMI is hindered by the lack of consensus regarding appropriate BMI cut-offs for SA, mostly due to variation within SA themselves (405).

A further limitation of my analyses in this Chapter is the lack of metabolic data (such as glucose, TG, insulin) which would have provided a means of assessing the ethnic specific association between fat depots, lean mass and clinical outcomes. While the available literature provides comprehensive details on the relationships between metabolic syndrome associated markers and both ethnicity and fat depots, it was not possible to draw any direct conclusions regarding metabolic risk in the cohorts here. Together with the observational nature of the study, my data therefore, represents a means of assessing ethnic differences in body fat distribution using the gold standard techniques of MRI in the UK biobank and MRI plus MRS in TWLO study for AT and liver fat analysis respectively. Despite the differences in the method for measuring liver fat, the results in TWLO and UK biobank were similar in female but not in male, which might be due to very small number of BA in TWLO study (n=14). From the analysis here, it was only possible to be able to speculate on the relationships between body composition and the development of adverse phenotypes.

The strengths of my study include the availability of both anthropometric and body composition data measured via MRI and MRS in two relatively large data sets in the UK. The consistency of results between the two studies is promising but my results are more confirmatory than novel.

In conclusion, the data here demonstrates significant ethnic differences in AT that are strongly associated with metabolic risk. Further work will be required

to determine how the nature of these individual fat stores influences the development of metabolic syndrome associated features.

Chapter 5
Conclusions and Future work

Chapter 5 Conclusions

The aim of this thesis was to provide an in-depth examination of the ethnic differences in compartmental adiposity. Specific adipose depots are causally linked to the development of features of the metabolic syndrome associated and, in terms of obesity-associated metabolic diseases such as T2D and CVD. SA populations have at least double the burden in the prevalence of T2D and CVD compared to Cau (224). Furthermore, SA develops T2D ten years earlier than Cau in developed countries, and with earlier progression rate in developing countries such as the UK (20). In this thesis, it was possible to examine the body fat phenotype of SA who reside in their country of origin (India) and SA living in the UK.

Unanswered questions regarding why SA have a higher susceptibility of developing obesity-related metabolic disorders highlight the importance of quantifying and assessing the ethnic differences in obesity associated metabolic diseases. Quantifying ethnic differences in body adiposity requires the use of accurate phenotyping, given the established differences between separate AT compartments and their association with metabolic disease development (91). Answering these questions should help prevention, detection, and treatment of metabolic diseases and allow new insight into racial disparities of fat metabolism and the pathophysiology of obesity-associated metabolic diseases as well as provide the scientific community with ethnic-specific guidelines to improve global metabolic health.

Findings from the present thesis have demonstrated:

- Assessing adult body fat distribution in separate UK-based populations confirmed distinct patterns in pre-diabetic compared to free-living (free of known-diseases) individuals. Despite total body adiposity being higher in free-living populations, pre-diabetics had more VAT (7.6% increase) and more ectopic fat in the liver (11.4% increase) compared to free-living population. These differences confirm the association between these depots and an adverse metabolic phenotype. Additional comparison revealed pre-diabetic

females to have more liver fat than pre-diabetic males, an effect directly opposed to what is observed in free-living population. These data suggest gender differences in fat metabolism may influence metabolic disease progression.

- In contrast to physical inactivity, day to day episodes of physical activity were associated with lower regional and ectopic fat in liver and pancreas. These observations were valid in both free-living and pre-diabetes populations. Based on these associations, physical activity assessed objectively by self-reported questionnaires appears effective.

- Applying updated BMI guidelines for SA (±2 kg/m^2 for each BMI category after the underweight category) did not offer any more insights when applied to a homogeneous SA population. The previous results are enforced by the remarkable number of lean SA who presented with unfavourable fat distribution as revealed by the TOFI phenotype. Interestingly, SA females who were hyperglycaemic showed a trend towards lower BMI compared to normal-glycaemic females. Taken together, these observations imply BMI is unable to accurately represent body fat in SA.

- Findings of this study demonstrated contrasting metabolic profiles in SA living in India compared to the UK; In India, lean SA presented a remarkably adverse phenotype compared to age equivalent Cau (21.6% TOFI in males, 37.1% TOFI in females). SA residents in the UK had very low presence of this adverse adiposity (8.1% only in female), despite that the fact that they were much older (18 compared to 39-years-old). This highlights a possible high metabolic vulnerability in SA towards environmental inputs. Furthermore, the results of this study confirm that the thin-fat phenotype presented in SA infants up to 6 years old, persists in 18-year-old.

- Data in adults indicate that even after adjusting for confounders such as age, gender and BMI, UK based SA did not have significantly higher VAT or liver fat compared to other ethnic groups. This important result was replicated in two, large, unrelated populations. While limited by the lack of metabolic data, these data suggest that increased VAT and IHCL may not be contributing factors to the increased prevalence of metabolic disease observed in SA. Furthermore, the PMNS cohort showed stronger correlations between fasting glucose and insulin in levels of SAT rather than VAT. This suggests gender and racial differences in the pathophysiology of insulin resistance where SAT may not play a protective role in SA, and therefore, phenotypes such as MHO, where individuals might have high amount of fat but low risk of metabolic disease dysregulation due to subcutaneous fat distribution, are less likely to occur in SA population.

- Regarding the pathophysiology of metabolic syndrome in SA, my analysis of the PMNS cohort indicate that a reduced muscle mass, associated with lower glucose uptake, increased insulin secretion and subsequent insulin resistance may play a key role.

- BA populations have a higher burden of metabolic diseases compared to Cau (231). However, my data indicate that BA also showed favourable body adiposity, with less VAT and less liver fat compared to SA and Cau. The data here, therefore, indicate distinct ethnic variations may exist in body fat metabolism and metabolic dysregulation.

5.1 What went wrong?

- During the research for this thesis, the metabolic data and blood biochemistry markers including blood glucose (fasting + 2 hours OGTT), insulin (fasting + 2 hours OGTT), and TG were planned to be revealed for the cohorts included in my thesis. These metabolic data would have provided in-depth understating into the metabolic pathophysiology in SA compared to Cau since SA have shown higher glucose response and lower insulin sensitivity compared to Cau after identical meals consumption (406). However, these data did not release by the time of writing the thesis. Therefore, one of the most significant limitations in the data presented here is the lack of metabolic data for the participants in two of my three results chapters, limiting my ability to draw conclusions regarding ethnic differences in the association(s) between metabolic dysregulation and body fat compartments. Certainly, once the metabolic data is available it requires further investigation.

- The data presented from the European pre-diabetes cohort (DIRECT) in Chapter 2 was scheduled to have ethnicity data released at the beginning of 2017. However, these data have not yet been released despite continuous follow up and will require further investigation once available.

5.2 Limitations

- A major limitation is the proportional lack of SA participants; the total percentage of SA in this study overall is 4.6%. Indeed, the UK Biobank had a lower percentage of SA at 1.2%. To overcome this obstacle, it was possible to obtain abdominal MR scans of SA participants from India, made available by our collaboration with the PMNS, who allowed analyse of their MR scans of SA (total SA in PMNS= 443, which is four times more than were available from the UK Biobank).

- The cross-sectional nature of the data in this thesis was a barrier as it limits discussion of causality. Longitudinal follow-up, which remains expensive and problematic, of the populations that were studied, would provide additional understanding of the dynamics of fat metabolism with time and diet and physical activity interventions.

- The relatively small number of obese SA in Chapter 3 limited the applicability of the results. Therefore for future studies, it may be beneficial to have a larger cohort that more comprehensively represents all BMI ranges. For example, recruitment of SA in India, the largest SA country, would benefit by recruiting from cities or states like Delhi and Punjab where the obesity prevalence is 27.8%, and perhaps avoid Tripura where the obesity prevalence is less than 5%.

5.3 Future work

- In future studies, more concentrated effort needs to be made to obtain an equal proportion of participants from all ethnicities studied. The low percentage of non-Cau participation in biomedical studies is a well-established obstacle facing medical committees in order to fully understand the metabolic importance of ethnic differences (407). Therefore, future work in this area is needed in order to identify the magnitude of the barriers (i.e. culture, religion) behind this obstacle and also for the relative entities (i.e. social) to develop proper strategies to overcome them.

