Ethnic differences in the role of ectopic fat in the development of type 2 diabetes between men of white European and black west African ethnicity

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Ethnic differences in the role of ectopic fat in the development of type 2 diabetes between men of white European and black west African ethnicity

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Abstract

Background: Ectopic fat is thought to play a central role in the pathophysiology of type 2 diabetes (T2D). Despite being disproportionately affected by T2D, black populations typically exhibit lower levels of ectopic fat compared to white populations; this paradox questions the role of ectopic fat in the development of T2D in black populations.

Aim: To assess ethnic differences in ectopic fat deposition and their relationships with metabolic parameters of T2D in white European (WE) and black west African (BWA) men at three glycaemic states: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D.

Methods: Fifty-one WE (23 NGT/10 IGT/18 T2D) and 50 BWA (20 NGT/10 IGT/20 T2D) men were recruited. All participants underwent: 1) a Dixon-magnetic resonance imaging scan to assess visceral adipose tissue (VAT), intrahepatic lipids (IHL) and intrapancreatic lipids (IPL); 2) a proton-magnetic resonance spectroscopy scan to assess intramyocellular lipids (IMCL); 3) a hyperinulinaemic-euglycaemic clamp, with the infusion of glucose and glycerol isotopes, to assess whole-body, hepatic, skeletal muscle and adipose tissue insulin sensitivity; and 4) a hyperglycaemic clamp to assess first- and second-phase insulin secretory function, insulin secretion rate and insulin clearance.

Results: In the combined glycaemic cohorts, VAT, IHL and IPL were significantly lower in the BWA men compared to the WE men (all $p<0.05$), however, IMCL did not differ by ethnicity ($p=0.74$). Differences in VAT, IHL, IPL and IMCL by glycaemic state were similar in both ethnic groups, indicated by non-significant ethnicity*glycaemic state interactions (all $p>0.05$). Relationships between ectopic fat depots and metabolic parameters were generally stronger in the WE men than BWA men. VAT, IHL and IPL showed significant inverse associations with adipose tissue insulin sensitivity in WE men but not BWA men (all $p<0.05$). Interrelationships between the ectopic fat depots were stronger in WE men than BWA men.

Conclusion: The greater ectopic fat levels in the T2D states compared to the NGT states in both WE and BWA men suggests ectopic fat plays a role in the development of T2D in both ethnic groups; however, the weaker relationships between ectopic fat depots and metabolic parameters of T2D in BWA men suggests ectopic fat may play a lesser role in T2D in BWA men compared with WE men. Furthermore, the lack of association between ectopic fat depots and adipose tissue insulin sensitivity in BWA men suggests that the role of adipose tissue dysfunction in driving ectopic fat storage may differ by ethnicity.

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Statement of Contribution

The Soul-Deep study was designed and formulated by Louise Goff (principal investigator), Stephanie Amiel, Margot Umpleby and Janet Peacock. Recruitment of participants and metabolic assessments were conducted by Cynthia Mohandas, Toyosi Bello, Chinmay Marathe and Meera Ladwa. Magnetic resonance imaging data acquisition was supervised by Geoffrey Charles-Edwards. Data on insulin sensitivity in chapters 3, 4, 5, and 6 were calculated by Toyosi Bello, Margot Umpleby, Fariba Shojaee-Moradie, Nicola Jackson and Lucy Coppin. Data on insulin secretion and insulin clearance in chapters 4 and 5 were calculated by Meera Ladwa, Linda Boselli and Riccardo Bonadonna.

The author analysed magnetic resonance imaging and magnetic resonance spectroscopy data, and conducted statistical analysis and interpretation of the MRI and metabolic data. The author interpreted data and composed the present thesis in discussion with Louise Goff.

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List of Abbreviations

^{1}H-MRS	Proton-Magnetic Resonance Spectroscopy
AIRg	Acute Insulin Response to Glucose
ANOVA	Analysis of Variance
ASAT	Abdominal Subcutaneous Adipose Tissue
BMI	Body Mass Index
BP	Blood Pressure
BSA	Body Surface Area
BWA	Black West African
CI	Confidence Interval
CT	Computed Tomography
CV	Coefficient of Variation
DEXA	Duel-Energy X-ray Absorptiometry
dSAT	Deep Subcutaneous Adipose Tissue
EGP	Endogenous Glucose Production
EMCL	Extramyocellular Lipid
FPG	Fasting Plasma Glucose
HbA1c	Glycated Haemoglobin
HDL	High Density Lipoprotein
HISI	Hepatic Insulin Sensitivity Index
iAUC	Incremental Area Under the Curve
IGT	Impaired Glucose Tolerance
IHL	Intrahepatic Lipid
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
IMCL	Intramyocellular Lipid
IPL	Intrapancreatic Lipid
IQR	Interquartile Range
ISF	Insulin Secretory Function
jMRUi	Java-based Magnetic Resonance User Interface
LDL	Low Density Lipoprotein
NASH	Non-Alcoholic Steatohepatitis

NAFLD	Non-Alcoholic Fatty Liver Disease
NEFA	Non-Esterified Fatty Acid
NGT	Normal Glucose Tolerance
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NMR	Nuclear Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
OGTT	Oral Glucose Tolerance Test
PRESS	Point Resolved Spectroscopy
PPM	Parts Per Million
Ra	Rate of Appearance
Rd	Rate of Disappearance
RF	Radio Frequency
ROI	Region of Interest
SAAM-II	Simulation, Analysis, and Modelling
SAT	Subcutaneous Adipose Tissue
SD	Standard Deviation
Soul-D	South London Diabetes
Soul-Deep	South London Diabetes and Ethnicity Phenotyping
sSAT	Superficial Subcutaneous Adipose Tissue
T2D	Type 2 Diabetes
TNF-α	Tumor Necrosis Factor-α
VAT	Visceral Adipose Tissue
VLDL-TG	Very Low Density Lipoprotien-Triglyceride
WE	White European

Chapter 1: Introduction

1.1 Thesis scope

Ectopic fat accumulation is emerging as one of the major factors that contributes to the development of type 2 diabetes (T2D) and appears to be a key feature that explains the well-known link between obesity and T2D. There is much evidence suggesting that the pathophysiology of T2D differs between populations of black and white ethnicity with several metabolic features differing between the two ethnic groups. Despite their greater prevalence of T2D, black populations typically exhibit lower levels of visceral and hepatic fat compared to their BMI-matched white counterparts, which has caused researchers to question the role of ectopic fat in the development of T2D in black populations. Some studies have suggested that black populations may be more sensitive to the detrimental effects of ectopic fat compared to white populations; however, others have suggested that ectopic fat plays a lesser role in the development of T2D in black populations. Addressing these speculations underlies the purpose of this thesis, which includes comprehensive analyses of ectopic fat depots and the metabolic parameters that are related to the development of T2D. Since T2D disproportionately affects populations of black ethnicity, it is of utmost importance to understand its pathophysiology in this high risk ethnic group, which may inform future ethnic-specific prevention and treatment strategies. Therefore, the overarching scope of this thesis is to investigate ethnic differences in the role of ectopic fat in the development of T2D between men of black west African and white European ethnicity across three glycaemic states: normal glucose tolerance, impaired glucose tolerance and T2D. The fat depots of interest include: visceral adipose tissue (VAT), intrahepatic lipids (IHL), intrapancreatic lipids (IPL) and intramyocellular lipids (IMCL).

1.2 Type 2 diabetes

Type 2 diabetes is a complex chronic metabolic disorder characterised by a persistent hyperglycaemic state caused by the inadequate production and utilisation of insulin (DeFronzo, 1988). T2D is detected clinically by the deterioration of several parameters including glycated haemoglobin (HbA1c), fasting plasma glucose (FPG) and postprandial glucose levels (Safai et al., 2018). The quality of life of T2D sufferers is highly affected due to the increased risk of macrovascular and microvascular damage, which can lead to chronic complications including cardiovascular disease, nephropathy, neuropathy and retinopathy (Muggeo, 1998, Jelinek et al., 2017). The treatment of T2D and its complications places a great financial strain on the National Health Service (NHS) in the UK where it is estimated to consume 10% of the total budget (Hex et al., 2012); therefore, there is strong emphasis on early intervention and prevention to minimise complications. The prevalence of T2D is increasing worldwide at an alarming rate (Zheng et al., 2017). According to data collected by the International Diabetes Federation in 2017, over 2.7 million adults in the UK have T2D with a further 0.5 million undiagnosed (IDF, 2017). The expected global increase in the prevalence of T2D by 2045 is presented in Figure 1.1, which shows the greatest increase is expected to occur in Africa (IDF, 2017) mainly due to the increase of westernisation and industrialisation (Cho et al., 2018). The current burden of T2D in Africa is largely underestimated due to the high percentage of undiagnosed cases of T2D in comparison to other regions; indeed, Africa has the highest percentage of undiagnosed cases of T2D worldwide with 69% of cases undiagnosed, which is almost double that of Europe (38%) (IDF, 2017).

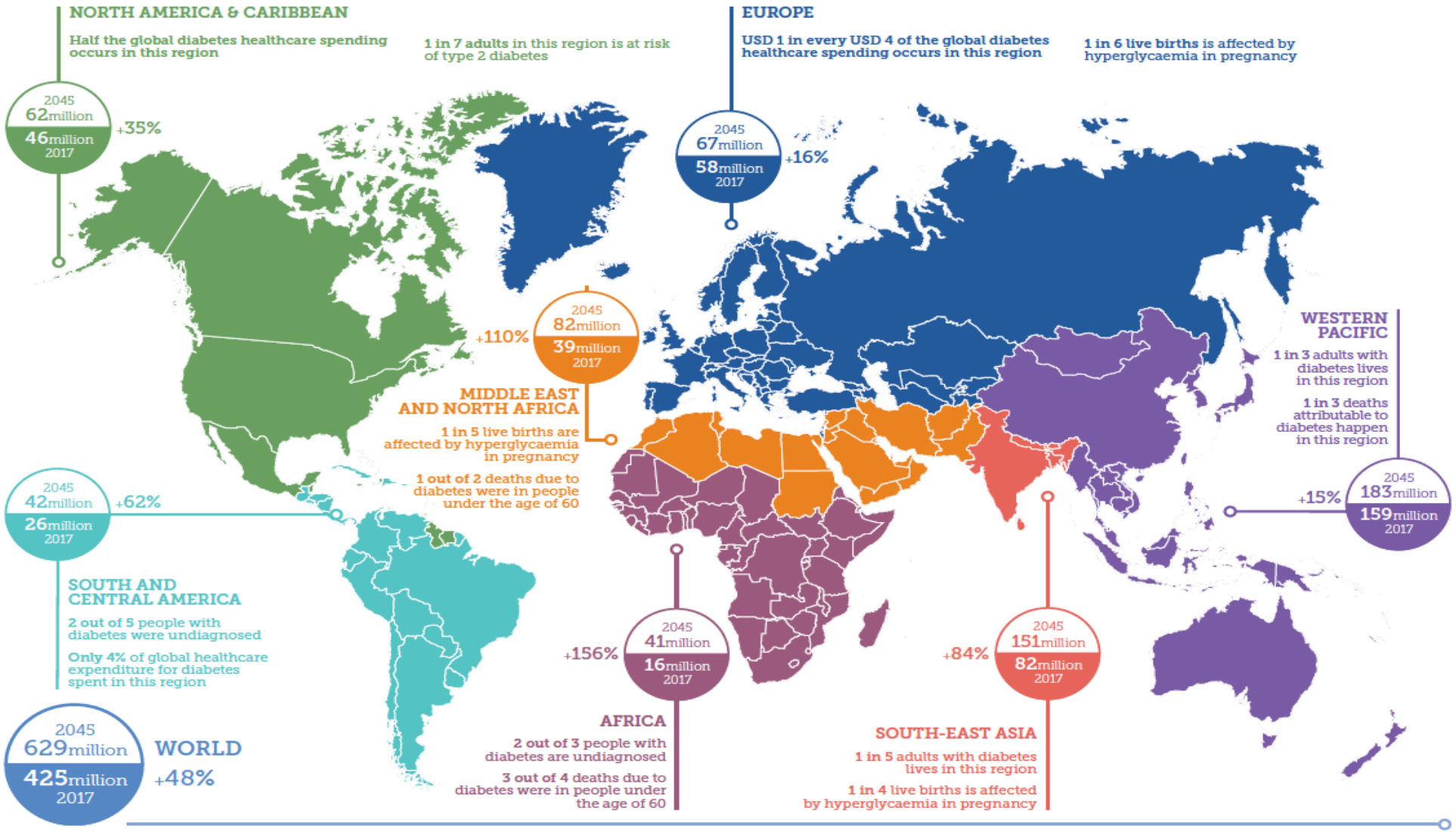

Figure 1.1: *Global estimations of the increase in prevalence of T2D. International Diabetes Federation - diabetes atlas 2017 (IDF, 2017).*

1.2.1 Type 2 diabetes in UK ethnic minority groups

The disproportionate burden of T2D is not limited to black populations in Africa, since, in the UK, black populations are also at greater risk of T2D compared to the general population. Individuals that identify as being from an ethnic minority background make up 14% of the UK population (ONS, 2011). Of those, 25% identify as being from a black African/black Caribbean/black British ethnic group, which is the second largest ethnic minority group in the UK (ONS, 2011). Considering the high percentage of ethnic minority groups in the UK, the Health Survey for England, conducted in 2004, took a specific focus on the health status of these ethnic minority groups (HSE, 2005). The resultant report showed that doctor-diagnosed diabetes is greater in several ethnic minority groups, including black African and black Caribbean groups, compared to the general population. Figure 1.2 shows the prevalence rates of diabetes stratified by ethnicity and age, which shows doctor-diagnosed diabetes is 2-3 times greater in black ethnic groups than the general population; this trend is also present within the younger age groups and is particularly evident in the black African men.

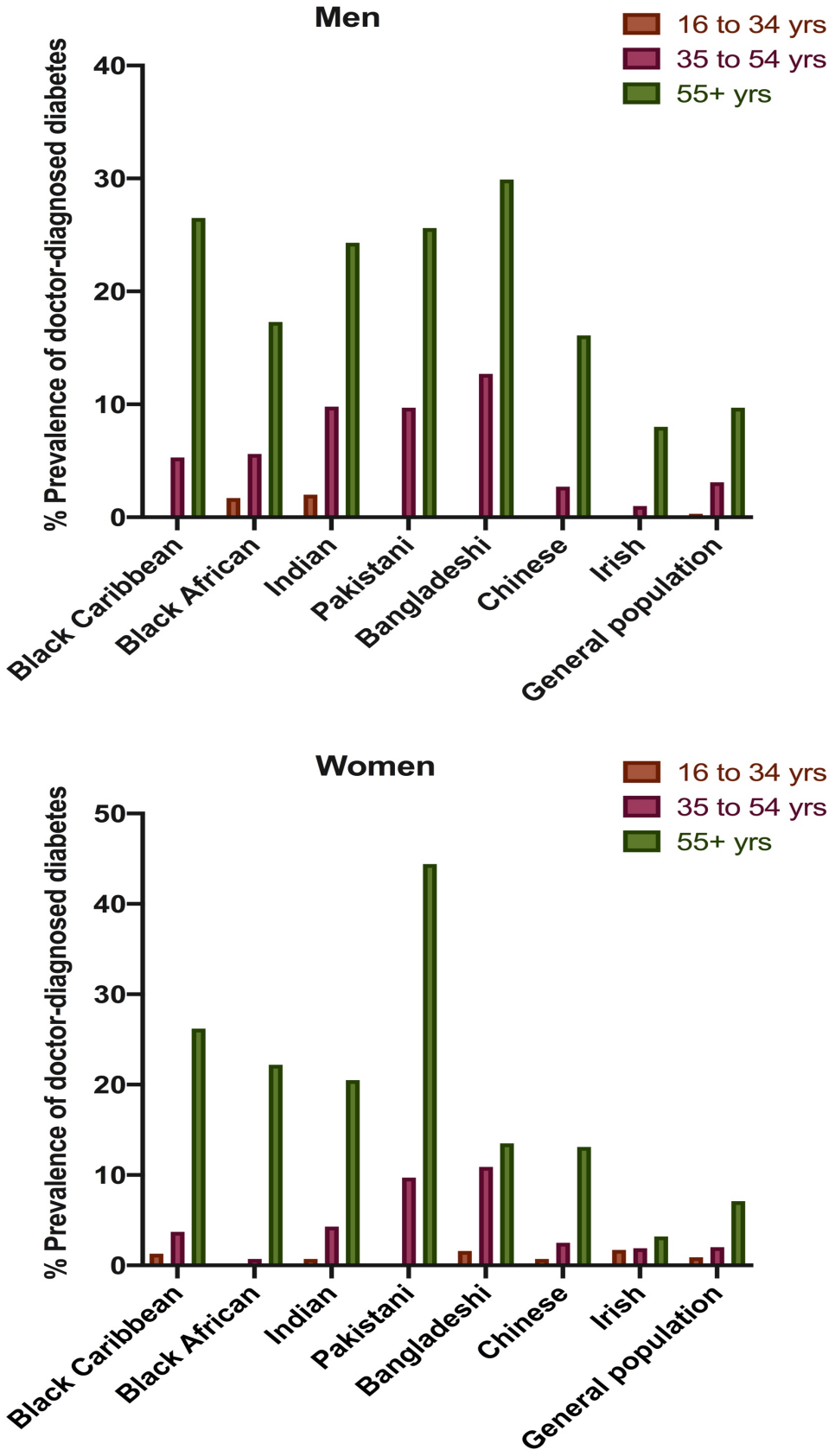

Figure 1.2: *Prevalence of doctor-diagnosed diabetes stratified by age and ethnicity in the UK population. Created from data extracted from the Health Survey for England, 2004 (HSE, 2005).*

1.2.2 Normal glucose homeostasis

To understand the pathophysiology of T2D, it is important to first consider normal glucose homeostasis in the human body. In an individual of normal glucose tolerance (NGT), when a meal is consumed blood glucose levels increase which signal for pancreatic beta-cells to secrete insulin into the circulation (Cernea and Dobreanu, 2013). Insulin signals to the peripheral organs, mainly the muscles and liver, to absorb glucose for storage as glycogen, as well as signalling to the liver to inhibit the breakdown of glycogen into glucose (glycogenolysis); these processes allow glucose to be utilised and stored effectively in the body and maintain a normoglycaemic state (Aronoff et al., 2004).

1.2.3 Pathophysiology of type 2 diabetes

There is a long-established understanding that T2D is characterised by hyperglycaemia that typically results from a combination of insulin resistance and insufficient beta-cell insulin secretory function to overcome the insulin resistance (DeFronzo, 1988); however, the understanding of the pathophysiology of T2D that underlies these features has evolved greatly over the past 100 years. In 1922, the discovery of insulin led researchers to propose that it was the inadequate production of insulin by pancreatic beta-cells that was the key driver of T2D (Polonsky, 2012). In an attempt to understand the cause of beta-cell failure, researchers discovered that insulin resistance precedes beta-cell failure, thus placing the muscles, liver and pancreas central to the pathophysiology of T2D (DeFronzo, 1988). However, more recent research suggests that dysfunctional adipose tissue is a major cause of insulin resistance and plays a key role in the pathophysiology of T2D (Smith and Kahn, 2016, Snijder et al., 2005, Longo et al., 2019).

Dysfunctional adipose tissue is characterised by several features including low-grade chronic inflammation, insulin resistance and a reduced ability to store excess energy as triglycerides in adipose tissues (Shulman, 2014). This leads to the storage of triglycerides

in non-adipose tissues including the muscles, liver and pancreas, which is termed ectopic fat. The lipid intermediates generated from the flux of these ectopic fat stores are toxic to the cells they reside in and are proposed to cause insulin resistance (Snel et al., 2012).

It is now accepted that the development of T2D is more complex than initially appreciated. Even though the muscles, liver and pancreas are the key organs that maintain normal glucose homeostasis (DeFronzo, 1988), it has now become apparent that several organs may play a role in the onset and progression of T2D (Cornell, 2015, Defronzo, 2009). Figure 1.3 shows the interaction of various organs with insulin resistance that may play a role in the pathophysiology of T2D (Cornell, 2015). The role of the muscles, liver, pancreas and adipose tissue in the pathophysiology of T2D are further addressed below while the role of other peripheral organs shown in Figure 1.3 are beyond the scope of this thesis.

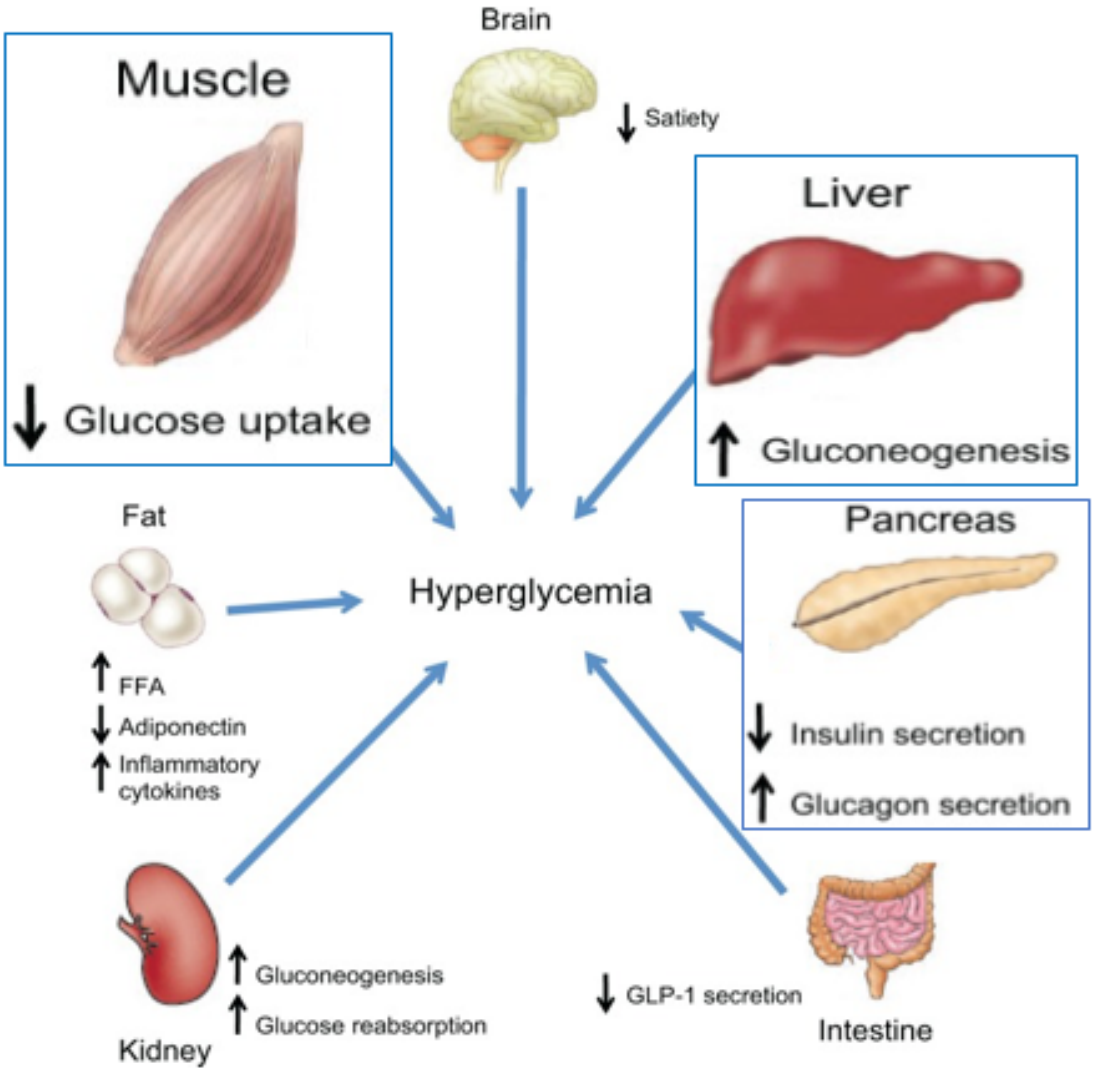

Figure 1.3: *The interaction of insulin resistance and a range of body organs which may play a role in the development of type 2 diabetes (Cornell, 2015).*

The established understanding of T2D is supported by longitudinal studies which have shown that T2D develops from the simultaneous effects of insulin resistance of the peripheral tissues and dysfunction of the pancreatic beta-cells (Martin et al., 1992). In the early stages of the development of T2D the peripheral tissues become less sensitive to insulin and their absorption of glucose is in turn reduced. In order to compensate for this insulin resistance, beta-cells increase the production of insulin to maintain normoglycaemia. Over time, with increasing insulin resistance of the peripheral organs,

and, in turn, a moderate increase in blood glucose levels, a state of impaired glucose tolerance (IGT), also called prediabetes, develops which is characterised by slight hyperglycaemia and marked hyperinsulinaemia. Prolonged overproduction of insulin eventually causes beta-cells to become debilitated and subsequently lose their function of sufficient insulin production, thus, leading to a further increase of hyperglycaemia and the onset of T2D. This progression is demonstrated in Figure 1.4, which shows the changes in glucose, insulin and insulin sensitivity levels during the typical progression from NGT to IGT to T2D (DeFronzo, 2004).

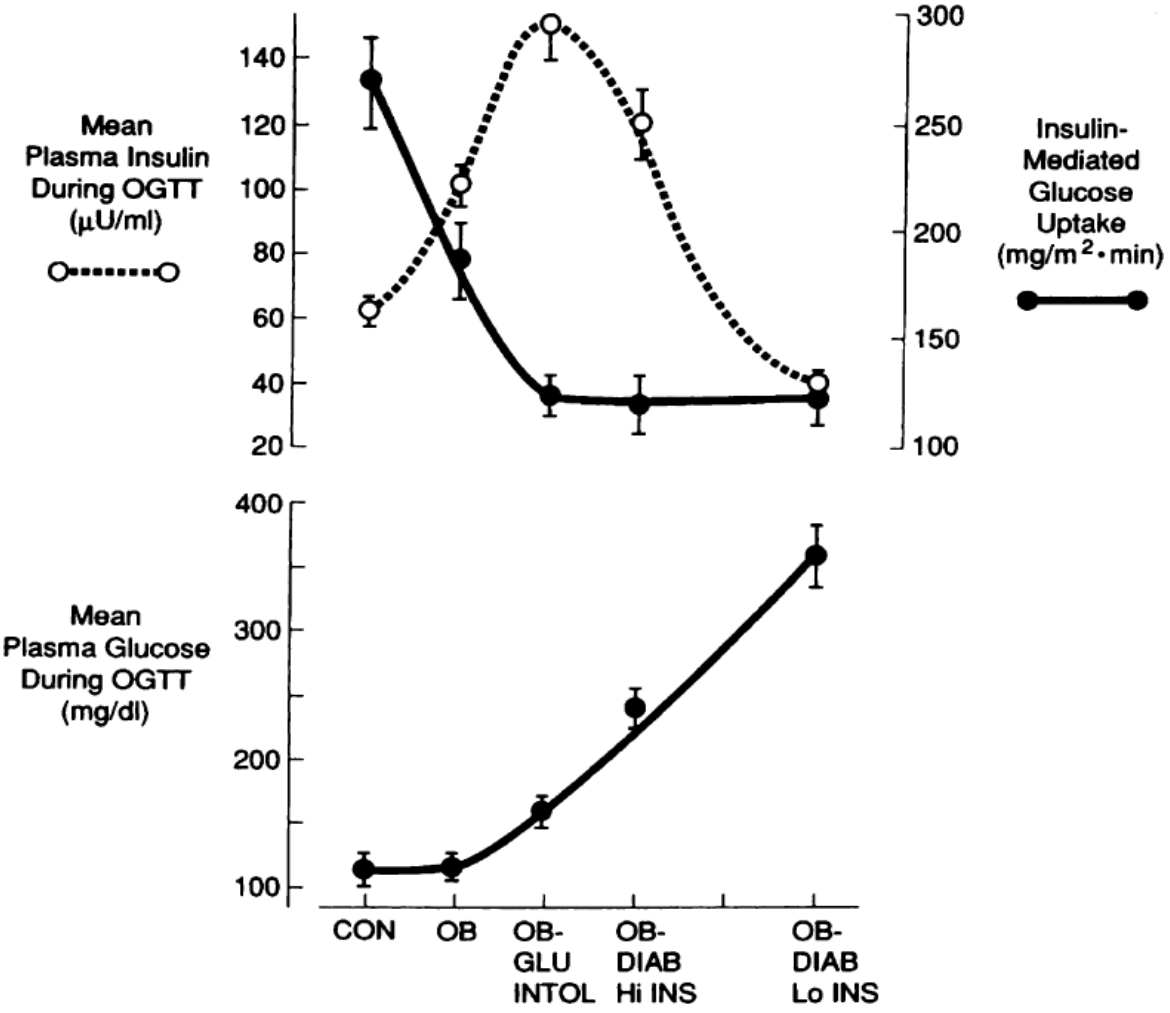

Figure 1.4: *Plasma glucose, insulin and insulin sensitivity measured during an oral glucose tolerance test. Comparison of groups at various glycaemic states including: healthy control (CON), obese (OB), obese glucose intolerant (OB- GLU INTOL), obese with T2D and high insulin (OB-DIAB Hi INS), and obese with T2D and low insulin (OB-DIAB Lo INS) (DeFronzo, 2004).*

The advancement from NGT to T2D is a progressive process, which usually occurs over several years (Meigs et al., 2003). It is believed that insulin resistance precedes beta-cell dysfunction; this has been supported by longitudinal studies which show specific features of insulin resistance, such as reduced glucose clearance and hyperinsulinaemia, are apparent 10-20 years prior to the onset of T2D (Warram et al., 1990). Similarly, during the development of T2D, beta-cell dysfunction begins as early as 12 years before diagnosis (UKPDS, 1995). Additionally, it is estimated that 50% of beta-cell function is lost by the time an individual has reached an impaired glucose tolerant state (Gastaldelli et al., 2004). While the features of the progression of T2D are well established, the role of adipose tissue dysfunction and ectopic fat accumulation have only recently gained attention.

1.3 Obesity, adipose tissue and type 2 diabetes

Obesity is a major risk factor for T2D (Bhupathiraju and Hu, 2016). In fact, the increasing prevalence of T2D is partly attributed to the increasing prevalence of obesity worldwide (Kopelman, 2000). Obese individuals have an estimated 6-8 times greater risk of developing T2D compared to individuals with a healthy body mass index (BMI) (Abdullah et al., 2010). Additionally, it is estimated that 90% of people with T2D in the UK are classified as clinically overweight or obese (Gatineau M, 2014).

Other major risk factors for T2D include age, family history and ethnicity; however, total body adiposity, determined by BMI, is estimated to account for a third to half of the weighting of the risk scores for T2D (Sattar and Gill, 2014). Stressing the importance of whole-body adiposity in T2D, studies investigating the relative risk of T2D have shown that current BMI, early obesity, weight gain throughout adulthood and waist circumference are all independent risk factors for T2D (Chan et al., 1994). Hence, due to

the evident link between obesity and T2D, the study of excess adiposity has been a long-standing area of interest in T2D research.

1.3.1 Body fat distribution and type 2 diabetes

There is much evidence demonstrating that the distribution of body fat is more important in determining metabolic risk than general measures of obesity (Meisinger et al., 2006, Li et al., 2012). BMI does not consider body fat distribution but is an anthropometric measure that is easy to undertake in almost any setting. Strong relationships have commonly been reported between BMI and risk of T2D (Colditz et al., 1990) and insulin resistance (Ferrannini et al., 1997). Although, despite the well-known link between obesity and T2D, about 30% of individuals with an obese BMI are metabolically healthy, while, 20-30% of individuals with a healthy BMI are metabolically unhealthy (Wildman et al., 2008). Another example of paradoxical relationships between whole-body adiposity and T2D risk is the well-known gender differences in T2D risk (Logue et al., 2011). Even though men typically have lower levels of whole-body fat compared to women at any given level of BMI, they are at greater risk of T2D (Kautzky-Willer et al., 2016). Hence, the relationship between adiposity and T2D is more complex than initially appreciated, which has encouraged research into the pattern of body fat distribution as opposed to general measures of adiposity such as whole-body fat or BMI (Meisinger et al., 2006, Li et al., 2012).

1.3.1.1 Android *versus* gynoid obesity

In 1947, Vague first proposed the classification of obesity into android (apple shape) and gynoid (pear shape) type by comparing the fat mass and distribution of men and women (Vague, 1947). He found that women could have twice the fat mass of men but still live longer while free of metabolic-related diseases, which he attributed to the pattern of fat distribution (Vague, 1956). Individuals who deposit fat around the central abdominal

region have a greater risk of cardiometabolic diseases compared to individuals who deposit fat in the lower part of the body and extremities (Wiklund et al., 2008, Samsell et al., 2014). Therefore, the phenomena of male *versus* female adiposity, or android *versus* gynoid adiposity, further supports the notion that rather than the amount of adipose tissue, its distribution appears to be a key influencer of metabolic health.

With the advancement of imaging techniques in the 1980s, researchers have been able to use computed tomography (CT) scanning to investigate associations between regional fat distribution and parameters of metabolic health (Thomas et al., 2013). Using these methods, Despres *et al.* showed that adipose tissue deposited within the visceral cavity, termed visceral adipose tissue (VAT), was negatively associated with glucose tolerance but this association was not present for subcutaneous adipose tissue (SAT) (Despres et al., 1989). This explained the link between android adiposity and T2D because android adiposity appears to be an indicator of high VAT deposition.

Since the discovery of the importance of abdominal obesity in T2D risk, waist circumference has been used as an important clinical tool to assess the risk of T2D since waist circumference is a marker of VAT deposition (Feller et al., 2010). Supporting the use of waist circumference to assess T2D risk, in a large cohort, Wang *et al.* found that waist circumference was a more sensitive predictor of T2D status than BMI and waist-to-hip ratio (Wang et al., 2005). Furthermore, waist circumference was a stronger independent predictor of insulin resistance compared to BMI and waist-to-hip ratio, and explained 50% of variation in insulin sensitivity (Wahrenberg et al., 2005). Therefore, even when using anthropometric measures, there is much evidence showing that abdominal obesity is an important factor in determining the risk of T2D (Li et al., 2012, Sanches et al., 2008).

In the 1990s, the development of duel-energy X-ray absorptiometry (DEXA) to determine body composition led to extensive studies that examined the relationship between body fat distribution and metabolic risk (Laskey, 1996, Shepherd et al., 2017). Using these methods, abdominal fat was shown to be a significantly stronger predictor of insulin resistance compared to peripheral fat (Carey et al., 1996). In contrast, thigh SAT showed inverse associations with insulin resistance indicating a possible metabolically protective role of peripheral SAT (Goodpaster et al., 2000). Therefore, the advancement of imaging techniques to assess body fat distribution have consistently confirmed that abdominal adiposity is closely linked to T2D risk.

1.3.2 Adipose tissue

Adipose tissue is a complex organ that functions as a storage compartment for triglycerides and is also the largest endocrine organ in the human body that secretes hormones and cytokines, termed adipokines (Unger, 2003). Remarkably, cells within adipose tissues, termed adipocytes, have the capacity to expand by several thousand-fold in volume to accommodate the storage of excess triglycerides, particularly in states of positive energy balance (Snel et al., 2012). However, when adipocytes become severely enlarged, such as in cases of obesity, they become dysfunctional and become unable to efficiently store triglycerides (Jernas et al., 2006). Adipose tissue dysfunction becomes progressively worse with increasing obesity; this state is particularly harmful during a positive energy balance due to the glycotoxic and lipotoxic effects of prolonged exposure to circulating glucose and lipids (Longo et al., 2019).

Dysfunctional adipose tissue has been closely related to metabolic disturbances that lead to T2D (Snel et al., 2012). Adipose tissue expands by increasing adipocyte numbers (hyperplasia) and increasing adipocyte size (hypertrophy) or a combination of both (Rutkowski et al., 2015). Adipose tissue expansion by hypertrophy is related to adipocyte

dysfunction and metabolic disturbances including insulin resistance, dyslipidaemia and inflammation which are individually addressed further below (Cuthbertson et al., 2017). Studies have shown that adipocyte hypertrophy is associated with insulin resistance (Cruz et al., 2002) and is an independent risk factor for T2D (Weyer et al., 2000). While investigating T2D in individuals of normal weight, Acosta *et al.* found that adipocytes isolated from patients with T2D were significantly larger in size compared to those isolated from the control group without T2D (Acosta et al., 2016). Even though adipose tissue dysfunction is closely related to its severe expansion, there is great inter-individual variability in the point at which adipose tissue becomes dysfunctional, a phenomenon coined the "personal fat threshold" (Cuthbertson et al., 2017, Taylor and Holman, 2015). Hence, there is much evidence showing that the way in which adipose tissue expands appears to be more crucial in determining the point at which it becomes dysfunctional rather than the actual level of expansion.

Generation of mature adipocytes from preadipocytes, called adipogenesis, is the underlying mechanism in adipose tissue hyperplasia (Ghaben and Scherer, 2019). Means by which adipogensis can be increased have been explored in an attempt to reduce metabolic disease risk (Ghaben and Scherer, 2019). For example, the genetic transcription factors involved in adipogenesis are therapeutic targets of some T2D drugs including thiazolidinediones (Sattar and Gill, 2014). These drugs stimulate adipose tissue transcription factors to promote hyperplasia and, in turn, reduce hypertrophy, which results in improved insulin sensitivity by the reduction of adipose tissue dysfunction (Hauner, 2002). Therefore, dysfunctional adipose tissue plays a crucial role in the pathophysiology of T2D and is proposed to be the key driver of insulin resistance (Longo et al., 2019).

1.3.2.1 Adipose tissue inflammation

Adipose tissue is a major endocrine organ that secretes a number of hormones which includes leptin, adiponectin, resistin as well as several cytokines, called adipokines (Guerre-Millo, 2002). When adipocytes become severely enlarged and dysfunctional, they release excess inflammatory adipokines that cause an immune reaction in the adipose tissue inducing a state of persistent chronic low-grade inflammation (Reilly and Saltiel, 2017). Part of the immune response is a visible increase of macrophages that infiltrate the adipose tissue and function to clear up debris produced from damaged adipocytes (Thomas and Apovian, 2017). Figure 1.5 shows the phenotypic modulation of adipocytes from normally functioning adipose tissue to enlarged and dysfunctional adipose tissue that typically occurs during increased adiposity (Ouchi et al., 2011).

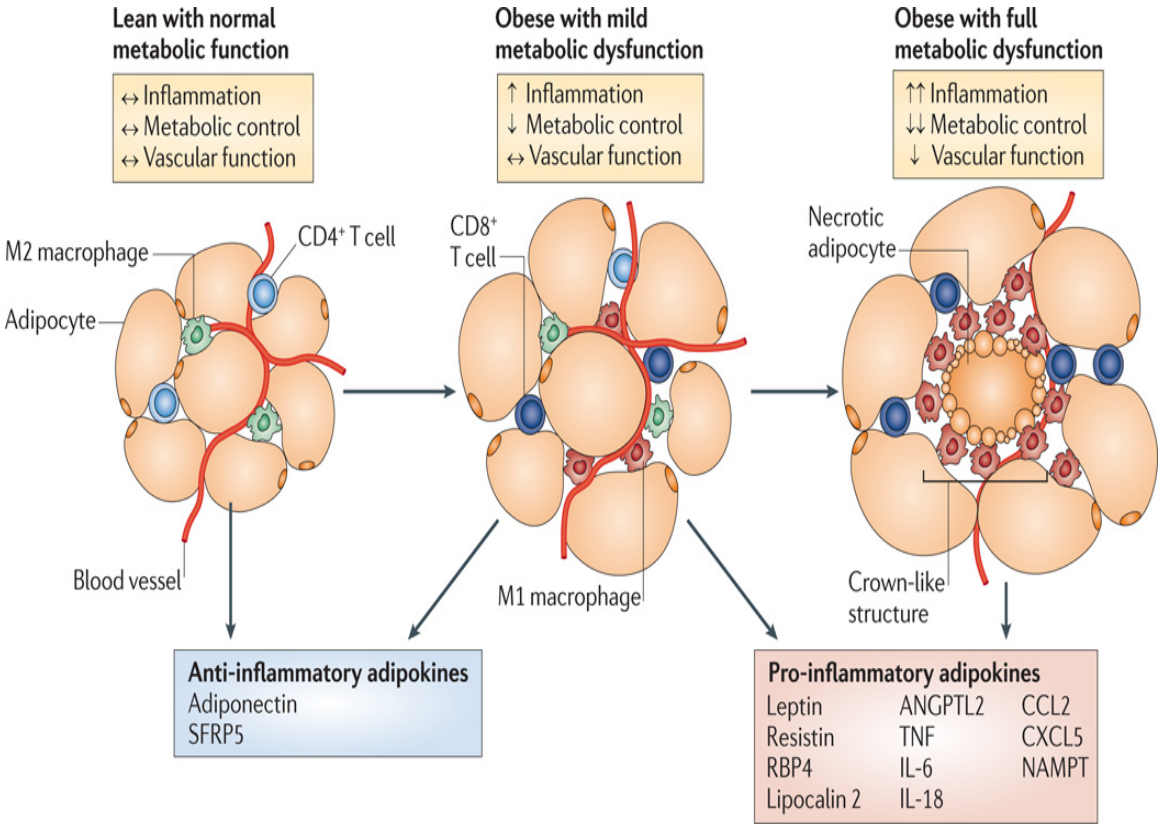

Figure 1.5: *Phenotypic modulation of adipocytes from normal to obese states. As adipose tissue becomes severely enlarged, pro-inflammatory cytokines are released in excess which induce an immune reaction resulting in recruitment of macrophages and other immune cells (Ouchi et al., 2011).*

Several inflammatory cytokines have been reported to be increased in individuals with obesity compared to those of a healthy weight which include interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) (Almuraikhy et al., 2016). These adipokines are also found to be elevated in patients with T2D confirming a link between adipose tissue inflammation and glucometabolic disorders. Population-wide studies have found that Il-6 is associated with incident diabetes independent of obesity and is an independent risk factor for T2D (Goldberg et al., 2019, Wannamethee et al., 2007). Furthermore, Il-6 has been causally linked to T2D since it has been shown to impair adipose tissue differentiation (Snel et al., 2012), stimulate hepatic fatty acid synthesis, (Sattar et al., 2008) and interfere with insulin signalling of hepatocytes and adipocytes (Kristiansen and Mandrup-Poulsen, 2005).

1.3.2.2 Adipose tissue insulin resistance

Adipose tissue is one of the sites of insulin action (Sobczak et al., 2019); in adipocytes, insulin signals to suppress the breakdown of stored triglycerides (lipolysis) and promotes the uptake of non-esterified fatty acids (NEFA) to be stored as triglycerides (lipogenesis) (DeFronzo et al., 1985). Similar to the liver and muscle tissues, adipose tissue insulin sensitivity is reduced in individuals with T2D leading to an over-excretion of NEFAs (Gastaldelli et al., 2017). These excess NEFAs are directed to other tissues where they deposit as ectopic fat, which is also a key feature of adipose tissue dysfunction, and is suggested to cause insulin resistance in the organ it resides in (Sattar and Gill, 2014, Snel et al., 2012). The decrease of adipose tissue insulin sensitivity is a progressive process that occurs with increasing obesity (Kim et al., 2019, Kim et al., 2017). Furthermore, it has been suggested that adipose tissue insulin resistance resulting from dysfunctional adipocytes precedes peripheral insulin resistance and is therefore the key link between obesity and insulin resistance (Smith and Kahn, 2016).

1.3.3 Subcutaneous adipose tissue

Subcutaneous adipose tissue is the primary and metabolically safest compartment for storage of triglycerides (Ibrahim, 2010). In a healthy state, excess energy is ultimately stored as triglycerides in the SAT depots. The SAT compartment is considered to be the safest storage depot for triglycerides due to its avid capacity to absorb circulating NEFAs and triglycerides; this process maintains circulating lipid levels within normal ranges (Ibrahim, 2010).