- Further investigation into the effects of overall muscle mass (size and quality) and its contribution to the development of metabolic syndrome associated features is required. My work has indicated that such future studies be carried out in a longitudinal nature with ethnic and gender specific fashion for precise findings.

- Metabolic disorders and adverse fat adiposity manifest at an early stage in SA (328) and therefore, the policy guidelines and intervention studies using MR for SA should be aimed accordingly.

- Implementing current advanced technologies such as continues glucose monitoring and creating a longitudinal profile of subjects/participants can provide insights into the triggers underlying elevated blood glucose (dietary, physiological, etc.). This will enable the creation of clustering maps for elevated blood glucose triggers, insulin profiling and T2D progressions in young SA. Comparing such profiles with Cau would allow an in-depth understanding of the ethnic differences in the metabolic feedback loop and its short-term and long-term impact on body fat phenotyping.

- Furthermore, future interventions will highly benefit from social and religious aspects in combination with dietary patterns. This will allow not only the evaluation of micro and macro nutritional intake, which certainly showed an impact on body composition (408) but also the way of food preparation which does differ by ethnicities and religious practices (409). For example, elements such as cooking practices and certain edible oils usage have shown to impact adversely on body compositions in SA (409) and therefore require further investigations.

- Gut microbiome alteration (quantity and composition) has a role in metabolic disease establishment and the manifestation of insulin resistant, obesity and contributing to AT deposition (410). Although the exact underlying mechanism is not yet fully illustrated, ethnicity has been shown to be the strongest determinant of gut microbial makeup, even in the same geographical location (411). Hence, a greater understanding of alterations of the gut microbiota, in combination with dietary patterns, may provide insights into capturing the full magnitude of ethnicity-environment interaction and its impact on metabolic disorders.

- Obesity is associated with constant state of chronic low-grade inflammation leading to activation and infiltration of pro-inflammatory immune cells and a dysregulated production of high levels of pro-inflammatory cytokines, which contributes to insulin resistance and T2D progression (412). SA populations have shown worse inflammatory profile compared to Cau (389). Hence, future studies might benefit from adding inflammation markers (in particular the makers that enable the assessment of adipocytes function rather than volume only, for example, adiponectin and resisted) which would enable greater understanding of the crosstalk between ethnicity, immune system and adipocytes and may shed a light in better treatment modalities for obesity and obesity-related diseases. Furthermore, adipocytes size has been shown to account for the adverse metabolic profile and body composition in SA compared to Cau (389). However, adipocyte extraction is currently an invasive procedure (through biopsies) and therefore future biotechnological studies focusing on developing non-invasive tools for individualized assessment of adipocyte size may shed a light into the mechanism behind the increased prevalence of obesity associated diseases in SA.

- The contrast between Indian and UK based SA, highlights the need to identify the potentially different pathophysiology of metabolic syndrome associated features in native and immigrant populations. Furthermore, the impact of day to day events and the magnitude of environmental impact (i.e. food accessibility, food quality, deprivation, air pollution, transport infrastructure, sports facilities, green areas, etc.) in rural versus urban environments warrants further investigation. Cross-sectional studies focused on expats communities living in different countries could offer important insight into ethnic differences in fat metabolism and magnitude of environmental contribution.

Chapter 6
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Appendix 1 UK Biobank access application number 6569.

Cross-referenced Chapter 2 Page 66.

UK Biobank access application number 6569

Principal Investigator Dr Olof Dahlqvist Leinhard

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Summary of research Key words: Obesity, Biomarkers, Ectopic fat, Visceral fat

The aim of the proposed study is to investigate relationships between fat distribution and other factors related to the metabolic syndrome. Most population studies are today using BMI, Waist to Hip ratio or total amount of body fat as measurements and biomarkers for obesity. Imaging methods are now being introduced and can offer improved accuracy and reproducibility for the biomarkers indicating obesity related diseases. A new automatic analysis method is here proposed for the analysis of abdominal MR images acquired within the UK Biobank study, offering a unique possibility to quantify abdominal fat distribution. These measures of fat distribution will be returned to the UK Biobank enabling access to these biomarkers for the research community, thereby supporting obesity-related research in line with the purpose of the UK Biobank. We expect that the proposed work, to quantify and localize fat volume in specific organs of importance, will enable identification of new and more specific biomarkers for chronic diseases where body composition plays an important role. This will have great impact in many of the proposed research projects starting or already started within UK Biobank. After MR scanning, we will analyse the data and quantify abdominal fat and subcutaneous fat in the abdominal region, as well as thigh muscle volume, using automated image analysis. These measures will then be correlated to other factors related to obesity and the metabolic syndrome, such as genetic and demographic data, life style and dietary information, blood analysis data and metabolic information. We intend to analyze the full cohort of the UK Biobank imaging study.

UK Biobank access application number 9914

Dr Rajarshi Banerjee, Perspectum Diagnostics Ltd, Oxford Principal

Investigator: Dr Rajarshi Banerjee Department: Oxford Centre for Innovation Institution: Perspectum Diagnostics Ltd, Oxford Centre for Innovation, New Road, Oxford, OX1 1BY, United Kingdom

Tags: 9914, disease, Fat, Fibrosis, inflammation, Iron, Liver Summary:

Perspectum Diagnostics has developed a method of analysing magnetic resonance imaging (MRI) data that gives an accurate estimate of the amount of liver fat, the amount of liver iron, and the extent of inflammation and scarring in the liver. These three characteristics of the liver are also the most important in the diagnosis of liver disease. By analysing the abdominal MR images from all UK Biobank participants, we can determine approximately how many have abnormal liver composition, and the distribution of each of these measures in the population. Finally, and most importantly, we can examine the outcomes of the participants with liver disease, and determine which biomarkers are predictive of these outcomes. 1b: New, clinically meaningful data will be generated from the existing DICOM images, and fed back in to the UK Biobank data repository. These data will be directly

relevant to future health outcomes and of use to other researchers. Excess liver fat is associated with coronary artery atheroma and metabolic syndrome, and is strongly associated with obesity-related disease. Liver fibrosis and inflammation are both associated with adverse outcomes, which is especially relevant in those with fatty liver disease. We will be able to show which patients have liver disease, and future researchers can link these findings to specific outcomes. 1c: The MRI scans from the imaging enhancement study will be analysed by LiverMultiScan to determine liver fat, iron, inflammation and fibrosis (LIF score). These measures have separately been validated against liver biopsies from patients. These data will then be compared to measures of body composition, serum markers (lipid profile, iron stores, CRP and others) and habits associated with liver disease (e.g. alcohol intake, exercise and diet). We will follow up all patients and identify those with a liver-related clinical outcome (e.g. liver failure, hepatic encephalopathy), and determine which prognostic factors best predict these outcomes in this population. 1d: All 100,000 participants from the UK Biobank imaging enhancement study (i.e. the full cohort from the imaging enhancement study) will be analysed to determine the baseline liver health profiles of the population. Clinical outcomes data will be collected, with the aim of capturing – every liver-related death – every episode of oesophageal variceal bleeding – every new diagnosis of cirrhosis – every new diagnosis of liver failure or gross ascites due to liver disease (excluding malignant ascites) – every new primary hepatocellular carcinoma and cholangiocarcinoma – every new pancreatic carcinoma

Appendix 2 UK Biobank Physical Activity Questionnaire (IPAQ) and Guidelines for Data Processing and Analysis of the International (IPAQ).

Cross-referenced in Chapter 2 Page 66.

		The touch screen question	Hint given for the participants	Field ID
Physical Activity Measures	Days/weeks walked 10+ minutes	In a typical WEEK, on how many days did you walk for at least 10 minutes at a time?	Include walking that you do at work, travelling to and from work, and for sport or leisure.	864
	Duration of Walks	How many minutes did you usually spend walking on a typical DAY?	If the time spent walking on each day of the week varies a Lot, provide an average of the walking time	874
	Days/wk moderate physical activity 10+ min	In a typical WEEK, on how many days did you do 10 minutes or more of moderate physical activities like carrying light loads, cycling at normal pace? (Do not include walking)?	Moderate activities examples: walking upstairs, going to the gym(push-ups, weight lifting, dynamic yoga), jogging, energetic dancing, aerobics and gardening Remember to include activities that you do for work, leisure, travel and around the house.	884
	Duration of moderate activity min	How many minutes did you usually spend doing moderate activities on a typical DAY?		894
	Days/weeks vigorous physical activity 10+ min	In a typical WEEK, how many days did you do 10 minutes or more of vigorous physical activity? (These are activities that make you sweat or breathe hard such as fast cycling, aerobics, heavy lifting	Vigorous activities examples: running (not slow > 5 mph), cycling uphill, carrying heavy furniture upstairs, martial arts, competitive sports or intensive exercise Remember to include heavy activities that you do for work, leisure, travel and around the house.	904
	Duration of vigorous activity	How many minutes did you usually spend doing vigorous activities on a typical DAY?		914
	Usual walking pace	How would you describe your usual walking pace?	Slow pace is defined as less than 3 miles per hour (6000 steps/hour) Steady average pace is defined as between 3-4 miles per hour (7500 steps per hour). Fast pace is defined as more than 4 miles per hour (10000 steps per hour)	924

	Freq of stair climbing in last 4 weeks	At home, during the last 4 weeks, about how many times a DAY do you climb a flight of stairs? (approx. 10 steps)	For all participants except those who indicated they were unable to walk.	943
	Freq of walking for pleasure in last 4 weeks	How many times in the last 4 weeks did you go walking for pleasure?		971
	Duration of walking for pleasure	Each time you went walking for pleasure, about how long did you spend doing it?		981
	Freq of strenuous sports in last 4 weeks	How many times in the last 4 weeks did you do strenuous sports	For all participants who indicated that they spent time doing strenuous sports in the previous 4 weeks	991
	Duration of strenuous sports	Each time you did strenuous sports, about how long did you spend doing it?	Examples of strenuous sports: Heavy DIY includes chopping wood, home or car maintenance, lifting heavy objects or using heavy tools.	1001
	Freq of light DIY in last 4 weeks	How many times in the last 4 weeks did you do light DIY?	Examples of light DIY: pruning, watering the lawn and carpentry) in the previous 4 weeks	1011
	Duration of light DIY	Each time you did light DIY, about how long did you spend doing it?		1021
	Freq of heavy DIY in last 4 weeks	How many times in the last 4 weeks did you do heavy DIY?		2624
	Duration of heavy DIY	Each time you did heavy DIY, about how long did you spend doing it?		2634
	Freq of other exercises in last 4 weeks	How many times in the last 4 weeks did you do other exercises such as swimming, cycling, keep fit?		3637
	Duration of other exercises	Each time you did other exercises such as swimming, cycling, keep fit, about how long did you spend doing them		3647

	Types of physical activity in past 4 weeks	In the last 4 weeks did you spend any time doing the following: walking, other exercise, strenuous sport, light DIY, heavy DIY	Strenuous sports include sports that make you sweat or breathe hard. Heavy DIY includes chopping wood, home or car maintenance, lifting heavy objects or using heavy tools.	6164
	Job involves heavy lifting	Does your work involve heavy manual or physical work?	Physical work includes work that involves handling of heavy objects and use of heavy tools.	816
	Time spent doing vigorous physical activity	**Yesterday**, about how long did you spend doing activities that needed vigorous effort, making you breathe hard?		104900
	Time spent doing moderate physical activity	**Yesterda**y, about how long did you spend doing activities that needed moderate effort, making you somewhat short of breath?		104910
	Time spent doing light physical activity	**Yesterday**, about how long did you spend doing activities that needed some light effort, involving movement but not making you short of breath	Hatha yoga and siling not for competition	104920
Physical Inactivity Measures	Time spent watching television	In a typical DAY, how many hours do you spend watching TV?		1070
	Time spent using computer	In a typical DAY, how many hours do you spend using the computer?		1080
	Time spent driving	In a typical DAY, how many hours do you spend driving?		1090

Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)

– Short and Long Forms

November 2005

Contents

Revised November 2005 2

1. Introduction

This document describes recommended methods of scoring the data derived from the telephone / interview administered and self-administered IPAQ short and long form instruments. The methods outlined provide a revision to earlier scoring protocols
for the IPAQ short form and provide for the first time a comparable scoring method for IPAQ long form. Latest versions of IPAQ instruments are available from www.ipaq.ki.se.

Although there are many different ways to analyse physical activity data, to date there is no formal consensus on a 'correct' method for defining or describing levels of
physical activity based on self–report population surveys. The use of different scoring
protocols makes it very difficult to compare within and between countries, even when
the same instrument has been used. Use of these scoring methods will enhance the
comparability between surveys, provided identical sampling and survey methods have been used.

2. Uses of IPAQ Instruments

IPAQ short form is an instrument designed primarily for population surveillance of physical activity among adults. It has been developed and tested for use in adults (age range of 15-69 years) and until further development and testing is undertaken the use of IPAQ with older and younger age groups is not recommended.

IPAQ short and long forms are sometimes being used as an evaluation tool in intervention studies, but this was not the intended purpose of IPAQ. Users should carefully note the range of domains and types of activities included in IPAQ before using it in this context. Use as an outcome measure in small scale intervention studies is not recommended.

3. Summary Characteristics of IPAQ Short and Long Forms

1. IPAQ assesses physical activity undertaken across a comprehensive set of domains including:
a. leisure time physical activity
b. domestic and gardening (yard) activities
c. work-related physical activity
d. transport-related physical activity;
2. The IPAQ **short** form asks about three specific types of activity undertaken in the four domains introduced above. The specific types of activity that are

assessed are walking, moderate-intensity activities and vigorous-intensity activities.
3. The items in the **short** IPAQ form were structured to provide separate scores on walking, moderate-intensity and vigorous-intensity activity. Computation of the total score for the short form requires summation of the duration (in minutes) and frequency (days) of walking, moderate-intensity and vigorous-intensity activities. Domain specific estimates cannot be estimated.

4. The IPAQ **long** form asks details about the specific types of activities undertaken within each of the four domains. Examples include walking for transportation and moderate-intensity leisure-time activity.
5. The items in the **long** IPAQ form were structured to provide separate domain specific scores for walking, moderate-intensity and vigorous-intensity activity within each of the work, transportation, domestic chores and gardening (yard) and leisure-time domains. Computation of the total scores for the long form requires summation of the duration (in minutes) and frequency (days) for all the types of activities in all domains. Domain specific scores or activity specific sub scores
may be calculated. Domain specific scores require summation of the scores for walking, moderate-intensity and vigorous-intensity activities within the specific domain, whereas activity-specific scores require summation of the scores for the specific type of activity across domains.

4. Overview of Continuous and Categorical Analyses of IPAQ

Both categorical and continuous indicators of physical activity are possible from both
IPAQ forms. However, given the non-normal distribution of energy expenditure in many populations, it is suggested that the continuous indicator be presented as median minutes/week or median MET–minutes/week rather than means (such as mean minutes/week or mean MET-minutes/week).

4.1 Continuous Variables

Data collected with IPAQ can be reported as a continuous measure. One measure of
the volume of activity can be computed by weighting each type of activity by its energy requirements defined in METs to yield a score in MET–minutes. METs are multiples of the resting metabolic rate and a MET-minute is computed by multiplying
the MET score of an activity by the minutes performed. MET-minute scores are equivalent to kilocalories for a 60 kilogram person. Kilocalories may be computed from MET-minutes using the following equation: MET-min x (weight in kilograms/60 kilograms). MET-minutes/day or MET-minutes/week can be presented although the
latter is more frequently used and is thus suggested.
Details for the computation for summary variables from IPAQ short and long forms are detailed below. As there are no established thresholds for presenting METminutes,
the IPAQ Research Committee propose that these data are reported as comparisons of median values and interquartile ranges for different populations.

4.2 Categorical Variable: Rationale for Cut Point Values

There are three levels of physical activity proposed to classify populations:
1. Low
2. Moderate
3. High

The algorithms for the short and long forms are defined in more detail in Sections 5.3

and 6.3, respectively. Rules for data cleaning and processing prior to computing the
algorithms appear in Section 7.
Regular participation is a key concept included in current public health guidelines for
physical activity.1 Therefore, both the total volume and the number of days/sessions
are included in the IPAQ analysis algorithms.
The criteria for these levels have been set taking into account that IPAQ asks questions in all domains of daily life, resulting in higher median MET-minutes estimates than would have been estimated from leisure-time participation alone. The
criteria for these three levels are shown below.
Given that measures such as IPAQ assess total physical activity in all domains, the "leisure time physical activity" based public health recommendation of 30 minutes on
most days will be achieved by most adults in a population. Although widely accepted
as a goal, in absolute terms 30 minutes of moderate-intensity activity is low and broadly equivalent to the background or basal levels of activity adult individuals would accumulate in a day. Therefore a new, higher cutpoint is needed to describe the levels of physical activity associated with health benefits for measures such as IPAQ, which report on a broad range of domains of physical activity.