1.3.3.1 Abdominal *versus* peripheral subcutaneous adipose tissue

Even though the storage of triglycerides in the SAT compartment is more favourable in comparison to VAT and ectopic depots, abdominal SAT (ASAT) appears to be more harmful than peripheral SAT (Patel and Abate, 2013a). An increase of ASAT has been more strongly related to T2D compared to peripheral SAT (Patel and Abate, 2013b, Abate et al., 1996). Individuals with T2D have a greater amount of ASAT compared to controls without T2D (Abate et al., 1996). Considering peripheral SAT, a large cohort study by Snijder *et al.* showed that thigh SAT was independently associated with favourable circulating glucose and lipid levels while ASAT was associated with unfavourable glucose and lipid levels (Snijder et al., 2005). Additionally, gluteofemoral fat was related to favourable circulating lipid profiles including lower total cholesterol, low density lipoprotein (LDL) cholesterol and greater high-density lipoprotein (HDL) cholesterol (Terry et al., 1991, Williams et al., 1997). A lower ratio of ASAT to peripheral fat is also considered favourable; an increase of gluteofemoral fat relative to ASAT is protective against insulin resistance and serves as a "sink" for circulatory non-esterified fatty acids (Manolopoulos et al., 2010).

The metabolic differences in the influence of peripheral SAT and ASAT on T2D may be explained by their distinct structural and functional properties. Azuma *et al.* found that

adipocytes isolated from ASAT were larger in size than those isolated from leg SAT which indicates that abdominal SAT are more likely to be hypertrophic and dysfunctional (Azuma et al., 2007). Therefore, there is much evidence suggesting that increased ASAT plays a role in metabolic dysregulation and T2D.

1.3.3.2 Deep and superficial subcutaneous adipose tissue

To add to the complexity in the field of body-fat distribution and T2D risk, the SAT compartment itself is heterogeneous and the distribution of adipocytes within the SAT depot is also related to T2D risk. SAT is composed of two distinct compartments: superficial SAT (sSAT) and deep SAT (dSAT) which are separated by the fascia-superficialis; the dSAT is located below the fascia with the sSAT above (Sniderman et al., 2007). Structural and functional properties differ between sSAT and dSAT as well as their contribution to metabolic disease risk. The dSAT is more metabolically active than sSAT; adipocytes isolated from dSAT show greater lipolytic activity compared to those from sSAT (Monzon et al., 2002). Furthermore, with increasing adiposity, as the SAT increases there is a disproportionate expansion of dSAT compared to sSAT (Marinou et al., 2014). Hence, the differences between dSAT and sSAT suggest that an increased ratio of dSAT to sSAT is an indicator of dysfunctional adipose tissue. Indeed, dSAT is commonly likened to VAT and shows strong associations with VAT but this relationship is not present between sSAT and VAT (Marinou et al., 2014). The similarities between dSAT and VAT are also evident in their relationships with metabolic risk since dSAT and VAT, but not sSAT, showed strong associations with insulin resistance (Kelley et al., 2000). The disparities between dSAT and sSAT may be due to their structural differences since adipocytes from dSAT are large and loosely organised while those of the sSAT are smaller and tightly packed (Markman and Barton, 1987).

1.3.4 Visceral adipose tissue

As previously mentioned, central adiposity is known to be related to T2D due to the increase of VAT. Indeed, increased VAT is an independent risk factor for T2D irrespective of overall adiposity and is usually indicated by waist circumference or waist to hip ratio (Chan et al., 1994, Despres, 1993). Furthermore, increased VAT is associated with insulin resistance and hyperglycaemia and is considered to be detrimental to the development of T2D (Despres, 2006, Gastaldelli, 2008). Increased VAT is also related to several other features of poor metabolic health such as increased LDL-cholesterol, and triglyceride levels (Fujioka et al., 1987, Fujimoto et al., 1994).

1.3.4.1 Visceral adipose tissue *versus* subcutaneous adipose tissue

Several large cohort studies comparing VAT and SAT fat depots have shown that VAT is more strongly related to T2D compared to SAT (Wagenknecht et al., 2003, Muller et al., 2012). In the Framingham Heart Study, Fox *et al.* showed that, even though both VAT and SAT were associated with metabolic markers of T2D, VAT showed a greater association than SAT (Fox et al., 2007). From the same cohort, Porter *et al.* investigated these associations while dividing the data by tertiles of VAT and SAT; they found that when stratified across tertiles of VAT, SAT was not associated with a linear increase of metabolic risk factors across the tertiles (Porter et al., 2009). This suggests that while overall adiposity increases absolute risk of T2D, an increased amount of SAT at the same BMI may have protective qualities compared to VAT. Therefore, the ratio of VAT to SAT is a sensitive and informative marker of T2D risk in comparison to either measure alone. This notion is supported by several studies. When compared to controls without T2D, individuals with T2D had significantly lower SAT and greater VAT suggesting that an increase of VAT to SAT ratio plays a crucial role in the pathophysiology of T2D (Gallagher et al., 2009). In addition, Kim *et al.* showed that with a decrease in SAT to

VAT ratio there was an increase in fasting glucose and triglyceride levels and a decrease in HDL cholesterol levels (Kim et al., 2011).

1.3.4.2 Mechanisms linking visceral adipose tissue to type 2 diabetes

The negative metabolic effects of VAT compared with SAT has partly been attributed to the differences in their structural and functional properties. The cellular structure of adipocytes from VAT are larger than those of SAT (Schoettl et al., 2018). VAT adipocytes are morphologically and functionally similar to hypertrophic SAT adipocytes. As previously mentioned, hypertrophic SAT adipocytes become dysfunctional with specific detrimental metabolic characteristics which include: increased release of inflammatory adipokines, increased output of NEFAs and reduced insulin sensitivity; these characteristics have been extensively reported in studies investigating VAT and its role in T2D (Ibrahim, 2010, Wronska and Kmiec, 2012).

VAT is considered to be more metabolically active compared to SAT showing greater vascularisation per kg (Virtanen et al., 2002) and greater lipolytic activity (Petrus et al., 2017); this results in greater release of NEFAs into the circulation that are subsequently deposited as ectopic fat (Gastaldelli, 2008). An increase of VAT has also been linked with an increase of inflammatory markers and a greater concentration of immune cells within the adipocytes. Therefore, VAT has shown to have great pathogenic potential, which is of interest in T2D research due to its link with the promotion of ectopic fat deposition (Bays et al., 2008).

1.4 Ectopic fat and type 2 diabetes

Ectopic fat refers to the deposition of triglycerides in non-adipose tissue depots including the liver, pancreas and muscles (Snel et al., 2012). The deposition of ectopic fat is thought to occur due to the reduced capacity of the subcutaneous adipocytes to expand leading to a dysfunction of the adipocytes (Sattar and Gill, 2014). The dysfunctional adipose tissue becomes unable to store excess triglycerides which, in turn, get redirected and stored as ectopic fat deposits; this process is summarised in Figure 1.6 (Snel et al., 2012, Brons and Grunnet, 2017). Along with low-grade chronic inflammation and insulin resistance, ectopic fat deposition is a key feature of dysfunctional adipose tissue that is closely related to T2D (Paniagua, 2016).

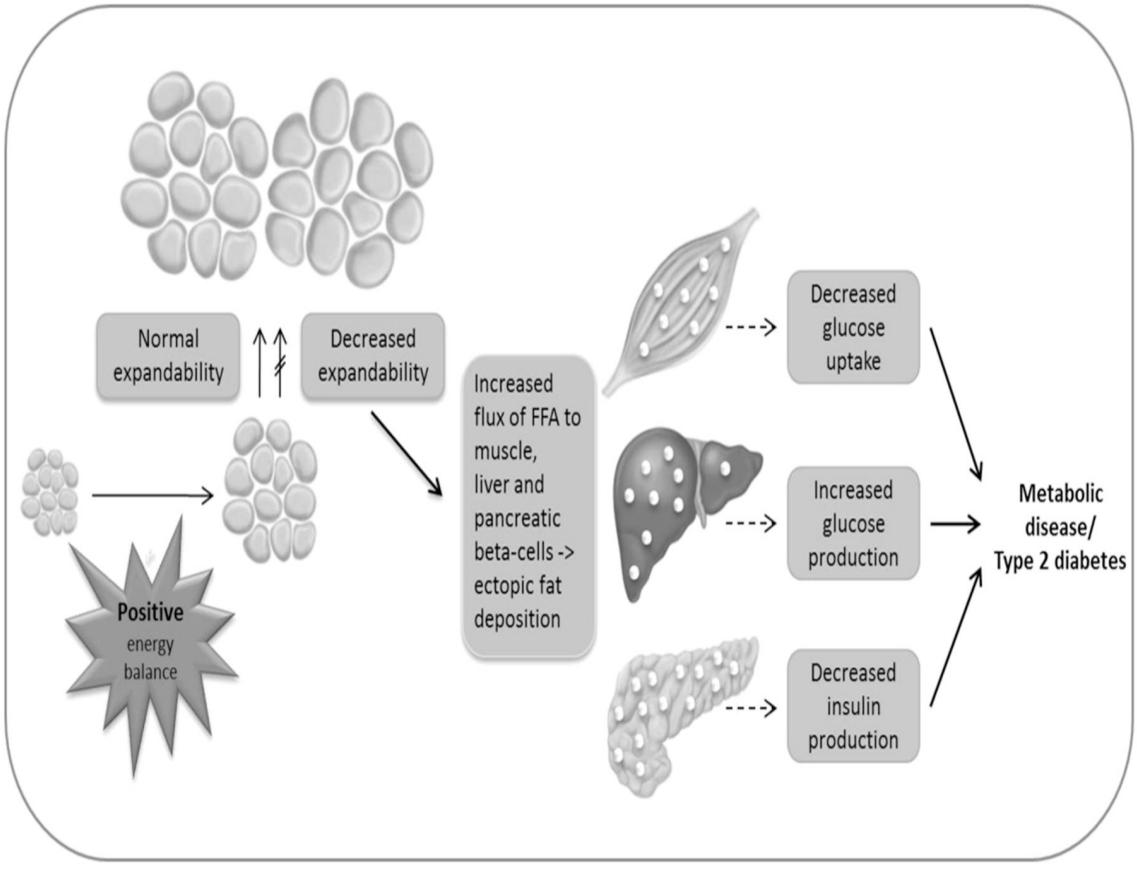

Figure 1.6: *Proposed theory of ectopic fat deposition. The reduced expandability of subcutaneous fat depots during a positive energy balance leads to a spill-over of fatty acids that are stored in the liver, muscles and pancreas (Brons and Grunnet, 2017).*

Ectopic fat deposits play a role in the pathophysiology of T2D as they are considered to interfere with the normal function of the cells and decrease the insulin sensitivity of the organ they reside in, a phenomenon called *lipotoxicty* (Unger, 2003). Ectopic fat relates to triglycerides stored within (intra) as well as between (inter) the cells of an organ, both of which are considered to contribute to organ lipotoxicity (Brookheart et al., 2009). To understand the mechanisms of ectopic fat induced lipotoxicity, several studies have examined the effects of triglycerides on various cellular functions. These studies have consistently shown that it is not the triglycerides themselves but the lipid intermediates produced during the breakdown of triglycerides, such as ceramides, fatty acyl-CoAs and diacylglycerol, that have toxic effects on nearby cellular functions (Daemen et al., 2018, Snel et al., 2012, Brons and Grunnet, 2017).

1.4.1 Intrahepatic lipids

1.4.1.1 The role of the liver in type 2 diabetes

The liver plays a crucial role in maintaining normal glucose homeostasis since, depending on insulin signalling, the liver stores or releases glucose (Defronzo, 2009). During normal glucose tolerance, insulin signals to the liver to uptake glucose from the circulation and store it as glycogen. The liver breaks down these glycogen stores (glycogenolysis) to produce glucose from other sources (gluconeogenesis) and releases it into the circulation when blood glucose levels are low and energy is required (Defronzo, 2009). The liver also has an important role of maintaining normal circulating glucose levels during sleep, since glucose released by the liver accounts for 90% of the total glucose supply to the brain during sleep (DeFronzo, 2004).

1.4.1.2 Hepatic insulin resistance

During the pathophysiology of T2D, the liver becomes insulin resistant resulting in the reduced suppression of glycogenolysis by insulin even when blood glucose levels are

high, which adds to the hyperglycaemia. During early T2D, the reduced uptake of glucose by skeletal muscles accounts for postprandial hyperglycaemia, however, as T2D worsens, hepatic glucose production becomes the major contributor to elevated fasting glucose levels (DeFronzo et al., 1989). Individuals with impaired glucose tolerance and T2D have reduced hepatic insulin sensitivity during the fasting and insulin-stimulated states compared to normal glucose tolerant controls (DeFronzo et al., 1982). There is a strong correlation between whole-body insulin sensitivity and hepatic insulin sensitivity suggesting the mechanisms involved in insulin resistance occur simultaneously in the muscles and liver (DeFronzo et al., 1985).

1.4.1.3 Hepatic insulin clearance

In order to prevent hypoglycaemia, the liver has a crucial function of removing insulin from the circulation and it is estimated that approximately 80% of insulin clearance occurs in the liver (Kotronen et al., 2007). Hepatic insulin clearance is reduced in individuals with T2D (Pivovarova et al., 2013). Inverse associations have been reported between insulin clearance and insulin secretion during the progression of T2D since, as insulin secretion increases to overcome insulin resistance, insulin clearance reduces to maintain high circulating insulin levels (Najjar and Perdomo, 2019); this has been considered a protective mechanism to raise blood insulin levels and reduce the burden of insulin secretion on the beta-cells (Piccinini et al., 2017).

1.4.1.4 Intrahepatic lipids in type 2 diabetes

Ectopic fat accumulation in the liver, termed intrahepatic lipid (IHL), plays an important role in the pathophysiology of T2D (Bosy-Westphal et al., 2019, Geisler and Renquist, 2017). IHL is elevated in individuals with T2D, furthermore, greater levels of non-alcoholic liver disease (NAFLD) are reported in patients with T2D compared with BMI-matched controls (Dai et al., 2017). NAFLD is associated with insulin resistance,

metabolic syndrome and atherosclerosis (Adams et al., 2005, Ou et al., 2013). Complications relating to excess liver fat are more prevalent in patients with T2D because NAFLD can progress to more severe conditions including non-alcoholic hepatosteatosis (NASH), cirrhosis and hepatocellular carcinoma (Bhatt and Smith, 2015).

Intrahepatic lipid is believed to affect the metabolic function of the liver by causing lipotoxicity to the cells it resides in. IHL is associated with hepatic and peripheral insulin resistance (Hwang et al., 2007); this relationship is independent of BMI, VAT and overall adiposity. On a cellular level, IHL is associated with defects in the cellular signalling processes that result in the suppression of glycogenolysis by insulin in the liver (Seppala-Lindroos et al., 2002). IHL is strongly associated with VAT independent of overall adiposity (Korenblat et al., 2008); however, while considering the consistent evidence linking VAT to increased risk of T2D, it has been suggested that IHL may be more closely related to T2D than VAT (Fabbrini et al., 2009).

The importance of IHL in T2D risk has been demonstrated in a recent study by Okamura *et al.* who investigated the effect of three phenotypes, obesity (BMI >30), high VAT and high IHL, individually on incident T2D. The percentage incidence of T2D was presented in eight groups that were divided based on the presence of each phenotype shown in Figure 1.7 (Okamura et al., 2019). They found that the incidence of T2D was greater in the group that had high IHL in the absence of the other two phenotypes compared with the groups that had only one of the other phenotypes. This supports the notion that IHL plays a greater role in the development of T2D compared to VAT and obesity.

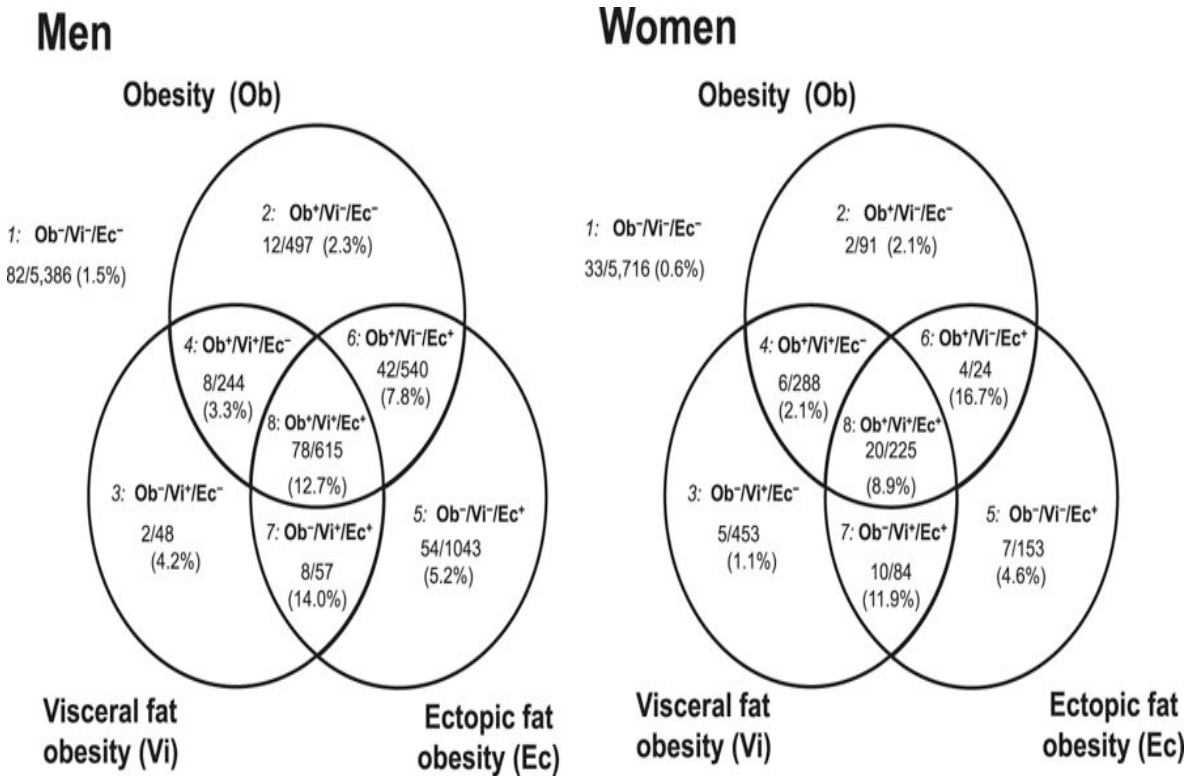

Figure 1.7: *Venn diagram showing eight groups divided based on the presence of three phenotypes of obesity. The incident of type 2 diabetes in each group is indicated by the number of participants with type 2 diabetes (percentage)/all participants in the group (Okamura et al., 2019).*

1.4.1.5 Portal theory

One of the key theories that describes the main mechanism of IHL accumulation is the *portal theory* (Item and Konrad, 2012). This theory proposes that excess NEFAs are secreted by VAT directly into the portal vein, which subsequently deposit into the liver and, over time, accumulate as IHL. The mechanisms of the portal theory are summarised in Figure 1.8 (Item and Konrad, 2012). Early studies in the 1980s linked the increased flux of NEFAs from VAT to the liver, via the portal vein, to hepatic metabolic disturbances (Stromblad and Bjorntorp, 1986). Portal NEFAs have been linked to an increase of hepatic gluconeogenesis, a reduction of insulin clearance and the excess production of hepatic lipoproteins (Bjorntorp, 1990).

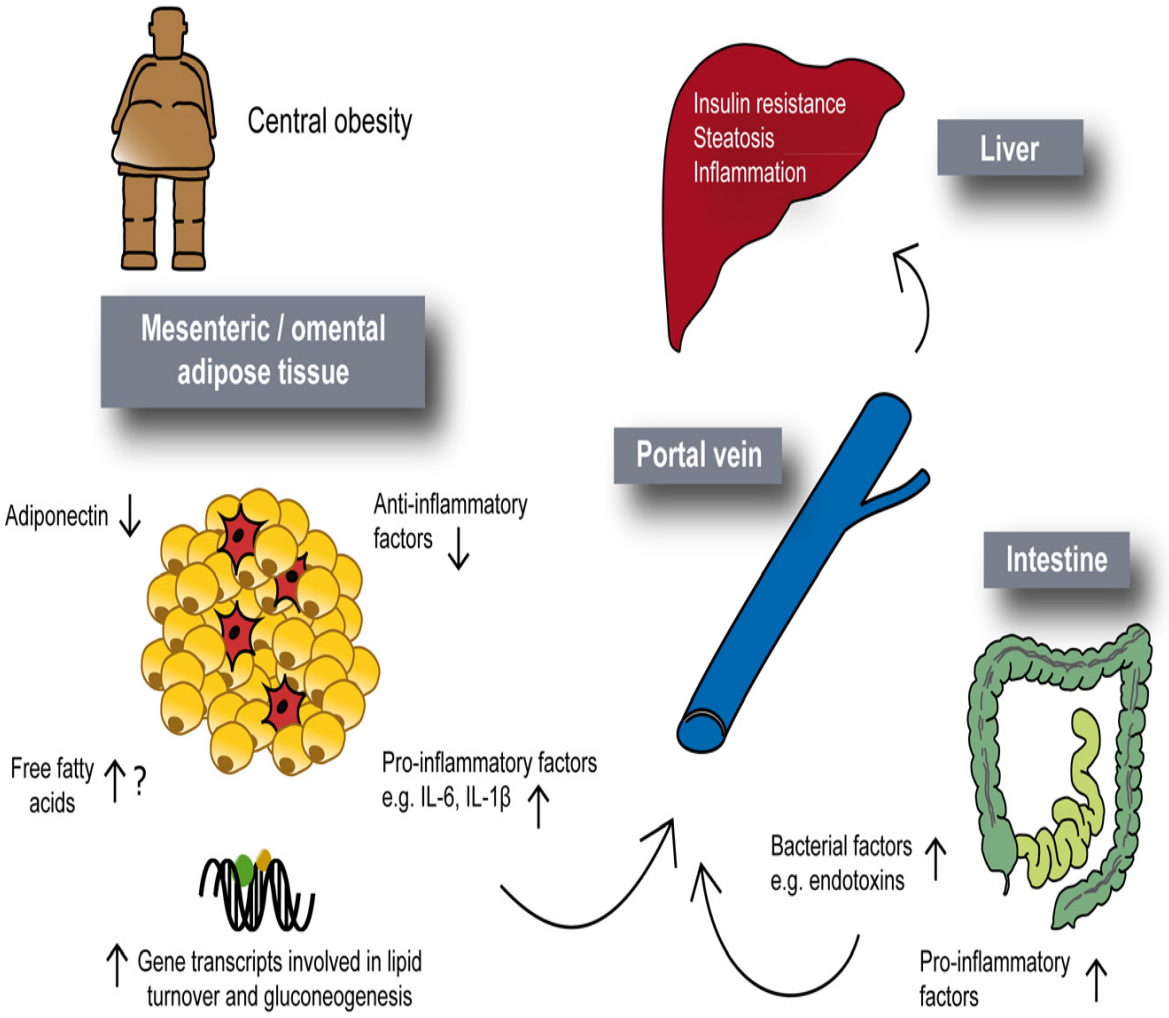

Figure 1.8: *Mechanisms of visceral adipose tissue-induced metabolic disturbances of the liver termed the 'portal theory'. Increased release of non-esterified fatty acids and pro-inflammatory factors are transported to the liver via the portal vein (Item and Konrad, 2012).*

Over the past two decades, with the increased interest in IHL and its role in T2D, there has been increasing evidence to support the *portal theory* by showing that the deposition of IHL is directly linked to VAT (Item and Konrad, 2012, Bergman and Ader, 2000). Several studies have shown that IHL strongly correlates with VAT (Gastaldelli et al., 2007, Guerrero et al., 2009, Item and Konrad, 2012, Nazare et al., 2012). The high lipolysis rate of VAT increases the concentration of NEFAs in the portal circulation, which has shown to reduce hepatic insulin sensitivity and increase hepatic

gluconeogenesis. Item *et al.* showed hepatic insulin sensitivity decreases in response to an increase in NEFAs in the portal circulation; however, no differences in hepatic insulin sensitivity were detected in response to an increase of NEFAs in the systemic circulation (Item and Konrad, 2012). This provides direct evidence to support the *portal theory* showing that it is portal NEFAs that are detrimental to hepatic metabolic functions.

1.4.2 Intrapancreatic lipids

During increased adiposity, like the liver, excess storage of triglycerides also occurs in the pancreas which is termed intrapancreatic lipids (IPL). The pathological process of triglyceride storage in the pancreas was described in 1933 by Ogilvie who found markedly greater fat deposition within the pancreas of cadavers with obesity compared to lean controls (Ogilvie, 1933). However, it is only the past decade that there has been an increased interest in triglyceride storage in the pancreas due to its posited influence on beta-cell dysfunction (Yu and Wang, 2017). Additionally, more recent research in IPL has been assisted by the advancement of imaging techniques that allow the non-invasive measurement of IPL (Al-Mrabeh et al., 2017).

Early studies investigating the lipotoxicity of beta-cells in rats with T2D were carried out by Lee *et al.* in 1994 (Lee et al., 1994). They showed that an increase in the accumulation of IPL preceded the dysfunction of beta-cells prior to the onset of T2D (Lee et al., 1994). Pascoe *et al.* showed that in-vitro exposure of beta-cells to fatty acids blocked glucose-stimulated proliferation of the beta-cells indicating that obesity is directly damaging to normal beta-cell function (Pascoe et al., 2012).

More recent studies in humans have shown links between IPL and common clinical metabolic parameters related to T2D (Yu and Wang, 2017). Tushuizen *et al.* found that IPL was higher in patients with T2D than age and BMI-matched controls and that IPL was independently associated with beta-cell dysfunction (Tushuizen et al., 2007).

Furthermore, they also reported that IPL was associated with common measures of adiposity including BMI, VAT, IHL, NEFAs and triglycerides. This indicates that with increasing whole-body adiposity, there appears to be strong interrelations in the processes that drive VAT, IHL and IPL accumulation.

1.4.2.1 Twin-cycle theory

The *twin-cycle theory* describes the possible mechanism that leads to IPL accumulation and was first proposed by Roy Taylor in 2008 (Taylor, 2013, Taylor, 2008). This theory states that during a prolonged period of positive energy balance, excess glucose is metabolised by the liver, which metabolises it into lipids that are released in very low density lipoproteins (VLDL). These VLDLs are released into the circulation and are deposited as triglycerides in the pancreas where they have detrimental lipotoxic effects on the beta-cells. This results in reduced insulin secretion, which leads to an increase of blood glucose levels that are subsequently redirected to the liver and metabolised, hence, leading to a vicious twin-cycle between the liver and pancreas, which is summarised in Figure 1.9 (Taylor and Barnes, 2018).

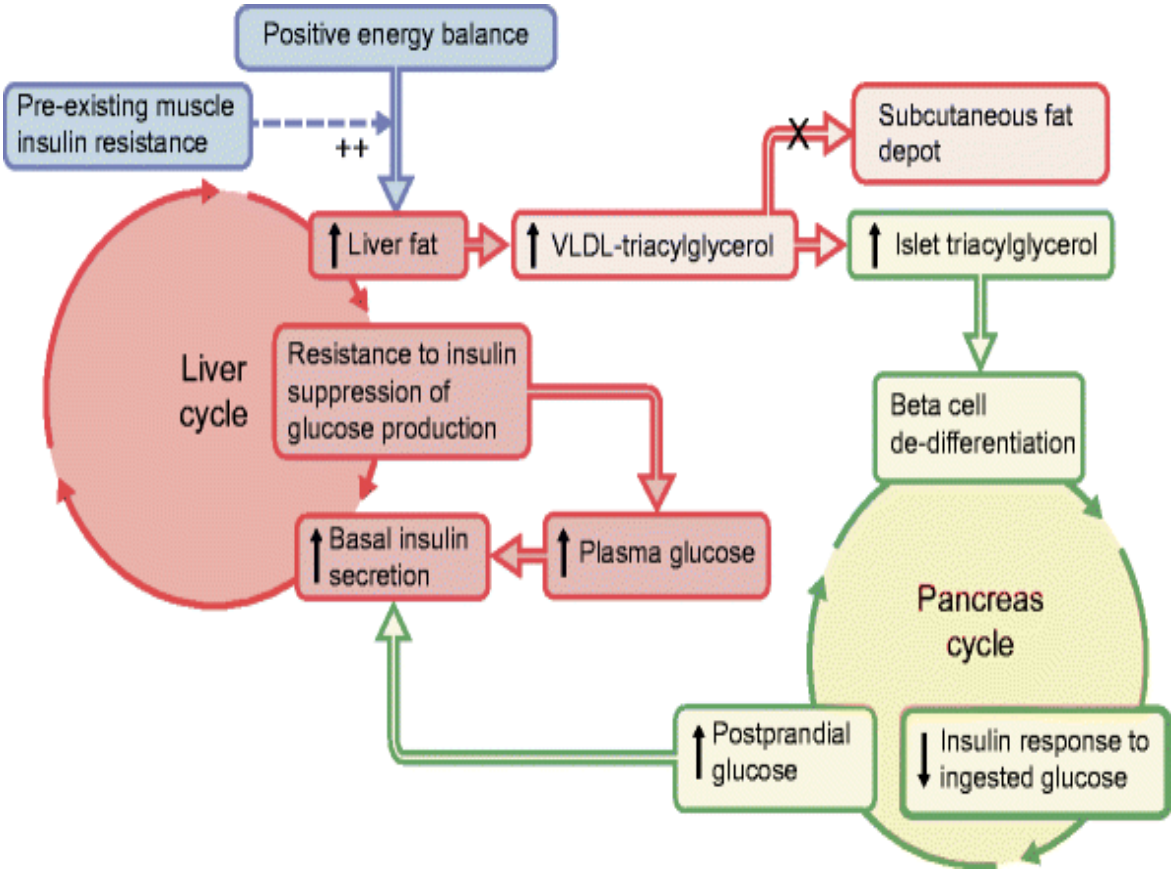

Figure 1.9: *Twin-cycle theory of pancreatic and hepatic lipotoxicity. Pancreatic fat storage is driven by the release of lipids from the liver during a positive energy balance (Taylor and Barnes, 2018).*

1.4.3 Intramyocellular lipids

Similarly to IHL and IPL, elevated triglycerides stored within the muscle cells, known as intramyocellular lipids (IMCL), are also linked with T2D. Increased IMCL levels were first linked to insulin resistance in 1980 when Standl *et al.* used muscle biopsies to quantify IMCL depots in individuals with T2D and healthy controls (Standl et al., 1980). In a healthy state, the skeletal muscle accounts for approximately 90% of insulin-mediated glucose disposal (DeFronzo et al., 1985). Insulin resistance of the muscles is the major contributor to whole-body insulin resistance during the development of T2D. IMCL has been shown to interfere with cellular pathways of glucose uptake in the muscle cells thus reducing their insulin sensitivity (Bjornholm and Zierath, 2005). Several

studies have confirmed a direct association between IMCL accumulation and insulin resistance indicating that IMCL may play a role in reducing muscle insulin sensitivity (Ingram et al., 2011, Krssak et al., 1999, Sinha et al., 2002, Thamer et al., 2003).

1.5 Magnetic resonance imaging for the assessment of fat deposition

Several techniques have been developed for the assessment of total and regional adipose tissue in the body which include skinfold measurement, underwater weighing, air displacement, bioelectrical impedance, CT, DEXA and magnetic resonance imaging (MRI) (Thomas et al., 2013). Technological advancements that led to the development of MRI and CT scanning have allowed the in-depth assessment of individual body composition components including fat deposition (Borga et al., 2018). These techniques overcome more traditional methods as they are non-invasive, relatively fast to perform and provide a direct accurate measure of various body composition components (Thomas et al., 2013).

To understand the metabolic implications of excess adiposity, several body composition studies have focused on the regional distribution of fat in the human body including SAT, VAT and ectopic fat stores (Wang et al., 2014). Currently, MRI remains the most accurate non-invasive technique for quantifying fat depots in the human body, and has been validated against invasive methods in human and animal cadavers (Abate et al., 1994, Fowler et al., 1992). MRI is superior to CT as it provides high resolution images that are more detailed, particularly with respect to soft tissue organs, compared to images obtained by CT (Hu, 2012). Furthermore, MRI does not subject individuals to ionising radiation and can therefore, be used in children and during pregnancy.

1.5.1.1 Magnetic resonance imaging for the assessment of ectopic fat

The use of MRI to assess ectopic fat has been crucial in advancing the current understanding of the importance of ectopic fat in T2D (Thomas et al., 2012b, Yu and

Wang, 2017). Until the advent of MRI, the assessment of ectopic fat relied on the ability to obtain tissue biopsies, which had considerable limitations and was clearly impossible for tissues such as the heart and pancreas (Thomas et al., 2013).

MRI is particularly useful for assessing adipose tissue due to its unique ability to differentiate between fat and non-fat tissues; this separation relies on the natural chemical shift differences between the water molecules within fat and non-fat tissues in the human body (Springer et al., 2010, Proctor and Yu, 1950). By using certain MRI sequences such as the "DIXON" sequence, fat-only and water-only images can be produced, which allow clear visual separation between fat tissues and other tissues (Dixon, 1984, Karstaedt et al., 1983). Therefore, MRI images can be used to quantify regional and ectopic fat depots including SAT, VAT, IHL and IPL. Figure 1.10 shows two fat-only abdominal MRI images of an individual with a healthy BMI (A) and an obese BMI (B); the greater SAT and IHL are clearly visible in the subject with an obese BMI (Springer et al., 2010).

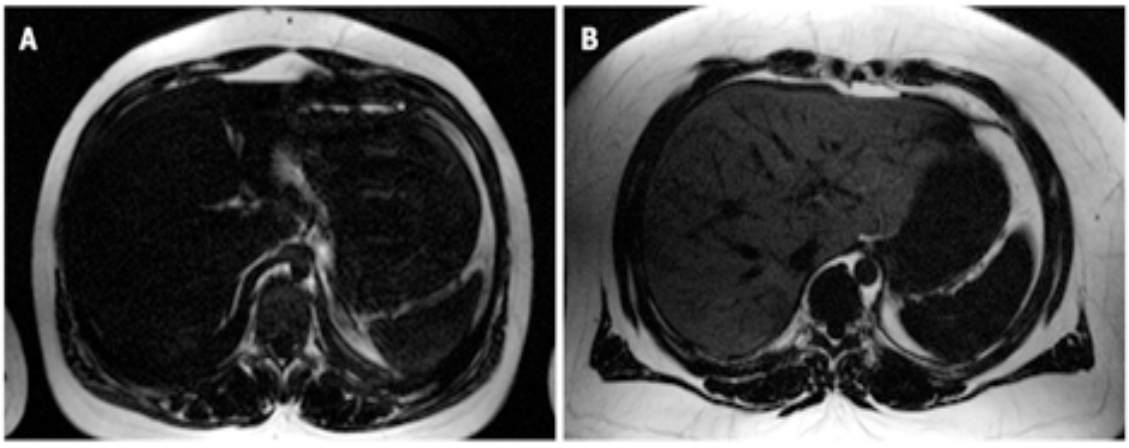

Figure 1.10: *Fat-only axial MRI images of the abdomen. DIXON-MRI sequences allow the acquisition of fat-only images which allows visual comparison of a lean individual with low intrahepatic lipid and subcutaneous fat (A) with an obese individual with high intrahepatic lipid and subcutaneous fat (B) (Springer et al., 2010).*

1.5.1.2 Magnetic resonance spectroscopy for the assessment of intramyocellular lipids

The development of magnetic resonance spectroscopy (MRS) is closely related to that of MRI as both use the fundamental principles of nuclear magnetic resonance (NMR) to differentiate between molecules. Proton-MRS (^{1}H-MRS) uses these principles to produce spectra while MRI produces images (Tognarelli et al., 2015). Figure 1.11 shows the underlying principles of NMR used in MRI and MRS to differentiate between different molecules in vivo (Hwang and Choi, 2015).

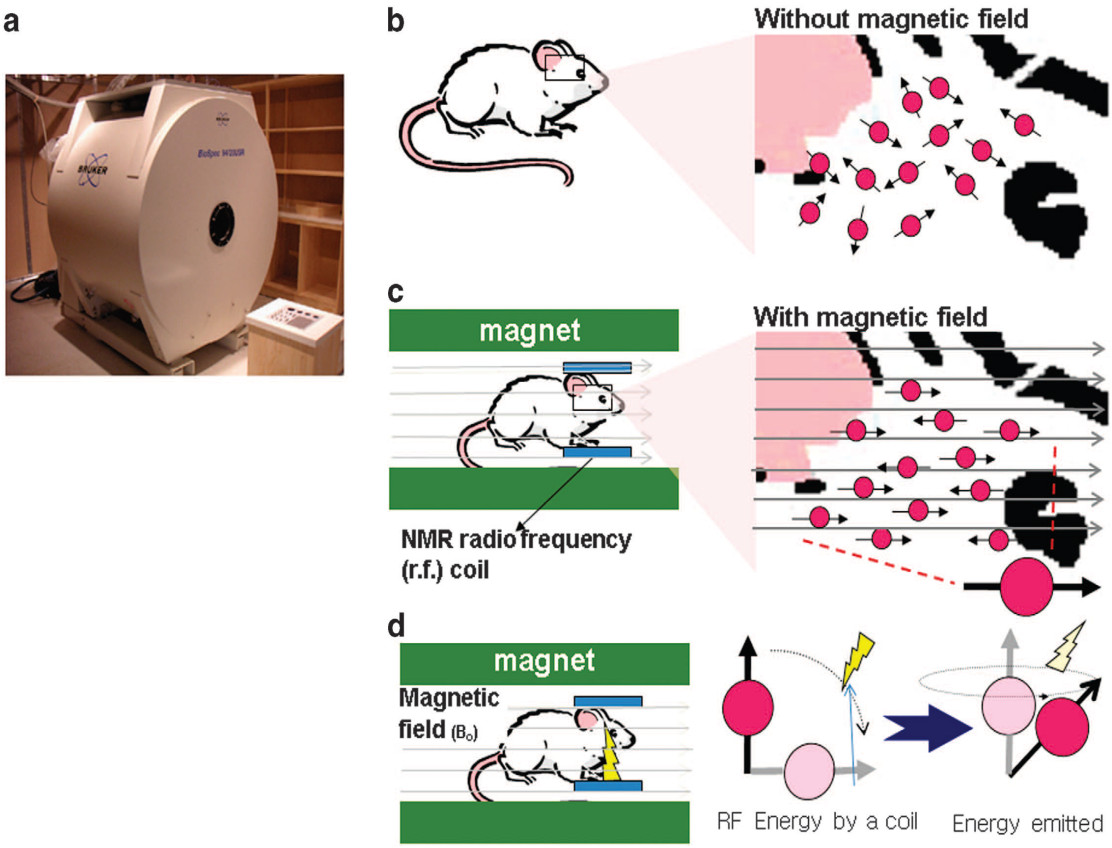

Figure 1.11: *Principles of nuclear magnetic resonance. Magnetic resonance spectrometer (a). Random orientation of molecules outside of a magnetic field (b), however, under a magnetic field molecules become aligned (c). a longitudinal magnetization is generated parallel to the magnetic field by the radiofrequency (RF) coil (d) causing molecules to undergo precession and emit energy which is detected by the receiver (Hwang and Choi, 2015).*

Hydrogen atoms are present in huge abundance in all tissues of the human body. The protons within the nuclei of hydrogen atoms have an electrical charge and a natural spin, so there is a tiny magnetic field associated with each spin. Each proton has a natural orientation depending on the chemical structure and polarisation of the molecules within its local environment. When a strong magnetic field is applied, such as that within an MRI scanner, all the protons tend to align with the direction of the external magnetic field, thereby creating a net magnetisation that is in the same direction as the external magnetic field. This is described as the longitudinal magnetisation. When the protons align with the longitudinal magnetization, they precess at a frequency determined by the Larmor equation (Rigden, 1986). In order to measure this magnetisation, a second magnetic field is applied orthogonal to the longitudinal magnetisation at the Larmor frequency, which is at radio frequencies (RF); this induces a current in the coil. The successive pulsing of magnetisation causes the molecules to resonate due to their continuous transition between being completely aligned to returning to their natural alignment. This continuous precession releases energy, which differs from one molecule to another, and is detected by the receiver and converted into a spectra. The resulting spectra is composed of separate peaks which represent different components of molecules that are separated due to a chemical shift in their energy while under a magnetic field (Boesch et al., 1997).

Proton-magnetic resonance spectroscopy is a valuable tool for quantifying skeletal muscle lipids because it is non-invasive and allows the differentiation between lipid molecules deposited between myocytes, called extramyocellular lipids (EMCL), and lipids stored within myocytes, IMCL (Schick et al., 1993, Boesch et al., 2006). EMCL is considered to be metabolically relatively inert, whereas, IMCL has been linked with insulin resistance as mentioned previously (Li et al., 2015).

1.6 Type 2 diabetes in populations of black ethnicity

As previously mentioned, populations of African ancestry suffer disproportionately from T2D. In South London, black ethnic groups make up about 20% of the population, however, they make up 40% of T2D patients registered at diabetes clinics in the local boroughs (Winkley et al., 2013). Furthermore, it is estimated that black populations develop T2D at a younger age, up to 10 years earlier, and at lower BMIs than white populations (Goff, 2019). Black populations bear a disproportionate burden of the morbidity and mortality associated with T2D, with higher rates of retinopathy, microalbuminuria, kidney failure, lower extremity amputation and mortality due to T2D compared white populations (Marshall, 2005, Harris et al., 1998b, Young et al., 2003). The high rates of diabetes complications in black patients with T2D has been attributed to poorer glycaemic control with common treatments of T2D being less effective in black populations (Marshall, 2005).

Some studies have attributed the greater prevalence of T2D in black populations to the greater prevalence of obesity and a tendency for lower socioeconomic status (Carter et al., 1996, Signorello et al., 2007). However, when obesity, socioeconomic status, and behavioural factors are adjusted for in statistical models, the greater prevalence of T2D persists in black groups compared to white groups; this indicates that not only environmental factors but genetic factors are also responsible for the ethnic disparities in T2D risk (Bonham and Brock, 1985, O'Brien et al., 1989, McWilliams et al., 2009). This notion is supported in a study by Cheng *et al.* who performed a genetic admixture analysis using over 2000 ancestry-informative markers on a large cohort of 7021 African Americans (Cheng et al., 2012). They showed that a greater percentage of African ancestry was associated with T2D even after adjustments for BMI and socioeconomic status shown in Figure 1.12; interestingly, this association was far stronger than non-

genetic factors for T2D in African Americans. Hence, not only environmental but biological factors are also related to the increased risk of T2D in black populations.

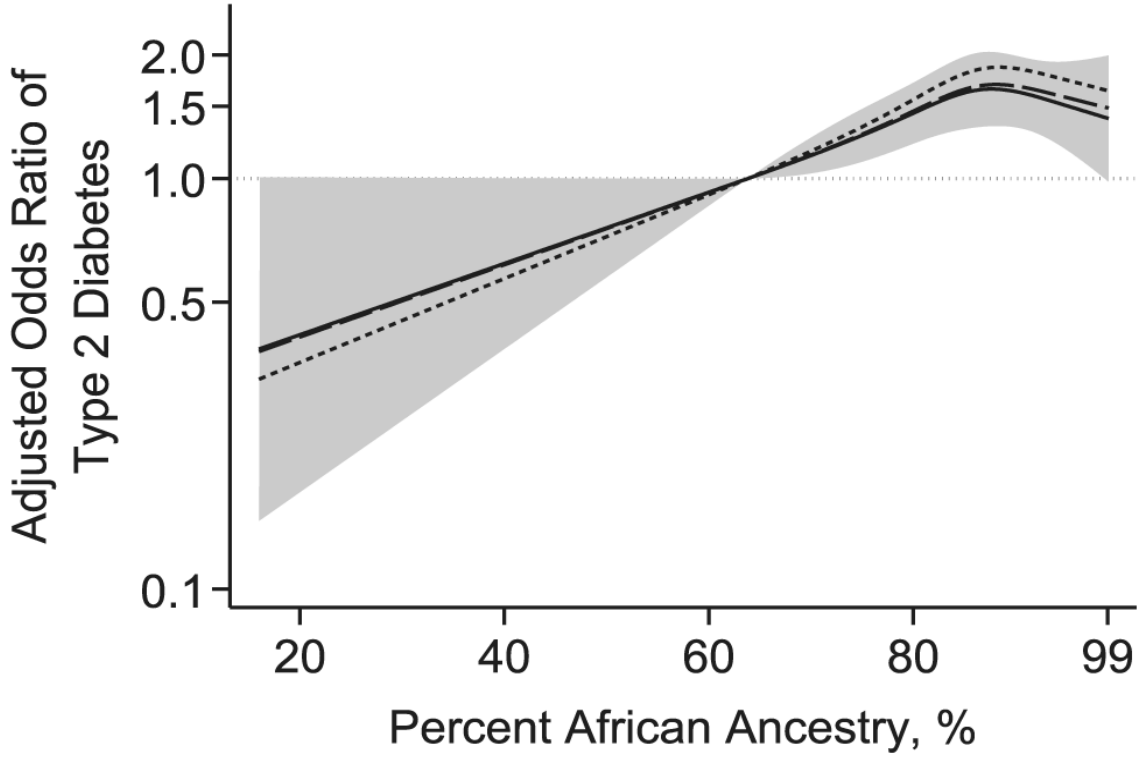

Figure 1.12: *Relationships between African ancestry and T2D. In a study of over 2000 African American individuals, a greater percentage of African ancestry was positively related to greater odds ratio of T2D (Cheng et al., 2012).*

1.6.1 Pathophysiology of type 2 diabetes in black populations

Due to the greater burden of T2D in black populations, research of potential ethnic differences in the pathophysiology of T2D between black and white groups has become an area of increasing interest. It has become well established that there are several metabolic features that are related to T2D that differ between black and white populations which include: insulin sensitivity, insulin secretion, insulin clearance, ectopic fat deposition, adipose tissue function and lipid metabolism (Goedecke et al., 2017, Alderete et al., 2014, Goran, 2008, Bergman et al., 2019).

1.6.1.1 Insulin sensitivity and insulin secretion in black populations

Increased insulin resistance and reduced insulin secretory function are two key features of T2D regardless of ethnicity, however, black populations generally have lower insulin sensitivity and greater insulin secretion compared to their white counterparts (Alderete et al., 2014, Goedecke et al., 2017, Hasson et al., 2015). Data from the Insulin Resistance Atherosclerosis Study (IRAS), showed that black populations have greater insulin resistance and insulin secretion compared to white populations (Haffner et al., 1996). Several other studies have also reported lower insulin sensitivity in black groups compared to their white counterparts (Uwaifo et al., 2002b, Uwaifo et al., 2002a, Tillin et al., 2006).

Several studies have shown increased insulin secretion in black populations compared to white populations (Hughan et al., 2013, Osei and Schuster, 1994). This was also demonstrated in a systematic review and meta-analysis conducted by Kodama *et al.* who investigated ethnic differences in relationships between insulin sensitivity and insulin response (Kodama et al., 2013). As presented in Figure 1.13, the results showed that even though there was a moderate overlap between the study outcomes from populations of Caucasian and African ethnicity, the African groups tended to have lower insulin sensitivity and greater insulin secretory response compared to the Caucasian groups.

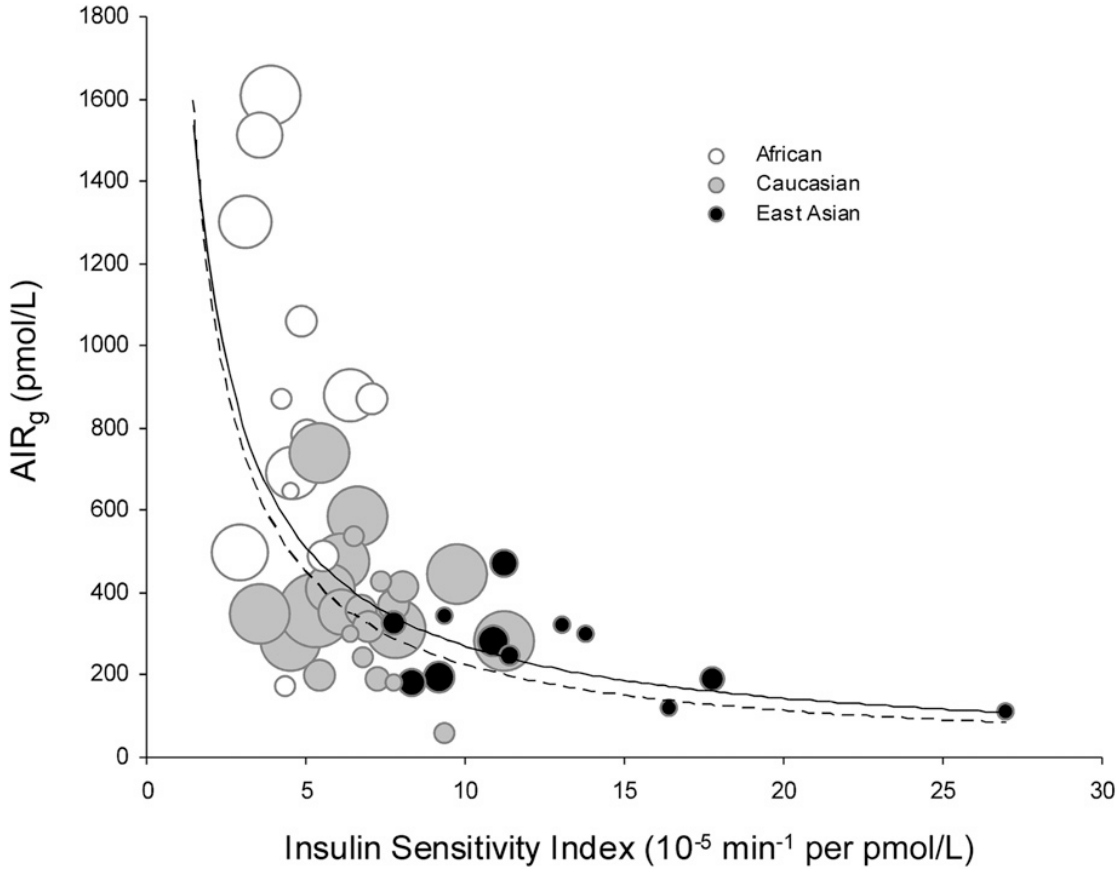

Figure 1.13: *Relationships between insulin sensitivity and insulin response. Each circle represents a study population with the size of the study population proportional to circle size (Kodama et al., 2013).*

The increased insulin secretory function in black populations has been suggested to be a compensatory mechanism for the reduced insulin sensitivity compared to whites. However, Hannon *et al.* showed, when matched for insulin sensitivity, African American adolescents exhibited greater first-phase insulin secretion compared to white adolescents, Figure 1.14 (Hannon et al., 2008). Therefore, there is much evidence showing that there are ethnic differences in insulin sensitivity and insulin secretion between black and white populations which appears to be present from as early as childhood. However, the reasons behind these differences are yet to be fully understood.

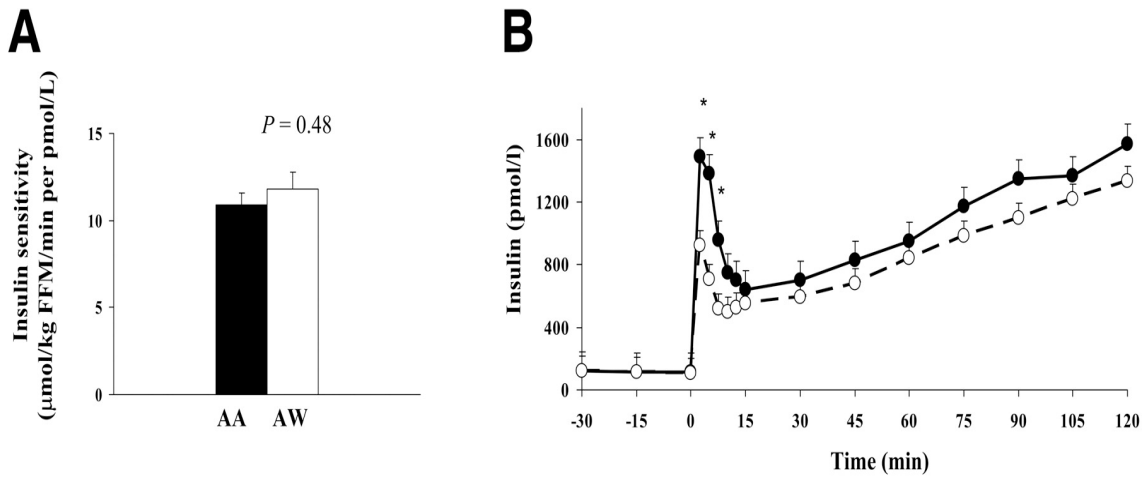

Figure 1.14: *Insulin sensitivity (A) and insulin secretion (B) in African American (AA) and American white (AW) adolescents (Hannon et al., 2008).*

1.6.1.2 Insulin clearance in black populations

Early studies from the 1990s, investigating insulin secretion in black populations showed that, although insulin levels were higher in black populations compared to whites, the levels of c-peptide were no different (Osei and Schuster, 1994, Cruickshank et al., 1991). This suggested that there were ethnic differences in insulin clearance. Since then, several studies have confirmed that insulin clearance is lower in black populations since the ratio of c-peptide to insulin is consistently reported to be lower in blacks during the fasting and glucose-stimulated states (Harris et al., 2002, Bergman et al., 2019).

Studies have shown that hepatic insulin clearance correlates with insulin secretion as well as insulin sensitivity (Pivovarova et al., 2013). Therefore, prior to frank T2D, with the long-term progression of insulin resistance there is an increase of insulin secretion as well as a reduction of insulin clearance; both are considered to be mechanisms to prolong higher levels of insulin in the circulation in order to overcome insulin resistance. While the lower levels of insulin clearance may be a protective mechanism to preserve beta-cell insulin secretory function in black populations, it may be another sign of increased insulin resistance in this ethnic group (Piccinini et al., 2017, Piccinini et al., 2018).

1.6.1.3 Lipid metabolism in black populations

Clinically unfavourable blood lipid profiles, characterised by high circulating triglycerides and LDL-cholesterol, and low HDL-cholesterol, are key features related to T2D (Reaven, 2006). It has been well documented that patients with T2D have high circulating lipids and low HDL-cholesterol levels; this is an indication of ineffective storage of excess energy in safe adipose tissue compartments as well as an over-production of cholesterol by the liver due to a positive energy balance (Chung and Parks, 2016).

Despite their greater risk for T2D, black populations consistently exhibit more favourable lipids profiles than white populations, indicated by lower circulating triglycerides, LDL- and VLDL-cholesterol levels, as well as higher HDL-cholesterol levels (Bentley and Rotimi, 2017, D'Adamo et al., 2010, Miljkovic-Gacic et al., 2006). The ratio of triglycerides to HDL is considered a sensitive marker for T2D risk, however, it has consistently been found to be lower in black populations (Yu et al., 2012). Furthermore, triglyceride to HDL ratio did not correlate with measures of insulin resistance in blacks as it did in whites (Sumner, 2009, Sumner et al., 2005). Therefore, it has been suggested that common clinical lipid markers are not sensitive at detecting T2D risk in black populations (Sumner, 2009, Giannini et al., 2011). Overall, this evidence suggests that lipid metabolism differs between black and white populations and may impact the development of T2D differently in these two ethnic groups.

1.6.1.4 Obesity in black populations

Given the greater risk of T2D in black populations, it is expected that they would exhibit greater levels of obesity. Indeed, several studies conducted in the US have highlighted a greater prevalence of obesity in African Americans compared to white Americans (Arroyo-Johnson and Mincey, 2016). Increased body fat determined by BMI, waist

circumference and DEXA scans have confirmed a greater level of obesity in African Americans observed in children, adolescents and adults (Cossrow and Falkner, 2004, Maligie et al., 2012, Gower et al., 1998). However, in the UK, ethnic differences in obesity between black and white groups is less straightforward with distinct gender differences present. As shown in Figure 1.15, black African and black Caribbean women have a greater level of obesity compared to the general female population in the UK (Gatineau, 2011). However, black African and black Caribbean men, unexpectedly, show slightly lower levels of obesity compared to the general male population, which is particularly evident in the black African men (Gatineau, 2011). Therefore, the greater levels of obesity among black women may partially explain their greater risk of T2D; however, the same cannot be said for black men, which suggests gender differences may exist in the role of adiposity in the pathophysiology of T2D within black populations.

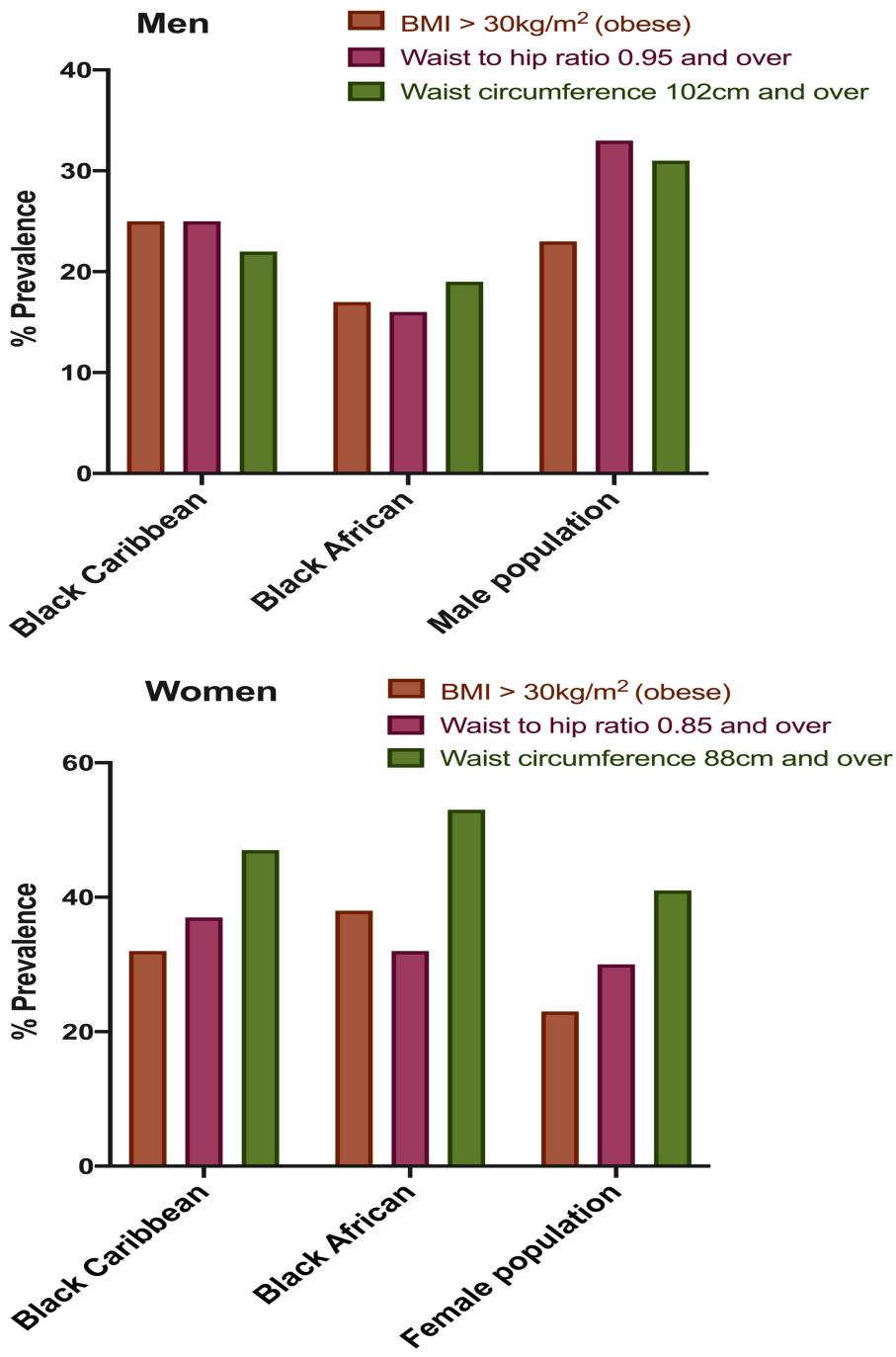

Figure 1.15: *Prevalence of obesity in black ethnic groups in the UK in comparison to the general population. Created from data extracted from the National Obesity Observatory, NHS, 2011 (Gatineau, 2011).*

1.6.1.5 Body-fat distribution in black populations

As mentioned previously, black populations have a greater prevalence of T2D compared to white populations, however, investigating this further Zhang *et al.* reported the

prevalence of T2D by ethnicity and by level of obesity using a large cohort from the National Health and Nutrition Examination Survey (NHANES) (Zhang et al., 2009). He found that, in the normal weight category, the prevalence of T2D was 5% in blacks which was three times greater than in whites who had a 1.5% prevalence. However, this disparity decreased greatly as BMI increased; in fact, in the over 35 BMI category, the prevalence of T2D was only slightly greater in blacks (20%) compared to whites (18%). This suggests that the influence of obesity in the pathophysiology of T2D may differ by ethnicity and obesity may not be such a strong determinant in black populations.

1.7 Ectopic fat deposition in black populations

Despite the greater prevalence of T2D in black populations, they typically have lower levels of ectopic fat compared to white populations, which creates a paradox unique to black ethnic groups (Goran, 2008, Guerrero et al., 2009, Goedecke et al., 2017). It is well established that, similar to black populations, the prevalence of T2D is also greater in South Asian, Native American and Hispanic populations compared to white populations (Alderete et al., 2014). Extensive research has also been carried out in the above ethnic groups in an attempt to investigate the reasons for their greater prevalence of T2D. Studies comparing ectopic fat between each individual ethnic group (South Asian, Native American and Hispanic) and white populations have shown a common report: a greater level of ectopic fat in these ethnic groups compared to white populations (Alderete et al., 2014, Chandalia et al., 2007, Sniderman et al., 2007). The greater level of ectopic fat in these ethnic minority populations has been used to explain their greater prevalence of T2D as they appear to have a greater susceptibility to store ectopic fat at lower levels of BMI compared to white populations. However, such cannot be said for black populations who consistently exhibit lower levels of ectopic fat (Alderete et al., 2014); this is commonly referred to as the ectopic fat paradox in black populations (Goran, 2008).

While some researchers have suggested that black populations may be more sensitive to the lipotoxic effects of ectopic fat others suggest that ectopic fat may play a lesser role in the development of T2D in black populations (Ingram et al., 2011, Alderete et al., 2013). These speculations are yet to be clarified, therefore, the study of ectopic fat and its role in the development of T2D in black populations continues to be an area of increasing interest.

1.7.1 Visceral adipose tissue in black populations

Visceral adipose tissue has consistently been shown to be lower in black populations compared to white populations (Alderete et al., 2014, Goedecke et al., 2017). In 1995, Conway *et al.* were the first to directly quantify VAT area using CT scanning, which they used to conduct an ethnic comparison of VAT between black and white women (Conway et al., 1995). Contrary to their expectations, they found that the black women had significantly lower VAT compared to the white women. Their findings were further confirmed by Lovejoy *et al.* who reported lower VAT in black women compared to BMI and waist-to-hip ratio matched white women; furthermore they found VAT was associated with waist-to-hip ratio in white women but not black women (Lovejoy et al., 1996). The lower levels of VAT in black populations have since been confirmed from more recent studies using larger cohorts in both men and women as well as youths (Alderete et al., 2014, Ronn et al., 2017). Furthermore, with an increase of whole body adiposity there is a lesser increase in VAT in black populations compared to white populations. This was demonstrated by Despres *et al.* who showed that VAT increased to a greater extent with increasing body fat in whites compared to blacks as shown in Figure 1.16 (Despres et al., 2000).

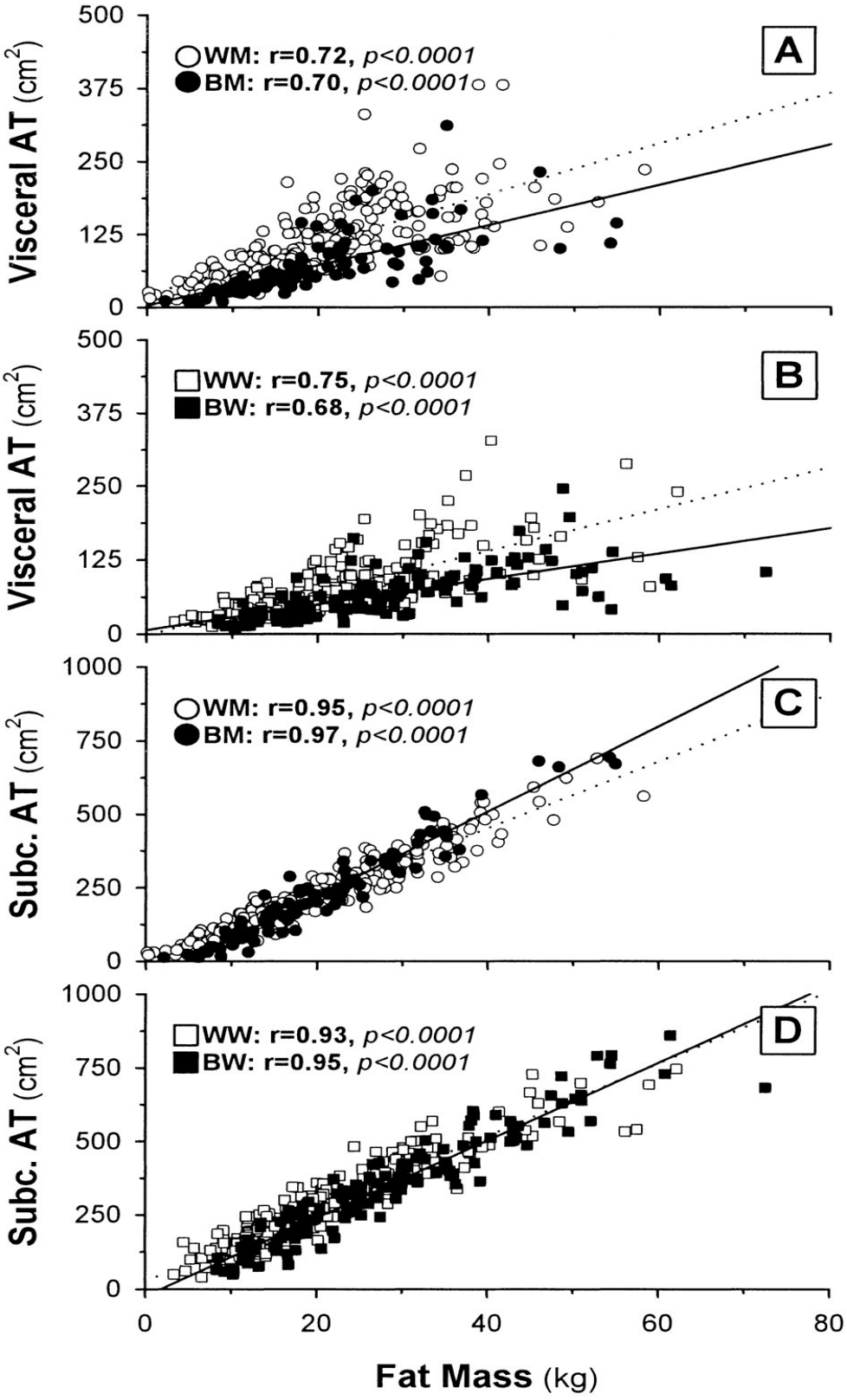

Figure 1.16: *Relationships between fat mass and subcutaneous and visceral adiposity in white (white circles) and black (black circles) populations. In both men (A and C) and women (B and D), with an increase in whole-body adiposity there were similar increases in subcutaneous fat in all groups but lower increases in visceral fat in the black groups compared to the white groups (Despres et al., 2000).*

1.7.2 Visceral and subcutaneous adipose tissue in black populations

While black populations have lower VAT levels in comparison to white populations they have higher levels of SAT deposition (Goran et al., 1997, Staiano et al., 2013, Katzmarzyk et al., 2010). Considering ethnicity and glycaemic state, Gallagher *et al.* showed VAT was greater, while SAT was lower, in the groups with T2D compared to controls in both white and black adults (Gallagher et al., 2009). Not only was VAT lower in the blacks compared to the whites, this ethnic difference was present between the healthy and T2D groups, shown in Figure 1.17. However, this was not the case for SAT which was significantly greater in the black groups than the white groups at both glycaemic states. This suggests that during the progression of T2D, as whole body adiposity increases, there is a disproportionate increase of VAT in white compared to black individuals. Furthermore, black ethnic groups appear to store excess fat more efficiently in the metabolically safer SAT depot compared to white populations. Therefore, this adds to the paradox that, while black populations exhibit several features of favourable adipose tissue expansion, they suffer disproportionately from T2D.

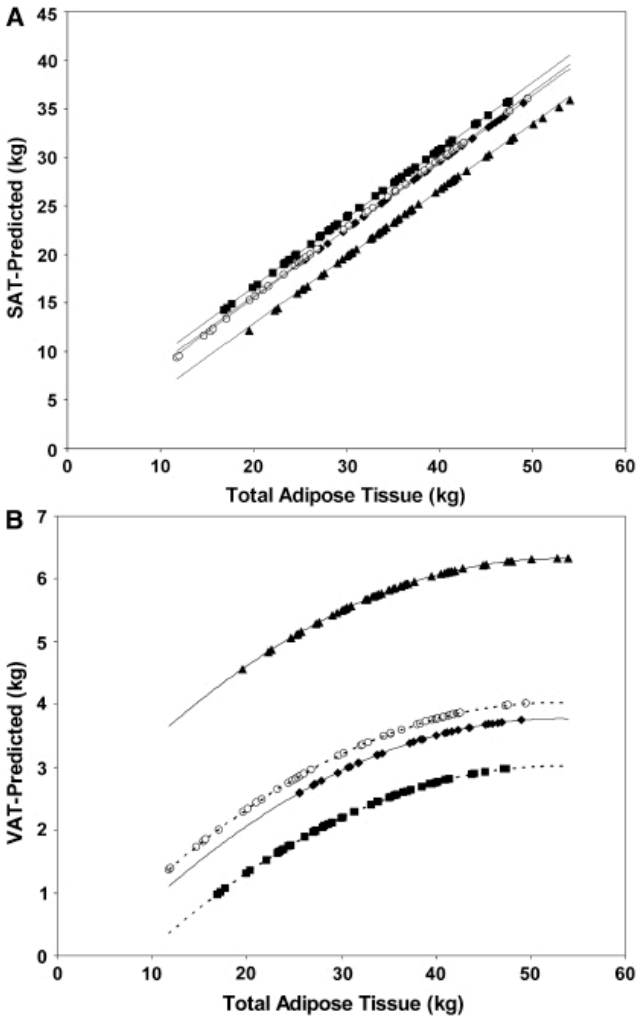

Figure 1.17: *Prediction models for relationships between total adipose tissue and subcutaneous fat (A) and visceral fat (B). Type 2 diabetes (solid lines: ◆, African Americans; ▲, whites) and control (dashed lines: ▪, African Americans; ○, whites (Gallagher et al., 2009).*

1.7.3 Deep and superficial subcutaneous adipose tissue in black populations

While VAT and SAT deposition have been extensively studied in black populations, dSAT and sSAT are only recently gaining attention. Current investigations of dSAT and sSAT in black populations have shown inconsistent conclusions which may be explained by glycaemic state, level of obesity or gender of the population studied. Liska *et al.* showed no ethnic differences in dSAT between white and black adolescents, however,

sSAT was higher in the black group (Liska et al., 2007). Similar findings were reported in women by Goedecke *et al.* (Goedecke et al., 2009) and Lovejoy *et al.* (Lovejoy et al., 2001).

In a large cohort study, Nazare *et al.* found no ethnic differences in dSAT and sSAT between white and black men; however, differing results were reported in the women where dSAT was lower in the black women compared to the white women, with no ethnic differences in sSAT (Nazare et al., 2012). In contrast, Evans *et al.* found no ethnic differences in dSAT and sSAT between black and white women (Evans et al., 2011). Therefore, ethnic differences in dSAT and sSAT appear to be influenced by gender and may impact the pathophysiology of T2D differently by ethnicity.

1.7.4 Intrahepatic lipids in black populations

Similar to VAT in black populations, it is widely reported that blacks have lower IHL deposition compared to their white counterparts (Goedecke et al., 2015, Schwimmer et al., 2005, Szczepaniak et al., 2012). Goedecke *et al.* found IHL was lower in black women compared to their white counterparts (Goedecke et al., 2015). Similar findings were reported by Naran *et al.* who also reported a negative association between IHL and SAT, indicating that an increase of SAT may act as a protective reservoir for excess triglycerides in blacks (Naran et al., 2018). Guerrero *et al.* reported that with increasing total adiposity, IHL increases to a lesser degree in blacks compared to whites (Guerrero et al., 2009).

Ethnic differences in NAFLD have also been commonly reported with lower prevalence rates being reported in black populations compared to white populations (Pan and Fallon, 2014). In a large cohort study, the prevalence of hepatic steatosis was significantly lower in black men compared to white men (Browning et al., 2004).

There is increasing evidence supporting the detrimental role of IHL in the development of T2D with some studies showing a greater link between IHL and T2D than VAT (Alderete et al., 2013, Lee et al., 2012). Considering this, it is highly paradoxical that black populations typically have low levels of IHL. Adding to this paradox, Liska *et al.* found not only was IHL lower in black compared to white adolescents, but in some black subjects, IHL deposition was undetectable despite the presence of obesity (Liska et al., 2007). Figure 1.18 shows the protective circulating lipids and ectopic fat characteristics that are typically found in black individuals, which, hypothetically, should result in lower T2D risk (D'Adamo et al., 2010). Therefore, there is a lack of clarity in the role of IHL in T2D in black populations.

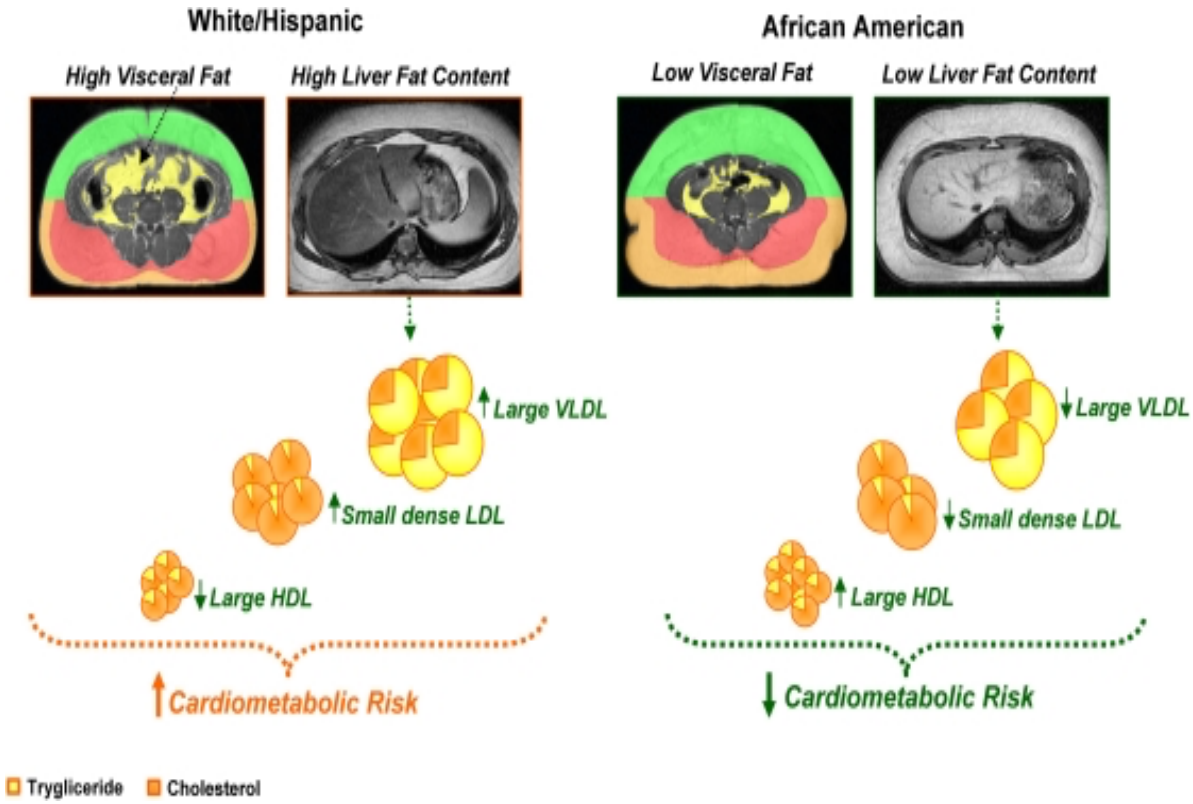

Figure 1.18: *Hypothetical origin of cardiometabolic risk profiles of white and African American individuals (D'Adamo et al., 2010)*

1.7.5 Intrapancreatic lipids in black populations

Unlike VAT and IHL, far fewer studies have investigated IPL in black populations. To date, only 5 studies have conducted ethnic comparisons of IPL between black and white/Hispanic groups.

Two of the studies of IPL in black populations compared IPL between African American and Hispanic adolescents. Le *et al.* showed that IPL was significantly lower in African American adolescents compared to their Hispanic counterparts (Le et al., 2011). In a study on adolescents with prediabetes, Toledo-Corral *et al.* showed that IHL predicts prediabetes status in Hispanics but not African Americans, however, IPL predicted prediabetes status in African Americans but not Hispanics (Toledo-Corral et al., 2013); this suggests there are ethnic differences in the role of IPL in the development of T2D between African American and Hispanic youths.

Studies of IPL in black adults showed a lower IPL in black women compared to white/Hispanic women with a linear relationship reported between IPL and disposition index in the black women but not white women (Lingvay I, 2014). Similar to previous findings, Szczepaniak *et al.* showed IPL was lower in black adults compared to white adults with positive associations between IPL and disposition index in both ethnic groups (Szczepaniak et al., 2012). To date, no ethnic comparison studies of IPL have been conducted in black *versus* white men. Hence, while studies of IPL have been few, they suggest that IPL may be more dominant in the development of T2D in comparison to IHL in black populations.

1.7.6 Intramyocellular lipids in black populations

Similarly to IPL, few studies have investigated IMCL in black populations. To current knowledge, all studies that investigated ethnic differences in IMCL deposition between black and white populations showed no ethnic differences (Alderete et al., 2014,

Goedecke et al., 2017). Liska et al. showed no ethnic differences in IMCL between white and black adolescents with obesity (Liska et al., 2007). While, in a large cohort study, Maligie *et al.* showed IMCL was higher in blacks compared to whites, however, this difference diminished after adjustment for confounding factors including BMI (Maligie et al., 2012).

Some studies have suggested that, rather than the amount of IMCL, it may be its relationship with insulin sensitivity that may be more crucial in unfolding its influence in T2D. Considering this, Lawrence *et al.* showed even though there were no ethnic differences in IMCL, it was inversely associated with insulin sensitivity in whites but not blacks, indicating that IMCL may be a significant determinant of insulin sensitivity in white but not black populations (Lawrence et al., 2011). Similar findings were reported by Ingram *et al.* who showed no ethnic difference in IMCL between white and black adults; but IMCL inversely correlated with insulin sensitivity in white but not black adults with a significant interaction by ethnicity (Ingram et al., 2011). Like IPL, to date, no ethnic comparison studies of IMCL have been conducted in black *versus* white men.

1.7.7 Limitations to current knowledge of type 2 diabetes in black populations

Of note, most ethnicity studies, which focus on the role of ectopic fat on the pathophysiology of T2D, have been mainly limited to youths, women and African American populations with very few investigating men. Studies of T2D in youth focus mainly on severe cases of obesity which induces insulin resistance, thought to be mediated primarily from severe lipotoxicity. However, most cases of T2D occur in middle-aged populations where the pathophysiology is more complex with a combination of insulin resistance and beta-cell dysfunction as well as many other factors which differ from T2D in adolescents. Therefore, despite the large body of literature on T2D in black

adolescents, conducted mainly in African American populations, an understanding of T2D in black adults is highly warranted.

1.7.8 Sex differences in type 2 diabetes in black populations

Studies have shown that the development of T2D differs between men and women even in black populations (Høeg et al., 2011). Findings by Goedecke *et al.* support this, which show that insulin sensitivity is lower while insulin secretion is greater in black women compared to black men even after adjustments for measures of body fat (Goedecke et al., 2016). Furthermore, it is widely accepted that the distribution of body fat differs between men and women, particularly in ectopic fat deposition as women have consistently been shown to have lower VAT and IHL (Schorr et al., 2018, Gillen et al., 2018, Nissen et al., 2016, Despres et al., 2000). Women have a greater capacity to store fat safely in the SAT depot while men have consistently been shown to have greater VAT as well as ectopic fat deposition compared to women (Karastergiou et al., 2012, Blaak, 2001). Figure 1.19 summarises gender differences in parameters that are related to T2D and shows the vast and complex differences that must be appreciated when considering the pathophysiology of T2D between men and women (Kautzky-Willer et al., 2016).

Studies specifically investigating black women have shown they exhibit lower levels of ectopic fat; however, despite this, there is much evidence to suggest that the severe expansion of SAT may be the key driver of T2D in black women compared to white women (Goedecke et al., 2013, Goedecke et al., 2017). Additionally, while SAT was a more sensitive predictor of metabolic health in black women, VAT was the more sensitive predictor in the white women (Goedecke et al., 2013). However, there is a lack of evidence to suggest that this is the case in black men due to few studies being conducted in them.

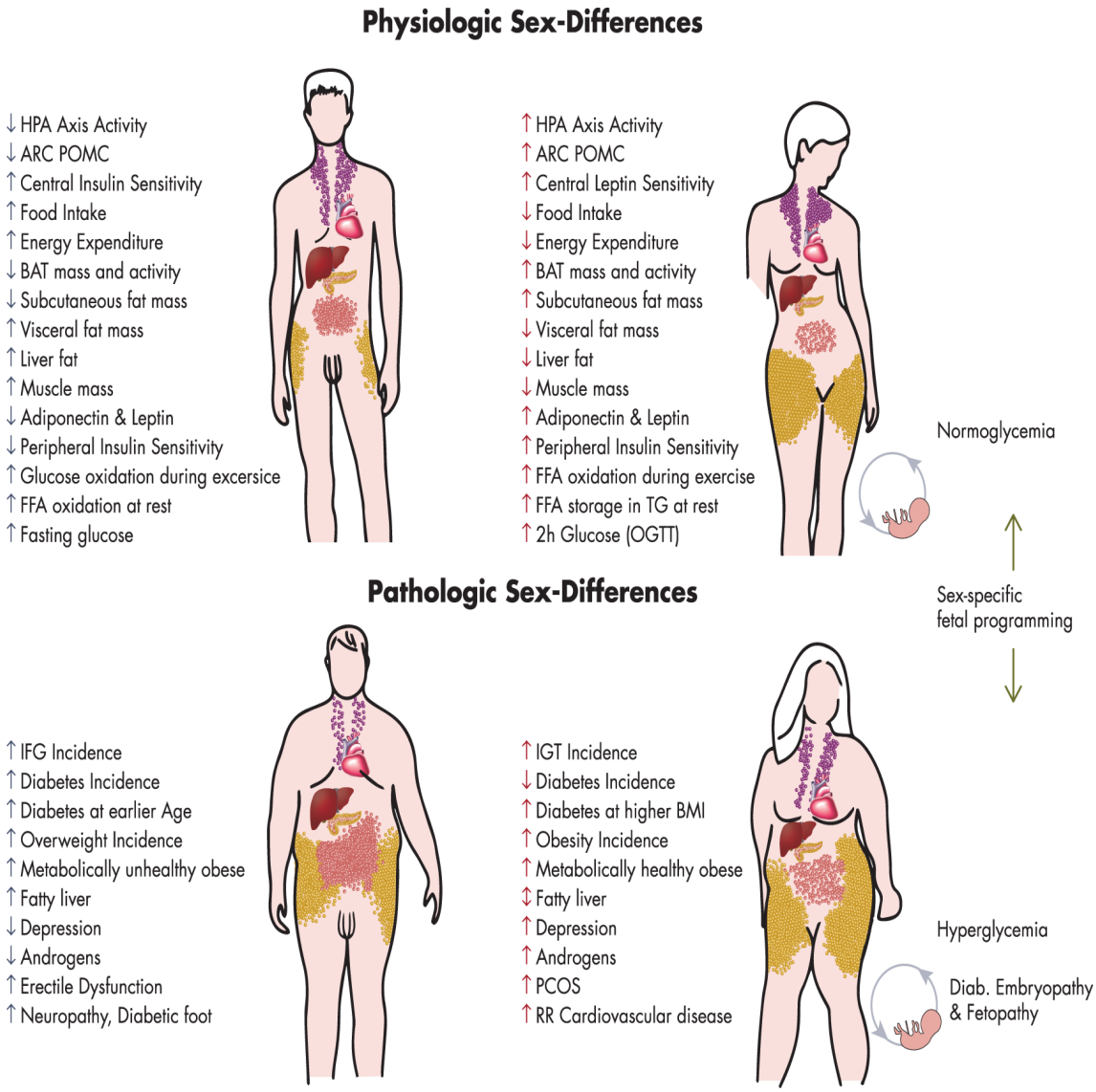

Figure 1.19: *General sex differences in T2D risk and related characteristics. Blue arrows indicate differences compared to women and red arrows indicate differences compared to men (Kautzky-Willer et al., 2016).*

1.8 Summary

Ectopic fat deposition is one of the key factors contributing to the development of T2D and explains the well-known link between obesity and T2D. Advancements in MRI technologies have allowed the accurate quantification of regional body fat distribution and the detailed analysis of ectopic fat depots. It has since been established that ectopic fat is detrimental to the function of the organ it resides in and causes insulin resistance when deposited in the liver and muscles, and disturbs insulin secretion in the pancreas. Even though black populations have a greater prevalence of T2D than white populations, they typically exhibit lower levels of ectopic fat which creates a paradox unique to black ethnic groups. Investigations of relationships between ectopic fat depots and metabolic parameters of T2D in black vs white populations have produced inconsistent conclusions. While some studies suggest that black populations may be more sensitive to ectopic fat storage and experience their detrimental effects at lower levels others suggest that ectopic fat may play a lesser role in T2D in black populations compared to their white counterparts. Furthermore, there are some suggestions that the pathophysiology of T2D may differ between black and white populations, which has mainly been investigated in youths and women, and in studies mostly conducted in the US, with a lack of investigations conducted in black men. Clinically, black populations are less responsive to common treatments of T2D which leads to greater T2D-related complications compared to white populations. This is partly attributed to the limited understanding of the pathophysiology of T2D in populations of African ancestry, including the role of ectopic fat. Understanding the role of ectopic fat in the development of T2D in black populations may assist in informing ethnically-tailored treatment and prevention strategies to reduce the burden of T2D this high-risk ethnic group.

1.9 Hypotheses

It is hypothesised that black west African men will have lower ectopic fat deposition compared to white European men at normal glucose tolerance, impaired glucose tolerance and type 2 diabetes glycaemic states. Furthermore, there will be stronger relationships between individual ectopic fat depots and the relevant metabolic parameters of type 2 diabetes in the black west African men compared to the white European men.

1.10 Aims

The primary aim of this thesis is to assess and compare ectopic fat depots between black west African (BWA) and white European (WE) men through the progressive stages of type 2 diabetes by comparing three glycaemic states: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D). The secondary aim is to investigate relationships between individual ectopic fat depots of interest with the relevant metabolic parameters of T2D in each ethnic group at each glycaemic state.

Within each objective a comparison will be undertaken between the BWA and WE ethnic groups as well as between the NGT, IGT and T2D groups.

Primary objectives - to investigate ethnic differences in:

1. Regional abdominal adipose tissue including visceral adipose tissue, abdominal subcutaneous adipose tissue (SAT), deep SAT and superficial SAT (Chapter 3)
2. Intrahepatic lipid and its relationships with hepatic insulin sensitivity and insulin clearance (Chapter 4)
3. Intrapancreatic lipid and its relationship with beta-cell insulin secretory function (Chapter 5)
4. Intramyocellular lipid and its relationship with insulin sensitivity (Chapter 6)

Chapter 2: Methods

2.1 Overview of the South London Diabetes and Ethnicity Phenotyping study

The work reported in this thesis was conducted as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) study, which is an observational study investigating ethnic differences in the pathophysiology of T2D between men of BWA and WE ethnicity. Within each ethnic group, three glycaemic states are assessed: NGT, IGT and T2D; the sample sizes of each are shown in Table 2.1. The Soul-Deep study assessed the following parameters: beta-cell insulin secretory function (ISF), insulin sensitivity (whole-body, skeletal muscle, hepatic and adipose tissue), regional fat distribution (SAT, dSAT, sSAT and VAT) and ectopic fat accumulation (IHL, IPL and IMCL). This thesis focuses primarily on the ectopic fat data and the relationships between ectopic fat depots and the metabolic parameters that were measured.