'High'

This category was developed to describe higher levels of participation. Although it is
known that greater health benefits are associated with increased levels of activity there is no consensus on the exact amount of activity for maximal benefit. In the absence of any established criteria, the IPAQ Research Committee proposes a measure which equates to approximately at least one hour per day or more, of at least moderate-intensity activity above the basal level of physical activity Considering
that basal activity may be considered to be equivalent to approximately 5000 steps per day, it is proposed that "high active" category be considered as those who move
at least 12,500 steps per day, or the equivalent in moderate and vigorous activities. This represents at least an hour more moderate-intensity activity over and above the
basal level of activity, or half an hour of vigorous-intensity activity over and above basal levels daily. These calculations were based on emerging results of pedometers
studies.2
This category provides a higher threshold of measures of total physical activity and is
a useful mechanism to distinguish variation in population groups. Also it could be used to set population targets for health-enhancing physical activity when multidomain
instruments, such as IPAQ are used.

1 Pate RR, Pratt M, Blair SN, Haskell WL , Macera CA, Bouchard C et al. Physical activity and public health. A recommendation
from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *Journal of American
Medical Association* 1995; 273(5):402-7. and U.S. Department of Health and Human Services. *Physical Activity and Health: A*

Report of the Surgeon General. Department of Health and Human Services, Centers for Disease Control and Prevention,
National Center for Chronic Disease Prevention and Health Promotion, The Presidents' Council on Physical Fitness and Sports:
Atlanta, GA:USA. 1996.
2 Tudor-Locke C, Bassett DR Jr. How many steps/day are enough? Preliminary pedometer indices for public health. Sports
Med. 2004;34(1):1-8.

'Moderate'

This category is defined as doing some activity, more than the low active category. It
is proposed that it is a level of activity equivalent to "half an hour of at least moderate-intensity PA on most days", the former leisure time-based physical activity
population health recommendation.

'Low'

This category is simply defined as not meeting any of the criteria for either of the previous categories.

5. Protocol for IPAQ Short Form

5.1 Continuous Scores

Median values and interquartile ranges can be computed for walking (W), moderateintensity
activities (M), vigorous-intensity activities (V) and a combined total physical activity score. All continuous scores are expressed in MET-minutes/week as defined
below.

5.2 MET Values and Formula for Computation of MET-minutes/week

The selected MET values were derived from work undertaken during the IPAQ Reliability Study undertaken in 2000-20013. Using the Ainsworth et al. Compendium
(*Med Sci Sports Med* 2000) an average MET score was derived for each type of activity. For example; all types of walking were included and an average MET value
for walking was created. The same procedure was undertaken for moderate-intensity
activities and vigorous-intensity activities. The following values continue to be used for the analysis of IPAQ data: Walking = 3.3 METs, Moderate PA = 4.0 METs and Vigorous PA = 8.0 METs. Using these values, four continuous scores are defined:

Walking MET-minutes/week = 3.3 * walking minutes * walking days
Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate days
Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days
Total physical activity MET-minutes/week = sum of Walking + Moderate + Vigorous METminutes/
week scores.

5.3 Categorical Score

Category 1 Low

This is the lowest level of physical activity. Those individuals who not meet criteria for Categories 2 or 3 are considered to have a 'low' physical activity level.
3 Craig CL,Marshall A , Sjostrom M et al. International Physical Activity Questionnaire: 12 country reliability and
validity *Med Sci Sports Exerc* 2003;August

Category 2 Moderate

The pattern of activity to be classified as 'moderate' is either of the following criteria:

a) 3 or more days of vigorous-intensity activity of at least 20 minutes per day

OR

b) 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day

OR

c) 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum Total physical activity of at least 600 MET-minutes/week.

Individuals meeting at least one of the above criteria would be defined as accumulating a minimum level of activity and therefore be classified as 'moderate'.

See Section 7.5 for information about combining days across categories.

Category 3 High

A separate category labelled 'high' can be computed to describe higher levels of participation.

The two criteria for classification as 'high' are:

a) vigorous-intensity activity on at least 3 days achieving a minimum Total physical activity of at least 1500 MET-minutes/week

OR

b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum Total physical activity of at least 3000 MET-minutes/week.

See Section 7.5 for information about combining days across categories.

5.4 Sitting Question in IPAQ Short Form

The IPAQ sitting question is an additional indicator variable of time spent in sedentary activity and is not included as part of any summary score of physical activity. Data on sitting should be reported as median values and interquartile ranges.

To-date there are few data on sedentary (sitting) behaviours and no well-accepted thresholds for data presented as categorical levels.

6. Protocol for IPAQ Long Form

The long form of IPAQ asks in detail about walking, moderate-intensity and vigorousintensity

physical activity in each of the four domains. Note: asking more detailed questions regarding physical activity within domains is likely to produce higher prevalence estimates than the more generic IPAQ short form.

6.1 Continuous Score

Data collected with the IPAQ long form can be reported as a continuous measure and reported as median MET-minutes. Median values and interquartile ranges can be computed for walking (W), moderate-intensity activities (M), and vigorous-intensity

activities (V) within each domain using the formulas below. Total scores may also be

calculated for walking (W), moderate-intensity activities (M), and vigorous-intensity activities (V); for each domain (work, transport, domestic and garden, and leisure) and for an overall grand total.

6.2 MET Values and Formula for Computation of MET-minutes

Work Domain

Walking MET-minutes/week at work = 3.3 * walking minutes * walking days at work

Moderate MET-minutes/week at work= 4.0 * moderate-intensity activity minutes * moderate-intensity

days at work

Vigorous MET-minutes/week at work= 8.0 * vigorous-intensity activity minutes * vigorous-intensity
days at work
Total Work MET-minutes/week =sum of Walking + Moderate + Vigorous MET-minutes/week scores at
work.

Active Transportation Domain

Walking MET-minutes/week for transport = 3.3 * walking minutes * walking days for transportation
Cycle MET-minutes/week for transport= 6.0 * cycling minutes * cycle days for transportation
Total Transport MET-minutes/week = sum of Walking + Cycling MET-minutes/week scores for
transportation.

Domestic and Garden [Yard Work] Domain

Vigorous MET-minutes/week yard chores= 5.5 * vigorous-intensity activity minutes * vigorous-intensity
days doing yard work (**Note**: the MET value of 5.5 indicates that vigorous garden/yard work should
be considered a moderate-intensity activity for scoring and computing total moderate intensity
activities.)
Moderate MET-minutes/week yard chores= 4.0 * moderate-intensity activity minutes * moderateintensity
days doing yard work
Moderate MET-minutes/week inside chores= 3.0* moderate-intensity activity minutes * moderateintensity
days doing inside chores.
Total Domestic and Garden MET-minutes/week =sum of Vigorous yard + Moderate yard + Moderate
inside chores MET-minutes/week scores.

Leisure-Time Domain

Walking MET-minutes/week leisure = 3.3 * walking minutes * walking days in leisure
Moderate MET-minutes/week leisure = 4.0 * moderate-intensity activity minutes * moderate-intensity
days in leisure
Vigorous MET-minutes/week leisure = 8.0 * vigorous-intensity activity minutes * vigorous-intensity
days in leisure
Total Leisure-Time MET-minutes/week = sum of Walking + Moderate + Vigorous MET-minutes/week
scores in leisure.

Total Scores for all Walking, Moderate and Vigorous Physical Activities

Total Walking MET-minutes/week = Walking MET-minutes/week (at Work + for Transport + in Leisure)
Total Moderate MET-minutes/week total = Moderate MET-minutes/week (at Work + Yard chores +
inside chores + in Leisure time) + Cycling Met-minutes/week for Transport + Vigorous Yard chores
MET-minutes/week
Total Vigorous MET-minutes/week = Vigorous MET-minutes/week (at Work + in Leisure)

Note: Cycling MET value and Vigorous garden/yard work MET value fall within the coding range of
moderate-intensity activities.