Table 2.1: *Sample size of each comparison group of the Soul-Deep study.*

	NGT	**IGT**	**T2D**
WE	**23**	**10**	**18**
BWA	**20**	**10**	**20**

Soul-Deep was conducted between April 2013 and April 2019. The study was reviewed and approved by the London Bridge National Research Ethics Committee (references: 15/LO/1121 and 12/LO/1859). Prior to participation, all participants provided written, informed consent. The metabolic assessments were carried out at the Clinical Research Facility at King's College Hospital and the MRI visit was conducted at Guy's hospital, London Bridge.

2.2 Participants

Soul-Deep participants were recruited by advertisements in local newspapers, general practices, Facebook as well as leaflet distribution in the South London boroughs of Lambeth, Southwark, Lewisham and Bromley. The participants with T2D were recruited mainly from the database of the South London Diabetes (Soul-D) study, which included individuals with a recent diagnosis of T2D that gave consent to be contacted for future studies (Winkley et al., 2013). Individuals that showed interest in taking part were invited for a screening assessment at the clinical research facility at King's College Hospital. The screening assessments and metabolic visits for the NGT and IGT participants were carried out by Toyosi Bello, Dr Meera Ladwa and Dr Chinmay Marathe while, for the participants with T2D, they were carried out by Dr Cynthia Mohandas. All MRI and ^{1}H-MRS assessments were carried out by research radiographers at Guy's Hospital Radiology Department and supervised by Dr Geoff Charles-Edwards. Figure 2.1 shows a schematic of the study design and study visits.

2.2.1 Screening assessments

Potential participants underwent a screening assessment to assess eligibility. Participants completed a screening health questionnaire to report age, self-declared ethnicity of self, parents and grandparents, date of diabetes diagnosis if applicable, medical history, current medication and contraindications for MRI. A fasting blood test measured full blood count, renal and liver function, HbA1c, lipid profile, sickle cell trait and auto-antibodies (anti-insulin, anti-GAD and anti-A2). Anthropometric measurements were taken and included height, weight, waist circumference (measured at the midpoint between the lowest rib and the iliac crest) and seated blood pressure measured using an automated sphygmomanometer (taking the average of three measurements) (WHO, 2011).

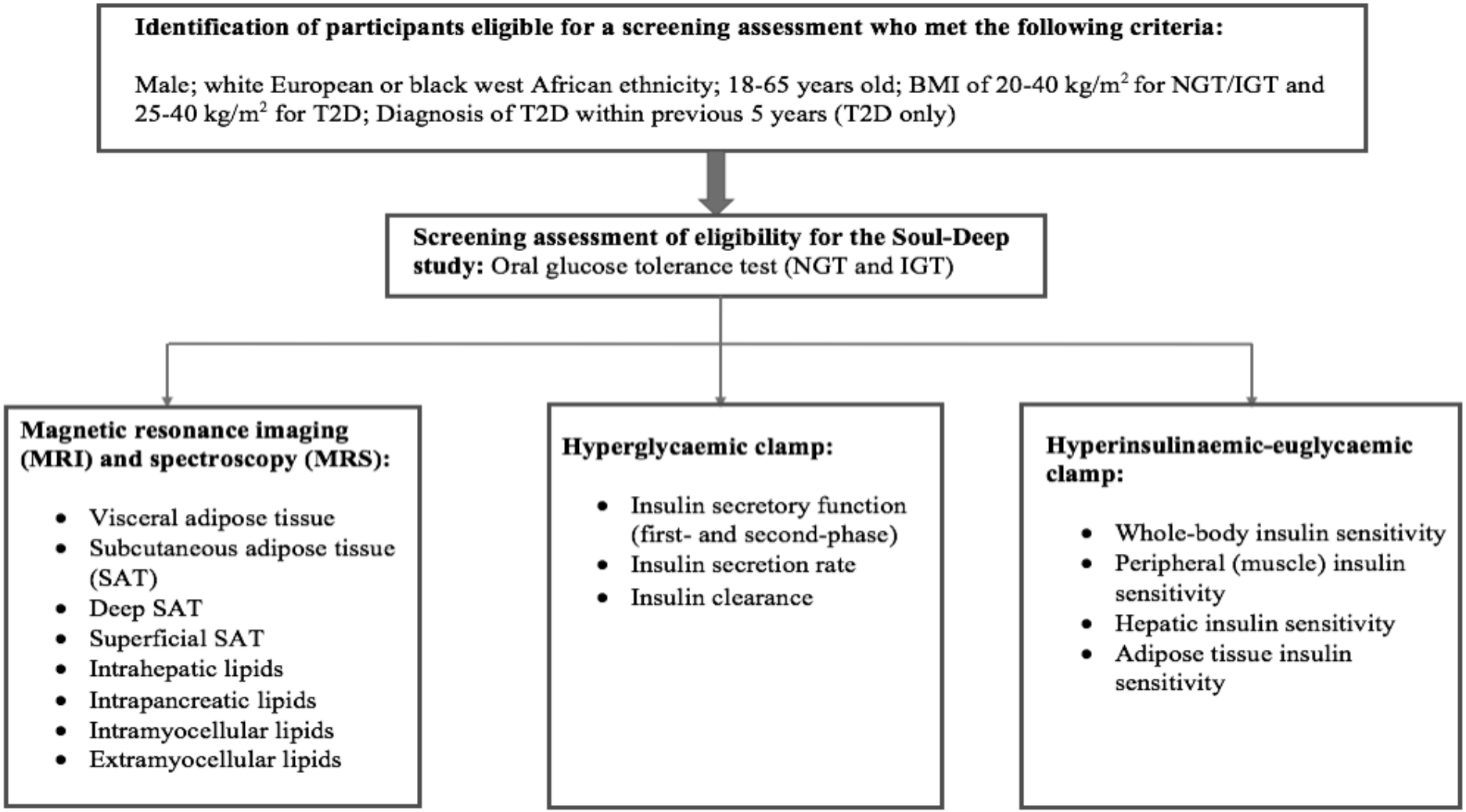

Figure 2.1: *Schematic of the Soul-Deep study participant recruitment process and parameters assessed during each visit.*

2.2.2 Oral glucose tolerance test

The NGT and IGT participants underwent a 75g oral glucose tolerance test (OGTT) carried out according to standard procedures outlined by WHO to determine their glucose tolerance (WHO, 2003). Prior to the OGTT, participants were instructed to consume at least 50g of carbohydrates during their meal on the evening before the OGTT and to attend the visit after an overnight fast (10 hours). A cannula was inserted in the antecubital fossa vein for blood sampling then a 75g oral glucose load was consumed. Plasma glucose was assessed at fasting (-10 and 0 minutes) and at 120 minutes. The participants with T2D were not required to have an OGTT; glycaemic state was identified by a documented diagnosis of T2D.

2.2.3 Inclusion and exclusion criteria

The following inclusion criteria were applied to assess eligibility: NGT participants were eligible to take part if they were 1) male, 2) 18-65 years old, 3) had a BMI of 20-40 kg/m^2, 4) were of WE or BWA ethnicity (identified through self-reported birthplace of self, parents and grandparents), 5) had a HbA1c level <47.5 mmol/mol (6.5%), 6) had normal glucose tolerance determined by a 2-hour plasma glucose <7.8 mmol/l during a 75g OGTT. IGT participants were eligible to take part if they were 1) male, 2) 18-65 years old, 3) had a BMI of 20-40 kg/m^2, 4) were of WE or BWA ethnicity (identified through self-reported birthplace of self, parents and grandparents), 5) had a positive family history of T2D determined by a first degree relative with T2D, 6) had a HbA1c level ≥38.8 mmol/mol (5.7%) if WE or ≥41.0 mmol/mol (5.9%) if BWA , 7) had impaired glucose tolerance determined by a 2-hour plasma glucose >7.8 <11.1 mmol/l during a 75g OGTT. Participants with T2D were eligible to take part if they were 1) male, 2) 18-65 years old, 3) had a BMI of 25-40 kg/m^2, 4) were of WE or BWA ethnicity (identified through self-reported birthplace of self, parents and grandparents), 5) had a recent diagnosis of type 2

diabetes (less than 5 years prior to the study), 6) had a HbA1c level of ≤63.9 mmol/mol (8%), and 7) treated with lifestyle and/or metformin only.

Participants were excluded from the study if they had contraindications for MRI (such as metal implants or claustrophobia), were receiving treatment with thiazolidinedione, insulin, oral steroids, beta-blockers or any other medication that could affect the study outcomes. Participants were also excluded if they showed evidence of liver or kidney damage, determined from a serum alanine aminotransferase (ALT) level of 2.5-fold above upper limit of the reference range or serum creatinine level above 150 mmol/l, respectively, or tested positive to anti-insulin, anti-GAD or anti-A2 auto-antibodies or had sickle cell disease.

2.3 Study design and procedures

After confirmation of eligibility, participants attended four assessment visits (Figure 2.1), which included a MRI and ^{1}H-MRS scan, a hyperglycaemic clamp and a hyperinsulinaemic-euglycaemic clamp. Prior to the visits, participants were instructed to refrain from 1) strenuous exercise and physical activity for 48-hours, 2) alcohol consumption for 24-hours, and 3) food and drink after 22:00 on the evening before. Participants with T2D on metformin therapy were instructed to cease taking it for 7-days prior to each visit. On the day prior to the three metabolic assessment visits, participants were instructed to consume a standardized diet which aimed to provide approximately 50% of calories from carbohydrates, 30-35% from fat and 15-20% from protein, spread evenly throughout the day to ensure no more than 30% of the daily carbohydrates were consumed in the evening meal. Assessments were completed in random order within a maximum period of 6 months between the first and last visit.

2.3.1 Magnetic resonance imaging scan

Participants underwent a MRI scan for the assessment of VAT, SAT, dSAT, sSAT, IHL and IPL. The MRI and ^{1}H-MRS scan (detailed below) were conducted on a single visit between 7:30am and 8:30am at the Clinical Imaging Department at Guy's Hospital, London. Participants first completed an MRI health safety screening form and changed into a hospital gown to ensure no metal objects were on the subjects clothing. Each participant was scanned, using a 2-point Dixon-based MRI sequence on a 1.5 Tesla Siemens Aera scanner, from the neck to the knee (excluding the arms) while lying in the supine position with surface coils placed around the scanned body area. Participants were instructed by the radiographer to hold their breath for three bouts of 15 seconds while abdominal images were acquired to reduce motion artefacts. Each MRI scan produced 320 contiguous, axial, T1-weighted transverse spin-echo images (repetition time: 6.77ms; echo time: 4.77ms (in-phase), 2.39ms (out-of-phase), flip angle: 10°) each with a slice thickness of 3mm. The combination of in-phase and out-of-phase images allowed the acquisition of water-only and fat-only MRI images. All MRI data were analysed using the open source image analysis software HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017) detailed below.

2.3.1.1 Analysis of regional abdominal adipose tissue

Areas of VAT, ASAT, dSAT and sSAT were determined at the L4-5 anatomical position from a single fat-only MRI image. Fat tissue is easily identifiable in a fat-only MRI image because fat tissue is white while non-fat tissue is black. The L4-5 cross-sectional position (between the fourth and fifth lumbar vertebrae) is a commonly used landmark for assessing abdominal adipose tissue and roughly corresponds to the position of the umbilicus. Axial L4-5 images were identified by using the sagittal view of the abdomen as shown in Figure 2.2.

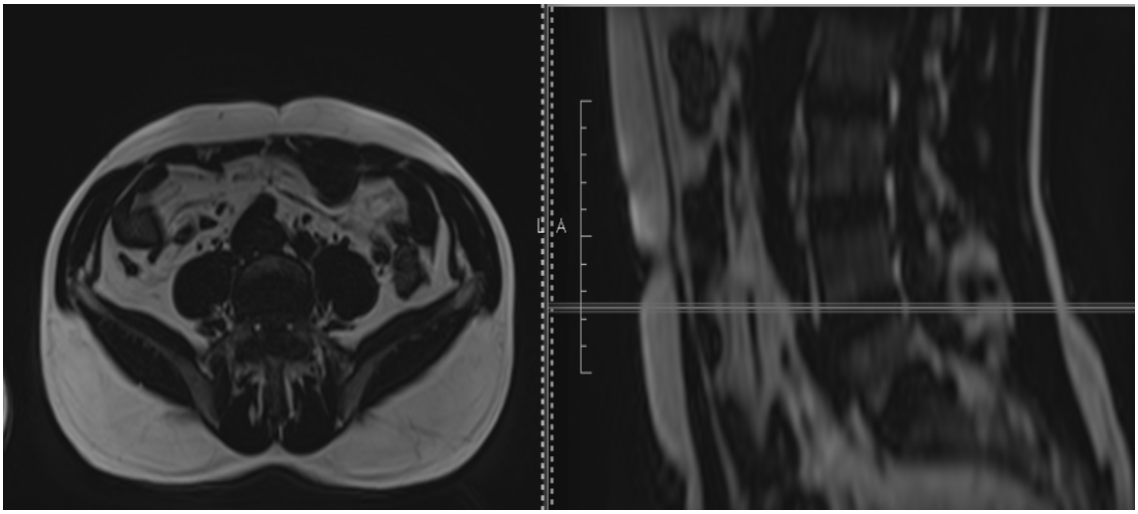

Figure 2.2: *Identification of the axial MRI image that corresponds to the L4-5 anatomical position using a sagittal image. The horizontal line on the sagittal image (right) depicts the position of the axial image (left).*

Visceral adipose tissue and ASAT were distinguishable as VAT is contained within the abdominal cavity, in the centre of the abdominal cross-section, while ASAT is on the outer layer of the abdomen. The dSAT and sSAT were distinguishable by identification of the fascia superficialis that is visible in a cross-sectional MRI image as indicated by arrows in Figure 2.3; dSAT is situated below the fascia while sSAT is above.

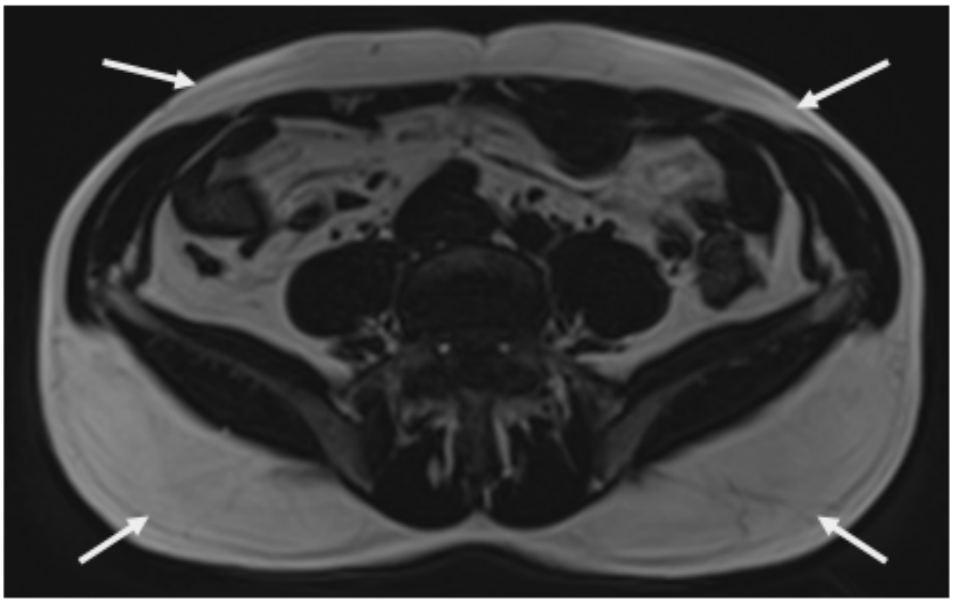

Figure 2.3: *An axial MRI image at the L4-5 anatomical position with the arrows referring to the fascia superficialis that separates dSAT from sSAT.*

In order to differentiate between adipose tissue and non-adipose tissue the tool 'Grow Region (2D Segmentation)' was used, which assisted in highlighting all pixels with a high intensity which mostly represent fat tissue. Then the areas of VAT, ASAT, dSAT and sSAT were determined by manually correcting the highlighted adipose tissue regions using the 'brush' tool as shown in Figure 2.4. Each of the adipose tissue depots were assigned regions of interest to determine their respective areas.

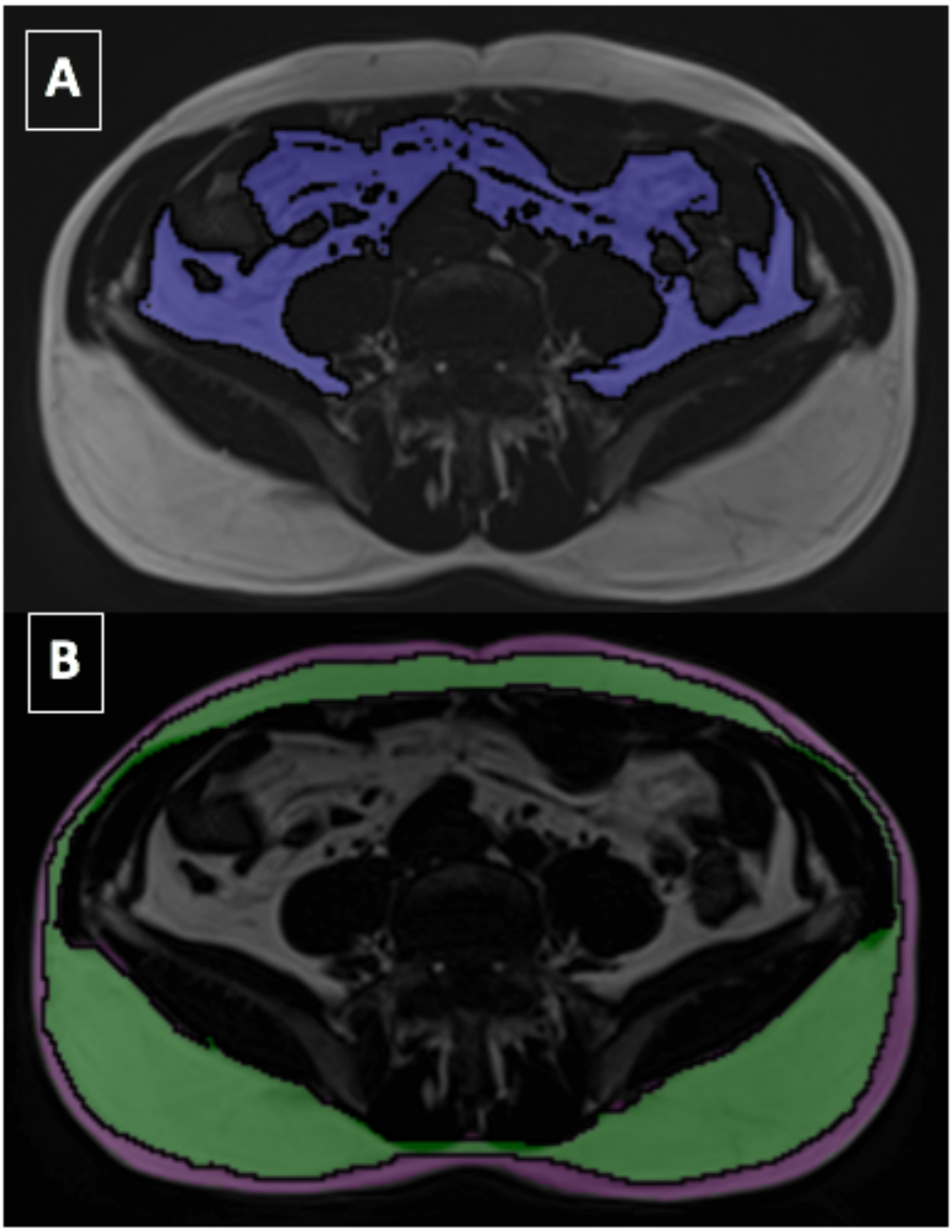

Figure 2.4: *An axial MRI image at the L4-5 anatomical position showing the highlighting of VAT (blue), A; and highlighting of dSAT (green) and sSAT (magenta), panel B.*

2.3.1.2 Analysis of intrahepatic lipids

Intrahepatic lipid was quantified in each participant by selecting two abdominal MRI images approximately 25-35mm apart which represent the superior and inferior parts of the liver as shown in Figure 2.5. The horizontal line in panels B and D represent the location of the axial images A and C, respectively. The fat-only and water-only images were selected for both the superior and inferior MRI images.

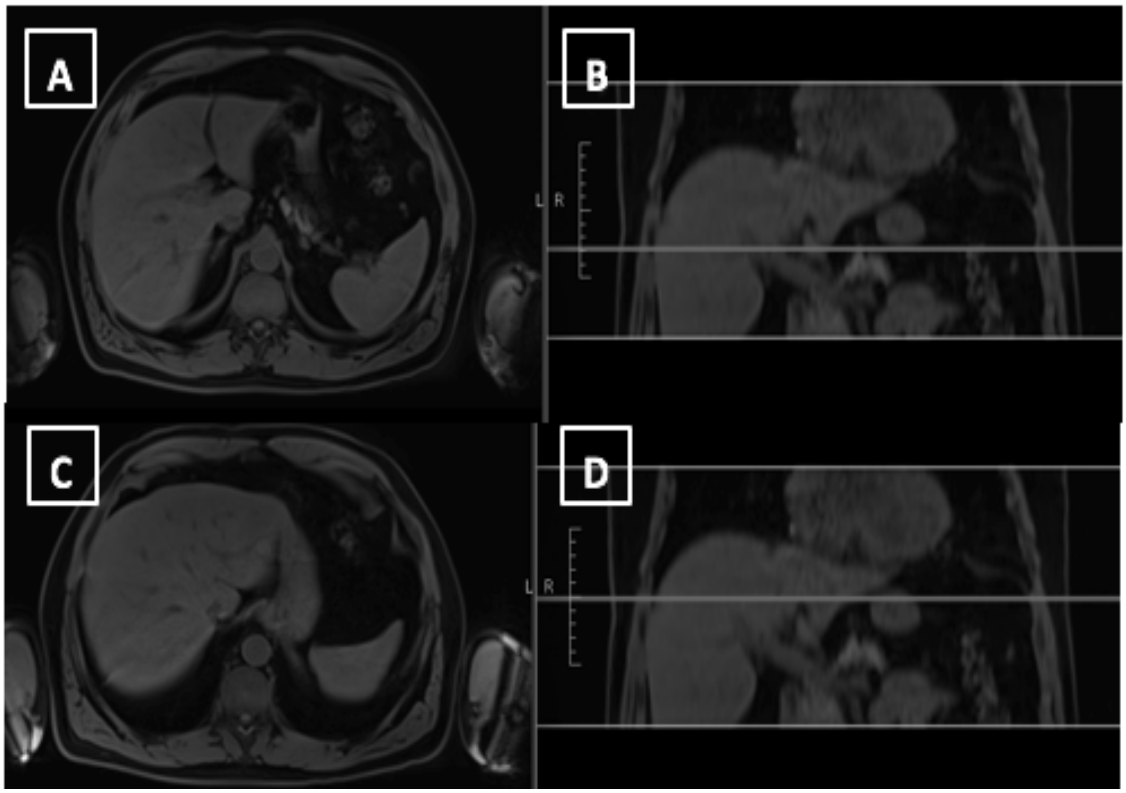

Figure 2.5: *Inferior and posterior MRI images of the liver. Coronal MRI images of the abdomen B and D with the horizontal line depicting the location of the axial MRI images A and C, respectively.*

In each pair of fat and water images, four circular regions of interest (ROI) were manually drawn in the liver tissue, by using the 'oval' tool, in identical locations in the fat and water images. ROIs were positioned with the purpose of including the anterior, posterior, medial and lateral segments of the liver. The ROI areas ranged from 10-20cm^2 and covered as large an area of liver tissue as possible while avoiding blood vessels, bile ducts and obvious artefacts as previously recommended (Campo et al.,

2017). Figure 2.6 shows an example of the ROI placement in two pairs of fat and water images that represent two axial locations in the abdomen. In each participant, a total of 8 ROIs were placed in the liver tissue; IHL was quantified within each ROI and a mean of all 8 ROIs was taken.

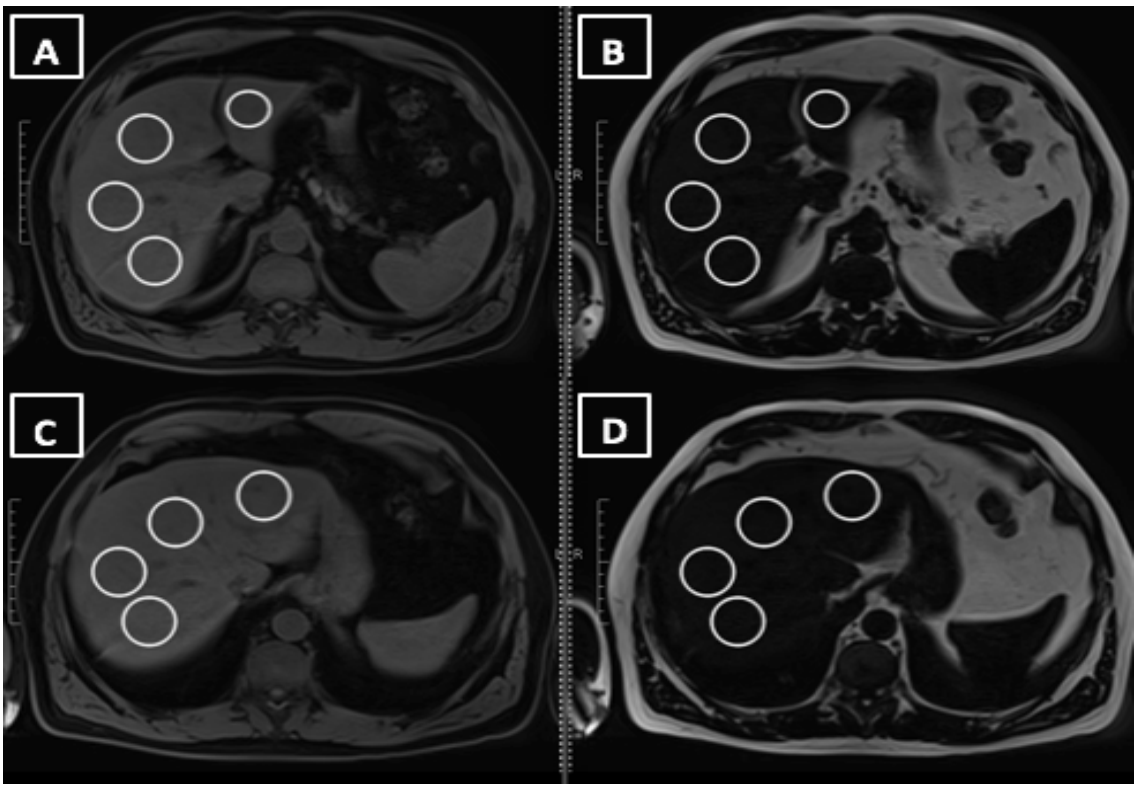

Figure 2.6: *Pairs of fat-only and water-only images with four identical regions of interest located in identical positions in each pair of images.*

For each ROI, a pixel signal intensity value is given in both the fat-only and water-only images. Figure 2.7 shows a representation of the information provided for each ROI which includes the area of the ROI and the signal intensity values which are used to calculate the percentage of IHL within each ROI by using the following formula:

$$\text{Fat fraction (\%)} = \frac{F}{F + W} \times 100$$

Where F is the signal intensity of the ROI in the fat-only image and W is the pixel signal intensity of the ROI in the water-only image.

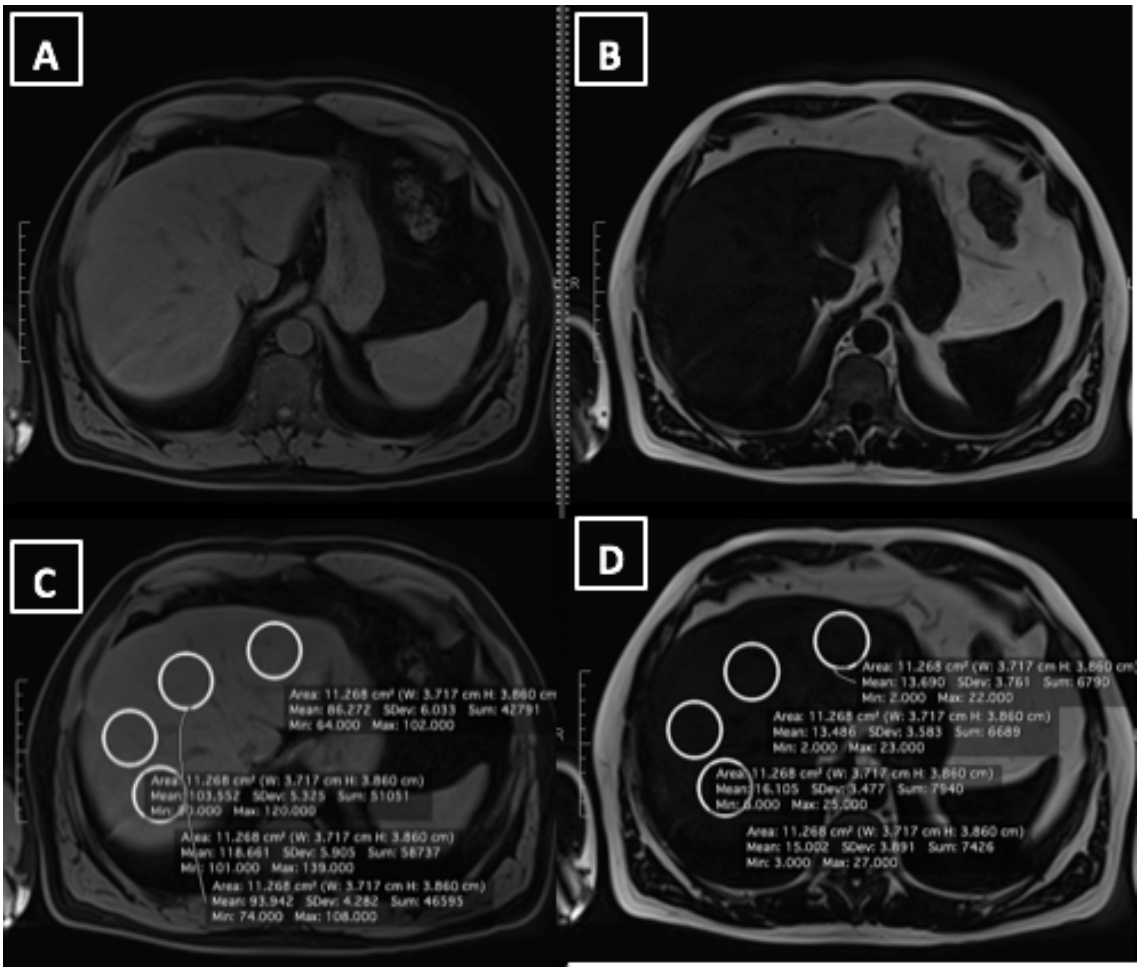

Figure 2.7: *MRI images showing the information provided in the HOROS software for each region of interest from which the pixel signal intensities are used to determine percentage fat fraction of each ROI. Panels A and B represent images C and D respectively.*

Due to the subjective nature of the MRI analysis of IHL, particularly with regards to the selection of appropriate MRI images and the positioning of ROIs within the liver tissue, the inter-observer variability in IHL was assessed. For this analysis, MRI data from 42 participants were analysed by a blinded independent observer (Esma Bulut, KCL student) to quantify IHL. A comparison analysis between the IHL results presented in this thesis and those from the second independent observer showed a mean coefficient of variation (CV) of 7.1% (SD 6.5%). Furthermore, there was a strong correlation between the IHL values between the two analyses (r= 0.99, P<0.001) indicating high agreement between the two analyses.

2.3.1.3 Analysis of intrapancreatic lipids

Intrapancreatic lipid was quantified in each participant by first identifying the pancreas, which is located adjacent to the inferior section of the liver, below the stomach and above the small intestines, as shown in Figure 2.8. Fat-only and water-only MRI images were used to quantify IPL. Figure 2.9 shows the location of the pancreas on axial MRI images of corresponding fat and water MRI images.

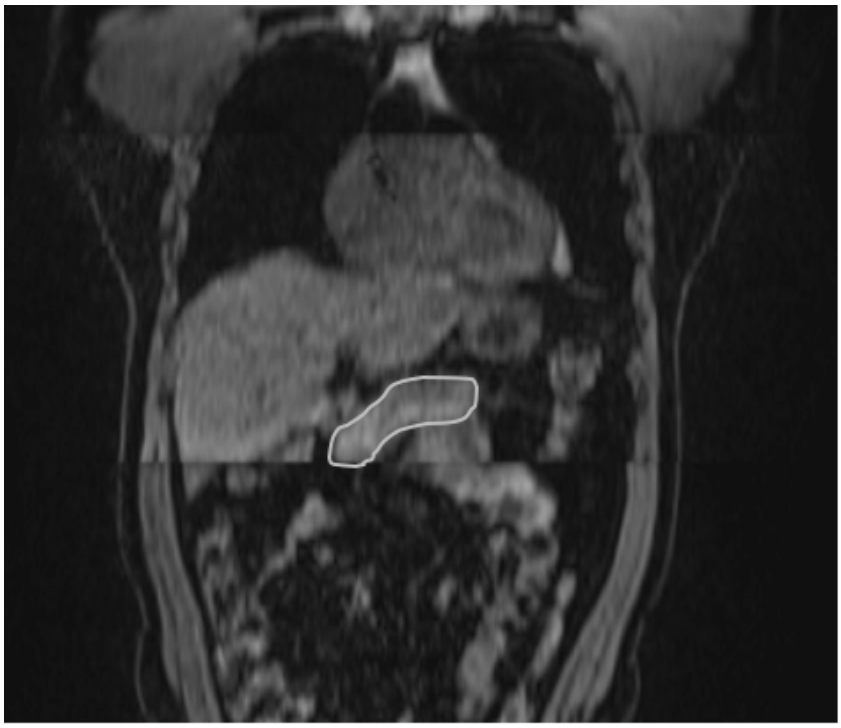

Figure 2.8: *Anatomical location of the pancreas shown on a sagittal MRI image.*

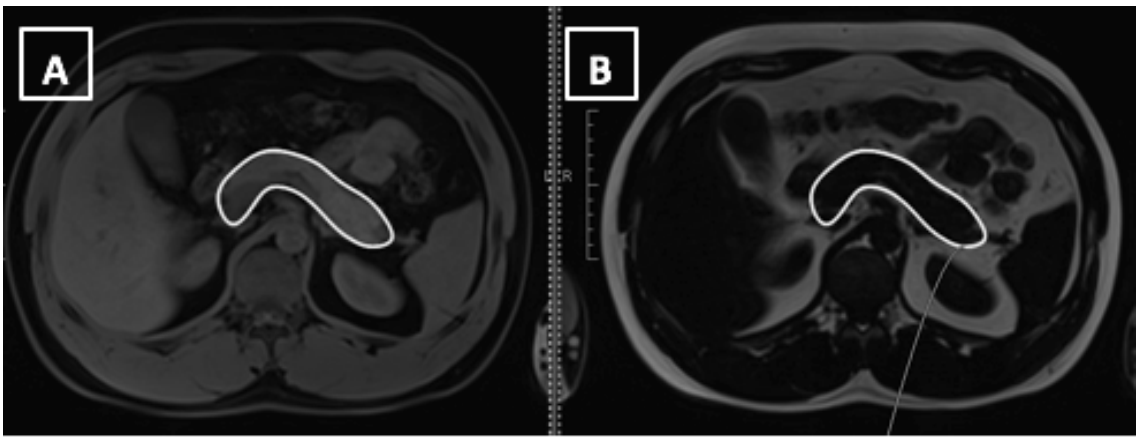

Figure 2.9: *Location of the pancreas on corresponding water (A) and fat (B) axial MRI images.*

Once the pancreas was identified, one circular ROI of area 1cm^2 was positioned on each of the head, body and tail regions in identical locations of fat and water MRI images. Due to the curved shape of the pancreas, different MRI slices were used with the greatest area of pancreatic tissue on the head, body and tail regions. An example of the positioning of the three ROIs within the pancreas is shown in Figure 2.10. The relatively small areas of ROIs was used due to recommendation by a recent review of magnetic resonance methods used to determine IPL; this ensures the ROIs are within the pancreatic borders and avoids contamination of the ROIs with VAT and the splenic vein (Al-Mrabeh et al., 2017). Additionally, to ensure the accurate positioning of the ROIs in the head, body and tail regions of the pancreas, the MRI analysis of IPL was conducted alongside a consultant radiologist with expertise of MRI pancreas anatomy (Dimitra Christodoulou).

The following formula was used to calculate percentage IPL within each ROI:

$$\text{Fat fraction (\%)} = \frac{F}{F + W} \times 100$$

Where F is the signal intensity of the ROI in the fat-only image and W is the pixel signal intensity of the ROI in the water-only image.

Using the pixel signal intensity data of each ROI, the pancreatic fat fraction was calculated in each region: IPL_{HEAD}, IPL_{BODY}, and IPL_{TAIL}; IPL_{MEAN} was calculated as the average of the head, body and tail regions. Due to the subjective nature of the IPL analysis with respect to positioning the ROIs on the head, body and tail of the pancreas, IPL quantification was repeated by a second independent investigator (Zoya Billoo, KCL student). Comparison of the IPL results presented in this thesis and those of the independent investigator showed an inter-observer CV of 14% and a significant correlation (r=0.62, P<0.001) indicating satisfactory agreement between the two analyses.

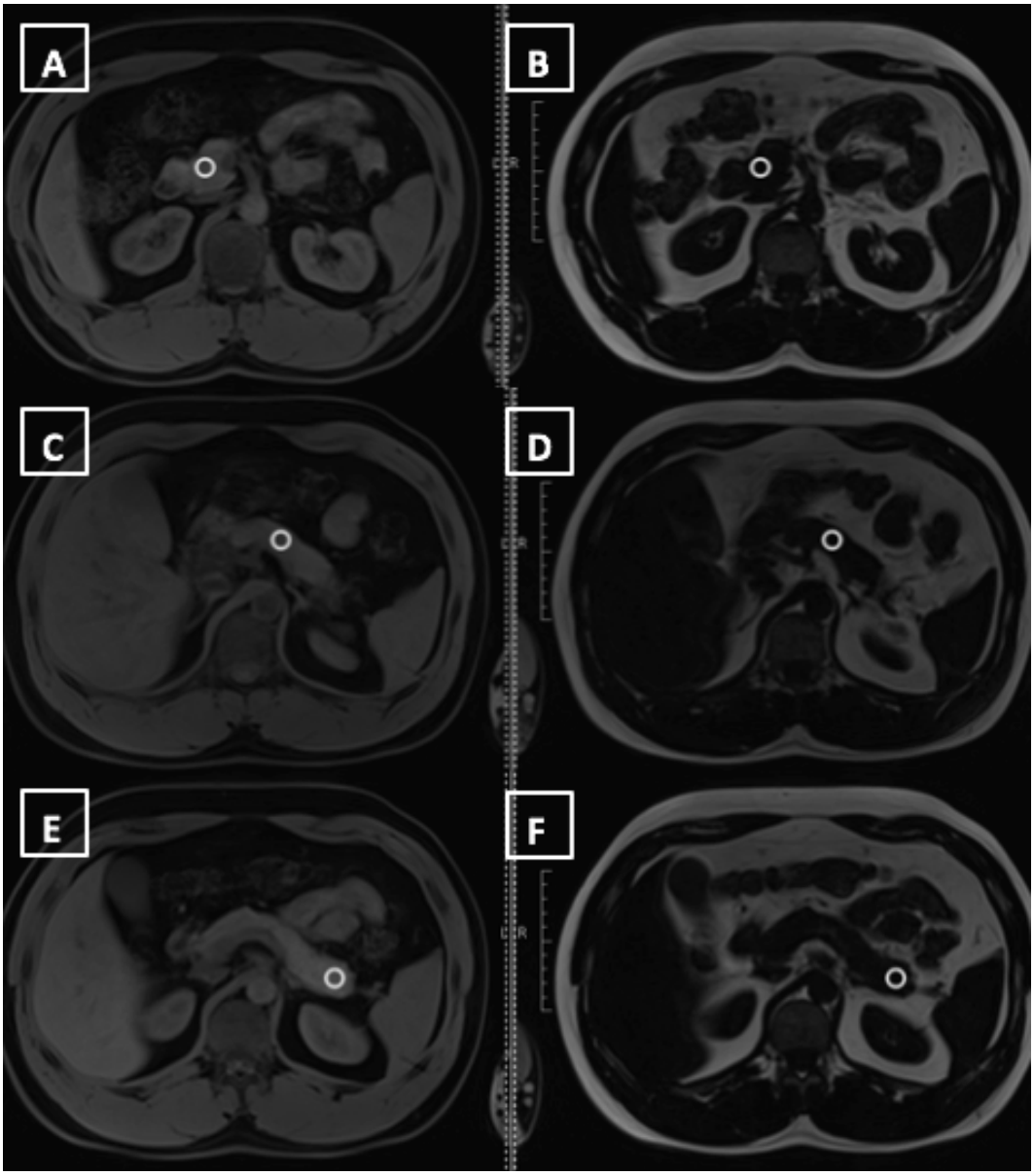

Figure 2.10: *Positioning of regions of interest in corresponding fat and water images of the pancreas head, A and B, body, C and D, and tail, E and F, regions.*

2.3.2 Magnetic resonance spectroscopy

Each participant underwent a ^{1}H-MRS scan of the soleus muscle (located in the posterior of the calf) on a 1.5T Seimens system to assess IMCL and EMCL. While lying in the supine position, an extremity radiofrequency coil was placed on the right leg to obtain images of the soleus muscle. A radiographer used these images to identify a volume of interest and placed a voxel (dimension: 1.3x1.3x3.0 mm^3) in the soleus muscle while avoiding gross marbling of fat. Localized proton spectra were acquired using a point

resolved spectroscopy (PRESS) sequence (TR 2000 ms; TE 30 ms) to obtain two spectra: a water-suppressed lipid spectra and a lipid-suppressed water spectra. The water resonance was set to 4.7ppm, which resulted in the IMCL and EMCL resonances to occur at frequencies of 1.3ppm and 1.5ppm, respectively.

2.3.2.1 Analysis of intramyocellular lipids

Proton-magnetic resonance spectroscopy spectra obtained from the ^{1}H-MRS scans of all participants were analysed on the Java-based Magnetic Resonance User Interface (jMRUI) version 5.0. Figure 2.11 shows a typical ^{1}H-MRS spectrum obtained from a ^{1}H-MRS analysis with the four main lipid peaks identified. These lipid peaks correspond to resonances of the hydrogen bonds from the methylene (CH_2) and methyl (CH_3) groups of IMCL and EMCL which are merged together between 0.8-1.7ppm. The large lipid peak shown in the original spectra is separated into the 4 lipid components by a deconvolution process with the use of prior knowledge of the peaks that is entered to the software to assist with identification of the individual peaks. The prior knowledge information that was added to the software was taken from previously published lipid fitting models (Rico-Sanz et al., 1998). This included knowledge of individual resonance frequencies of the EMCL CH_2, IMCL CH_2, EMCL CH_3 and IMCL CH_3 peaks which are 1.5, 1.3, 1.1 and 0.9 ppm, respectively (Schick et al., 1993). Also, the line width (width of the peaks) of the EMCL CH_2 and IMCL CH_2 peaks and the EMCL CH_3 and IMCL CH_3 peaks were set to equal ratios.

After deconvolution of the lipid peaks, the amplitude of each peak is generated by the software which corresponds to the amount of the specific metabolite that the peak corresponds to. IMCL and EMCL were expressed in arbitrary units as the relative ratio of the methylene IMCL or EMCL peaks to internal water; which is commonly used to express IMCL with the assumption that internal water is equivalent between subjects

(Ingram et al., 2011). The amplitude of the internal water peak was determined by deconvolution of the water peak (resonance frequency 4.7ppm) from the unsuppressed water spectra.

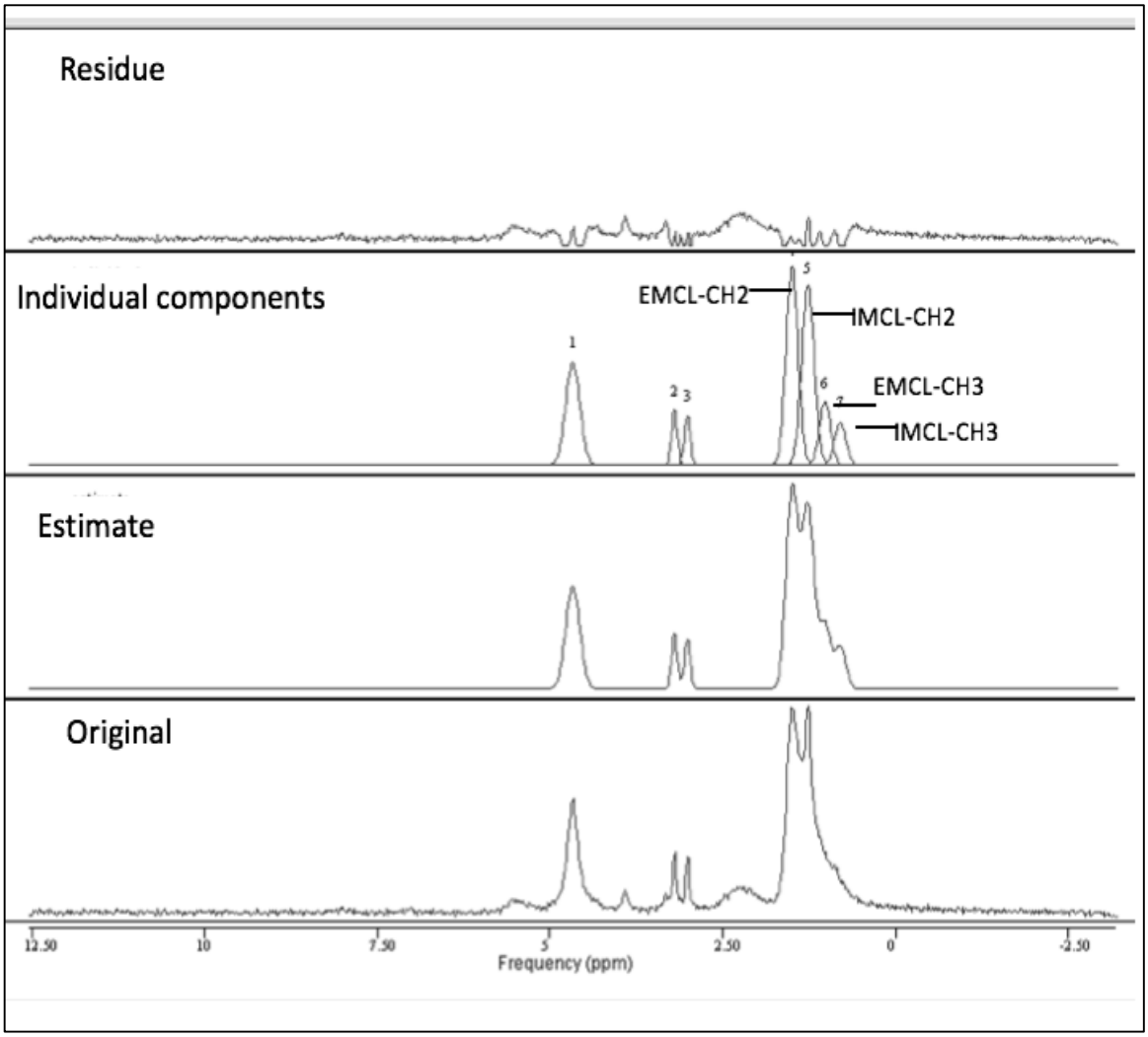

Figure 2.11: *^{1}H-MRS spectrum of the soleus muscle showing the deconvolution of the main lipids peaks.*

2.3.3 Assessment of insulin secretory function: hyperglycaemic clamp

A 2-hour hyperglycaemic clamp was conducted to assess intravenously stimulated insulin secretory function (ISF) and insulin clearance; this assessment was conducted according to the protocol of DeFronzo *et al.* 1979 (DeFronzo et al., 1979). Participants had a cannula inserted into an antecubital fossa vein for the infusion of glucose and a second cannula

inserted retrogradely into the dorsum of the hand for blood sampling. During this assessment participants were administered with a primed infusion of 20% dextrose to achieve a hyperglycaemic state of 6.9mmol/l above basal; this hyperglycemic state was maintained for 120 minutes by adjusting the glucose infusion rate every 2 minutes for the first 10 minutes and monitored every 5 minutes thereafter and adjusted where necessary. Blood samples were collected at -20, -10, 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes to measure glucose, insulin and c-peptide concentrations.

2.3.3.1 Calculations to determine measures of insulin secretory function

Measures of ISF and insulin clearance determined from the hyperglycaemic clamp test were modelled by Professor Riccardo Bonadonna, Dr Linda Boselli and Dr Meera Ladwa using previously described methods, which are summarised below (Cobelli et al., 2007, Cali et al., 2008, Malandrucco et al., 2012). Insulin secretion rate was calculated using a pre-defined two compartment minimal model of c-peptide kinetics called deconvolution; this model uses plasma c-peptide concentrations to determine insulin secretion rate during the intravenous glucose challenge and while considering c-peptide kinetics. C-peptide is used in the modelling of ISF because it is co-secreted with insulin in equimolar amounts but has a longer half-life in the circulation and has relatively constant kinetics that are well defined. Hence, plasma c-peptide concentrations are truly reflective of pancreatic insulin secretion.

To determine measures of glucose sensitivity, minimal modelling was performed while considering both insulin secretion rate and plasma glucose concentrations. This modelling method is a mathematical representation of the dynamic relationship between glucose concentrations and insulin secretion. First, the incremental area under the curve (iAUC) for insulin, glucose and c-peptide during the intravenous (hyperglycaemic clamp) glucose

challenge were calculated using the trapezoidal rule. ISF, which is a measure of beta-cell glucose sensitivity, is divided into first-phase and second-phase insulin secretion. To obtain an index of first- and second-phase insulin secretion during the hyperglycaemic clamp, the iAUC for c-peptide was calculated at 0-10 minutes for first-phase and 10-120 minutes for second-phase insulin secretory functions (DeFronzo et al., 1979). Using c-peptide, glucose and insulin iAUC data, ISF was modelled on the simulation, analysis, and modelling software (SAAM-II 1.2 software; SAAM Institute, Seattle, WA) (Barrett et al., 1998). The main outputs from the software that are presented in this thesis include: 1) glucose sensitivity of first-phase secretion (first-phase ISF), expressed as the amount of insulin secreted in response to a rate of increase in glucose of 1 mmol/l between time 0 and 1 minutes of the intravenous glucose challenge, in $(pmol.m^{-2}BSA)/(mmol.l^{-1}.min^{-1})$; 2) glucose sensitivity of second-phase secretion (second-phase ISF), expressed as the steady state insulin secretion rate in response to a step increase in glucose of 1 mmol/l above baseline, in $(pmol.min^{-1}.m^{-2}BSA)/(mmol.l^{-1})$.

2.3.3.2 Calculation to determine insulin clearance

Average insulin clearance was determined from the minimal modelling using the following formula (Mohandas et al., 2018):

$$Clearance_{Ins} = \frac{AUC_{ISR}}{AUC_1 + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

Where AUC_{ISR} is the area under the curve of insulin secretion rate, AUC_I is the area under the curve of insulin concentration, I_{Final} is the insulin concentration at the end of the clamp, I_{Basal} is the insulin concentration at the beginning of the clamp and MRT_{Ins} is the mean residence time of insulin, which was assumed to be 27 minutes as reported by Navalesi *et al.* (Navalesi et al., 1978).

2.3.4 Assessment of insulin sensitivity: hyperinsulinaemic euglycaemic clamp

A two-step hyperinsulinaemic-euglycaemic clamp with the infusion of stable glucose and glycerol isotopes was used to assess whole-body, peripheral (primarily skeletal muscle), hepatic and adipose tissue insulin sensitivity. The hyperinsulinaemic-euglycaemic clamp was conducted according to the protocol of DeFronzo *et al.* 1979 (DeFronzo et al., 1979). A cannula was inserted into an antecubital fossa vein for infusions and a second cannula was inserted retrogradely into the dorsum of the hand for blood sampling.

Participants were first administered with primed continuous infusions of [6,6 $^2H_2$] glucose (concentration: 2.0 mg/kg, rate of infusion: 0.02 mg kg^{-1} min^{-1}) and [$^2H_5$] glycerol (concentration: 0.12 mg/kg, rate of infusion: 0.0067 mg kg^{-1} min^{-1}) (CK Gases Ltd, UK) for 120 minutes before the first insulin infusion. After the infusion of the isotope tracers during the basal period, a 2-step hyperinsulinaemic-euglycaemic clamp was started. Upon starting the clamp, participants were administered with a 120-minute insulin infusion at a rate of 10mU/m^2 BSA/min (low dose) followed by 120-minute insulin infusion at a rate of 40mU/m^2 BSA/min (high dose).

Throughout the clamp, plasma glucose concentrations were maintained at 5mmol/l by adjusting the infusion of 20% dextrose enriched with [6,6 $^2H_2$] glucose according to plasma glucose readings taken every 5 minutes using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA). Blood samples were taken at -30, -20, -10, 0, 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 minutes, where time 0 represents the start of insulin low dose infusion, to measure plasma insulin, glucose and glycerol concentrations and enrichments. Figure 2.12 shows a timeline of infusions and blood sampling during the clamp procedure (Goff, 2013). Hepatic and adipose tissue insulin sensitivity were determined during the low-dose step

of the clamp, while, whole-body and peripheral insulin sensitivity were determined during the high-dose step of the clamp. The glucose infusion rate during the last 30 minutes of the high insulin dose was used as a measure of whole-body insulin sensitivity (M-value).

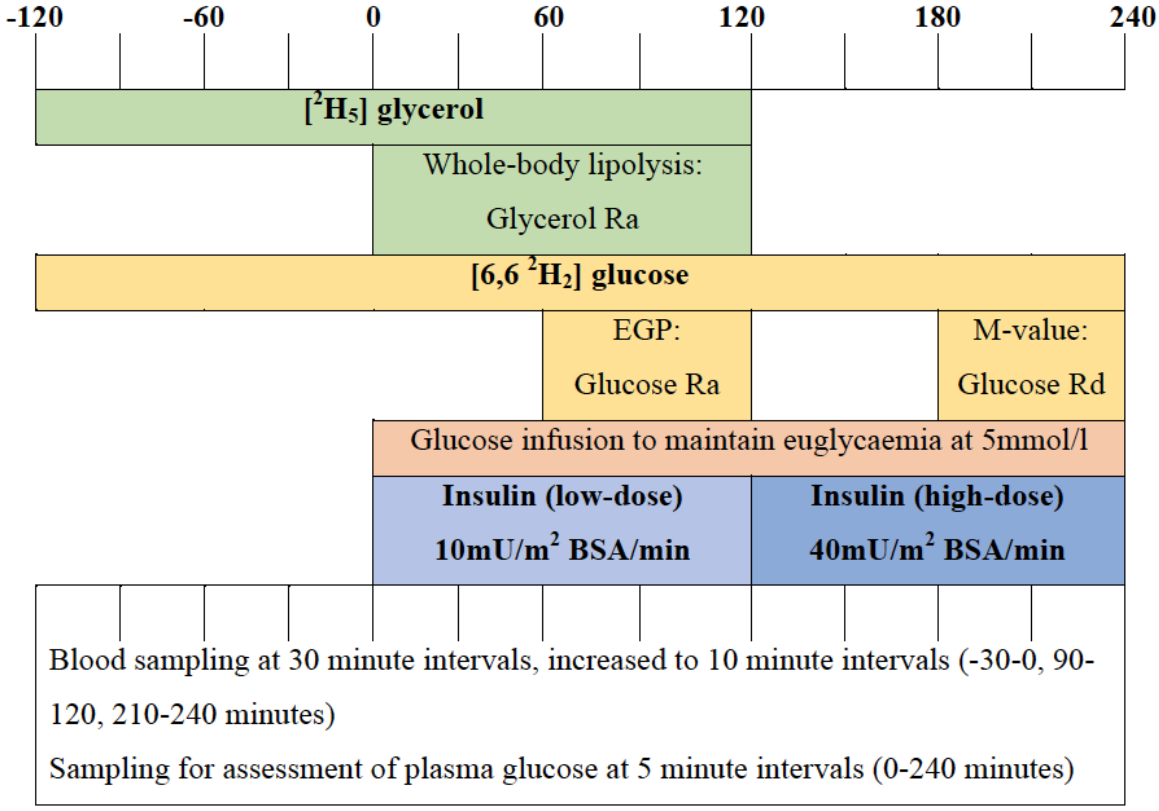

Figure 2.12: *Schematic of the timeline of infusions and blood sampling during the hyperinsulinaemic-euglycaemic clamp (Goff, 2013).*

2.3.4.1 Calculations to determine measures of insulin sensitivity

The total insulin mediated glucose disposal represents whole-body insulin sensitivity (M-value) and was calculated as the mean of the glucose infusion rate (adjusted with a correction factor 'space correction') in the final 30 minutes of the hyperinsulinaemic-euglycemic clamp (DeFronzo et al., 1979). Peripheral glucose utilisation (glucose rate of disappearance, Rd), endogenous glucose production (EGP) (glucose rate of appearance,

Ra) and whole-body lipolysis (glycerol rate of appearance, Ra) were calculated using Steele's non-steady state equations modified for stable isotopes assuming a volume distribution of 22% body weight (Steele et al., 1956). The calculation of glucose kinetics was also modified for inclusion of [6,6-$^{2}H_{2}$]-glucose in the dextrose infusion (Finegood et al., 1987). Before the calculation of glucose kinetics, enrichment and concentrations were smoothed using optical segments analysis (Finegood and Bergman, 1983).

Peripheral glucose utilisation (glucose Rd) was calculated during the basal state and during the final 30 minutes of the high-dose stage of the hyperinsulinaemic-euglycaemic clamp. The percentage increase in glucose Rd (% increase glucose Rd) from the basal to the stimulated state was used as a measure of muscle insulin sensitivity (Fabbrini et al., 2009). Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous glucose infusion rate from total glucose Ra. Glucose Ra was calculated during the basal state and during the final 30 minutes of the low-dose stage of the clamp. The percentage suppression of glucose Ra from the basal to the low-dose stage was used as a measure of hepatic insulin sensitivity which is defined as the insulin mediated suppression of hepatic glucose production (Yoshino et al., 2012).

2.3.5 Biochemical analyses

Plasma glucose concentrations were determined using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA). Serum insulin concentrations were determined by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK); where the inter-assay and intra-assay CVs were ≤ 5.9% and 4.6%, respectively. Serum c-peptide concentrations were determined using a radioimmunoassay (Millipore Ltd, Hertfordshire, UK). The glucose and glycerol enrichment in plasma were determined by gas chromatography-mass spectrometry on Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham,

UK) using selected ion monitoring. The isotopic enrichment of glucose was determined as the penta–O-trimethylsilyl-D-glucose-O-methyloxime derivative (Shojaee-Moradie et al., 1996). The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl glycerol derivative (Flakoll et al., 2000).

2.4 Statistical analyses

The Soul-Deep study was designed to include 20 participants per group (two ethnic groups at three glycaemic states) to allow a difference of one standard deviation to be detected with a power of 90% and two-sided significance for the primary outcome variable (beta-cell insulin secretory function). Variables were tested for normality using Shapiro-Wilks test, histograms and assessment of the Q-Q plots. Variables that followed a non-parametric distribution were log-transformed to achieve a normal distribution with the purpose of using parametric tests on them. A 2-way between-within groups analysis of variance (ANOVA) was used to test differences in variables of interest by ethnicity and glycaemic state. Significance of differences in variables of interest between the two ethnic groups at a specific glycaemic state were tested using an independent samples t-test on numeric parametrically-distributed data or chi-squared test on ordinal data. Data are presented as mean with standard deviation (SD) for normally-distributed data, geometric mean with 95% confidence interval (CI) for log transformed data or median with interquartile range (IQR) for non-parametric data. Data presented in box plots express the median and IQR. Relationships between variables of interest were assessed using Pearson correlations with partial correlations used when adjustments for confounders were required. Statistical analyses were conducted with SPSS version 25.0 and P values < 0.05 were considered statistically significant.

Chapter 3: Ethnic differences in regional abdominal adipose tissue deposition between men of black and white ethnicity

Data presented in this chapter have been published in (appendix I):

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3.1 Introduction

Obesity is a major risk factor for T2D (Bonham and Brock, 1985). The worldwide prevalence of obesity is reaching epidemic proportions which is directly impacting the prevalence rates of T2D (Bhupathiraju and Hu, 2016). While the link between obesity and T2D is well established, the mechanisms of adiposity induced insulin resistance are not fully understood. Considering the worldwide epidemics of T2D and obesity, the study of adipose tissue has become an area of paramount importance. Whole-body adiposity is commonly assessed using BMI, however, since BMI is not a biological trait but a calculated value used to characterise the obese phenotype, it is essential to look beyond the BMI to understand the role of excess adiposity in the development of T2D (Muller et al., 2016).

While obesity is known to cause metabolic dysfunctions, it is estimated that approximately 55% of individuals with obesity are metabolically healthy i.e. do not have hypertension, dyslipidaemia or disturbances in glucose metabolism (Jung et al., 2017). Conversely, approximately 30% of individuals with a healthy BMI are metabolically unhealthy (Wildman et al., 2008). Further investigations of these paradoxical phenomena have shown that not only the amount of adiposity but also the location of adipose tissue is important in determining metabolic risk. There is a growing body of evidence showing that increased central deposition of adipose tissue is strongly linked to several obesity-related morbidities including T2D (Despres, 2006). Indeed, studies investigating individuals with the obese but metabolically healthy phenotype have shown they characteristically have lower levels of VAT than their counterparts with obesity and metabolic diseases (Stefan et al., 2008b). Furthermore, normal weight individuals that are metabolically unhealthy often have high VAT and low SAT levels; thus, such phenotypes have been termed 'thin on the outside, fat on the inside' (Thomas et al., 2012a). Therefore, there is much evidence showing that regional distribution of adipose tissue is important in determining risk of T2D.

With the advancement of imaging technologies such as CT and MRI, regional distribution of adiposity has gained much traction within T2D research (Thomas et al., 2013). The deposition of adipose tissue in the central body region is more closely linked to T2D than other measures of whole-body adiposity and gluteofemoral adiposity (Despres, 2006, Smith et al., 2012). Demonstrating the importance of direct measurement of regional adipose tissue, Figure 3.1 shows cross-sectional abdominal MRI images from 9 individuals with equivalent BMI levels but noticeably different SAT and VAT levels (Thomas et al., 2012a). These differences can only be appreciated using imaging methods that directly quantify regional adipose tissue.

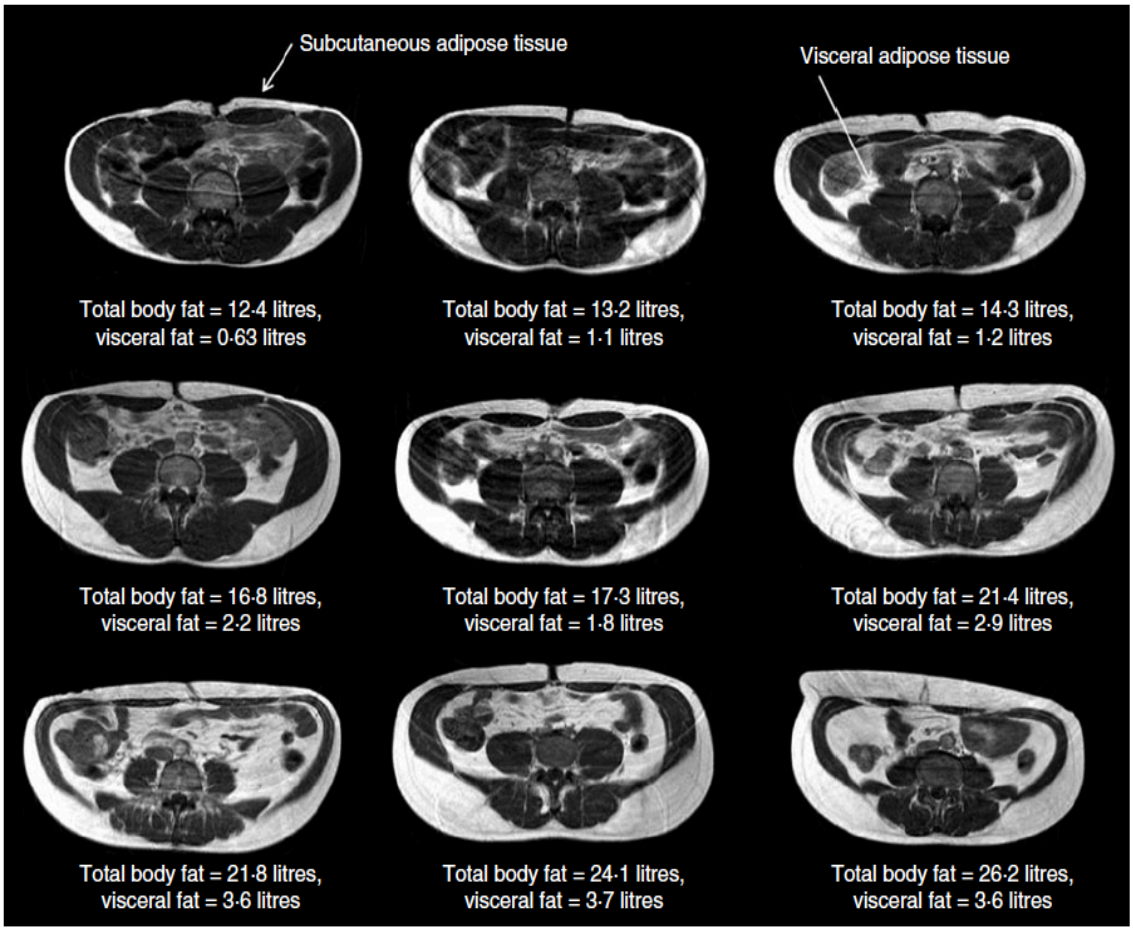

Figure 3.1: *Axial MRI images at the level of the umbilicus of 9 males who have equivalent BMI levels of 24 but greatly varying SAT and VAT levels (Thomas et al., 2012a).*

Increased central adiposity occurs due to increased VAT or abdominal SAT (ASAT) or a combination of both. Furthermore, the ASAT compartment can be subdivided into deep (dSAT) and superficial SAT (sSAT) which are separated by a fascial plane called the fascial superficialis (Despres et al., 1989).

Studies conducted on sSAT, dSAT and VAT have shown distinct structural and functional differences between the three fat depots. The sSAT is the primary source of storage of triglycerides and is considered to be the most metabolically safe fat storage compartment. During excess energy intake, the sSAT compartment increases and during its severe increase there is a disproportional increase of dSAT, which is a secondary

storage compartment of fat. The adipocytes within the dSAT are typically larger than those of sSAT indicative of dysfunctional hypertrophic expansion of adipocytes (Lundbom et al., 2013). During obese states, there is a disproportionate expansion of dSAT with the increase of SAT, which indicates that sSAT has a finite capacity after which it becomes overwhelmed and the increase of SAT occurs mainly by the increase of dSAT (Golan et al., 2012). As the SAT compartment increases and with further increase of dSAT, the adipocytes become dysfunctional and unable to adequately store excess triglycerides during energy surplus. This causes a spill-over of NEFAs into the circulation which deposit within the VAT compartment (Sattar and Gill, 2014).

The properties of VAT are similar to those of dSAT, however, are more deleteriously exaggerated. The properties of sSAT, dSAT and VAT are summarised in Table 3.1, which shows the metabolically safest fat storage compartment is sSAT followed by dSAT followed by VAT, which is considered to have the greatest pathogenic potential (Sniderman et al., 2007). Marinou *et al.* showed that dSAT is associated with VAT, however, sSAT is not, suggesting that both dSAT and VAT expand due to similar influences in response to the dysfunctional state of SAT adipocytes (Marinou et al., 2014).

Table 3.1: *Characteristics of adipose tissue compartments, adapted from a review by Sniderman et al., 2007 (Sniderman et al., 2007).*

	SSAT	**DSAT**	**VAT**
Development sequence	Primary	Secondary	Secondary
Demarcation of lobules	Best demarcated	Intermediate	Least demarcated
Vascularity of lobules	Least vascular	Intermediate	Most vascular
Stability of triglyceride stores	Most stable	Intermediate	Least stable
Atherogenic dyslipidaemia	Moderate association	(Very) strong association	Very strong association
Dysglycaemia	Moderate association	Very strong association	Very strong association
Cytokine secretion	Least adverse	Intermediate	Most adverse

Investigations of regional adipose tissue deposition in black populations have mostly focused on VAT and SAT and their associations with metabolic risk markers of T2D. It is well accepted that VAT is lower in black populations compared to their white counterparts (Alderete et al., 2014, Goedecke et al., 2017), which is present from as early as childhood (Goran, 1999, Goran et al., 1997). However, studies of SAT are inconclusive with some reporting greater levels of SAT in black populations (Hasson et al., 2010, Lovejoy et al., 2001) while others report no difference in comparison to white populations (Szczepaniak et al., 2012, Bacha et al., 2003, Hughan et al., 2013, Liska et al., 2007, van der Merwe et al., 2000).

The study of dSAT and sSAT in black populations has been limited; generally greater levels of sSAT in blacks compared to whites have been reported with no ethnic differences in dSAT (Liska et al., 2007, Evans et al., 2011, Goedecke et al., 2011, Lovejoy et al., 2001). While the relationships between VAT and insulin sensitivity have been extensively studied in white and black populations, no current reports have investigated the relationships between dSAT and sSAT with insulin sensitivity in a black population. Therefore, the contributions of dSAT and sSAT in the progression of T2D are understudied in black populations.

The illogical phenomenon of low levels of VAT but greater prevalence of T2D in black populations has caused speculation that black populations may be more sensitive to the detrimental effects of VAT and become more insulin resistant at lower levels. To elucidate these speculations, investigations of the relationships between VAT and metabolic parameters of T2D in black populations are necessary; such investigations are limited in the literature but will be addressed in this chapter. Additionally, investigations between ASAT, dSAT and sSAT with metabolic parameters are also warranted in black populations to elucidate their relevance in the pathophysiology of T2D.

3.2 Aim

The primary aim is to assess ethnic differences in VAT, ASAT, dSAT and sSAT between WE and BWA men of NGT, IGT and T2D glycaemic states. The secondary aim is to investigate ethnic differences in the relationships between 1) VAT and whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity; and 2) ASAT, dSAT and sSAT with whole-body insulin sensitivity.

3.3 Methods

3.3.1 Data acquirement

The data presented in this chapter were acquired from the methods described in chapter 2 (sections 2.3.1, 2.3.1.1, 2.3.4, 2.3.4.1). In brief, areas of VAT, ASAT, dSAT and sSAT were determined from a single axial MRI image at the L4-5 anatomical position. VAT and SAT areas determined at the L4-5 position correlate strongly with the respective volumes determined using consecutive MRI images from the whole abdominal cavity in both black and white populations (Demerath et al., 2007). Measures of insulin sensitivity were determined using a hyperinsulinaemic-euglycaemic clamp test with the infusion of stable glucose and glycerol isotopes to determine whole-body insulin sensitivity (M-value), hepatic insulin sensitivity (% suppression of endogenous glucose production [EGP]), muscle insulin sensitivity (% change in glucose rate of disappearance [Rd]) and adipose tissue insulin sensitivity (% suppression of lipolysis).

3.3.2 Statistical analysis

Participant characteristics, insulin sensitivity and regional adiposity data presented in this chapter for the WE and BWA men of NGT, IGT and T2D groups were compared using 2-way between-groups ANOVA. Ethnicity and glycaemic state were included in the 2-way between-groups ANOVA as independent variables to assess the main effect of each of the independent variables, as well as their interaction (ethnicity*glycaemic state), on the outcome measures (dependent variables). Ethnic differences between regional fat depots of interest were tested using independent samples t-tests.

Strength of relationships between variables of interest were tested using Pearson's correlation test; partial correlation was used when assessing relationships while adjusting for confounding variables. Ethnic differences in the strength of the relationships were

examined by fitting a regression with an interaction term for ethnicity and VAT as an independent variable.

3.4 Results

3.4.1 Participant characteristics

The clinical characteristics of the WE and BWA men of NGT, IGT and T2D groups are presented in Table 3.2. There were no ethnic differences in age, weight, height, BMI, BSA, blood pressure and fasting plasma glucose. Waist circumference was significantly greater in the WE men. HbA1c was greater in the BWA men which was driven by the greater HbA1c in BWA men with IGT. Considering measures of fasting plasma lipids, the WE men had greater total cholesterol levels (trend towards significance) compared to the BWA men with no differences in LDL- and HDL-cholesterol. Furthermore, fasting triglycerides were significantly greater in the WE men. In the T2D glycaemic group, the WE and BWA men had similar duration of diabetes, metformin use and anti-hypertensive drug use. The number of participants taking statins was slightly greater in the WE men, which approached statistical significance. All clinical characteristics differed significantly by glycaemic state except for height and HDL-cholesterol; these are indicative of the typical pattern of deterioration in several parameters related to T2D in the progression from NGT to T2D. The lack of significant ethnicity*glycaemic state interaction for waist circumference and triglycerides indicates that, although both these characteristics were significantly lower in the BWA men, this was present at all glycaemic states. This suggests that both lower waist circumference and plasma triglycerides are ethnic traits in populations of African descent in comparison to their European counterparts.

3.4.2 Metabolic characteristics

Measures of whole-body and tissue specific insulin sensitivity in the WE and BWA men of NGT, IGT and T2D are presented in Table 3.3. Even though all measures of insulin sensitivity were slightly greater in BWA men in the T2D group compared to their WE

counterparts, there were no statistically significant ethnic differences in all measures of insulin sensitivity between the WE and BWA men overall. As expected, all measures of insulin sensitivity were significantly lower in the T2D and IGT groups compared to the NGT groups, as indicated by significant main effects for glycaemic state.

Table 3.2: *Clinical characteristics of the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		**IGT**		**T2D**				
	WE (n=23)	**BWA (n=20)**	**WE (n=10)**	**BWA (n=10)**	**WE (n=18)**	**BWA (n=20)**	***P* eth**	***P* gly**	***P* *eth*gly***
Age (years)*	33.5 (28.4-39.5)	28.8 (24.6-33.7)	52.9 (45.8-61.0)	45.2 (41.7-49.1)	55.8 (52.6-59.2)	53.8 (50.2-57.8)	0.13	**<0.001**	0.76
Weight (kg)*	85.1 (78.3-92.4)	82.9 (77.2-89.2)	96.8 (86.5-108.3)	96.7 (87.4-107.1)	100.5 (92.7-108.8)	91.7 (86.4-97.3)	0.26	**<0.001**	0.69
Height (cm)	180.3 ± 5.8	177.4 ± 7.5	178.1 ± 5.6	177.0 ± 6.0	177.6 ± 5.7	175.5 ± 7.2	0.13	0.28	0.87
BMI (kg/m^2)	26.5 ± 4.5	27.0 ± 3.4	31.6 ± 3.1	31.1 ± 3.8	31.5 ± 4.1	30.0 ± 3.5	0.51	**<0.001**	0.53
Body surface area (m^2)	2.1 ± 0.2	2.0 ± 0.2	2.17 ± 0.18	2.18 ± 0.19	2.2 ± 0.20	2.1 ± 0.15	0.38	0.052	0.60
Waist circumference (cm)	93.8 ± 14.6	88.2 ± 8.9	109.2 ± 10.3	102.6 ± 11.5	111.9 ± 13.0	104.9 ± 9.7	**0.011**	**<0.001**	0.96
HbA1c (%)	5.45 ± 0.24	5.58 ± 0.47	5.67 ± 0.33	6.13 ± 0.39	6.64 ± 0.70	6.76 ± 0.70	**0.032**	**<0.001**	0.44
HbA1c (mmol/mol)	35.9 ± 2.9	37.5 ± 5.2	38.6 ± 3.5	43.5 ± 4.3	49.1 ± 7.61	50.4 ± 7.80	**0.033**	**<0.001**	0.49
Fasting plasma glucose (mmol/l)	5.20 ± 0.39	5.14 ± 0.47	5.88 ± 0.70	5.60 ± 0.44	6.88 ± 1.33	6.71 ± 0.96	0.32	**<0.001**	0.87
Systolic BP (mm Hg)	121.9 ± 9.1	122.3 ± 13.0	130.4 ±13.3	132.7 ± 10.0	130.9 ± 14.2	137.5 ± 13.7	0.24	**<0.001**	0.53
Diastolic BP (mm Hg)*	70.7 (67.2-74.4)	69.9 (65.5-74.6)	77.6 (73.6-81.9)	82.8 (78.5-87.4)	81.8 (77.8-85.9)	85.7 (82.0-89.6)	0.14	**<0.001**	0.56
Total cholesterol (mmol/l)	4.76 ± 1.1	4.36 ± 1.0	4.99 ± 0.67	4.50 ± 0.88	4.27 ± 0.70	4.09 ± 0.70	0.054	**0.042**	0.79

LDL-cholesterol (mmol/l)	2.99 ± 0.82	2.71 ± 0.81	3.12 ± 0.66	2.91 ± 0.65	2.28 ± 0.66	2.32 ± 0.53	0.31	**<0.001**	0.61
HDL-cholesterol (mmol/l)*	1.27 (1.14-1.40)	1.30 (1.11-1.47)	1.28 (1.03-1.55)	1.11 (0.91-1.31)	1.21 (1.09-1.32)	1.17 (1.00-1.34)	0.72	0.22	0.71
Triglyceride (mmol/l)*	1.09 (0.86-1.33)	0.68 (0.57-0.79)	1.38 (1.03-1.74)	1.09 (0.78-1.41)	1.78 (1.38-2.20)	1.27 (0.94-1.61)	**0.001**	**<0.001**	0.96
Diabetes duration (years)*	-	-	-	-	3.1 ± 1.0	2.7 ± 1.3	0.38		
Metformin use (number of participants (%))†	-	-	-	-	10 (40)	15 (60)	0.31		
Statin use (number of participants (%))†	-	-	-	-	16 (57)	12 (43)	0.067		
Antihypertensive drug use (number of participants (%))†	-	-	-	-	12 (44)	15 (56)	0.72		

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (*). *P* eth: main effect for ethnicity, *P* gly: main effect for glycaemic state, *P* eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA. *P*-values determined using Fisher's exact test for ordinal data (†).

Table 3.3: *Metabolic parameters of insulin sensitivity in the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		**IGT**		**T2D**				
	WE (n=23)	**BWA (n=18)**	**WE (n=9)**	**BWA (n=10)**	**WE (n=18)**	**BWA (n=18)**	***P* eth**	***P* gly**	***P* *eth*gly***
Whole-body insulin sensitivity (M-value) (mg/m^2 BSA min^{-1})	309.6 ± 127.5	313.5 ± 77.0	214.6 ± 99.8	197.0 ± 59.9	154.4 ± 76.2	191.0 ± 85.3	0.71	**<0.001**	0.56
Skeletal muscle insulin sensitivity (% increase in glucose Rd)	286.2 ± 138.4	299.4 ± 114.4	176.0 ± 127.0	183.2 ± 86.0	154.1 ± 107.5	203.5 ± 126.2	0.39	**<0.001**	0.79
Hepatic insulin sensitivity (% suppression of EGP)*	67.2 (58.5-77.2)[a]	61.4 (52.3-72.1)	45.5 (35.6-58.2)	55.3 (48.6-62.8)	28.6 (18.1-45.1)[e]	30.5 (21.1-44.1)[f]	0.60	**<0.001**	0.54
Adipose tissue insulin sensitivity (% suppression of lipolysis)	53.8 ± 17.4[b]	43.4 ± 25.2	47.8 ± 7.6[c]	46.8 ± 14.0[d]	35.5 ± 14.5[g]	37.2 ± 16.0[f]	0.45	**0.027**	0.39

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (*). *P* eth: main effect for ethnicity, *P* gly: main effect for glycaemic state, *P* eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA. [a]N=21, [b]n=22, [c]n=7, [d]n=9, [e]n=13, [f]n=15, [g]n=11

3.4.3 Abdominal adipose tissue deposition

Measures of regional abdominal adipose tissue deposition are presented in Table 3.4 for the WE and BWA men of NGT, IGT and T2D. Significant main effects for glycaemic state were present for all abdominal adipose depots, which were greater in the IGT and T2D groups compared to the NGT groups. There were significant ethnic differences in VAT, VAT:ASAT ratio and dSAT:sSAT ratio between the WE and BWA men, with no ethnic differences in ASAT, dSAT and sSAT. Ethnic comparisons were conducted for each of the regional abdominal adipose depots within each glycaemic state, which are addressed below.

3.4.3.1 Visceral adipose tissue and abdominal subcutaneous adipose tissue

As shown in Figure 3.2, VAT was significantly lower in the BWA men compared to the WE men which was present at all glycaemic states. As expected, VAT was greater in the IGT and T2D glycaemic states compared to the NGT groups in both WE and BWA men; however, this difference in VAT appeared to be greater in WE men indicated by a greater mean difference between in the T2D groups (mean difference = 59.7cm^2) compared to the NGT groups (mean difference = 51.5cm^2). However, there was no significant interaction by ethnicity*glycaemic state ($P_{interaction}$ =0.61) indicating that VAT appears to increase similarly in the WE and BWA men from NGT to T2D. There were no ethnic differences in ASAT at all glycaemic states as shown in Figure 3.3. Furthermore, there was no difference in ASAT by ethnicity*glycaemic state ($P_{interaction}$=1.00) indicating that ASAT is similarly greater in the IGT and T2D states than the NGT state in both ethnic groups. The ratio of VAT:ASAT was significantly lower in the BWA men in the NGT (P=0.034), IGT (P=0.009), and T2D (P=0.027) groups. This was mostly driven by the

significantly lower VAT in the BWA men since there were no ethnic differences in ASAT.

3.4.3.2 Deep and superficial subcutaneous adipose tissue

Individual values of dSAT and sSAT areas in the WE and BWA men of NGT, IGT and T2D groups are shown in Figures 3.4 and 3.5, respectively. Neither dSAT or sSAT differed by ethnicity in the NGT, IGT, or T2D glycaemic states. The ratio of dSAT to sSAT was significantly greater in the WE men than BWA men in the combined glycaemic groups, Table 3.4. Further investigation of dSAT:sSAT in each glycaemic group showed no ethnic differences in the NGT groups (P=0.24), a trend towards a significantly greater dSAT:sSAT in the WE men of IGT (P=089) and a significantly greater dSAT:sSAT in the WE men with T2D compared to the BWA men (P=0.029). No significant interactions by ethnicity*glycaemic state were present for dSAT ($P_{interaction}$=0.97), sSAT ($P_{interaction}$=0.95) and dSAT:sSAT ($P_{interaction}$=0.82).

Table 3.4: *Abdominal adipose tissue deposition in the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		**IGT**		**T2D**				
	WE (n=23)	**BWA (n=20)**	**WE (n=10)**	**BWA (n=10)**	**WE (n=17)**	**BWA (n=20)**	***P* eth**	***P* gly**	***P* *eth*gly***
VAT (cm^2)*	79.0 (55-112)	46.1 (34-61)	174.5 (126-241)	86.5 (63-119)	187.4 (152-230)	128.1 (103-159)	**<0.001**	**<0.001**	0.60
ASAT (cm^2)*	193.2 (149-249)	181.9 (136-243)	274 (227-330)	258.1 (191-348)	302.3 (251-364)	285.6 (247-330)	0.56	**<0.001**	0.99
VAT:ASAT*	0.46 (0.34-0.59)	0.30 (0.20-0.39)	0.69 (0.48-0.91)	0.37 (0.24-0.50)	0.68 (0.52-0.85)	0.48 (0.39-0.58)	**<0.001**	**0.002**	0.50
dSAT (cm^2)*	109.8 (76-159)	102.6 (73-145)	182.6 (147-227)	160.3 (116-221)	196.7 (164-236)	175.3 (150-205)	0.41	**<0.001**	0.98
sSAT (cm^2)*	75.5 (62-93)	76.6 (60-97)	88.6 (72-109)	96.4 (72-130)	103.7 (83-129)	108.8 (93-127)	0.59	**0.003**	0.95
dSAT:sSAT	1.68 ± 0.84	1.42 ± 0.49	2.16 ± 0.70	1.70 ± 0.38	1.95 ± 0.46	1.65 ± 0.35	**0.007**	**0.034**	0.82

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (*). *P* eth: main effect for ethnicity, *P* gly: main effect for glycaemic state, *P* eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA.