Total Physical Activity Scores

An overall total physical activity MET-minutes/week score can be computed as:
Total physical activity MET-minutes/week = sum of Total (Walking + Moderate + Vigorous) METminutes/
week scores.
This is equivalent to computing:
Total physical activity MET-minutes/week = sum of Total Work + Total Transport + Total Domestic and
Garden + Total Leisure-Time MET-minutes/week scores.
As there are no established thresholds for presenting MET-minutes, the IPAQ Research Committee proposes that these data are reported as comparisons of median values and interquartile ranges for different populations.

6.3 Categorical Score

As noted earlier, regular participation is a key concept included in current public health guidelines for physical activity.4 Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. There are three levels of physical activity proposed to classify populations – 'low', 'moderate', and 'high'. The criteria for these levels are the same as for the IPAQ short [described
earlier in Section 4.2]

Category 1 Low

This is the lowest level of physical activity. Those individuals who not meet criteria for Categories 2 or 3 are considered 'low'.

Category 2 Moderate

The pattern of activity to be classified as 'moderate' is either of the following criteria:
d) 3 or more days of vigorous-intensity activity of at least 20 minutes per day
OR
e) 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day
OR
4 Pate RR, Pratt M, Blair SN, Haskell WL , Macera CA, Bouchard C et al. Physical activity and public health. A recommendation
from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *Journal of American*
Medical Association 1995; 273(5):402-7. and U.S. Department of Health and Human Services. *Physical Activity and Health: A*
Report of the Surgeon General. Department of Health and Human Services, Centers for Disease Control and Prevention,
National Center for Chronic Disease Prevention and Health Promotion, The Presidents' Council on Physical Fitness and Sports:
Atlanta, GA:USA. 1996.

f) 5 or more days of any combination of walking, moderate-intensity or vigorousintensity
activities achieving a minimum Total physical activity of at least 600 MET-minutes/week.
Individuals meeting at least one of the above criteria would be defined as accumulating a moderate level of activity. See Section 7.5 for information about combining days across categories.

Category 3 High

A separate category labelled 'high' can be computed to describe higher levels of

participation.
The two criteria for classification as ‘high’ are:
a) vigorous-intensity activity on at least 3 days achieving a minimum Total physical activity of at least 1500 MET-minutes/week
OR
b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum Total physical activity of at least 3000 MET-minutes/week.
See Section 7.5 for information about combining days across categories.

6.4 IPAQ Sitting Question IPAQ Long Form

The IPAQ sitting question is an additional indicator variable and is not included as part of any summary score of physical activity. To-date there are few data on sedentary (sitting) behaviours and no well-accepted thresholds for data presented as
categorical levels. For the sitting question ‘Minutes’ is used as the indicator to reflect
time spent in sitting rather than MET-minutes which would suggest an estimate of energy expenditure.
IPAQ long assesses an estimate of sitting on a typical weekday, weekend day and time spent sitting during travel (see transport domain questions).

Summary sitting variables include

Sitting Total Minutes/week = weekday sitting minutes* 5 weekdays + weekend day sitting minutes* 2
weekend days
Average Sitting Total Minutes/day = (weekday sitting minutes* 5 weekdays + weekend day sitting
minutes* 2 weekend days) / 7
Note: The above calculation of ‘Sitting Total’ excludes time spent sitting during travel because the
introduction in IPAQ long directs the responder to NOT include this component as it would have
already been captured under the Transport section. If a summary sitting variable including time spent
sitting for transport is required, it should be calculated by adding the time reported (travelling in a
motor vehicle) under transport to the above formula. Care should be taken in reporting these alternate
data to clearly distinguish the ‘total sitting’ variable from a ‘total sitting – including transport’ variable.
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7. Data Processing Rules

In addition to a standardized approach to computing categorical and continuous measures of physical activity, it is necessary to undertake standard methods for the
cleaning and treatment of IPAQ datasets. The use of different approaches and rules
would introduce variability and reduce the comparability of data.
There are no established rules for data cleaning and processing on physical activity.
Thus, to allow more accurate comparisons across studies IPAQ Research Committee
has established and recommends the following guidelines:

7.1 Data Cleaning

I. Any responses to duration (time) provided in the hours and minutes response option should be converted from hours and minutes into minutes.

II. To ensure that responses in ‘minutes’ were not entered in the ‘hours’ column by mistake during self-completion or during data entry process, values of ‘15’, ‘30’, ‘45’, ‘60’ and ‘90’ in the ‘hours’ column should be converted to ‘15’, ‘30’, ‘45’, ‘60’ and ‘90’ minutes, respectively, in the minutes column.
III. In some cases duration (time) will be reported as weekly (not daily) e.g., VWHRS, VWMINS. These data should be converted into an average daily time by dividing by 7.
IV. If ‘don’t know’ or ‘refused ‘ or data are missing for time or days then that case is removed from analysis.
Note: Both the number of days *and* daily time are required for the creation of categorical and
continuous summary variables

7.2 Maximum Values for Excluding Outliers

This rule is to exclude data which are unreasonably high; these data are to be considered outliers and thus are excluded from analysis. All cases in which the sum
total of all Walking, Moderate and Vigorous time variables is greater than 960 minutes (16 hours) should be excluded from the analysis. This assumes that on average an individual of 8 hours per day is spent sleeping.
The ‘days’ variables can take the range 0-7 days, or 8, 9 (don’t know or refused); values greater than 9 should not be allowed and those cases excluded from analysis.

7.3 Minimum Values for Duration of Activity

Only values of 10 or more minutes of activity should be included in the calculation of
summary scores. The rationale being that the scientific evidence indicates that episodes or bouts of at least 10 minutes are required to achieve health benefits. Responses of less than 10 minutes [and their associated days] should be re-coded to
‘zero’.

7.4 Truncation of Data Rules

This rule attempts to normalize the distribution of levels of activity which are usually
skewed in national or large population data sets.
In IPAQ short - it is recommended that all Walking, Moderate and Vigorous time variables exceeding ‘ 3 hours’ or ‘180 minutes’ are truncated (that is re-coded) to be
equal to ‘180 minutes’ in a new variable. This rule permits a maximum of 21 hours of
activity in a week to be reported for each category (3 hours * 7 days).
In IPAQ long – the truncation process is more complicated, but to be consistent with
the approach for IPAQ short requires that the variables total Walking, total Moderateintensity
and total Vigorous-intensity activity are calculated and then, for each of these summed behaviours, the total value should be truncated to 3 hours (180 minutes).
When analysing the data as categorical variable or presenting median and interquartile ranges of the MET-minute scores, the application of the truncation rule will not affect the results. This rule does have the important effect of preventing misclassification in the ‘high’ category. For example, an individual who reports walking for 10 minutes on 6 days and 12 hours of moderate activity on one day could
be coded as ‘high’ because this pattern meets the ‘7 day” and “3000 MET-min”

criteria for ‘high’. However, this uncommon pattern of activity is unlikely to yield the health benefits that the ‘high’ category is intended to represent.
Although using median is recommended due to the skewed distribution of scores, if IPAQ data are analysed and presented as a continuous variable using mean values,
the application of the truncation rule will produce slightly lower mean values than would otherwise be obtained.

7.5 Calculating MET-minute/week Scores

Data processing rules 7.2, 7.3, and 7.4 deals first with excluding outlier data, then secondly, with recoding minimum values and then finally dealing with high values. These rules will ensure that highly active people remain classified as ‘high’, while decreasing the chances that less active individuals are misclassified and coded as ‘high’.
Using the resulting variables, convert time and days to MET-minute/week scores [see above Sections 5.2 and 6.2; METS x days x daily time].