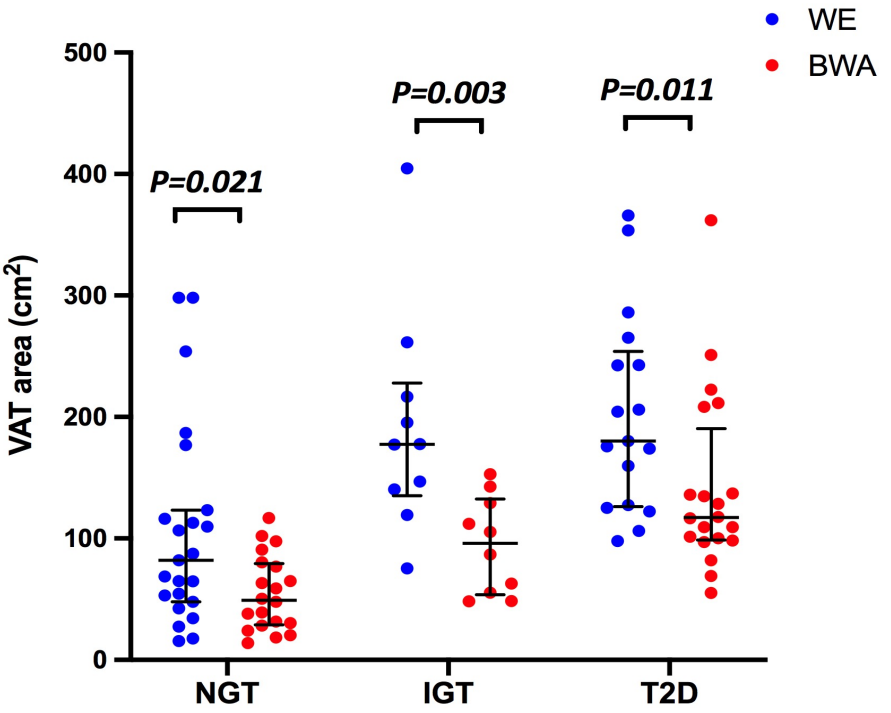

Figure 3.2: *Visceral adipose tissue (VAT) area determined at the L4-5 anatomical position in the WE and BWA men of NGT, IGT and T2D groups.*

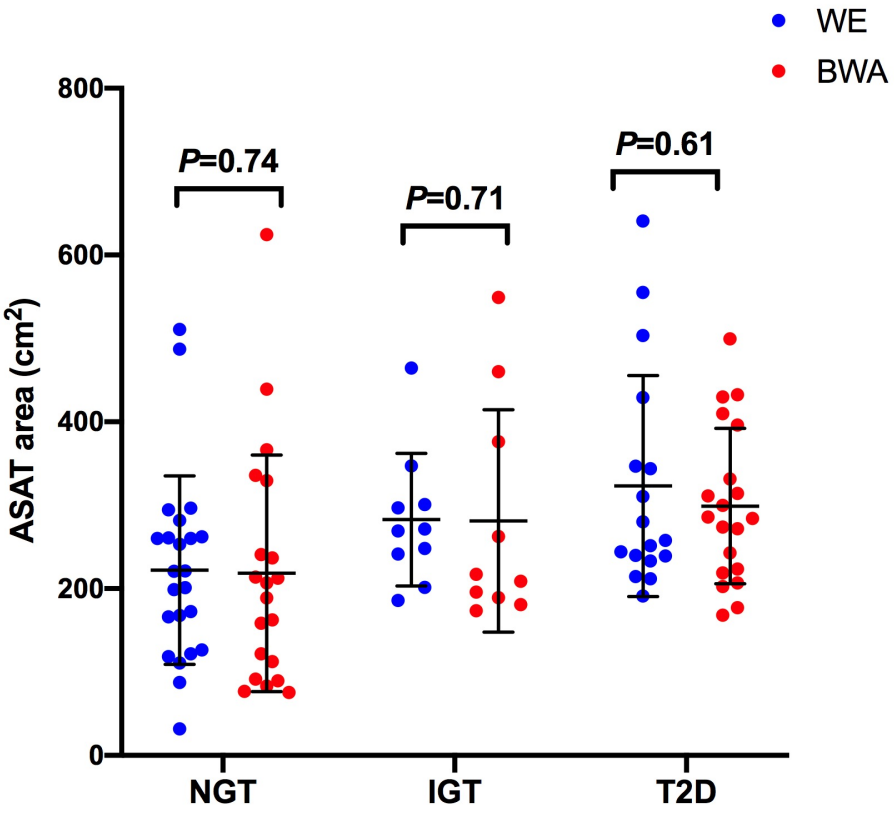

Figure 3.3: *Abdominal subcutaneous adipose tissue (ASAT) area determined at the L4-5 anatomical position in the WE and BWA men of NGT, IGT and T2D groups.*

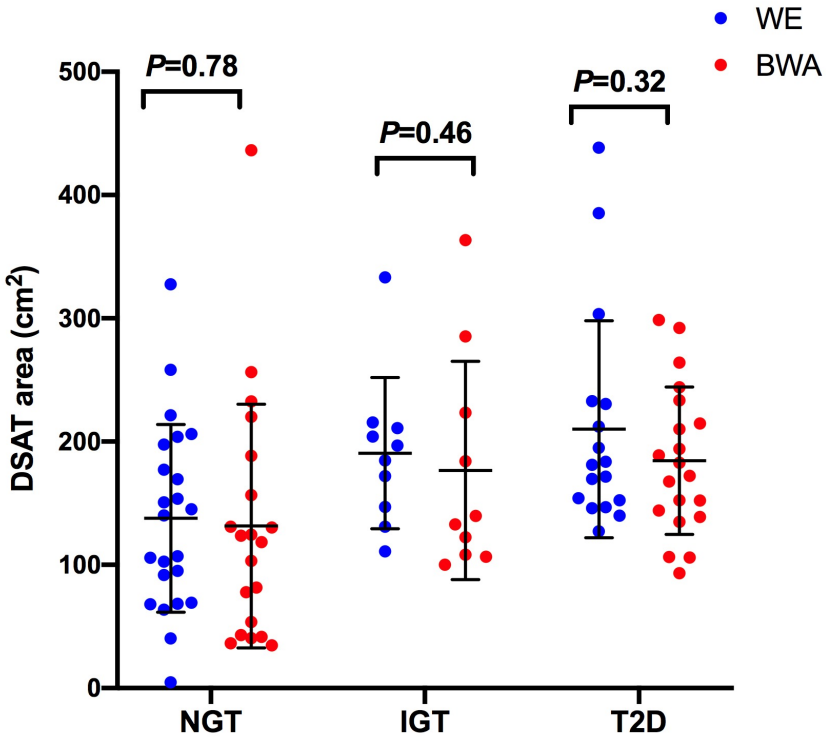

Figure 3.4: *Abdominal deep subcutaneous adipose tissue (dSAT) area determined at the L4-5 anatomical position in the WE and BWA men of NGT, IGT and T2D groups.*

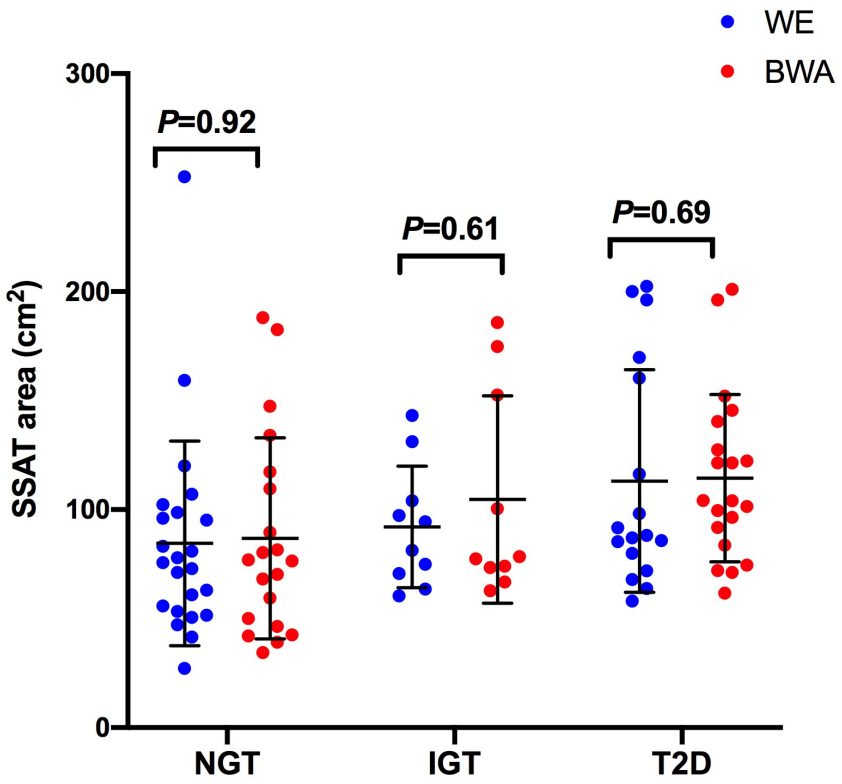

Figure 3.5: *Abdominal superficial subcutaneous adipose tissue (sSAT) area determined at the L4-5 anatomical position in the WE and BWA men of NGT, IGT and T2D groups.*

3.4.3.3 Total abdominal adipose tissue

Mean total abdominal adipose tissue is presented by the proportions of mean VAT, dSAT and sSAT for the WE and BWA men within each glycaemic state in Figure 3.6. While total abdominal adipose tissue was lower in the BWA men, this was not significant between the NGT groups (*P*=0.34) but approached significance between the IGT (*P*=0.091) and T2D (*P*=0.094) groups. This was mostly driven by the lower VAT in the BWA men in all glycaemic states.

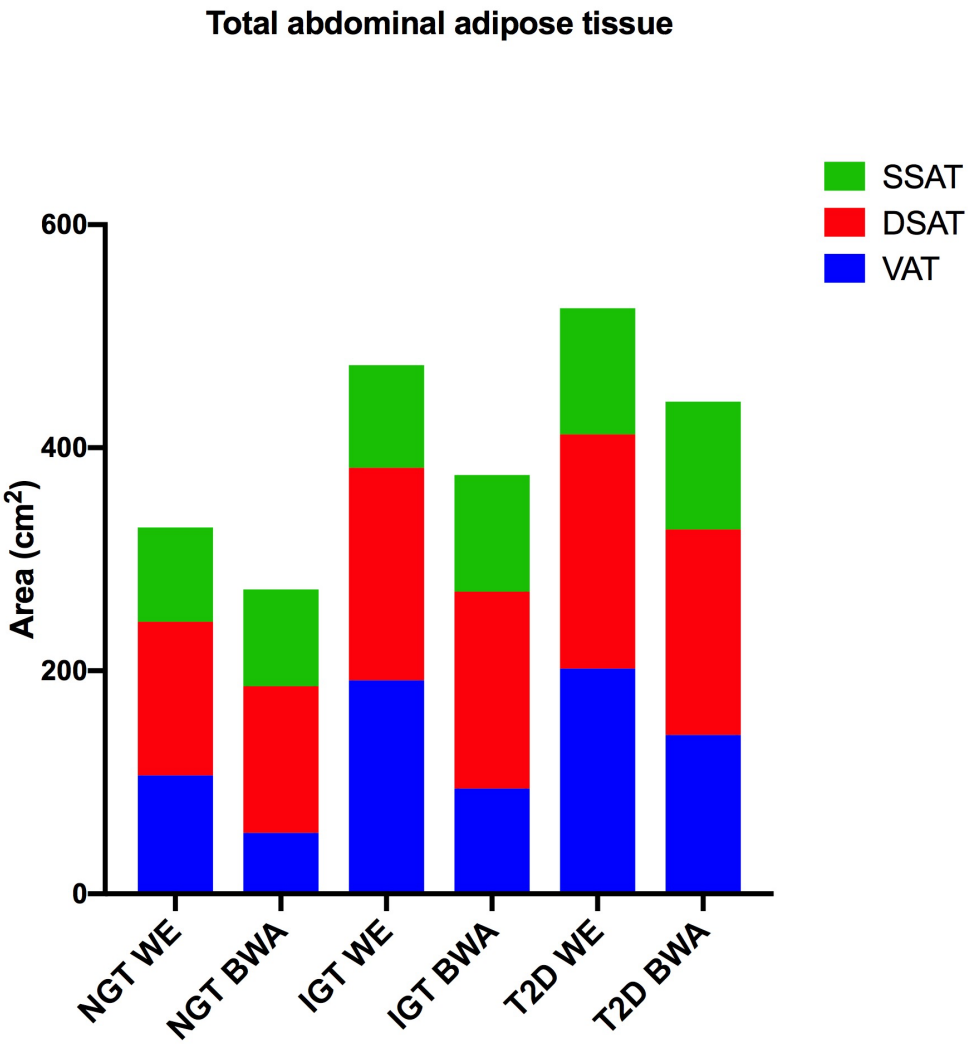

Figure 3.6: *Means of total abdominal adipose tissue showing the proportion of the three main components, VAT, DSAT, SSAT, in the 6 comparison groups.*

3.4.4 Interrelationships between regional adipose tissue depots

Interrelationships between the abdominal adipose tissue depots were investigated in the WE and BWA men in combined cohorts of all glycaemic states which are presented in Table 3.5. VAT was associated with dSAT and sSAT in both WE and BWA men. Since dSAT and sSAT are strongly associated with each other, to eliminate their possible influences on the relationships of VAT with dSAT and sSAT, partial correlation was conducted while adjusting for either dSAT or sSAT. After adjusting for dSAT, the associations between VAT and sSAT completely diminished in both ethnic groups; however, after adjusting for sSAT, the relationship between VAT and dSAT remained significant in the WE men but not the BWA men.

Table 3.5: *Pearson's correlation and partial correlation coefficients showing relationships between VAT and regional SAT depots in WE and BWA men of all glycaemic states.*

		WE (n=50)		**BWA (n=50)**	
VAT		**r**	***p***	**r**	***p***
dSAT		**0.57**	**<0.001**	**0.50**	**<0.001**
	Adjusted for sSAT	**0.39**	**0.006**	0.22	0.13
sSAT		**0.45**	**<0.001**	**0.46**	**0.001**
	Adjusted for dSAT	0.070	0.61	0.050	0.70

3.4.5 Relationships between visceral adipose tissue and measures of insulin sensitivity

Relationships between VAT and measures of insulin sensitivity are presented in Table 3.6 and Figures 3.7, 3.8 and 3.9 for the WE and BWA men in all glycaemic cohorts combined (panels A). Naturally, with increasing age and BMI, VAT increases while M-value decreases (Kurniawan et al., 2018, Hunter et al., 2010); therefore, to reduce their influence on the relationships between VAT and measures of insulin sensitivity, the above relationships were adjusted for both age and BMI, Table 3.6.

VAT was inversely associated with whole-body insulin sensitivity in the WE and BWA men, however, after adjusting for age and BMI, this relationship remained significant in the BWA but not the WE men. In a similar manner to the relationship with whole-body insulin sensitivity, VAT was inversely associated with hepatic insulin sensitivity in the WE and BWA men, however, after adjusting for age and BMI, this relationship remained significant in the BWA men but not the WE men. Interestingly, VAT was inversely associated with adipose tissue insulin sensitivity in the WE men but this relationship was not statistically significant in the BWA men. Furthermore, unlike the relationships of VAT with whole-body insulin sensitivity and hepatic insulin sensitivity, the relationship of VAT with adipose tissue insulin sensitivity remained significant in the WE men after adjusting for both age and BMI. Analysis of interaction by ethnicity for the above relationships showed the only significant ethnicity interaction was for the relationship between VAT and adipose tissue insulin sensitivity ($P_{interaction}$=0.030), Figure 3.9.

Relationships between VAT and measures of insulin sensitivity in the WE and BWA men analysed separately in the NGT and T2D glycaemic states are presented in Table 3.7 and Figures 3.7, 3.8 and 3.9 (panels B). While the sample sizes are markedly lower in comparison to all glycaemic cohorts combined, this analysis allows the elimination of the

influence of glycaemic state in the relationships between VAT and insulin sensitivity. This analysis showed similar trends with respect to ethnic differences in the NGT and T2D states. VAT was inversely associated with whole-body and hepatic insulin sensitivity in both WE and BWA men of NGT and T2D groups (except for VAT and hepatic insulin sensitivity in WE men with T2D). However, VAT was only associated with adipose tissue insulin sensitivity in the WE men but not BWA men within both NGT and T2D glycaemic groups.

Table 3.6: *Pearson's correlation and partial correlation coefficients showing relationships between VAT and measures of insulin sensitivity in WE and BWA men of all glycaemic states.*

	WE		**BWA**	
VAT	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity[a]	**-0.70**	**<0.001**	**-0.62**	**<0.001**
Adjusted for BMI	**-0.43**	**0.002**	**-0.41**	**0.005**
Adjusted for age	**-0.50**	**<0.001**	**-0.51**	**<0.001**
Adjusted for BMI and age	-0.22	0.14	**-0.34**	**0.025**
Hepatic insulin sensitivity[b]	**-0.51**	**0.001**	**-0.70**	**<0.001**
Adjusted for BMI	-0.27	0.085	**-0.58**	**<0.001**
Adjusted for age	-0.27	0.094	**-0.60**	**<0.001**
Adjusted for BMI and age	-0.10	0.53	**-0.50**	**0.001**
Adipose tissue insulin sensitivity[c]	**-0.68**	**<0.001**	-0.11	0.48
Adjusted for BMI	**-0.43**	**0.007**	-0.11	0.47
Adjusted for age	**-0.55**	**<0.001**	-0.16	0.40
Adjusted for BMI and age	**-0.36**	**0.031**	-0.14	0.40

[a]n: WE=49, BWA=46; [b]n: WE=42, BWA=43; [c]n: WE=39, BWA=42

Table 3.7: *Pearson's correlation coefficients showing relationships between VAT and measures of insulin sensitivity in WE and BWA men of NGT and T2D groups.*

	NGT				**T2D**			
	WE		**BWA**		**WE**		**BWA**	
VAT	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity	**-0.81**	**<0.001**	-0.45	0.058	**-0.53**	**0.028**	**-0.55**	**0.019**
n	23		18		17		18	
Hepatic insulin sensitivity	**-0.52**	**0.015**	**-0.57**	**0.013**	-0.41	0.19	-0.47	0.079
n	21		18		12		15	
Adipose tissue insulin sensitivity	**-0.58**	**0.005**	0.06	0.81	**-0.72**	**0.018**	-0.22	0.43
n	22		18		10		15	

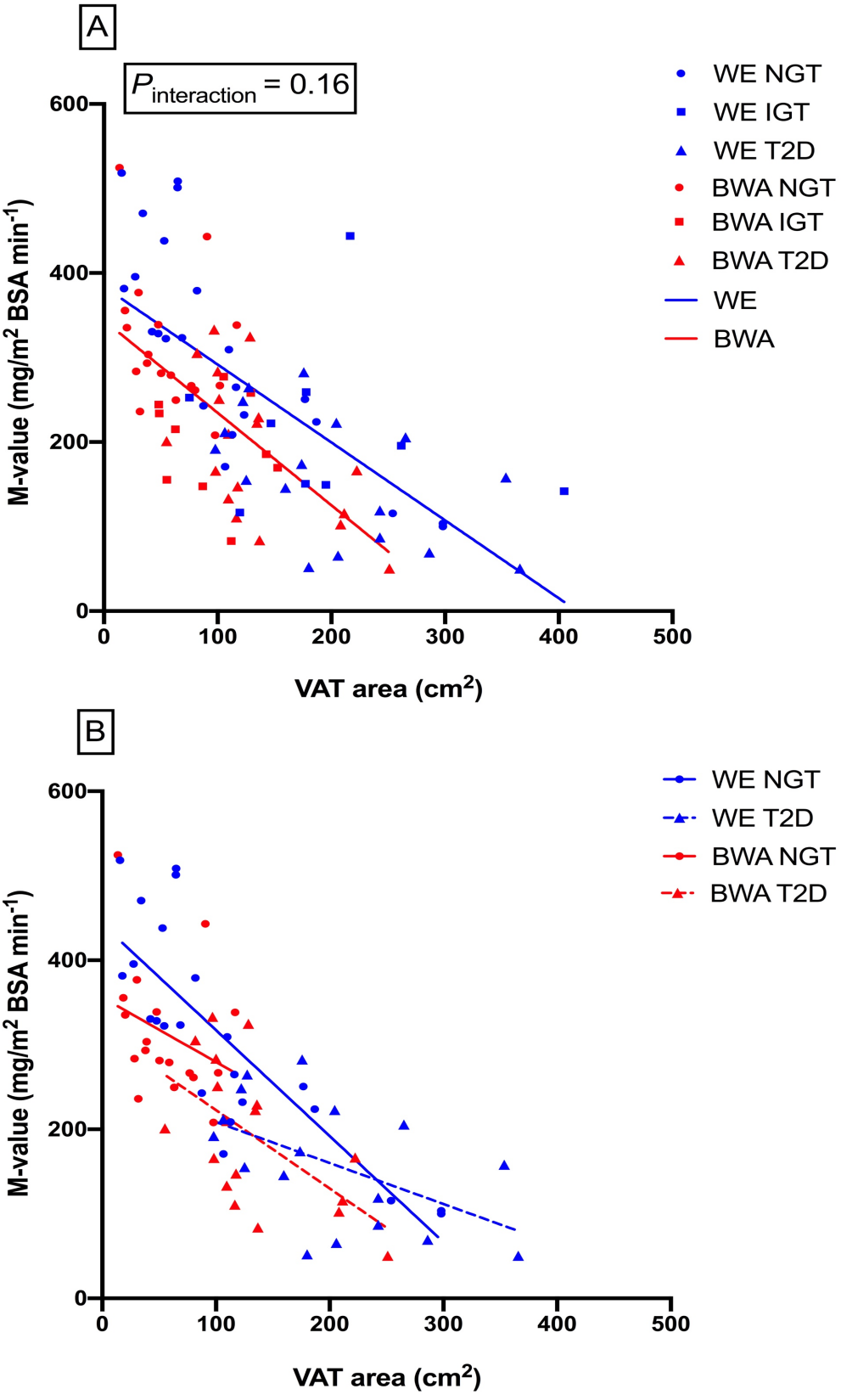

Figure 3.7: *Relationships between VAT and whole-body insulin sensitivity (M-value) in the WE and BWA men in the combined glycaemic cohorts (NGT, IGT and T2D), A; and in the NGT and T2D cohorts, B.*

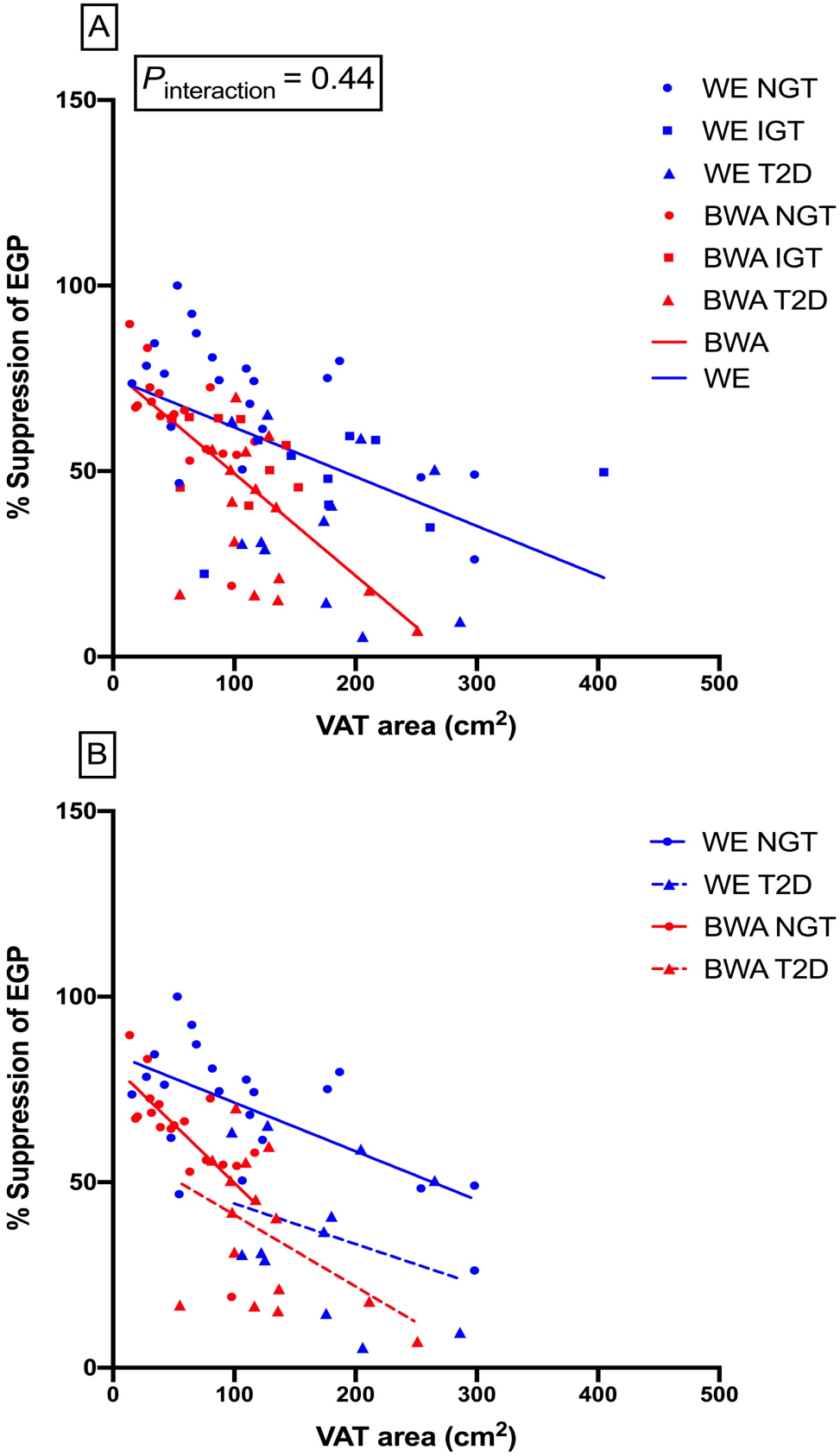

Figure 3.8: *Relationships between VAT and hepatic insulin sensitivity (% suppression of EGP) in the WE and BWA men in the combined glycaemic cohorts (NGT, IGT and T2D), A; and in the NGT and T2D cohorts, B.*

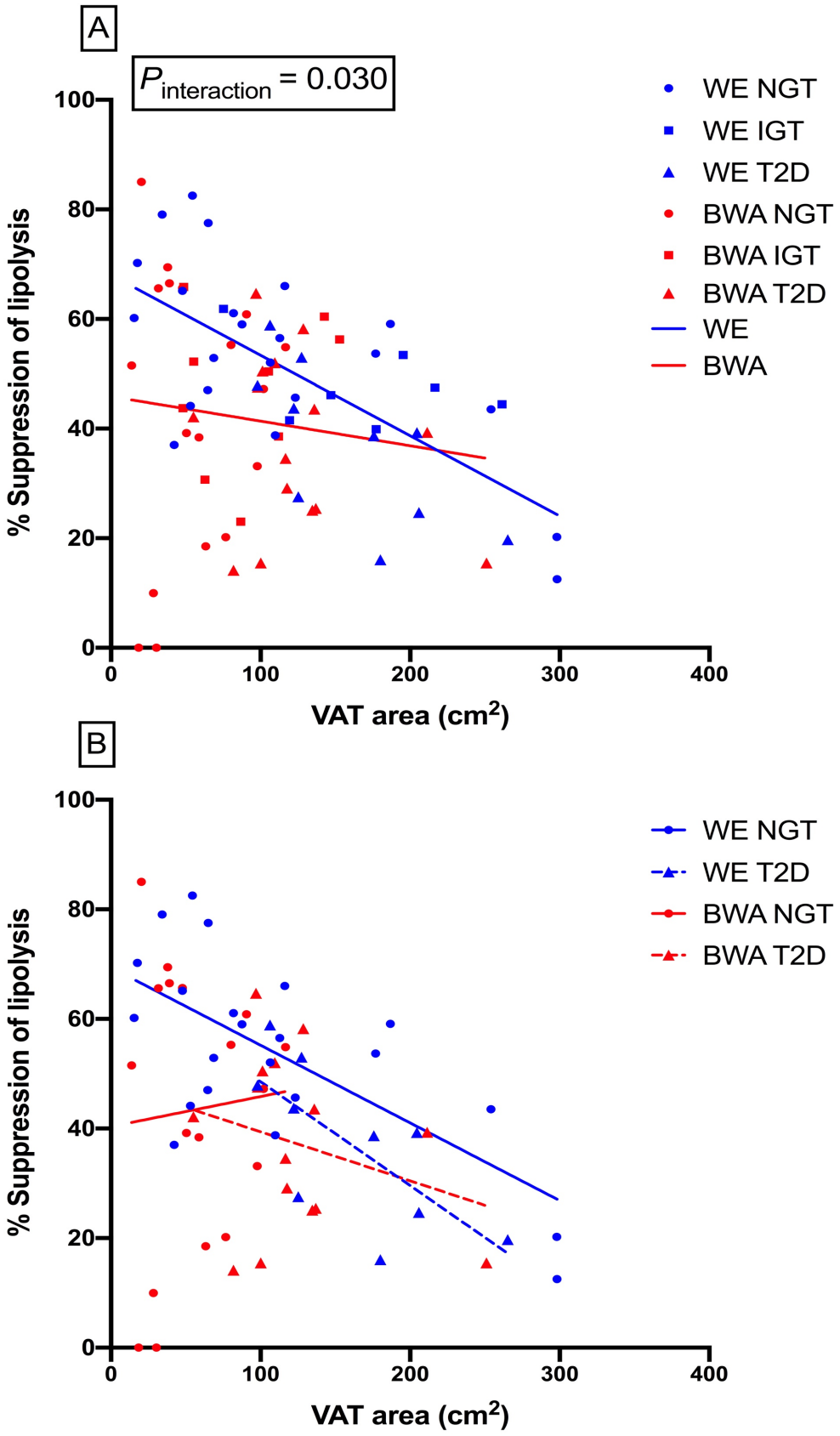

Figure 3.9: *Relationships between VAT and adipose tissue insulin sensitivity (% suppression of lipolysis) in the WE and BWA men in the combined glycaemic cohorts (NGT, IGT and T2D), A; and in the NGT and T2D cohorts, B.*

3.4.6 Relationships between regional subcutaneous adipose tissue depots and whole-body insulin sensitivity

Relationships between regional subcutaneous adipose tissue depots and whole-body insulin sensitivity are presented in Table 3.8 for the WE and BWA men in all glycaemic cohorts combined; partial correlation coefficients are shown for these relationships while adjusting for BMI and age. ASAT, dSAT and sSAT were inversely associated with whole-body insulin sensitivity in both WE and BWA men; however, these relationships diminished in both ethnic groups after adjustments for age and BMI. Of note, while there were significant relationships between all measures of abdominal adiposity and whole-body insulin sensitivity, including VAT, ASAT, dSAT and sSAT, in both ethnic groups, these relationships were consistently stronger in the WE men.

Relationships between regional abdominal adipose tissue depots and whole-body insulin sensitivity are presented separately for the NGT and T2D groups in Table 3.9. While the sample sizes are lower than those of the all glycaemic cohorts combined, this analysis showed some clear patterns. In the WE men, ASAT, dSAT and sSAT were inversely associated with whole-body insulin sensitivity in the NGT and T2D groups; however, in the BWA men, ASAT, dSAT and sSAT were inversely associated with whole-body insulin sensitivity in the NGT but not the T2D glycaemic group.

Table 3.8: *Pearson's correlation and partial correlation coefficients showing relationships between measures of regional subcutaneous adipose tissue and whole-body insulin sensitivity (M-value) in WE and BWA men of all glycaemic states.*

	WE (n=49)		**BWA (n=46)**	
M-value	**r**	***p***	**r**	***p***
ASAT	**-0.72**	**<0.001**	**-0.53**	**<0.001**
Adjusted for BMI and age	-0.24	0.10	-0.12	0.43
dSAT	**-0.73**	**<0.001**	**-0.53**	**<0.001**
Adjusted for BMI and age	-0.23	0.12	-0.11	0.47
sSAT	**-0.57**	**<0.001**	**-0.48**	**0.001**
Adjusted for BMI and age	-0.16	0.29	-0.11	0.47

Table 3.9: *Pearson's correlation coefficients showing relationships between regional adipose tissue depots and whole-body insulin sensitivity in WE and BWA men of NGT and T2D groups.*

	NGT				**T2D**			
	WE (n=23)		**BWA (n=18)**		**WE (n=17)**		**BWA (n=18)**	
M-Value	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
ASAT	**-0.61**	**0.002**	**-0.80**	**<0.001**	**-0.73**	**0.001**	-0.31	0.22
dSAT	**-0.52**	**0.011**	**-0.81**	**<0.001**	**-0.74**	**0.001**	-0.31	0.21
sSAT	**-0.54**	**0.007**	**-0.73**	**0.001**	**-0.63**	**0.007**	-0.25	0.33

3.5 Discussion

The purpose of this chapter was to investigate ethnic differences in regional abdominal adipose tissue deposition between WE and BWA men across three glycaemic states: NGT, IGT and T2D. Ethnic comparisons were conducted between WE and BWA men of all glycaemic states combined as well as within each of the glycaemic groups. The main findings recognise ethnic differences in regional adipose tissue distribution and the relationships between specific adipose tissue depots and measures of insulin sensitivity. The BWA men had significantly lower VAT levels compared to WE men in all glycaemic states, however, there were no ethnic differences in ASAT, dSAT and sSAT. Relationships between VAT and measures of insulin sensitivity showed similar patterns in both ethnic groups where VAT was inversely associated with whole-body and hepatic insulin sensitivity in WE and BWA men, however, VAT was significantly inversely associated with adipose tissue insulin sensitivity in only WE men.

3.5.1 Ethnic differences in regional adipose tissue deposition

The lower levels of VAT in the BWA men supports the findings of previous studies that have consistently shown VAT is lower in black populations compared to their white counterparts (Alderete et al., 2014, Goedecke et al., 2017). Also similar to previous studies, is the lack of ethnic differences in ASAT levels (Lee et al., 2011, Liska et al., 2007). Contrastingly, some studies report greater ASAT in black populations compared to whites, however, a commonality of these studies is that they were all conducted in women who have a greater capacity to store SAT compared to men. Indeed, several studies showed ASAT was significantly greater in black women compared to black men (De Lucia Rolfe et al., 2015, Tulloch-Reid et al., 2004). The consistently lower VAT levels, at similar levels of adiposity, in black populations compared to white populations is an indicator of a unique ethnic trait in populations of African descent, who appear to

have a reduced susceptibility to store excess triglycerides in the VAT depot at all glycaemic states.

The sSAT depot is the primary source of triglyceride storage and is the most metabolically safe storage location of excess triglycerides (Sniderman et al., 2007). With gradual expansion of sSAT, there is an expansion of dSAT which is composed of more disorganised and dysfunctional adipocytes that have greater lipolytic activity compared to those of sSAT. In this analysis, there were no ethnic differences in dSAT which is similar to previous reports from studies conducted in adolescents (Liska et al., 2007), women (Evans et al., 2011, Goedecke et al., 2011, Lovejoy et al., 2001, Nazare et al., 2012) and men (Nazare et al., 2012). Similarly, sSAT did not differ between the WE and BWA men which is similar to previous findings in men (Nazare et al., 2012) but opposes previous studies that showed greater sSAT in black women (Lovejoy et al., 2001, Liska et al., 2007, Evans et al., 2011, Goedecke et al., 2011) and children (Liska et al., 2007). This may be explained by gender or by differences in the level of whole-body adiposity because the studies that have shown sSAT to be greater in black populations investigated only groups with severe obesity while the participants in this study had an overweight or mildly obese BMI. In support of this explanation are findings by Evans *et al.* who reported significantly greater sSAT in black women with obesity but not lean black women in comparison to their BMI-matched white counterparts (Evans et al., 2011).

Increased ratios of VAT:SAT and dSAT:sSAT are indicative of unfavourable patterns of adipose tissue deposition, where, with increasing adiposity there is a greater susceptibility of triglyceride storage in the VAT and dSAT compartments. The analysis presented here showed VAT:SAT ratio was significantly lower in the BWA men compared to the WE men which was mostly driven by lower VAT in the BWA men. These findings are consistent with previous reports in the literature (Wagenknecht et al., 2003, Carroll et al.,

2008, Nazare et al., 2012, Liu et al., 2014). Furthermore, dSAT:sSAT was also significantly lower in the BWA men compared to WE men which is similar to previous findings in black men (Nazare et al., 2012). These findings indicate a preferential storage of triglycerides in the SAT compared to VAT, and sSAT compared to dSAT, in the BWA men. Hence, the lower VAT: SAT and dSAT:sSAT ratio in the BWA men suggests that the greater risk of T2D in black populations compared to white populations may not be explained by body fat distribution.

3.5.2 Interrelationships between abdominal adipose tissue depots

Previous studies have shown VAT associates with dSAT but not sSAT implying that the expansion of VAT occurs due to similar mechanisms related to the expansion of dSAT (Marinou et al., 2014, Kelley et al., 2000). In contrast to previous reports, the current analysis showed both dSAT and sSAT were associated with VAT in the WE and BWA men. However, the relationships between sSAT and VAT diminished after controlling for dSAT in both ethnic groups indicating that dSAT may be an influencer of VAT expansion. Interestingly, after controlling for sSAT, the relationship between dSAT and VAT remained in the WE men but not the BWA men indicating that dSAT may be more closely related to VAT expansion in WE men than BWA men. Furthermore, both dSAT and sSAT were associated with VAT in BWA men but not independently of each other; this may indicate that the overall expansion of SAT influences VAT in BWA men which is not dependent on dSAT.

3.5.3 Relationships between regional adipose tissue depots and insulin sensitivity

Since VAT is the adipose tissue depot that is most linked to T2D, but is paradoxically lower in black populations, the relationships between VAT and measures of insulin sensitivity were investigated in each ethnic group. This analysis showed VAT was

inversely associated with whole-body insulin sensitivity and hepatic insulin sensitivity in both ethnic groups; however, after adjusting for BMI and age, these relationships remained significant in the BWA but not WE men. Even though there is evidence suggesting that increased age and BMI are related to a decrease in insulin sensitivity in both white and black populations (Chandler-Laney et al., 2011), the above ethnic difference may indicate that an increase of age and BMI may result in a greater decrease of insulin sensitivity in white populations compared to black populations.

Increased accumulation of VAT is explained by the spill-over theory which suggests that with increasing SAT, adipocytes within SAT become dysfunctional and unable to store excess triglycerides efficiently which causes the release of excess NEFAs into the circulation that get deposited in the VAT depot (Sattar and Gill, 2014, Shulman, 2014). One of the features of dysfunctional SAT is reduced adipose tissue insulin sensitivity detected by greater lipolytic activity during the insulin-stimulated state (Gastaldelli et al., 2017). This analysis showed no ethnic differences in adipose tissue insulin sensitivity indicating that adipose tissue is equally responsive to the antilipoytic effects of insulin in both ethnic groups. However, VAT was significantly inversely associated with adipose tissue insulin sensitivity in the WE men but not BWA men. This suggests that VAT deposition in WE men occurs with increasing adipose tissue insulin resistance as suggested by the spill-over theory. Furthermore, the inverse relationship between adipose tissue insulin sensitivity and VAT remained significant after adjustment for age and BMI in the WE men; this indicates that the accumulation of VAT may be directly related to the dysfunction of SAT, independently of age and BMI. The lack of a significant inverse relationship between VAT and adipose tissue insulin sensitivity in the BWA men indicates that, unlike WE men, VAT deposition may be influenced to a lesser degree by adipose tissue insulin resistance in BWA men. Furthermore, the mechanisms of VAT

accumulation may differ between WE and BWA men and the spill-over theory may not apply to black populations.

Previous studies have shown that dSAT has a similar relationship with whole-body insulin resistance as VAT while sSAT shows no relationship or protective qualities in T2D (Golan et al., 2012). However, the data presented here showed that whole-body insulin sensitivity decreased with increasing dSAT and sSAT in both WE and BWA men. Despite the decrease in sample size, investigation of these relationships in the NGT and T2D states showed specific ethnic trends. While whole-body insulin sensitivity decreased with an increase of dSAT and sSAT in WE and BWA men in the NGT groups, in the T2D groups, these relationships were only present in the WE men. Also evident is the consistently stronger inverse relationships between all adipose depots investigated and measures of insulin sensitivity in WE men than BWA men. These findings further support the notion that the greater prevalence of T2D in black populations are not explained by patterns of body adiposity.

Visceral adipose tissue is thought to cause insulin resistance due to its greater release of NEFAs into the circulation particularly into the portal vein which has direct access to the liver; the excess NEFAs then cause lipotoxicity of the liver and reduce its sensitivity to insulin (Patel and Abate, 2013a). However, it has been postulated that the accumulation of VAT is a marker of dysfunctional SAT and, while VAT has several pathogenic features, it is strongly related to increased dysfunction of SAT (Smith, 2015). Dysfunctional SAT has an increased release of NEFAs that are toxic to all organs and cause several disturbances that underlie insulin resistance in the development of T2D (Smith and Kahn, 2016). Based on this view, it may be the dysfunction of adipose tissue that drives the progression from NGT to T2D in black populations who appear to be

protected from VAT accumulation. Hence, future studies should investigate ethnic differences in adipose tissue function which may untangle these speculations.

While VAT has greater lipolytic activity, SAT accounts for approximately 80% of total body adipose tissue in most individuals. Contrary to common belief, the SAT compartment contributes more so to the circulatory NEFA pool than VAT (Klein, 2004). Nielsen *et al.* showed this clearly using isotopic tracing of NEFAs from various adipose tissue compartments; the findings of this are summarised in Figure 3.10 (Nielsen et al., 2004). The accumulation of VAT and ASAT has been postulated to be a result of a metabolic state of insulin resistance as well as genetic and environmental factors that are exacerbated during a positive energy balance (Klein, 2004). This notion is supported by several studies attempting to reduce the burden of T2D and insulin resistance by surgically removing ASAT and VAT. Surprisingly these studies consistently showed that surgical removal of large percentages of ASAT or VAT did not improve insulin sensitivity (Fabbrini et al., 2010, Foster and Pagliassotti, 2012, Zhao et al., 2018). The lower VAT in BWA men may support these studies that suggest VAT is a marker but not a direct cause of dysfunctional adipose tissue which may drive T2D.

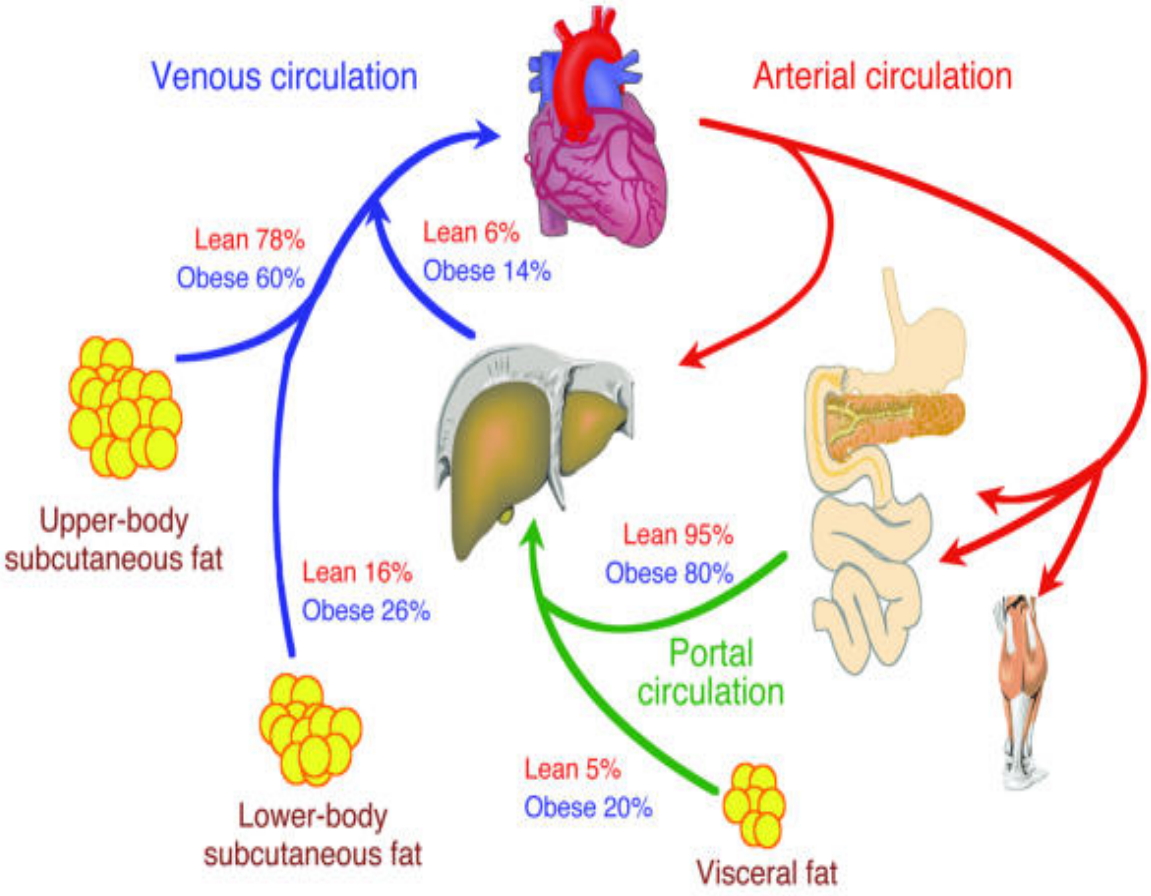

Figure 3.10: *Approximate contributions of visceral adipose tissue, upper-body and lower-body subcutaneous adipose tissue to the NEFA pool in the systemic and portal circulations (Nielsen et al., 2004).*

3.5.4 Strengths and limitations

This study has several strengths worth noting which include the use of MRI to determine measures of regional adipose tissue because MRI is considered to be the gold-standard non-invasive method of quantifying body composition components. However, a limitation is the analysis of a single MRI image as opposed to all MRI images of the whole abdominal cavity. This is of particular importance due to evidence from body composition studies that have shown black populations have lower trunk lengths and greater limb lengths compared to white populations (Bogin and Varela-Silva, 2010); therefore, the total volume of abdominal fat may have differed more greatly between the WE and BWA men.

Another strength is the assessment of dSAT and sSAT as well as their relationships with whole-body insulin sensitivity which has not previously been conducted in a black population. However, to fully understand whether functional, structural and genetic differences exist in dSAT and sSAT between WE and BWA men, further adipose tissue biopsy studies are required. The investigation of regional adipose tissue depots by ethnicity and glycaemic state is another novel aspect of this analysis; however, the smaller sample sizes of the ethnic groups within each glycaemic state, as opposed to all glycaemic states combined, may have reduced the power of detecting significant relationships.

3.6 Conclusion

This study has demonstrated that BWA men have lower VAT, VAT:ASAT and dSAT:sSAT compared to BMI-matched WE men while no ethnic differences in ASAT, dSAT and sSAT were detected. The lower VAT in the BWA men was present at all glycaemic states indicating it appears to be an ethnic characteristic which is unique to black populations regardless of T2D risk. Furthermore, VAT showed strong inverse relationships with whole-body and hepatic insulin sensitivity in both WE and BWA men. This suggests that despite the lower VAT in BWA men, it appears to have similar lipotoxic effects in black populations as it does in white population but this lipotoxicity occurs at a lower level of VAT in black populations. The relationship between VAT and adipose tissue insulin resistance differed by ethnicity where it was present in WE men but not BWA men. Hence, dysfunctional SAT, characterised by reduced adipose tissue insulin sensitivity, may drive VAT accumulation more so in WE men than BWA men.

Chapter 4: Ethnic differences in intrahepatic lipid and its relationship with hepatic insulin sensitivity and insulin clearance in men of black and white ethnicity

Data presented in this chapter have been published in (appendix I and appendix II):

Hakim O, Bello O, Ladwa M, Christodoulou D, Bulut E, Shuaib H, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity. *Diabetes Research and Clinical Practice.* 2019 Sep 19:107866.

Hakim O, Bello O, Bonadonna RC, Mohandas C, Shojaee-Moradie F, Jackson N, Boselli L, Whitcher B, Shuaib H, Alberti KG, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. Ethnic differences in intrahepatic lipid and its association with hepatic insulin sensitivity and insulin clearance between men of black and white ethnicity with early type 2 diabetes. *Diabetes, Obesity and Metabolism.* 2019 May 10.

4.1 Introduction

The liver plays an integral role in glucose metabolism and thus the development of T2D. In the NGT state, insulin acts on the liver to mediate both endogenous glucose production and insulin clearance; both these functions are altered in individuals with T2D (Defronzo, 2009, Iozzo et al., 2003). During the progression from NGT to T2D, the liver becomes insulin resistant and, therefore, the suppression of glucose production by insulin is decreased while the rate of hepatic insulin clearance is reduced. The reduction in insulin clearance has been considered a protective mechanism to increase circulating insulin levels during insulin resistance in order to preserve beta-cell function (Piccinini et al., 2017).

As well as the deterioration of the metabolic functions of the liver, ectopic storage of triglycerides in the liver, termed intrahepatic lipid (IHL), is found to be elevated in individuals with T2D (Shulman, 2014). Furthermore, the prevalence of non-alcoholic fatty liver disease (NAFLD) is alarmingly 65% in populations with T2D, which is estimated to be more than twice that of the general population (Doycheva et al., 2016). There has been increasing interest in IHL in T2D research particularly since IHL has been linked more closely with several metabolic parameters of T2D compared to other fat depots, including visceral adipose tissue (VAT) (Lee et al., 2012, Fabbrini et al., 2009, Hong et al., 2016). Several studies have compared IHL to VAT and concluded that IHL is more strongly linked to T2D than VAT (Kantartzis et al., 2010, Kadowaki et al., 2019). One of those studies was a longitudinal study of over 15,000 adults, which was conducted by Okamura *et al.*, who showed fatty liver presented a greater risk for incidence T2D compared to high VAT and obesity (Okamura et al., 2019). Similarly, Lee *et al.* showed that after, adjustment for age and sex, fatty liver was a significant independent risk factor for the metabolic syndrome but VAT was not (Lee et al., 2012). IHL is proposed to cause

lipotoxicity of the hepatocytes by interfering with cellular signalling functions including those mediated by insulin (Snel et al., 2012). Investigations of the role of IHL in the development of T2D have shown that IHL is inversely associated with both hepatic insulin sensitivity and insulin clearance (Kotronen et al., 2007, Kotronen et al., 2008, Seppala-Lindroos et al., 2002). Therefore, there is much evidence that highlights the detrimental role of IHL accumulation in the development of T2D.

The main theory that describes the mechanism behind IHL accumulation is the *portal theory*, which states that IHL deposition results from high flux of NEFAs that are transported via the portal vein (Item and Konrad, 2012). NEFAs are detrimental to metabolic functions of the liver by causing lipotoxicity to the local environment in which they reside as well as promoting the deposition of IHL (Liu et al., 2016). Since, VAT is the only adipose depot that has direct access to the portal vein, as well as being a highly lipolytic fat depot, an increase of NEFAs from VAT to the liver has been suggested to drive IHL accumulation (Rytka et al., 2011); this phenomenon is a key feature of the *portal theory*. However, it is also likely that excess circulating NEFAs from SAT that drive VAT accumulation, particularly in a state of dysfunctional SAT, also contribute to excess NEFA delivery to the liver (Klein, 2004, Nielsen et al., 2004). These suggestions highlight the interrelated nature of the spill-over and portal theories, which most likely occur simultaneously (Castro et al., 2014).

Ethnic differences in IHL, insulin clearance and hepatic insulin sensitivity have been reported between black and white populations. Despite the greater risk of T2D in black populations, they have consistently been shown to have lower levels of IHL compared to their white counterparts (Guerrero et al., 2009, Liska et al., 2007, Liu et al., 2016, Schwimmer et al., 2005). The lower levels of IHL in black individuals has been attributed to lower VAT which is also commonly reported in black populations with reference to

the above-mentioned portal theory (Guerrero et al., 2009). However, studies investigating relationships between various fat depots in black populations are few, hence, it is unclear if the mechanisms that drive IHL accumulation are similar in black and white individuals. Therefore, ethnic differences in the mechanisms that drive IHL accumulation will be explored in this chapter.

Investigations of ethnic differences in hepatic insulin sensitivity are few and have shown inconsistent conclusions. While some studies report greater hepatic insulin sensitivity in blacks compared to whites (Goedecke et al., 2015) others report lower (Ellis et al., 2012), however, most report no ethnic differences (DeLany et al., 2014, Arslanian et al., 2002, Hannon et al., 2008, Bacha et al., 2012, Lee et al., 2013, Stefan et al., 2004). Consistently, insulin clearance has been shown to be lower in black populations compared to white populations (Harris et al., 2002, Piccinini et al., 2017, Michaliszyn et al., 2017).

While several observational studies have investigated IHL in black populations, few have explored relationships between IHL and the metabolic parameters of T2D. At present, only one study has investigated relationships between IHL and hepatic insulin sensitivity (% suppression of EGP) in a black *vs* white population (Goedecke et al., 2015). Additionally, only one study has investigated relationships between IHL and insulin clearance in a black *vs* white population (Chung et al., 2019). Thus, studies of the role of IHL on metabolic functions of the liver are lacking in black populations.

Considering the strong link between IHL and T2D but the paradoxically lower IHL in black populations, some researchers have suggested that black individuals appear to be more sensitive to the detrimental effects of IHL (Alderete et al., 2013, Goedecke et al., 2015), while others have suggested that IHL appears to be less detrimental in black populations (Chung et al., 2018, Chung et al., 2019). Thus, with the lack of conclusive studies, it is crucial to investigate the role of IHL in the pathophysiology of T2D in black

populations. Furthermore, despite ethnic differences being reported in IHL, hepatic insulin sensitivity and insulin clearance between black and white populations, they have not been previously investigated in a single study to understand the impact of IHL on the metabolic functions of the liver and its influence on the development of T2D in black populations. Due to the lower levels of IHL present in black populations compared to their white counterparts but similar hepatic insulin sensitivity, it is hypothesised that IHL would be more strongly related to hepatic insulin sensitivity in BWA men than WE men.

4.2 Aim

The primary aim of this chapter is to assess ethnic differences in intrahepatic lipids (IHL) between WE and BWA men of NGT, IGT and T2D glycaemic states. The secondary aim is to investigate ethnic differences in the relationships between IHL and hepatic insulin sensitivity and insulin clearance.

4.3 Methods

4.3.1 Data acquirement

The data presented in this chapter were acquired using the methods described in chapter 2 (sections 2.3.1, 2.3.1.2, 2.3.3, 2.3.3.2, 2.3.4, 2.3.4.1). In brief, IHL was determined by the analysis of two abdominal MRI images that have a large area of liver tissue in each participant. Four circular regions of interest were positioned on the liver tissue to determine % IHL in each region and a mean IHL, using all 8 regions, was calculated. Measures of insulin sensitivity were determined using a hyperinsulinaemic-euglycaemic clamp test with the infusion of stable glucose and glycerol isotopes to determine whole-body insulin sensitivity (M-value), hepatic insulin sensitivity (% suppression of EGP), and adipose tissue insulin sensitivity (% suppression of lipolysis). Insulin clearance was determined using an intravenous glucose challenge (hyperglycaemic clamp). Insulin clearance was modelled using c-peptide and insulin data during the intravenous glucose challenge.

4.3.2 Statistical analysis

IHL, insulin sensitivity and insulin clearance data presented in this chapter for the WE and BWA men of NGT, IGT and T2D groups were compared using 2-way between-groups ANOVA. Ethnicity and glycaemic state were included in the 2-way between-groups ANOVA as independent variables to assess the main effect of each of the independent variables, as well as their interaction (ethnicity*glycaemic state), on the outcome measures (dependent variables). Ethnic differences in IHL were tested within each glycaemic state using independent samples t-test for normally distributed data or Mann-Whitney test for non-parametric data. Strength of relationships between variables of interest were tested using Pearson's correlation test; partial correlation was used when assessing relationships while adjusting for confounding variables. Ethnic differences in

the strength of the relationships were examined by fitting a regression with an interaction term for ethnicity and IHL as an independent variable.

4.4 Results

4.4.1 Participant characteristics

The clinical characteristics of the WE and BWA men of NGT, IGT and T2D groups are presented in chapter 3, Table 3.2. The BWA men were slightly older than the WE men however there were statistically significant differences in clinical characteristics except for waist circumference, triglycerides, which were significantly lower in the BWA men; and HbA1c which was greater in the BWA men.

4.4.2 Intrahepatic lipids

IHL in the WE and BWA men of NGT, IGT and T2D are presented Table 4.1, which shows IHL was significantly lower in the BWA men than WE men, indicated by a significant main effect for ethnicity. Additionally, IHL was significantly lower in the NGT groups than the IGT and T2D groups, indicated by a significant main effect for glycaemic state. Analysis of ethnic differences in IHL within each glycaemic state showed IHL was significantly lower in the BWA men within each glycaemic state (NGT: p=0.044, IGT: p=0.049, and T2D: p=0.027), as presented in Figure 4.1, which shows the individual values of IHL within each ethnic and glycaemic group.

Non-alcoholic fatty liver disease (NAFLD) can be defined as an IHL level above 5% determined by Dixon-MRI (Noureddin et al., 2013); using this criteria, the percentage of participants with NAFLD is presented in Table 4.1. The presence of NAFLD was significantly lower in the BWA men compared to the WE men within each glycaemic state. There were no significant ethnicity by glycaemic state interactions for IHL and NAFLD, indicating the ethnic differences in these variables are similar within each glycaemic state, Table 4.1.

Table 4.1: *IHL and metabolic parameters in the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		IGT		T2D				
	WE (n=23)	**BWA (n=20)**	**WE (n=10)**	**BWA (n=10)**	**WE (n=18)**	**BWA (n=18)**	***P* Eth**	***P* Gly**	***P* Eth*Gly**
IHL (%)*	4.75 (3.54-6.36)	3.65 (3.22-4.14)	8.37 (4.82-14.5)	4.47 (3.15-6.33)	7.45 (5.36-10.37)	4.88 (3.56-6.69)	**0.001**	**0.010**	0.52
Non-alcoholic fatty liver disease (% of participants)	35	10	70	30	67	33	**0.001**	**0.014**	0.81
Metabolic parameters									
Whole-body insulin sensitivity (M-value) (mg/m^2 BSA min^{-1})[a]	309.6 ± 127.5	313.5 ± 77.0	214.6 ± 99.8	197.0 ± 59.9	154.4 ± 76.2	191.0 ± 85.3	0.71	**<0.001**	0.56
Hepatic insulin sensitivity (% suppression of EGP)*[b]	67.2 (58.5-77.2)	61.4 (52.3-72.1)	45.5 (35.6-58.2)	55.3 (48.6-62.8)	28.6 (18.1-45.1)	30.5 (21.1-44.1)	0.60	**<0.001**	0.54
Adipose tissue insulin sensitivity (% suppression of lipolysis)[c]	53.8 ± 17.4	43.4 ± 25.2	47.8 ± 7.6	46.8 ± 14.0	35.5 ± 14.5	37.2 ± 16.0	0.45	**0.027**	0.39
Insulin clearance (mL/m^2 BSA min^{-1})*[d]	1284 (1071-1538)	799 (663-963)	-	-	821 (649-1038)	785 (623-989)	**0.011**	**0.021**	**0.033**

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (*). P eth: main effect for ethnicity, P gly: main effect for glycaemic state, P eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA. Insulin clearance data not available for IGT.
[a]n: NGT: WE=23, BWA=18; IGT: WE=9, BWA=10; T2D: WE=18, BWA=18; [b]n: NGT: WE=21, BWA=18; IGT: WE=9, BWA=10; T2D: WE=13, BWA=15; [c]n: NGT: WE=22, BWA=18; IGT: WE=7, BWA=9; T2D: WE=11, BWA=15; [d]n: NGT: WE=21, BWA=19

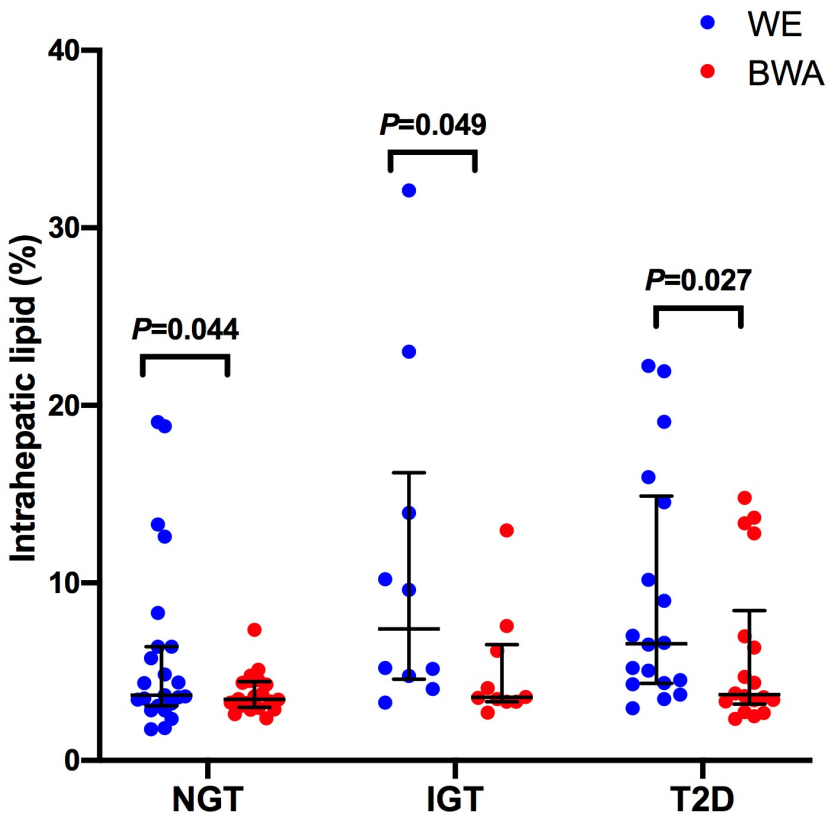

Figure 4.1: *Intrahepatic lipids in the WE and BWA men of NGT, IGT and T2D groups. Boxplots show median with interquartile range.*

4.4.3 Metabolic characteristics

Measures of insulin sensitivity and insulin clearance are presented in Table 4.1. There were no ethnic differences in whole-body, hepatic and adipose tissue insulin sensitivity between the WE and BWA men. All insulin sensitivity measures differed by glycaemic state and showed a deterioration between the NGT to IGT to T2D states. Insulin clearance data were available for the NGT and T2D groups, which showed significantly lower insulin clearance in the BWA men, indicated by a significant main effect for ethnicity. Furthermore, there was a significant ethnicity*glycaemic state interaction for insulin clearance. Post-hoc analysis showed this was driven by lower insulin clearance in the BWA men of NGT since insulin clearance did not differ between the T2D men (p=0.78) but differed significantly between the NGT men (p<0.001).

4.4.4 Relationships between intrahepatic lipids and hepatic insulin sensitivity

Relationships between IHL and hepatic insulin sensitivity are presented in Table 4.2 and Figure 4.2 (panel A) for the WE and BWA men in all glycaemic cohorts combined. Previous studies have shown with increasing age and BMI, IHL also increases (Ulbrich et al., 2015) while insulin sensitivity decreases (Cnop et al., 2003); therefore, to reduce their influence, the above relationships were adjusted for both age and BMI, Table 4.2. IHL was significantly inversely associated with hepatic insulin sensitivity in both WE and BWA men; these relationships diminished after adjustment for age and BMI. Furthermore, these relationships did not differ by ethnicity indicated by a non-significant ethnicity interaction (P=0.21).

The assessment of relationships in the combined glycaemic cohorts benefits from greater sample sizes, however, glycaemic state may influence these relationships. Therefore, the above relationships were investigated in the NGT and T2D glycaemic states, which are presented in Table 4.3 and Figure 4.2 (panel B). In the NGT groups, IHL was significantly inversely associated with hepatic insulin sensitivity in WE men but not BWA men. No significant relationships were present in the T2D groups, which may be due to the considerably lower sample sizes.

Table 4.2: *Pearson's correlation and partial correlation coefficients showing relationships between IHL and hepatic insulin sensitivity in WE and BWA men of all glycaemic states.*

	WE (n=43)		**BWA (n=42)**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Hepatic insulin sensitivity (% suppression of EGP)	**-0.37**	**0.014**	**-0.38**	**0.013**
Adjusted for age	-0.23	0.15	-0.28	0.072
Adjusted for BMI	-0.07	0.67	-0.19	0.25
Adjusted for age and BMI	-0.04	0.82	-0.14	0.40

Table 4.3: *Pearson's correlation coefficients showing relationships between IHL and hepatic insulin sensitivity in WE and BWA men of NGT and T2D groups.*

	WE		**BWA**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Hepatic insulin sensitivity (% suppression of EGP)				
NGT	**-0.55**	**0.010**	-0.17	0.49
n	21		18	
T2D	-0.06	0.85	-0.46	0.10
n	13		14	

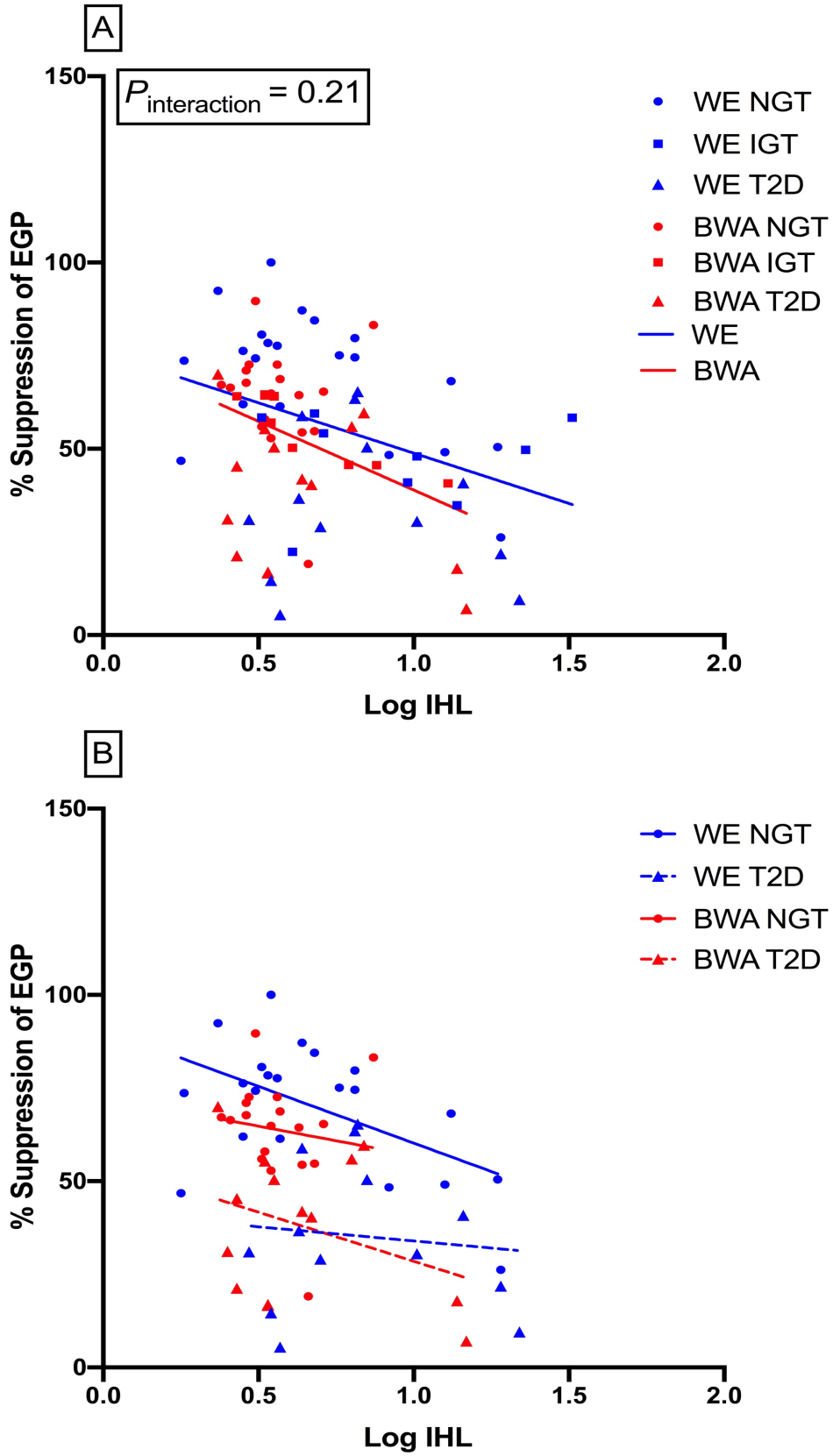

Figure 4.2: *Relationships between IHL and hepatic insulin sensitivity (% suppression of endogenous glucose production) in the WE and BWA men of all glycaemic states (NGT, IGT and T2D), A; and in the NGT and T2D glycaemic states, B.*

4.4.5 Relationships between intrahepatic lipids and whole-body and adipose tissue insulin sensitivity

Relationships between IHL and whole-body and adipose tissue insulin sensitivity are presented in Table 4.4 in all glycaemic cohorts combined. IHL was inversely associated with whole-body insulin sensitivity in both WE and BWA men; this relationship remained significant after adjustment for age and BMI in the WE men but completely diminished in the BWA men. IHL was inversely associated with adipose tissue insulin sensitivity in WE men but not BWA men. There were no significant ethnicity interactions for the relationships between IHL and whole-body insulin sensitivity (P=0.30) or adipose tissue insulin sensitivity (P=0.25).

To reduce the influence of possible confounders related to glycaemic state, the above relationships were investigated in the NGT and T2D groups, presented in Table 4.5. These relationships showed distinct ethnic differences where IHL was inversely associated with whole-body insulin sensitivity in the WE men but not BWA men of NGT and T2D states. Furthermore, IHL was inversely associated with adipose tissue insulin sensitivity in the WE men but not BWA men of NGT; however, no significant relationships were present in the T2D men which may be due to the low sample sizes. Taken together, the correlations between IHL and measures of insulin sensitivity in NGT and T2D groups show predominantly stronger relationships in the WE men than BWA men.

Table 4.4: *Pearson's correlation and partial correlation coefficients showing relationships between IHL and whole-body and adipose tissue insulin sensitivity in WE and BWA men of all glycaemic states.*

	WE		**BWA**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity (M-value)	**-0.70**	**<0.001**	**-0.43**	**0.004**
n	50		44	
Adjusted for age	**-0.65**	**<0.001**	**-0.35**	**0.023**
Adjusted for BMI	**-0.43**	**0.002**	-0.19	0.23
Adjusted for age and BMI	**-0.45**	**0.002**	-0.15	0.36
Adipose tissue insulin sensitivity (% suppression of lipolysis)	**-0.49**	**0.001**	-0.07	0.65
n	40		41	
Adjusted for age	**-0.38**	**0.020**	-0.08	0.64
Adjusted for BMI	-0.09	0.56	-0.07	0.69
Adjusted for age and BMI	-0.07	0.66	-0.07	0.68

Table 4.5: *Pearson's correlation coefficients showing relationships between IHL and whole-body and adipose tissue insulin sensitivity in WE and BWA men of NGT and T2D groups.*

	WE		**BWA**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity (M-value)				
NGT	**-0.75**	**<0.001**	-0.20	0.42
n	23		18	
T2D	**-0.62**	**0.006**	-0.34	0.20
n	18		16	
Adipose tissue insulin sensitivity (% suppression of lipolysis)				
NGT	**-0.56**	**0.007**	-0.04	0.89
n	22		18	
T2D	-0.27	0.42	-0.16	0.58
n	11		14	

4.4.6 Relationships between intrahepatic lipids and insulin clearance

Relationships between IHL insulin clearance are presented in Table 4.4 and Figure 4.3 (panel A) for the WE and BWA men in all glycaemic cohorts combined. As previously mentioned, IHL increases with increasing age and BMI, while insulin clearance decreases (Ehrhardt et al., 2019); therefore, to reduce their influence, the above relationships were adjusted for both age and BMI, Table 4.6. IHL was inversely associated with insulin clearance in the WE men but not the BWA men; however, this relationship diminished after adjustment for age and BMI. There was no significant ethnicity interaction present for this relationship (P=0.55).

Relationships between IHL and insulin clearance are presented in the WE and BWA men within the NGT and T2D glycaemic states in Table 4.7 and Figure 4.3 (panel B). In the NGT groups, IHL was inversely associated with insulin clearance in the WE men but not BWA men. However, no relationships were present in the T2D groups which may be related to physiological processes in T2D that may overpower these relationships.

Table 4.6: *Pearson's correlation and partial correlation coefficients showing relationships between IHL and insulin clearance in WE and BWA men of all glycaemic states.*

	WE (n=39)		**BWA (n=37)**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Insulin clearance	**-0.43**	**0.006**	-0.26	0.12
Adjusted for age	**-0.37**	**0.021**	**-0.35**	**0.039**
Adjusted for BMI	-0.18	0.28	-0.24	0.15
Adjusted for age and BMI	-0.24	0.16	-0.30	0.080

Table 4.7: *Pearson's correlation coefficients showing relationships between IHL and insulin clearance in WE and BWA men of NGT and T2D groups.*

	WE		**BWA**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
Insulin clearance				
NGT	**-0.78**	**<0.001**	-0.12	0.62
n	21		19	
T2D	-0.14	0.58	-0.42	0.085
n	18		18	

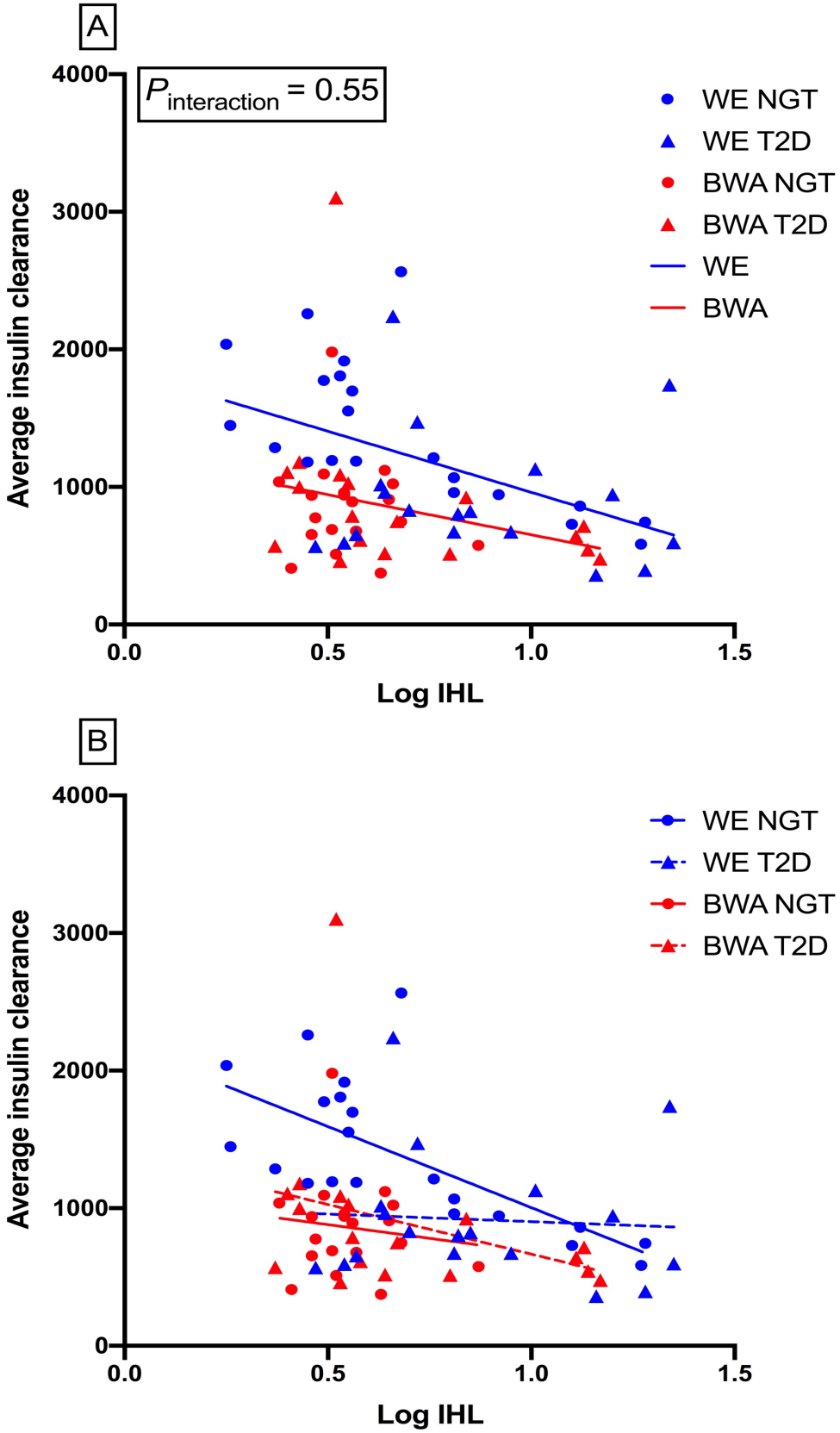

Figure 4.3: *Relationships between IHL and insulin clearance in the WE and BWA men of the combined NGT and T2D glycaemic states, A; and separately in the NGT and T2D glycaemic states, B.*

4.4.7 Relationships between intrahepatic lipids and measures of regional adiposity

To investigate ethnic differences in the measures of regional adiposity that may influence IHL deposition, relationships between IHL and measures regional adiposity are presented in Table 4.6 for the WE and BWA men in all glycaemic cohorts combined. IHL was associated with VAT and ASAT in both WE and BWA men. Further analysis of these relationships within each glycaemic state are shown in Table 4.7 which showed clear ethnic distinctions. In the NGT groups, IHL was associated with VAT and ASAT in WE men but not BWA men. However, in the T2D groups, IHL was associated with all measures of adiposity in both ethnic groups except for VAT in the WE men and ASAT in the BWA men.

Table 4.8: *Pearson's correlation coefficients showing relationships between IHL and measures of regional adiposity in WE and BWA men of all glycaemic states.*

	WE (n=50)		**BWA (n=48)**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
VAT	**0.48**	**<0.001**	**0.65**	**<0.001**
ASAT	**0.50**	**<0.001**	**0.33**	**0.022**

Table 4.9: *Pearson's correlation coefficients showing relationships between IHL and measures of whole-body and regional adiposity in WE and BWA men of NGT and T2D groups.*

	WE		**BWA**	
Intrahepatic lipids	**r**	***p***	**r**	***p***
NGT				
n	23		20	
VAT	**0.67**	**<0.001**	0.22	0.36
ASAT	**0.55**	**0.006**	0.16	0.51
T2D				
n	17		18	
VAT	0.30	0.24	**0.69**	**0.002**
ASAT	**0.56**	**0.021**	0.33	0.18

4.5 Discussion

The purpose of this chapter was to investigate ethnic differences in intrahepatic lipid accumulation between WE and BWA men across three glycaemic states: NGT, IGT and T2D. This chapter also explored ethnic differences in relationships between IHL and hepatic insulin sensitivity and insulin clearance as well as other parameters related to T2D. Ethnic comparisons were conducted between WE and BWA men of all glycaemic states combined as well as within each of the glycaemic groups. The present analysis has demonstrated that, despite the lower IHL in BWA men at all glycaemic states, it shows strong relationships with hepatic and whole-body insulin sensitivity in BWA men and WE men. However, IHL was significantly inversely related to adipose tissue insulin sensitivity in WE men but not BWA men.

The lower IHL in BWA men is consistent with previous findings from studies comparing black and white populations (Alderete et al., 2014, Goedecke et al., 2017, Guerrero et al., 2009). Additionally, the lower prevalence rates of NAFLD in the BWA men shown here are similar to those previously reported from studies comparing the prevalence of NAFLD in different ethnic groups (Browning et al., 2004). The reason for lower IHL in black populations may be explained by lower release of circulating NEFAs and triglycerides released by the SAT depot which may deposit in the liver. Indeed, White *et al.* reported that black women exhibited significantly lower rates of triglyceride synthesis and *de novo* lipogenesis of the SAT depot compared to white women (White et al., 2018). The liver is a principle site of lipid metabolism in the body and an increase of NEFAs, from dietary and non-dietary sources, promote hepatic lipid production pathways such as *de novo* lipogenesis and the production of VLDL particles (Berlanga et al., 2014). Since IHL is also related to excess hepatic lipid production (Stefan et al., 2008a), the lower IHL in BWA men may explain the commonly reported favourable lipid profiles in black

populations (Goff et al., 2013, Winkley et al., 2013). Indeed, in this study, the BWA men exhibited lower triglyceride levels than WE men. This notion has been suggested by other studies which have demonstrated direct relations between IHL and plasma lipoprotein levels in black and white adolescents (D'Adamo et al., 2010).

4.5.1 Intrahepatic lipids and hepatic insulin sensitivity

Since IHL is lower in BWA men than WE men, it is expected that they would exhibit greater hepatic insulin sensitivity as it has been extensively reported that IHL is associated with diminished hepatic insulin sensitivity (Kelley et al., 2003, Petersen et al., 2005, Seppala-Lindroos et al., 2002, Bajaj et al., 2004, Hwang et al., 2007, Gastaldelli et al., 2007). However, there were no ethnic differences in hepatic insulin sensitivity indicating IHL may not be as important in reducing insulin sensitivity in BWA men as WE men or that IHL reduces insulin sensitivity at lower levels in BWA men. To further explore these speculations, relationships between IHL and hepatic insulin sensitivity were investigated. This investigation showed IHL was inversely related to hepatic insulin sensitivity (% suppression of EGP) in both ethnic groups. Hence, it is evident that with an increase of IHL there is a similar decrease in hepatic insulin sensitivity in WE and BWA men indicating that BWA men appear to experience the detrimental effects of IHL at lower levels than WE men.

The advantage of the analysis of relationships in the combined glycaemic states is the greater power; however, the pathophysiology of T2D is better understood during an individual glycaemic state since many metabolic parameters, such as insulin sensitivity, do not follow a linear pattern in the progression from NGT to T2D (Ramlo-Halsted and Edelman, 2000). Furthermore, the understanding of the role of ectopic fat in the pathophysiology of T2D has been expanded by studies conducted individual glycaemic groups (Lim et al., 2011, Steven et al., 2016, Szczepaniak et al., 2012, Tushuizen et al.,

2007, van der Zijl et al., 2011), therefore, the above relationships were investigated in the NGT and T2D groups separately. Even though this analysis included considerably lower sample sizes, it may be more informative in untangling the role of IHL in the pathophysiology of T2D, particularly in the NGT state.

The analysis of relationships between IHL and hepatic insulin sensitivity by ethnicity in the NGT and T2D glycaemic states showed some ethnic distinctions. In the NGT state, IHL was significantly inversely related to hepatic insulin sensitivity in WE men but this relationship was not significant in the BWA men, while, in the T2D state, IHL showed no associations in either ethnic group. Therefore, due to the stronger inverse relationships between IHL and hepatic insulin sensitivity in WE men in the NGT state, it appears that IHL may play a lesser role in reducing hepatic insulin sensitivity in BWA men than WE men. Only one other study investigated relationships between IHL and hepatic insulin sensitivity, measured using the hyerinsulinaemic-euglycaemic clamp with the infusion of glucose isotopes, in a black *vs* white group (Goedecke et al., 2015). They found IHL was inversely associated with hepatic insulin sensitivity in NGT black but not white women with obesity. While, in a study that explored IHL and basal hepatic insulin sensitivity index (HISI), Chung *et al.* showed IHL was inversely related to basal HISI in black and white women (Chung et al., 2018). Both of these studies were conducted in NGT women with obesity but showed contrary findings to those in the NGT men in the analysis presented here. These disparities may be explained by gender differences as there is consistent evidence showing that the phenotype of T2D differs by gender within populations of African descent with T2D being more strongly driven by excess adiposity in black women compared to black men (Carnethon et al., 2002, Harris et al., 1998a).

4.5.2 Intrahepatic lipids and whole-body insulin sensitivity

Previous studies have shown that IHL is a stronger predictor of whole-body insulin sensitivity than other measures of adiposity including VAT, making a case for IHL being the most important ectopic fat depot in T2D risk (Kirchhoff et al., 2007, Hong et al., 2016, D'Adamo et al., 2010). Extensive studies have been conducted in both animal and human models to elucidate the mechanisms of IHL induced whole-body insulin resistance (Trouwborst et al., 2018). Current investigations show excess IHL deposition leads to accumulation of lipid intermediates that cause hepatic mitochondrial dysfunction, inflammation and increased VLDL-TG production, which may drive systemic insulin resistance (Trouwborst et al., 2018). In the present study, IHL was inversely related to whole-body insulin sensitivity in both ethnic groups, however, this relationship remained significant in only the WE men after adjustment for age and BMI. This indicates that individual susceptibility to IHL accumulation may influence whole-body insulin sensitivity independently of age and degree of whole-body adiposity in WE men but not BWA men. The ethnic disparities in these relationships extend to the individual glycaemic states where IHL was inversely related to whole-body insulin sensitivity in the WE men but not BWA men. This finding in WE men supports previous studies, which highlight the pivotal role of IHL in the pathophysiology of T2D, however, in BWA men the influence of IHL is less apparent than in WE men.

4.5.3 Intrahepatic lipids and adipose tissue insulin sensitivity

The deposition of IHL is thought to be explained by the *spillover theory* and the *portal theory* which have similarities in their features. Both these theories describe the excess release of NEFAs into the circulation, due to dysfunctional adipose tissue, that become deposited as IHL (Bosy-Westphal et al., 2019). Reduced adipose tissue insulin sensitivity is a major characteristic of dysfunctional adipose tissue, especially of VAT, which is

considered to be one of the drivers of IHL accumulation (Gastaldelli et al., 2017). Indeed, several reports have linked dysfunctional adipose tissue and adipose tissue insulin resistance to an increase of IHL accumulation (Lomonaco et al., 2012, Bell et al., 2012). Shown in this chapter is a significant inverse association between IHL and adipose tissue insulin sensitivity in the WE but not BWA men, which was also present in the NGT glycaemic state. This indicates that IHL deposition may be more strongly influenced by adipose tissue insulin resistance in WE men than BWA men. However, this relationship diminished after adjustment for BMI which may indicate that IHL accumulation resulting from adipose tissue insulin resistance is dependent upon adipose tissue expansion. Hence, considering IHL to be a major contributor to T2D, this finding supports the well-known link between increasing whole-body adiposity and risk of T2D. Furthermore, this notion may hold true more so in white men compared to black men who have a greater prevalence of T2D in the healthy BMI category compared to white populations (Zhang et al., 2009).

It is particularly noticeable that despite the reduction in sample size for the relationships presented in the individual glycaemic states, relationships between IHL and all measures of insulin sensitivity were stronger in the WE men of NGT but were weaker in the BWA men. This interesting phenomenon may be explained by lower sample size in the BWA men but may further suggest that IHL is less detrimental in the development of T2D in BWA men than WE men. Additionally, genetic predisposition to T2D may play a more important role in BWA men because the NGT men had a negative family history of T2D; future studies to elucidate these speculations are warranted.

4.5.4 Intrahepatic lipids and insulin clearance

Considering the role of IHL in insulin clearance, correlation analysis showed IHL was inversely associated with insulin clearance in the WE men but not BWA men; a similar

pattern was also evident in the NGT glycaemic state. The findings in the WE men support previous findings that suggest IHL may impact insulin clearance, however, in BWA men, IHL appears to have less influence on insulin clearance. Supporting the ethnic distinctions presented here, Chung *et al.* showed that IHL was inversely associated with hepatic insulin clearance in white but not black women (Chung et al., 2019). However, the data in this chapter along with those by Chung *et al.* are the only two studies that have investigated relationships between IHL and insulin clearance in a black population. Hence, since reduced insulin clearance has been suggested to be a defect that may play a crucial role in the development of T2D in black populations (Bergman et al., 2019), further studies are required to clarify whether the reduced insulin clearance is a result of hepatic lipotoxicity in black populations (Piccinini et al., 2018, Bergman et al., 2019).

4.5.5 Intrahepatic lipids and regional adiposity

To understand the possible drivers of IHL accumulation, relationships between IHL and regional measures of adipose tissue were investigated. This analysis showed that both abdominal VAT and ASAT were strongly related to IHL accumulation in both ethnic groups. Similar findings have previously been reported in black and white adolescents with obesity (Lee et al., 2017). IHL accumulation appears to be directly influenced by VAT and SAT accumulation, which is consistent with previous studies that show strong relationships between IHL and whole-body and abdominal adiposity (Rossi et al., 2011, Thomas et al., 2005, Eguchi et al., 2006). When investigated in each glycaemic state, despite the reduction in samples size, these relationships showed clear distinctions by ethnicity. In the T2D groups, IHL was related to VAT and ASAT in both ethnic groups; however, in the NGT groups these relationships were present in only the WE men. It appears that in the NGT state, BWA men deposit IHL independently of VAT and SAT

deposition. Furthermore, the portal theory, which places VAT as the major determinant of IHL accumulation, may not to apply to NGT BWA men.

4.5.6 Strengths and limitations

This chapter has several strengths worth noting including the direct quantification of IHL using MRI. Another strength is the measurement of whole-body, hepatic and adipose tissue insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp with the infusion of stable glucose and glycerol isotopes, which is considered to be the gold-standard method for determining these measures.

A limitation of the presented measure of insulin clearance is that it represents whole-body insulin clearance and is not specific to the liver, however, approximately 80% of insulin is cleared by the liver (Ferrannini et al., 1983). Another limitation is the slightly greater statin use in the WE men with T2D than BWA men which may have impacted the metabolic functions of the liver more so in WE men as statins have been shown to reduce IHL and limit their lipotoxic effects (Schierwagen et al., 2017, Sigler et al., 2018). The lack of data on alcohol consumption in the participants is also a limitation as alcohol is known to affect IHL accumulation and disturb several functions of the liver. However, tests were conducted to exclude participants with liver damage as determined by alanine aminotransferase (ALT) levels 2.5-fold above the upper limit of the reference range.

4.6 Conclusion

This chapter shows that IHL is lower in BWA men at all glycaemic states with a lower presence of NAFLD in BWA men compared to WE men. While IHL was inversely related to hepatic and whole-body insulin sensitivity in both WE and BWA men, these relationships were consistently stronger in the WE men. Furthermore, the inverse relationship between IHL and insulin clearance was significant in only the WE men. Also, IHL was significantly inversely related to adipose tissue insulin sensitivity in WE men but not BWA men. These ethnic discrepancies may be explained by the lower range of IHL in the BWA men, however, they may suggest that IHL plays a lesser role in the development of T2D in BWA men compared to WE men. To understand the role of IHL in T2D in black populations, future studies ought to compare the effects of IHL lowering therapies on T2D between black and white populations.

Chapter 5: Ethnic differences in intrapancreatic lipid and its relationship with beta-cell function in men of black and white ethnicity

Data presented in this chapter have been published in (appendix I and appendix III):

Hakim O, Bello O, Ladwa M, Christodoulou D, Bulut E, Shuaib H, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity. *Diabetes Research and Clinical Practice.* 2019 Sep 19:107866.

Hakim O, Bonadonna RC, Mohandas C, Billoo Z, Sunderland A, Boselli L, Alberti KG, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. Associations between pancreatic lipids and β-cell function in Black African and White European men with type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism.* 2018 Nov 7;104(4):1201-10.