7.6 Calculating Total Days for Presenting Categorical Data on Moderate and High Levels

Presenting IPAQ data using categorical variables requires the total number of ‘days’
on which all physical activity was undertaken to be assessed. This is difficult because
frequency in ‘days’ is asked separately for walking, moderate-intensity and vigorousintensity
activities, thus allowing the total number of ‘days’ to range from a minimum

of 0 to a maximum of 21’days’ per week in IPAQ short and higher in IPAQ long. The
IPAQ instrument does not record if different types of activity are undertaken on the same day.
In calculating ‘moderately active’, the primary requirement is to identify those individuals who undertake activity on at least ‘5 days’/week [see Sections 4.2 and 5.3]. Individuals who meet this criterion should be coded in a new variable called “*at*
least five days” and this variable should be used to identify those meeting criterion b)
at least 30 minutes of moderate-intensity activity and/or walking; and those meeting
criterion c) any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of 600 MET-minutes/week.
Below are two examples showing this coding in practice:
i) an individual who reports ‘2 days of moderate-intensity’ and ‘3 days of walking’ should be coded as a value indicating “*at least five days”*;
ii) an individual reporting ‘2 days of vigorous-intensity’, ‘2 days of moderateintensity’
and ‘2 days of walking should be coded as a value to indicate “*at*
least five days” [even though the actual total is 6].
The original frequency of ‘days’ for each type of activity should remain in the data file
for use in the other calculations.
The same approach as described above is used to calculate total days for computing
the ‘high’ category. The primary requirement according to the stated criteria is to identify those individuals who undertake a combination of walking, moderate-intensity
and or vigorous-intensity activity on at least 7 days/week [See section 4.2].

Individuals who meet this criterion should be coded as a value in a new variable to reflect *"at least 7 days*".
Below are two examples showing this coding in practice:
i) an individual who reports '4 days of moderate-intensity' and '3 days of walking' should be coded as the new variable *"at least 7 days*".
ii) an individual reporting '3 days of vigorous-intensity', '3 days moderateintensity' and '3 days walking' should be coded as *"at least 7 days*" [even
though the total adds to 9] .

8. Summary algorithms

The algorithms in Appendix 1 and Appendix 2 to this document show how these rules
work in an analysis plan, to develop the categories 1 [Low], 2 [Moderate], and 3 [High] levels of activity.

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IPAQ Scoring Protocol (Short Forms)
Continuous Score
Expressed as MET-min per week: MET level x minutes of activity/day x days per week
Sample Calculation
MET levels MET-minutes/week for 30 min/day, 5 days
Walking = 3.3 METs 3.3*30*5 = 495 MET-minutes/week
Moderate Intensity = 4.0 METs 4.0*30*5 = 600 MET-minutes/week
Vigorous Intensity = 8.0 METs 8.0*30*5 = 1,200 MET-minutes/week

_____
TOTAL = 2,295 MET-minutes/week
Total MET-minutes/week = Walk (METs*min*days) + Mod (METs*min*days) + Vig (METs*min*days)
Categorical Score- three levels of physical activity are proposed
1. Low
□□No activity is reported **OR**
□□Some activity is reported but not enough to meet Categories 2 or 3.
2. Moderate
Either of the following 3 criteria
□□3 or more days of vigorous activity of at least 20 minutes per day **OR**
□□5 or more days of moderate-intensity activity and/or walking of at least 30 minutes
per day **OR**
□□5 or more days of any combination of walking, moderate-intensity or vigorousintensity
activities achieving a minimum of at least 600 MET-minutes/week.
3. High
Any one of the following 2 criteria
□□Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR**
□□7 or more days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 MET-minutes/week
Please review the full document "Guidelines for the data processing and analysis of the International
Physical Activity Questionnaire" for more detailed description of IPAQ analysis and recommendations for
data cleaning and processing [www.ipaq.ki.se].
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IPAQ Scoring Protocol (Long Forms)
Continuous Score
Expressed as MET-minutes per week: MET level x minutes of activity/day x days per week
Sample Calculation
MET levels MET-minutes/week for 30 min/day, 5 days
Walking at work= 3.3 METs 3.3*30*5 = 495 MET-minutes/week
Cycling for transportation= 6.0 METs 6.0*30*5 = 900 MET-minutes/week
Moderate yard work= 4.0 METs 4.0*30*5 = 600 MET-minutes/week
Vigorous intensity in leisure= 8.0 METs 8.0*30*5 = 1,200 MET-minutes/week

_____
TOTAL = 3,195 MET-minutes/week
Domain Sub Scores
Total MET-minutes/week at **work** = Walk (METs*min*days) + Mod (METs*min*days) + Vig
(METs*min*days) at work
Total MET-minutes/week for **transportation** = Walk (METs*min*days) + Cycle (METs*min*days) for transportation
Total MET-minutes/week from **domestic and garden** = Vig (METs*min*days) yard work +
Mod (METs*min*days) yard work + Mod (METs*min*days) inside chores
Total MET-minutes/week in **leisure-time** = Walk (METs*min*days) + Mod (METs*min*days)
+ Vig (METs*min*days) in leisure-time
Walking, Moderate-Intensity and Vigorous-Intensity Sub Scores
Total **Walking** MET-minutes/week = Walk MET-minutes/week (at Work + for Transport + in
Leisure)
Total **Moderate** MET-minutes/week = Cycle MET-minutes/week for Transport + Mod METminutes/
week (Work + Yard chores + Inside chores + Leisure) + Vigorous Yard chores METminutes
Note: The above is a total moderate activities only score. If you require a total of all moderate-intensity
physical activities you would sum Total Walking and Total Moderate
Total **Vigorous** MET-minutes/week = Vig MET-minutes/week (at Work + in Leisure)
Total Physical Activity Score
Total Physical Activity MET-minutes/week = **Walking** MET-minutes/week + **Moderate** METminutes/
week + Total **Vigorous** MET-minutes/week
Continued……..

Also
Total Physical Activity MET-minutes/week = Total MET-minutes/week (at Work + for
Transport + in Chores + in Leisure)
Categorical Score- three levels of physical activity are proposed
1. Low
No activity is reported **OR**
a. Some activity is reported but not enough to meet Categories 2 or 3.
2. Moderate
Either of the following 3 criteria
a. 3 or more days of vigorous-intensity activity of at least 20 minutes per day **OR**

b. 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day **OR**
c. 5 or more days of any combination of walking, moderate-intensity or vigorousintensity
activities achieving a minimum of at least 600 MET-min/week.

3. High

Any one of the following 2 criteria

- Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR**
- 7 or more days of any combination of walking, moderate- or vigorous- intensity activities accumulating at least 3000 MET-minutes/week

Please review the full document "Guidelines for the data processing and analysis of the International Physical Activity Questionnaire" for more detailed description of IPAQ analysis and recommendations for data cleaning and processing [www.ipaq.ki.se].

Appendix 3 Matlab codes for liver and pancreas quantification

Cross-referenced in Chapter 2 Page 72

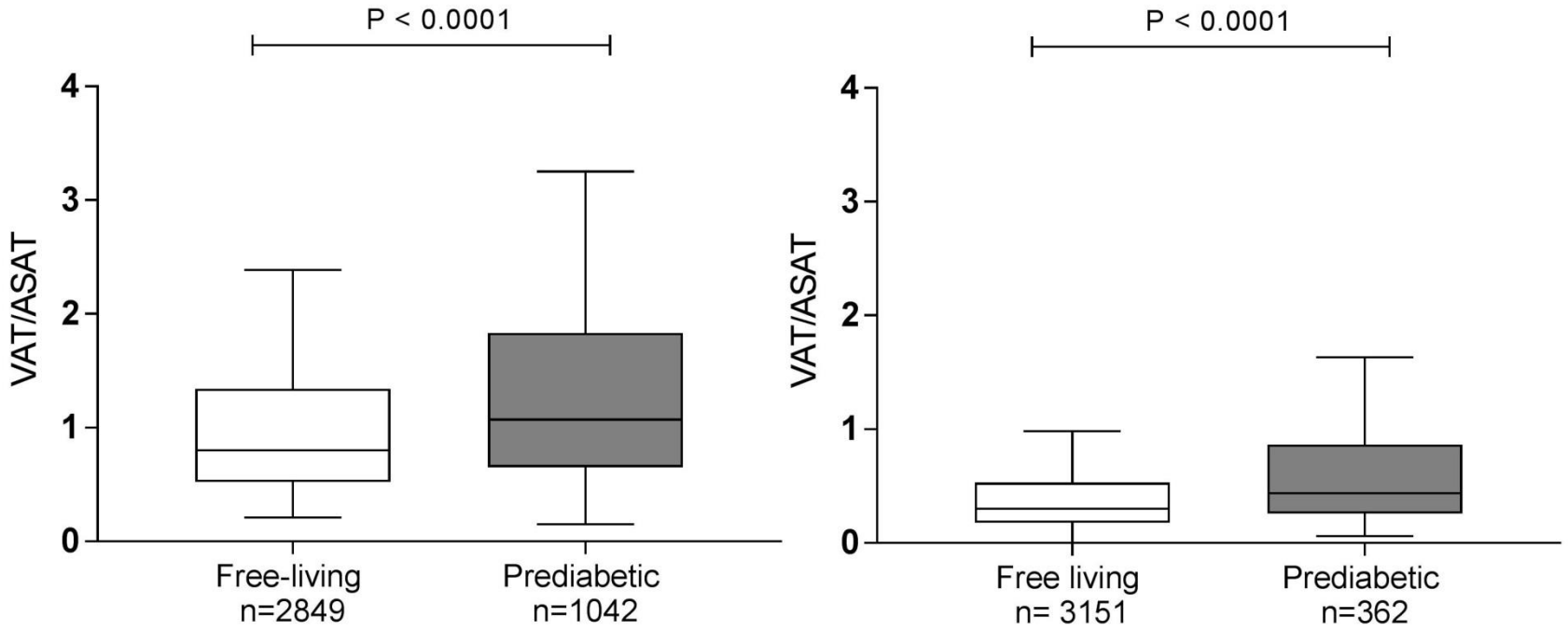
Matlab codes for liver and pancreas quantification

```
%% Create fat map
a = x(:,:,1);
b = x(:,:,2);
fatfrac =b./(a+b);
%fatfrac=a./(a+b);

figure('Name','Fat Fraction','NumberTitle','on');imagesc(fatfrac,
[0 0.5]);
axis image;

%mask adipose tissue
%fprintf(1,'Draw Adipose Mask (please)\n');

%%% 23-5-13: Ask user to draw NOT fat (before drew fat)
fprintf(1,'Please draw Adipose Mask (draw around everything that is
NOT FAT)\n');

mask3=roipoly;close all
mask3 = ~mask3; %<---- flip definition of mask (23-5-13)

fatfrac = fatfrac.*abs(1-mask3);
x(:,:,3) = x(:,:,3).*abs(1-mask3);



%% mask background and blood vessels

%2sd threshold for vessels and exclude outliers (T2*>50)
x3=x(:,:,3);
t2_threshold = median(x3((x3>0)&(x3<50)))+2*std
(x3((x3>0)&(x3<50)));

m1 = (x(:,:,3)<t2_threshold)&(x(:,:,3)>0);

%erode mask around blood vessels
erodedmask = imerode(m1,strel('disk',1));
%figure('Name','T2* mask','NumberTitle','on');imagesc(erodedmask);
%axis image;


%liver only image and histogram
liveronly = fatfrac.*erodedmask;
figure('Name','Masked fat','NumberTitle','on');imagesc(liveronly,
[0 0.4]);
axis image;
mean_liver_fat = mean(liveronly(erodedmask));
stan_dev_liver_fat = std (liveronly(erodedmask));
fprintf(1,'Liver fat = %1.2f +/- %1.2f
percent\n',mean_liver_fat*100,stan_dev_liver_fat*100);

%%% 18-6-13: At this point, calculate R2*
t2star = x(:,:,3);
```

```
r2star = 1e3./t2star;
r2star(isinf(r2star))=0;

%T2* map
nr=1;nc=2;
figure('Name','T2*','NumberTitle','on');
subplot(nr,nc,1)
imagesc(t2star, [0 80]);colorbar
axis image;title('T2*/ms')
subplot(nr,nc,2)
imagesc(r2star, [0 50]);colorbar


%Liver only T2* map
nr=1;nc=2;
figure('Name','Masked T2*','NumberTitle','on');
subplot(nr,nc,1)
imagesc(t2star.*erodedmask, [0 80]);colorbar
axis image;title('T2*/ms')
subplot(nr,nc,2)
imagesc(r2star.*erodedmask, [0 50]);colorbar
axis image;title('R2*/s^{')



mean_liver_t2star = mean(t2star(erodedmask));
stan_dev_liver_t2star = std (t2star(erodedmask));
mean_liver_r2star = mean(r2star(erodedmask));
stan_dev_liver_r2star = std (r2star(erodedmask));

fprintf(1,'Liver T2* = %1.2f +/- %1.2f
ms\n',mean_liver_t2star,stan_dev_liver_t2star);
fprintf(1,'Liver R2* = %1.2f +/- %1.2f s^-
1\n',mean_liver_r2star,stan_dev_liver_r2star);

%% fat histogram
[h, bin] = histc(liveronly(erodedmask),0:0.01:0.5);
figure('Name','Fat
histogram','NumberTitle','on');plot(0:0.01:0.5,h);

%% T2 hist
edges = 1:1:150;
x3=x(:,:,3);[h, bin] = histc(x3(:),edges);
figure('Name','T2* histogram','NumberTitle','on');plot(edges,h);
```

Appendix 4 Detailed Gender specific phenotyping of VAT, ASAT and liver fat between free-living and pre-diabetic population

Cross-references in Chapter 2 Page 120



Gender specific phenotyping of VAT/ASAT ratio between free-living and pre-diabetic population in (**A**) men and (**B**) women. Data presented as box and whisper plots: where error bars are min/max range, upper and lower edges are 25th and 75th percentiles. *p* values are calculated from nonparametric Mann-Whitney in SPSS (v.23). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. VAT; visceral adipose tissue, ASAT; abdominal subcutaneous adipose tissue. Graphs were performed using GraphPad Prism version 5.0. This graph shows that overall the ratio of VAT/ASAT is higher in males (A) than in females (B) in both population which might be partially due to the males has higher percentage in particular in pre-diabetic populations. The gender pattern between two populations are consistent with what was seeing in VAT and ASAT separately.

Detailed Gender specific phenotyping of VAT, ASAT and liver fat between free-living and pre-diabetic population in. Data presented as mean ± Standard deviations. ¶ indicates significance of p>0.001 calculated from nonparametric Mann-Whitney in SPSS (v.23). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. VAT; visceral adipose tissue, ASAT; abdominal subcutaneous adipose tissue

	Male						**Female**					
	VAT		ASAT		Liver fat		VAT		ASAT		Liver fat	
	Free-living	Pre-diabetic	Free-living	Pre-diabetic	Free-living	Pre-diabetic	Free-living	Pre-diabetic	Free-living	Pre-diabetic	Free-living	Pre-diabetic
N number	2849	1045	2849	1042	2839	1120	3136	362	3136	362	3132	428
Mean ± SD	4.9 ± 2.3	6.0 ± 2.3¶	5.8 ± 2.5	5.7 ± 2.4¶	4.7 ± 4.7	5.9 ± 5.4¶	2.1 ± 1.5	4.2 ± 1.9¶	8.0 ± 3.4	9.3 ± 3.5¶	3.6 ± 4.5	7.3 ± 7.0¶
Minimum	0.35	0.4191	0.65	0.9088	0.65	0.26	0.1	0.2036	0.77	1.882	0.45	0.35
25% Percentile	3.255	4.303	4.27	4.122	1.74	2.15	1.46	2.824	5.57	6.564	1.32	2.17
Median	4.63	5.971	5.5	5.397	2.87	4.105	2.33	4.002	7.5	8.688	1.96	4.615
75% Percentile	6.39	7.567	7.07	6.873	5.69	7.805	3.51	5.361	9.93	11.89	3.698	9.66
Maximum	14.41	14.49	22.32	21.94	34.04	37.59	12.09	10.79	23.48	20.89	34.5	34.81

Detailed Gender specific phenotyping of VAT, ASAT and liver fat between free-living and pre-diabetic population in. Data presented as mean ± Standard deviations. ¶ indicates significance of p>0.001 calculated from nonparametric Mann-Whitney in SPSS (v.23). Free living population data obtained from UK biobank and pre-diabetic data obtained from DIRECT IMI. VAT; visceral adipose tissue, ASAT; abdominal subcutaneous adipose tissue.

	Free living population	**Pre-diabetic population**	***p* value**	**Mean differences between free-living and pre-diabetic groups (95% confidence interval)**
Age (years)	61.7 ± 7.1 (44-73)	61.0 ± 7.2 (30 – 75)	ns	-
Height (cm)	169.5 ± 9.2	173.2 ± 8.7	< 0.0001	-3.70 (-4.21 to -3.18)
Weight (kg)	75.8 ± 15.1	86.4 ± 14.4	< 0.0001	-10.6 (-11.2 to -9.99)
BMI (kg/m^2)	26.7 ± 4.40	28.8 ± 4.50	<0.0001	-2.10 (-2.24 to -1.96)
WHR	0.86 ± 1.37	0.95 ± 0.07	0.009	0.09 (0.0217 to 0.1583)
SBP (mmHg)	133.9 ± 17.7	129.5 ± 18.1	<0.0001	-4.9 (-5.92 to -3.87)
DBP (mmHg)	78.7 ± 10.0	95.8 ± 12.2	<0.0001	16 (15.1 to 17.2)

Appendix 5 UK Biobank ethnicity collaborator manuscript (Genome-wide genetic data on ~500,000 UK Biobank participants)
Cross-referenced in Chapter 4 page 195

Manuscript title: Genome-wide genetic data on ~500,000 UK Biobank participants

Clare Bycroft et al

Abstract

The UK Biobank project is a large prospective cohort study of ~500,000 individuals from across the United Kingdom, aged between 40-69 at recruitment. A rich variety of phenotypic and health-related information is available on each participant, making the resource unprecedented in its size and scope. Here we describe the genome-wide genotype data (~805,000 markers) collected on all individuals in the cohort and its quality control procedures. Genotype data on this scale offers novel opportunities for assessing quality issues, although the wide range of ancestries of the individuals in the cohort also creates particular challenges. We also conducted a set of analyses that reveal properties of the genetic data – such as population structure and relatedness – that can be important for downstream analyses. In addition, we phased and imputed genotypes into the dataset, using computationally efficient methods combined with the Haplotype Reference Consortium (HRC) and UK10K haplotype resource. This increases the number of testable variants by over 100-fold to ~96 million variants. We also imputed classical allelic variation at 11 human leukocyte antigen (HLA) genes, and as a quality control check of this imputation, we replicate signals of known associations between HLA alleles and many common diseases. We describe tools that allow efficient genome-wide association studies (GWAS) of multiple traits and fast phenome-wide association studies (PheWAS), which work together with a new compressed file format that has been used to distribute the dataset. As a further check of the genotyped and imputed datasets, we performed a test-case genome-wide association scan on a well-studied human trait, standing height.

Appendix 6.1 List of methods of calculation / definitions used through the thesis according to chapters' appearance to facilte interpretation of the results through all chapters

Not cross-referenced as it is a summary.

List of methods of calculation / definitions used through the thesis according to chapters' appearance

1. Chapter One

1.1 Fat markers:

1.1.1 Visceral adipose tissue: defined as the abdominal adipose tissue deposition in the abdominal cavity excluding abdominal subcutaneous adipose tissue.

1.1.2 Subcutaneous adipose tissue: defined as the adipose tissue deposition subcutaneously (under the skin).

1.1.3 Ectopic fat: defined as deposition of fat (triglycerides) in lean tissues such as liver and pancreas.

1.1.4 Liver fat: defined as the deposition of fat (triglycerides) within the liver cells (hepatocytes) – it is different from adipose tissue which is located within adipocytes

1.1.4 Pancreas fat: defined as the deposition of fat (triglycerides) within the pancreas cells - it is different from adipose tissue which is located within adipocytes

2. Chapter Two

2.1 Fat markers:

2.1.1 Visceral adipose tissue volume was recorded in litres (l) and defined as the volume of the adipose tissue within the abdominal cavity, excluding adipose tissue outside the abdominal skeletal muscles and adipose tissue and lipids within and posterior of the spine and posterior of the back muscles.

2.1.2 Abdominal subcutaneous adipose tissue volume was recorded in litres (l) and defined as subcutaneous adipose tissue in the abdomen from the top of the femoral head to the top of the thoracic vertebrae T9.

2.1.3 Liver fat : measured as a percentage (%) and defined as relative proportion of fat to water in the liver using the calculation = fat / (water + fat)

2.1.4 Pancreatic fat: measured as a percentage (%) and defined as relative proportion of fat to water in the pancreas using the calculation = fat / (water + fat).

2.2 Blood Glucose:

2.2.1 Pre-diabetes: (mmol/mml) defines as HbAc1 values ranging from 5.7 to 6.4% or 40-48 mmol/mol.

3. Chapter Three

3.1 Fat markers:

3.1.1 Visceral adipose tissue area was recorded in (cm^2) and represents the number of pixels within the abdominal cavity multiplied by the total area excluding the abdominal subcutaneous adipose tissue area.

3.1.2 Abdominal subcutaneous adipose tissue area was recorded in (cm^2) and represents the number of pixels within the subcutaneous abdominal cavity multiplied by the total area excluding the internal abdominal adipose tissue area.

3.2 Blood Glucose:

3.2.1 Normal Blood Glucose or normogycaemic: defined as blood glucose ranging between 4.5 to 5.5 mmol/L

3.2.2 Pre-diabetes or hyperglycemea: defined as impaired fasting glucose between 5.6 and 6.9 mmol/L or impaired glucose tolerance of > 7.0 mmol/L.

4. Chapter Four

4.1 Fat Markers

4.1.1 Visceral adipose tissue: volume was recorded in litres (l) is the volume of the adipose tissue within the abdominal cavity, excluding adipose tissue outside the abdominal skeletal muscles and adipose tissue and lipids within and posterior of the spine and posterior of the back muscles.

4.1.2 Abdominal subcutaneous adipose tissue volume was recorded in litres (l) subcutaneous adipose tissue in the abdomen from the top of the femoral head to the top of the thoracic vertebrae T9.

4.1.3 Liver fat:

4.1.3.1 The West London Observation Study, liver fat was measured as intra-hepatocellular lipid (IHCL) content as percentage ratio of the -CH2- (part of a chain of CH2 groups lipid resonances with references to water resonance. The value refer to the peak area of the IHCL peak with reference to the water peak after correcting for T1 and T2.

4.1.3.2 The UK Biobank Ethnicity project, liver fat was measured as a percentage (%) and defined as relative proportion of fat to water in the liver using the calculation = fat / (water + fat).

Appendix 6.2 List of main calculations/ definitions used in fat/glucose measurements

Not cross-referenced as it is a summary.

		measurement definition	unit
fat deposit	Liver fat	Measured via magnetic resonance spectroscopy as intra-hepatocellular lipid (IHCL) content as percentage ratio of the -CH2- (part of a chain of CH2 groups lipid resonances with references to water resonance. The value refer to the peak area of the IHCL peak with reference to the water peak after correcting for T1 and T2.	no unit as it is a ratio, some papers use %
	Liver fat	Measured via magnetic resonance imaging as the relative proportion of fat to water in the liver using the calculation = fat / (water + fat).	%
	Visceral adipose tissue	Measured via magentic resonance imaging as the volume of the adipose tissue within the abdominal cavity, excluding adipose tissue outside the abdominal skeletal muscles and adipose tissue and lipids within and posterior of the spine and posterior of the back muscles.	litre
	Visceral adipose tissue area	Measured measured via magentic resonance imaging as the number of pixels within the abdominal cavity multiplied by the total area excluding the abdominal subcutaneous adipose tissue area.	cm^2
	Abdominal subcutaneous adipose tissue	Measured measured via magentic resonance imaging as the subcutaneous adipose tissue in the abdomen from the top of the femoral head to the top of the thoracic vertebrae T9.	litre
	Abdominal subcutaneous adipose tissue area	Measured via magnetic resonance imaging as the number of pixels within the subcutaneous abdominal cavity multiplied by the total area excluding the internal abdominal adipose tissue area	cm^2
	Pancreatic fat	Measured via magnetic resonance imaging as the relative proportion of fat to water in the pancreas using the calculation = fat / (water + fat).	%
Biochemistry markers	Normal Blood Glucose or normogycaemic	blood plasma glucose ranging between 4.5 to 5.5	mmol/L
	Pre-diabetes or hyperglycemea	Impaired fasting plasma glucose between 5.6 and 6.9 mmol/L or impaired glucose tolerance of > 7.0	mmol/L
	Pre-diabetes	HbAc1 values ranging from 5.7 to 6.4%	mmol/mml