5.1 Introduction

Beta-cell dysfunction is an integral component in the development of T2D. The prolonged exertion of the beta-cells to secrete sufficient insulin to overcome insulin resistance leads to exhaustion of beta-cells and impairment of their insulin secretory function (ISF) (Defronzo, 2009). Pancreatic abnormalities are evident in patients with T2D which include a reduction in pancreatic volume (Macauley et al., 2015, Garcia et al., 2017, DeSouza et al., 2018), irregular pancreatic borders (Macauley et al., 2015), substantial loss of beta-cell mass (Guillausseau et al., 2008) and a reduction in ISF (Kahn, 1998). The abnormalities typically observed in the structure and function of the pancreas during the development of T2D have been related to glucotoxicity, lipotoxicity and oxidative stress, mediated by excess fat in the pancreas; however, the exact mechanisms that link them are poorly understood (Poitout et al., 2010). While the link between VAT and IHL with T2D have been well documented, the role of the accumulation of triglycerides in the pancreas, termed intrapancreatic lipids (IPL), in the development of T2D has only recently gained increasing attention.

Compared to healthy controls, patients with T2D have been shown to have greater accumulation of IPL (Tushuizen et al., 2007, van der Zijl et al., 2011, Ou et al., 2013, Begovatz et al., 2015). Following animal studies in the 1990s that showed IPL was related to beta-cell dysfunction (Lee et al., 1994), there were several suggestions that IPL is related to the development of T2D and pancreatic lipotoxicy by IPL may cause or accelerate the deterioration of beta-cell ISF (Yu and Wang, 2017). In the early 2000s, the advancement of magnetic resonance technologies made the non-invasive assessment of IPL possible in humans; since then, several studies have suggested that IPL may be related to beta-cell dysfunction in the development of T2D (Lingvay et al., 2009). More recently, studies have shown that a reduction of IPL, achieved through weight loss, is

related to improved beta-cell ISF, even in patients with T2D (Lim et al., 2011, Taylor et al., 2019). IPL is believed to cause beta-cell damage by releasing lipid intermediates and NEFAs, which interfere with cellular signalling processes and cause beta-cell apoptosis (Sharma and Alonso, 2014). Therefore, there is much evidence that suggests that IPL accumulation is an important fat depot in the progression of T2D that deserves further attention.

To elucidate ethnic differences in the pathophysiology of T2D between black and white populations, several studies have considered measures of beta-cell ISF. Black populations consistently present with greater insulin levels compared to their white counterparts (Osei and Schuster, 1994, Hannon et al., 2008). Furthermore, in a meta-analysis, Kodama *et al.* showed black populations had greater beta-cell ISF, determined by the acute insulin response, compared to white populations (Kodama et al., 2013). Interestingly, this exaggerated insulin secretion in response to a glucose challenge in black populations is also present when the black and white groups were matched for insulin sensitivity (Kodama et al., 2013, Bacha et al., 2012). However, the possible reasons for the commonly reported ethnic differences in beta-cell ISF between black and white populations are poorly understood.

Unlike VAT and IHL, studies of IPL in black *versus* white populations are more limited and report inconsistent conclusions, while some studies report lower levels of IPL in black populations (Szczepaniak et al., 2012), others report no difference compared to their white counterparts (Trout et al., 2019). While in a study by Lingvay *et al.,* there were no ethnic differences in IPL between NGT women within the healthy or obese BMI groups, but there was lower IPL in black women with T2D compared to their white/Hispanic counterparts (Lingvay I, 2014). With VAT and IHL being consistently lower in black populations some reports have suggested that, considering the more rapid deterioration

of beta-cell ISF in black population, IPL may be a more detrimental fat depot in black populations compared to white populations. Supporting this notion is a study by Toledo-Corral *et al.* who showed that IPL predicted prediabetes status in black adolescents but IHL did not (Toledo-Corral et al., 2013). Meanwhile, in adults, IPL was more strongly related to ISF in black adults compared to their white counterparts (Szczepaniak et al., 2012, Lingvay I, 2014). However, with limited studies conducted on IPL in black populations, it is poorly understood whether ethnic differences exist in the role of IPL in beta-cell ISF.

Investigations of regional deposition of IPL have suggested that the distribution of IPL is not uniform within the pancreas and may differ between the head, body and tail regions of the pancreas. Some studies report greater IPL in the head of the pancreas (Livingstone et al., 2014, Chai et al., 2016) while others have shown IPL to be greater in the tail region (Idilman et al., 2015, Patel et al., 2013, Pezzilli and Calculli, 2014). Regional IPL distribution may be of importance in understanding beta-cell lipotoxicity in the development of T2D. Human studies have shown that there is approximately 2-fold greater density of beta-cells in the tail of the pancreas compared to the head and body (Wang et al., 2013). Furthermore, during the progression of T2D, there is greater loss of beta-cells from the head of the pancreas compared to the body and tail (Wang et al., 2013). However, it is unknown if IPL impacts beta-cell loss or function differently in the various regions of the pancreas. Therefore, regional distribution of IPL is an area that warrants investigation in order to increase the understanding of the mechanisms of beta-cell lipotoxicity.

The main theory that describes the mechanism of IPL accumulation is the twin-cycle theory which proposes that, during a state of excess energy consumption, excess glucose and lipids are metabolised in the liver resulting in an increased secretion of VLDL-TG

from the liver into the circulation. The excess VLDL-TG in the circulation then deposit in the pancreas as IPL, which accelerates beta-cell dysfunction via lipotoxicity, which in turn further increases blood glucose levels and drives further production of VLDL-TG in the liver (Taylor, 2013). Black populations have been shown to exhibit favourable lipid profiles (Gaillard and Osei, 2016, D'Adamo et al., 2010) as well as lower IHL levels (Guerrero et al., 2009, Alderete et al., 2014) compared to white populations indicating possible lower output of lipids by the liver; however, the impact of this on IPL accumulation has not previously been considered in black populations. Thus, the possible mechanisms of IPL accumulation will be addressed in the present chapter.

Due to lower levels of VAT and IHL in black populations compared to white populations, it is reasonable to hypothesise that IPL will be lower too. To date, there are only two studies that have investigated relationships between IPL and beta-cell ISF in a black population (Lingvay I, 2014, Szczepaniak et al., 2012). In these studies, ISF was assessed using the intravenous glucose tolerance test to measure the acute insulin response to glucose (AIRg) (Lingvay I, 2014, Szczepaniak et al., 2012). However, the AIRg is an indirect measure of ISF because it does not typically use c-peptide and therefore cannot account for insulin clearance, which is known to differ by ethnicity (Piccinini et al., 2017). Therefore, there is a lack of studies investigating the role of IPL on ISF in black populations particularly using comprehensive methods to assess beta-cell ISF. This chapter will present relationships between IPL and measures of ISF, determined using the hyperglycaemic clamp test, which has not previously been conducted in a black *versus* white population.

5.2 Aim

The primary aim of this chapter is to assess and compare ethnic differences in intrapancreatic lipids (IPL) between WE and BWA men of NGT, IGT and T2D glycaemic states. The secondary aim is to investigate ethnic differences in the relationships between IPL and beta-cell insulin secretory function.

5.3 Methods

5.3.1 Data acquirement

The data presented in this chapter were acquired from the methods described in chapter 2 (sections 2.3.1, 2.3.1.3, 2.3.3, 2.3.3.1). In brief, IPL was determined by the analysis of abdominal MRI images, which have the greatest area of intrapancreatic tissue, for each participant. A single circular region of interest was positioned on each of the head, body and tail of the pancreas to determine % IPL in each region and a mean IPL, using all three regions, was calculated. Measures of insulin secretory function (ISF) were determined using an intravenous glucose challenge (hyperglycaemic clamp) method. The measures of beta-cell ISF derived from this assessment include insulin secretion rate as well as modelled first- and second-phase ISF.

5.3.2 Statistical analysis

IPL and beta-cell ISF data presented in this chapter for the WE and BWA men of NGT, IGT and T2D groups were compared using 2-way between-groups ANOVA. Ethnicity and glycaemic state were included in the 2-way between-groups ANOVA as independent variables to assess the main effect of each of the independent variables, as well as their interaction (ethnicity*glycaemic state), on the outcome measures (dependent variables). Assessment of ethnic differences in measures of IPL within each glycaemic state were conducted using independent samples t-test. ISF data were available for only the NGT and T2D glycaemic states and were compared between the WE and BWA men using a 2-way between-groups ANOVA as described above. Strength of relationships between variables of interest were tested using Pearson's correlation test; partial correlation was used when assessing relationships while adjusting for confounding variables. Ethnic differences in the strength of the relationships were examined by fitting a regression with an interaction term for ethnicity and IPL as an independent variable.

5.4 Results

5.4.1 Participant characteristics

The clinical characteristics of the WE and BWA men of NGT, IGT and T2D are presented in chapter 3, Table 3.2. The two ethnic groups were well matched for age and BMI and showed no significant differences in clinical characteristics except for waist circumference, triglycerides and HbA1c, which were significantly lower in the BWA men.

5.4.2 Intrapancreatic lipids

IPL of the head (IPL_{HEAD}), body (IPL_{BODY}), tail (IPL_{TAIL}) and the mean of all three regions (IPL_{MEAN}) in the WE and BWA men of NGT, IGT and T2D glycaemic states are presented Table 5.1. All measures of IPL differed significantly by glycaemic state and were greater in the IGT and T2D states compared to the NGT state. The BWA men exhibited significantly lower IPL_{MEAN} compared to the WE men which was due to lower IPL_{HEAD} (P=0.007) and IPL_{BODY} (P=0.080) in the BWA men. IPL_{HEAD} showed a near significant ethnicity by glycaemic state interaction, indicating that the differences in IPL_{HEAD} between the glycaemic states differed by ethnicity.

Analysis of IPL_{MEAN} within each glycaemic state showed it was significantly lower in the BWA men in the IGT and T2D glycaemic states but did not differ between the NGT men, as presented in Figure 5.1, which shows the individual values of IPL_{MEAN} within each ethnic and glycaemic group. Regional measures of IPL are presented for the NGT, IGT and T2D glycaemic states in Figures 5.2, 5.3 and 5.4, respectively. Specific patterns of regional IPL deposition were evident in the individual glycaemic states. In the NGT state there were no ethnic differences in IPL deposition of the head, body and tail; however, in both IGT and T2D states, IPL_{HEAD} was significantly lower in the BWA men with no ethnic differences in IPL_{BODY} and IPL_{TAIL}.

Table 5.1: *Measures of regional IPL and mean IPL in the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		**IGT**		**T2D**				
	WE (n=23)	**BWA (n=20)**	**WE (n=10)**	**BWA (n=10)**	**WE (n=18)**	**BWA (n=19)**	***P* Eth**	***P* Gly**	***P* Eth*Gly**
IPL_{MEAN} (%)	6.60 (5.56-7.84)	6.52 (5.41-7.87)	10.36 (7.35-14.59)	7.26 (6.43-8.19)	9.79 (8.63-11.10)	7.88 (6.84-9.09)	**0.010**	**0.001**	0.16
IPL_{HEAD} (%)	5.46 (4.41-6.76)	5.61 (4.40-7.14)	9.16 (6.44-13.03)	5.86 (4.84-7.10)	9.21 (7.86-10.79)	6.58 (5.48-7.90)	**0.007**	**0.002**	0.069
IPL_{BODY} (%)	6.24 (5.11-7.62)	6.33 (5.24-7.65)	9.42 (7.29-12.18)	7.30 (6.35-8.38)	9.44 (8.01-11.13)	7.86 (6.61-9.35)	0.080	**0.001**	0.33
IPL_{TAIL} (%)	7.56 (6.31-9.04)	7.25 (5.91-8.89)	9.82 (7.20-13.40)	8.08 (6.20-10.51)	10.14 (8.64-11.90)	8.83 (7.52-10.37)	0.13	**0.017**	0.73

Data presented as geometric mean (95% CI) as all data was log transformed to achieve a normal distribution. P eth: main effect for ethnicity, P gly: main effect for glycaemic state, P eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA.

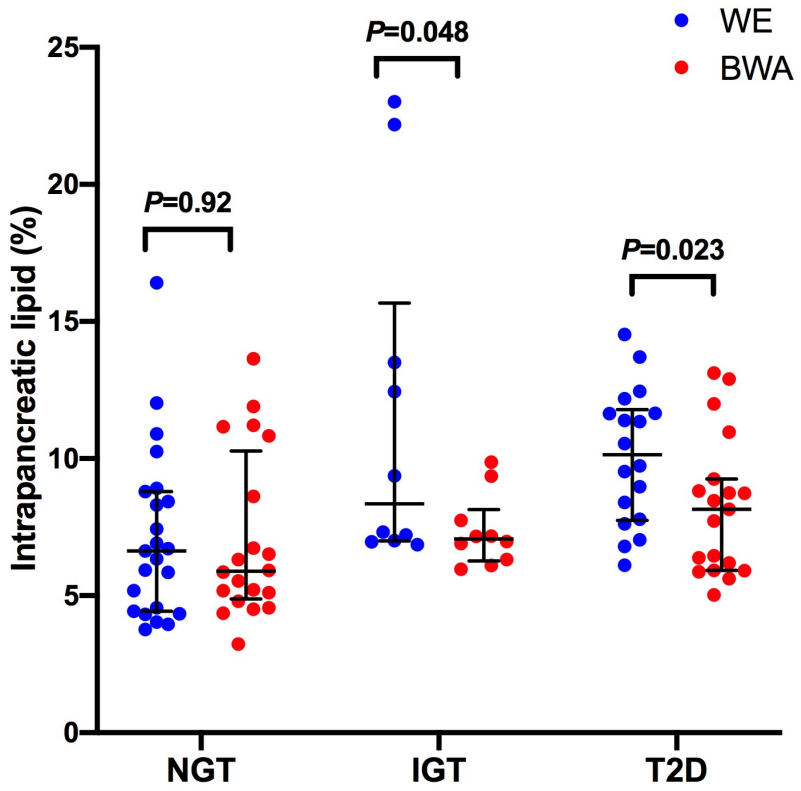

Figure 5.1: *Mean intrapancreatic lipid in the WE and BWA men of NGT, IGT and T2D groups. Boxplots show median (IQR).*

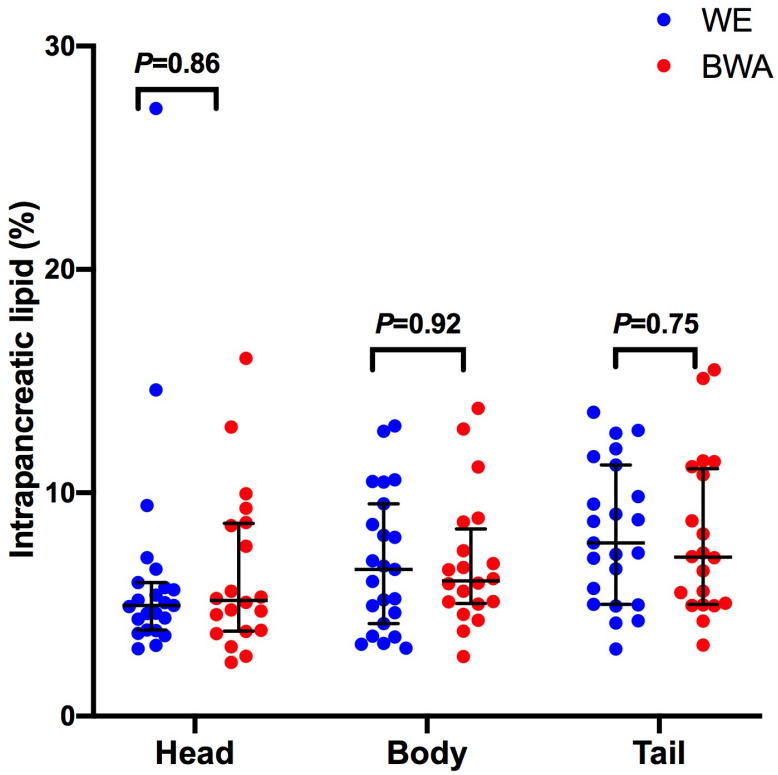

Figure 5.2: *Regional deposition of intrapancreatic lipid in the WE and BWA men of NGT glycaemic state. Boxplots show median (IQR).*

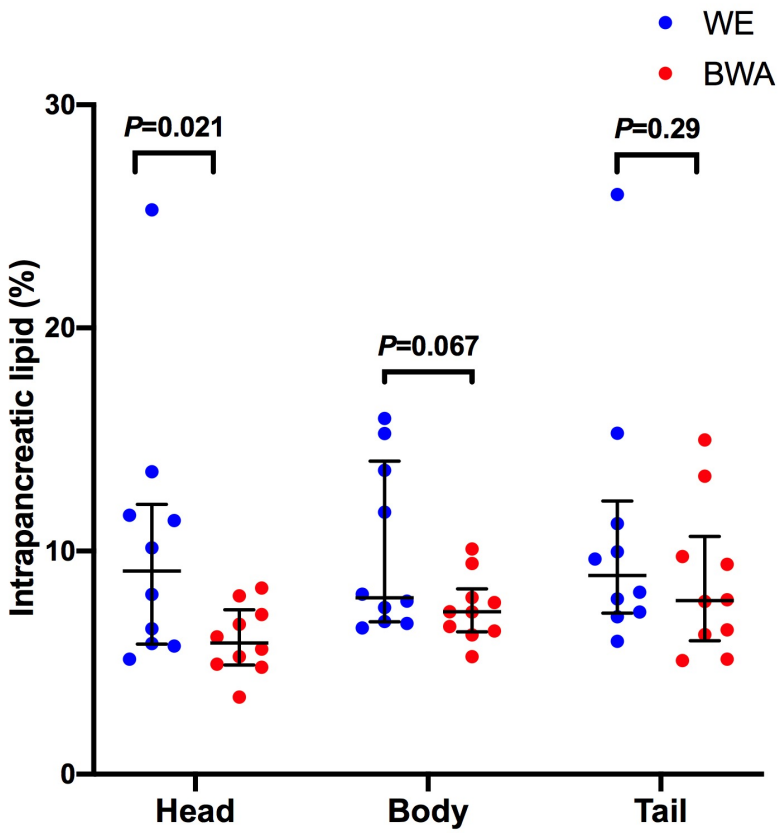

Figure 5.3: *Regional deposition of intrapancreatic lipid in the WE and BWA men of IGT glycaemic state. Boxplots show median (IQR).*

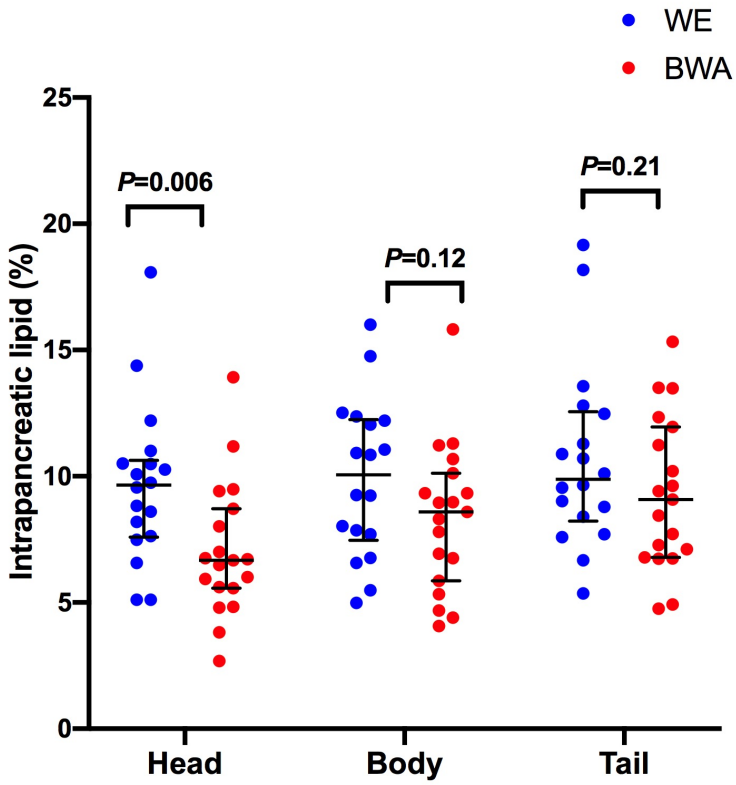

Figure 5.4: *Regional deposition of intrapancreatic lipid in the WE and BWA men with T2D. Boxplots show median (IQR).*

5.4.3 Measures of insulin secretory function

Metabolic measures of basal and stimulated ISF in the WE and BWA men of NGT and T2D glycaemic states are presented in Table 5.2. ISF data were not available for the IGT glycaemic state due to on-going assessment of these measures. All measures of ISF differed significantly by glycaemic state and, as expected, indicated poorer ISF in the T2D state. In the basal state, insulin did not differ by ethnicity however c-peptide was significantly lower in the BWA men indicting ethnic differences in basal insulin clearance.

C-peptide iAUC determined during the first-phase (0-10 minutes) period was similar in the WE and BWA men, however, c-peptide iAUC during the second-phase (10-120 minutes) was significantly lower in the BWA men. Post-hoc analysis showed this was mainly present in the men with T2D, suggesting poorer ISF in the BWA men with T2D compared to the WE men. However, when c-peptide was used along with glucose concentrations in the modelling of first- and second-phase ISF, no ethnic differences were present in both modelled measures of ISF. The insulin secretion rate, determined by c-peptide concentrations during the stimulated period, was also lower in the BWA men, which was driven by lower insulin secretion rate in the BWA men in the T2D glycaemic state. Taken together, while absolute insulin secretion was lower in the BWA men, there were no ethnic differences in glucose sensitivity of the beta-cells (modelled first- and second-phase ISF).

Table 5.2: *Measures insulin secretory function (ISF) determined during a 2-hour hyperglycaemic clamp test in the WE and BWA men of NGT and T2D glycaemic states.*

	NGT		T2D				
	WE (n=23)	**BWA (n=20)**	**WE (n=18)**	**BWA (n=19)**	***P* Eth**	***P* Gly**	***P* Eth*Gly**
Basal insulin (pmol/l)	39.4 (30.1-51.6)	44.9 (36.9-54.5)	100.1 (68.8-145.7)	71.2 (54.6-92.7)	0.43	**<0.001**	0.082
Basal c-peptide (nmol/l)	0.69 (0.54-0.83)	0.56 (0.48-0.64)	0.93 (0.75-1.11)	0.57 (0.48-0.66)	**<0.001**	**0.050**	0.073
c-peptide iAUC 0-10 mins (nmol/l min^{-1})[a]	7.17 (6.10-8.31)	8.48 (5.94-11.42)	0.90 (0.33-1.50)	0.55 (0.04-1.08)	0.64	**<0.001**	0.22
c-peptide iAUC 10-120 mins (nmol/l min^{-1})[a]	203 (176-235)	213 (170-267)	108 (86-136)	56 (39-79)	**0.009**	**<0.001**	**0.003**
First-phase ISF [(pmol/m^2 BSA)/(mmol/l min^{-1})][a]	551.2 (465.6-652.4)	629.6 (449.4-880.2)	49.6 (15.0-131.9)	51.7 (27.4-139.3)	0.53	**<0.001**	0.22
Second-phase ISF [(pmol min^{-1} m^2 BSA)/mmol/l)][a]	43.0 (35.1-52.8)	44.2 (34.8-56.3)	13.1 (8.02-21.5)	7.4 (4.5-12.1)	0.77	**<0.001**	0.87
Insulin secretion rate (120 minutes)† (pmol/L/m^2 BSA)[a]	51500 (43800-60500)	53100 (43100-65400)	33700 (28200-40100)	19700 (16400-23700)	**0.005**	**<0.001**	**0.002**

Data presented as geometric mean (95% CI) as all data was log transformed to achieve a normal distribution. P eth: main effect for ethnicity, P gly: main effect for glycaemic state, P eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA.
[a]n: NGT WE=22

5.4.4 Relationships between intrapancreatic lipids and measures of insulin secretory function

Relationships between mean IPL and measures of ISF are presented in Table 5.3 for the WE and BWA men of NGT and T2D glycaemic states combined. IPL_{MEAN} was inversely associated with first-phase ISF in WE men but this relationship was only trending towards significance in BWA men. IPL_{MEAN} was inversely associated with second-phase ISF in WE men but not BWA men. There were no relationships between IPL_{MEAN} and insulin secretion rate in both ethnic groups. There were no significant interactions by ethnicity for the relationships between IPL_{MEAN} and all measures of ISF (all $P_{interaction}>0.05$). All associations between IPL_{MEAN} and measures of ISF diminished after adjustment for age and BMI (all $P>0.05$). Investigating these relationships for the regional measures of IPL showed distinct ethnic patterns; in WE men, the relationships with first-phase and second-phase ISF were stronger in the head than the body and tail of the pancreas. In contrast, in the BWA men, the relationships between regional measures of IPL with first-phase ISF were stronger for the body and tail regions (data not shown).

Table 5.3: *Pearson's correlation coefficients showing relationships between mean IPL and measures of insulin secretory function in WE and BWA men of NGT and T2D glycaemic states combined.*

	WE (n=40)		**BWA (n=39)**	
IPL_{MEAN}	**r**	***p***	**r**	***p***
First-phase ISF	**-0.42**	**0.006**	-0.30	0.067
Second-phase ISF	**-0.34**	**0.031**	-0.15	0.37
Insulin secretion rate	-0.05	0.78	-0.16	0.35

Relationships between mean IPL and measures of ISF are presented for the WE and BWA men within the NGT and T2D glycaemic state in Table 5.4. While, there is considerably lower sample sizes in these analyses, a specific trend was evident: all significant relationships in the combined ethnic cohorts were not present in the individual glycaemic states. Furthermore, the only significant relationship present was that of IPL_{MEAN} and insulin secretion rate in NGT WE men but not BWA men.

Table 5.4: *Pearson's correlation coefficients showing relationships between mean IPL and measures of insulin secretory function in WE and BWA men of NGT and T2D glycaemic states.*

	WE		**BWA**	
IPL_{MEAN}	**r**	***p***	**r**	***p***
NGT				
n	22		20	
First-phase ISF	0.001	0.99	-0.12	0.61
Second-phase ISF	0.32	0.14	0.35	0.13
Insulin secretion rate	**0.46**	**0.033**	0.22	0.36
T2D				
n	18		19	
First-phase ISF	-0.13	0.61	-0.27	0.27
Second-phase ISF	-0.31	0.21	-0.19	0.43
Insulin secretion rate	0.19	0.44	-0.20	0.42

5.4.5 Relationships between intrapancreatic lipids and measures of regional adiposity

To investigate ethnic differences in the measures of adiposity that may influence IPL deposition, relationships between IPL_{MEAN} and measures of regional adiposity are presented in Table 5.5 for the WE and BWA men in all glycaemic cohorts combined. IPL_{MEAN} was associated with VAT, ASAT and IHL in both WE and BWA men.

Table 5.5: *Pearson's correlation coefficients showing relationships between mean IPL and measures of whole-body and regional adiposity in WE and BWA men of all glycaemic states.*

	WE (n=51)		**BWA (n=49)**	
IPL_{MEAN}	**r**	***p***	**r**	***p***
VAT[a]	**0.58**	**<0.001**	**0.43**	**0.002**
ASAT[a]	**0.58**	**<0.001**	**0.44**	**0.002**
Intrahepatic lipid[b]	**0.59**	**<0.001**	**0.38**	**0.008**

N: [a]WE=50; [b]BWA=48

Further analysis of the above relationships within the NGT and T2D glycaemic states are shown in Table 5.6 which shows clear distinctions by glycaemic state. In the NGT men, IPL_{MEAN} was associated with VAT and ASAT in the WE and BWA men. Whereas, IPL_{MEAN} was associated with IHL in the NGT WE men but not BWA men. In complete contrast, in the T2D state, there were no relationships between IPL_{MEAN} and all measures of regional adiposity except for IHL in the BWA men.

Table 5.6: *Pearson's correlation coefficients showing relationships between mean IPL and measures regional adiposity in WE and BWA men of NGT and T2D groups.*

	WE		BWA	
IPL_{MEAN}	**r**	***p***	**r**	***p***
NGT				
n	23		20	
VAT	**0.78**	**<0.001**	**0.65**	**0.002**
ASAT	**0.80**	**<0.001**	**0.59**	**0.007**
Intrahepatic lipid	**0.85**	**<0.001**	0.24	0.31
T2D				
n	18		19	
VAT[a]	-0.16	0.54	0.38	0.11
ASAT[a]	0.16	0.54	0.34	0.16
Intrahepatic lipid[b]	0.24	0.33	**0.53**	**0.025**

N: T2D: [a]WE=17; [b]BWA=18

5.4.6 Relationships between adipose tissue insulin sensitivity and intrapancreatic lipids

Relationships between adipose tissue insulin sensitivity and IPL_{MEAN} were investigated to elucidate the influence of adipose tissue dysfunction on IPL accumulation. In the combined glycaemic cohorts, adipose tissue insulin sensitivity was inversely associated with IPL_{MEAN} in the WE men (n=51, r=-0.50, *P*=0.001) but not BWA men (n=49, r=-0.14, *P*=0.37). Further analysis of these relationships within the NGT and T2D glycaemic states show similar patterns to those of the whole cohorts where adipose tissue insulin sensitivity was inversely associated with IPL_{MEAN} in NGT WE men (n=22, r=-0.50, *P*=0.001) but not BWA men (n=18, r=-0.14, *p*=0.57). No relationships were present in the T2D groups which may have been due to low sample sizes (n: WE=11 and BWA=15).

5.5 Discussion

The purpose of this chapter was to investigate ethnic differences in intrapancreatic lipid accumulation between WE and BWA men across three glycaemic states: NGT, IGT and T2D. The present chapter demonstrates distinct ethnic differences in IPL and its relationship with measures of ISF which differ by both ethnicity and glycaemic state. Generally, IPL was lower in BWA men and was more strongly related to measures of ISF in the WE men.

Intrapancreatic lipid was lower in BWA men in the IGT and T2D glycaemic states, however, there were no ethnic differences in IPL in the NGT state. The lack of ethnic differences in IPL in the NGT state may be due to the lower level of adiposity in comparison to the IGT and T2D groups. Generally, BWA men appear to be less susceptible to ectopic fat storage with increasing overall adiposity. This finding is supported by a study by Lingvay *et al.* who reported lower IPL in black women with obesity and T2D compared to their white/Hispanic counterparts; however, no ethnic difference was found in the lean NGT women (Lingvay I, 2014). In contrast to these findings, Szczepaniak *et al.* reported lower IPL in black adults compared to white adults who had mild obesity (Szczepaniak et al., 2012). However, Trout *et al.* showed no ethnic differences in IPL between black and white adults with obesity; although, the black group had a significantly greater BMI than the white group which may have influenced IPL deposition (Trout et al., 2019).

Previous chapters showed both VAT and IHL were lower in the BWA men of NGT but this was not the case for IPL. This suggests that the pancreas may be more susceptible to ectopic fat storage compared to the liver in black populations. Furthermore, this may indicate that beta-cell lipotoxicity might play a greater role in the pathophysiology of T2D in black populations compared to hepatic lipotoxicity. This notion is supported by the

findings of Toledo-Corral *et al.* who showed that IPL predicted prediabetes status in black adolescents, however, IHL did not (Toledo-Corral et al., 2013).

5.5.1 Intrapancreatic lipids and insulin secretory function

Previous studies have indicated that IPL contributes to the progressive beta-cell dysfunction that occurs during the pathophysiology of T2D. In the NGT state, IPL deposition and circulating triglycerides proposedly cause lipotoxicity and promote insulin secretion thereby exerting an additional stress to the beta-cells in addition to glucose (Rebelos et al., 2015, Sharma and Alonso, 2014, Hughan et al., 2013, Szczepaniak et al., 2012). While in T2D, IPL is related to reduced insulin secretion whereby lipotoxicity interferes with the cellular process that enhance insulin secretion (Gaborit et al., 2015, Giacca et al., 2011). Even though the BWA men had lower levels of IPL than WE men, there were no ethnic differences in beta-cell glucose sensitivity (modelled ISF) while absolute insulin secretion was lower in the BWA men. This suggests that there may be ethnic differences in the influence of IPL on beta-cell function between WE and BWA men, which may be clarified by examining relationships between IPL and measures of ISF.

Intrapancreatic lipid was significantly related to first-phase ISF in WE men, while, in BWA men, this relationship trended towards significance which may be explained by a lower range of IPL in the BWA men. During the progression from NGT to T2D, the loss of first-phase ISF is the critical step that ultimately determines the onset of T2D (Gerich, 2002), hence, most studies that investigate the influence of IPL on beta-cell function, focus on first-phase ISF. Considering this, the stronger relationship between IPL and first-phase ISF in the WE men suggests that IPL may impair first-phase ISF and, in turn, play a greater role in the development of T2D in WE men compared to BWA men. Furthermore, in the NGT state, IPL was related to insulin secretion rate in WE men but

not BWA men. The insulin secretion rate is determined by c-peptide concentrations during the glucose stimulated period and depict the amount of insulin secretion, irrespective of glucose concentrations. Previous studies have suggested that IPL enhances beta-cell insulin secretion even in the absence of glucose stimulation (Cen et al., 2016, Giacca et al., 2011, Steven et al., 2016, Gaborit et al., 2015). The lack of relationship between IPL and insulin secretion rate in the BWA men may indicate that IPL exposure may have a lesser influence on insulin secretion in BWA men compared to WE men.

Only two previous studies have investigated relationships between IPL and metabolic parameters in a black *versus* white population which showed stronger relationships in black populations compared to their white (Szczepaniak et al., 2012) and white/Hispanic (Lingvay I, 2014) counterparts. The differences between those findings and the data presented here may be explained by differences in the techniques used to determine IPL since both the above studies used MRS. While MRS was once considered to be a valid method to determine IPL, advancements in imaging methods have confirmed that MRI is superior to MRS due to the high risk of contamination with VAT when measuring IPL with MRS (Hu et al., 2010, Al-Mrabeh et al., 2017). Furthermore, in both the aforementioned studies, ISF was determined by measuring insulin concentrations, however, this study measured c-peptide levels which is a more accurate indicator of insulin secretion (Polonsky et al., 1984, Polonsky and Rubenstein, 1986).

5.5.2 Regional intrapancreatic lipids and insulin secretory function

Ethnic differences were present in the deposition of regional IPL where, in both the IGT and T2D states, the lower mean IPL was driven by lower IPL in the head of the pancreas. Considering the relationships between regional measures of IPL and the various measures of ISF, there were specific patterns evident in each ethnic group. In the WE men, stronger relationships were evident between measures of ISF and IPL of the head compared to

those with the body and tail regions. Increased IPL has been linked with increased beta-cell apoptosis (Oh et al., 2018, Sharma and Alonso, 2014). Additionally, during the progression from NGT to T2D, there is greater loss of beta-cells from the head of the pancreas compared to the body and tail regions (Wang et al., 2013). Considering this, the stronger relationships between IPL of the head and measures of ISF in WE may indicate that IPL may be more toxic to the beta-cells within the pancreas head and may contribute to this loss of beta-cell mass. In contrast, in the BWA men, relationships with measures of ISF appeared to be stronger with IPL of the body and tail regions compared to the head. This may suggest that beta-cells within the body and tail regions may be more susceptible to lipotoxicity in BWA men. However, this area of research is novel and future studies should be conducted to untangle these speculations on regional pancreatic lipotoxicity.

5.5.3 Intrapancreatic lipids and measures of regional adiposity

As an ectopic fat depot, IPL is thought to accumulate as a result of the expansion and dysfunction of adipose tissue depots, which leads to an overspill of NEFAs into the circulation, that subsequently deposit as ectopic fat in various organs including the pancreas (Sattar and Gill, 2014). Additionally, IPL is believed to be strongly linked to IHL accumulation as described in the previously-described *twin-cycle hypothesis* (Taylor, 2013). Considering these hypotheses, relationships between IPL and various measures of adiposity were assessed to investigate ethnic differences in the mechanisms of IPL deposition. IPL was related to VAT, ASAT and IHL in both ethnic groups. This is in agreement with the finding of Le *et al.* who showed that IPL was related to SAT, VAT and IHL in a cohort of black and Hispanic adolescents (Le et al., 2011). In individual glycaemic states, despite the lower sample size, IPL was related to VAT and ASAT in the WE and BWA men of NGT. However, IPL was related to IHL in the NGT WE men but not BWA men. In contrast, in the T2D state IPL and IHL were related in the BWA

men but not WE men. The lack of association between IPL and IHL in NGT BWA men may indicate that the *twin-cycle hypothesis* occurs to a lesser extent in them; additionally, VAT and SAT may be greater influencers of IPL deposition in BWA men. However, the positive association between IHL and IPL in the BWA men with T2D may indicate that a greater level of obesity may drive the twin-cycle hypothesis of IPL deposition in black populations.

5.5.4 Intrapancreatic lipids and adipose tissue insulin sensitivity

One of the main features of adipose tissue dysfunction is insulin resistance which is believed to drive ectopic fat accumulation, including IPL. The inverse relationships between adipose tissue insulin sensitivity and IPL in the WE men appears to support the current belief that dysfunctional adiposity and ectopic fat storage are closely connected (Bosy-Westphal et al., 2019, Kim et al., 2017, Smith and Kahn, 2016). However, this relationship was not present in the BWA men which indicates that adipose tissue insulin resistance may not be a principle determinant of IPL accumulation as it appears to be in WE men. This further supports the notion that the mechanisms of IPL accumulation appear to differ by ethnicity.

To current knowledge, this is the first investigation of relationships between IPL and adipose tissue insulin sensitivity in a black population. However, Le *et al.* have investigated relationships between IPL and pro-inflammatory markers which are another feature of adipose tissue dysfunction (Le et al., 2011). They showed that IPL was related to several pro-inflammatory markers in a mixed black and Hispanic cohort, which indicates that adipose tissue inflammation may influence IPL accumulation in black populations. While the Soul-Deep study lacks data on pro-inflammatory markers, they may be of interest in future studies.

5.5.5 Strengths and limitations

This chapter has several strengths worth noting including the assessment of IPL using MRI which is considered to be superior to MRS. Previous studies have confirmed that IPL analysis using MRS carries a high risk of signal contamination from VAT particularly in subjects with T2D who typically have smaller and deformed pancreata (Hu et al., 2010, Al-Mrabeh et al., 2017). Furthermore, during the analysis of IPL by MRI, small regions of interest of $1cm^2$ were used to minimise the risk of contamination from VAT in the IPL analysis. Additionally, to ensure the positioning of the regions of interest in the head, body and tail regions of the pancreas were accurate, the MRI analysis of IPL was conducted alongside a consultant radiologist (Dimitra Christodoulou) with expertise of MRI pancreas anatomy. Another strength is the assessment of regional IPL deposition in a black population which is a novel aspect of this analysis.

This study is not without its limitations which include the inclusion of intraorgan fat in the measure of IPL that is presented here. Unlike the liver, where triglyceride accumulation occurs only within hepatocytes, histological studies have shown that fat accumulation in the pancreas is inhomogeneous. To expand, pancreatic triglycerides are deposited in peripancreatic, intralobular and interlobular adipose tissue (i.e. adipocyte infiltration in the pancreas) as well as within pancreatic endocrine and exocrine cells including beta-cells (i.e. parenchymal tissue). The measure of IPL presented in this report includes both parenchymal and adipocyte infiltration of the pancreas and is not specific to triglycerides deposited within pancreatic cells. However, previous studies in rats have confirmed that fat infiltration in the whole pancreas paralleled the triglyceride accumulation within the islets which may suggest that IPL as determined by the presented method is likely to represent IPL of the islets (Lee et al., 2010).

5.6 Conclusion

The BWA men exhibited lower IPL levels in the IGT and T2D states compared to WE men with no differences in the NGT state. While IPL was inversely related to first-phase ISF in both WE and BWA men, this relationship was stronger in the WE men, which may be explained by a lower range in IPL in the BWA men. Furthermore, in the NGT state, IPL was related to insulin secretion rate in WE men but not BWA men. Therefore, the lower levels of IPL and weaker relationships with ISF in BWA men suggests that IPL may play a lesser role in the deterioration of beta-cell ISF during the progression of T2D in BWA men compared to WE men. Furthermore, the lack of relationship between IHL and IPL in NGT BWA men, a relationship which was present in the WE men, may indicate that the twin-cycle hypothesis may occur to a lesser extent in BWA men. Since IPL was inversely related to adipose tissue insulin sensitivity in WE men but not in BWA men, dysfunctional adipose tissue may be a lesser determinant of IPL deposition in BWA men compared to WE men. To further understand the role of IPL in T2D in black populations, future studies that explore the effects of IPL lowering therapies on T2D in black *vs* white populations are highly warranted.

Chapter 6: Ethnic differences in intramyocellular lipid and its relationship with skeletal muscle insulin sensitivity in men of black and white ethnicity

Data presented in this chapter have been published in (appendix I):

Hakim O, Bello O, Ladwa M, Christodoulou D, Bulut E, Shuaib H, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity. *Diabetes Research and Clinical Practice.* 2019 Sep 19:107866.

6.1 Introduction

Muscle insulin resistance is a crucial defect in individuals with T2D since muscles are a major site of glucose disposal (approximately 70-80% under hyperinsulinaemic-euglycaemic clamp conditions but only 30% in the postprandial state) (DeFronzo and Tripathy, 2009, Taylor et al., 1993). Hence, understanding the mechanisms the lead to reduced insulin sensitivity of myocytes is of paramount importance in T2D research. There are several determinants of muscle insulin resistance which include increasing age (DeFronzo, 1979), a family history of diabetes (Petersen et al., 2004a) and many diseases such as polycystic ovary syndrome (Lankarani et al., 2009), chronic kidney failure

(Bailey et al., 2006), heart failure (Swan et al., 1997) and lipodystrophy (Simha and Garg, 2006). However, many investigations that consider the cellular mechanisms of muscle insulin resistance in obesity-driven T2D have focused on the role of lipids (Abdul-Ghani and DeFronzo, 2010).

In the 1920s researchers demonstrated that both glucose and fatty acids are oxidised in muscle cells to yield energy during muscle contraction (Li et al., 2015). However, excess lipids stored within myocytes have been proposed to play a role in T2D from as early as the 1960s when researchers described possible mechanisms of NEFA-induced muscle insulin resistance (Randle et al., 1963). Many of the early studies of the role of muscle lipids in T2D utilised muscle biopsy methods for the assessment of muscle lipids. During this era, it was hypothesised that NEFAs caused insulin resistance in the myocytes by competing with glucose for oxidation to produce energy, called Randle's hypothesis, named after Professor Philip Randle who led the research (Hue and Taegtmeyer, 2009). Recent research has disproved some features of Randle's hypothesis but have agreed that excess muscle lipids are related to insulin resistance, however, the mechanisms are more complex than initially understood (Li et al., 2015, Lewis et al., 2002, Petersen and Shulman, 2018).

With the advancement of proton-magnetic resonance spectroscopy (^{1}H-MRS) for the non-invasive analysis of muscle lipids in the 1990s, the study of muscle lipids in T2D became an area of increasing research (Boesch et al., 2006, Schick et al., 1993). The main feature of ^{1}H -MRS that has made it superior to other measures for measuring muscle lipids is its ability to distinguish between lipid droplets stored within myocytes, termed intramyocellular lipids (IMCL), and lipids stored in adipocytes located between the muscle fibres, termed extramyocellular lipids (EMCL) (Thomas et al., 2013). By using ^{1}H-MRS to assess muscle lipids, several studies have shown that IMCL is inversely

related to insulin sensitivity, while EMCL is considered to be metabolically inert due to its lack of relation to insulin sensitivity (Thamer et al., 2003, Sinha et al., 2002, Perseghin et al., 1999, Krssak et al., 1999, Jacob et al., 1999). Furthermore, IMCL is found to be elevated in individuals with T2D compared to healthy controls (Perseghin et al., 1999) and in healthy offspring of individuals with T2D compared to non-offspring controls (Jacob et al., 1999, Perseghin et al., 1999, Petersen et al., 2004b). From the above evidence, several studies have suggested that the accumulation of triglycerides within muscle cells precedes and causes insulin resistance and plays a crucial role in the development of T2D (Eriksson et al., 1999, Spalding et al., 2008, Kelley et al., 2002).

Prior to the study of IMCL in black populations, researchers hypothesised that since visceral and hepatic fat are lower in black populations compared to their white counterparts, IMCL might explain the greater prevalence of T2D among black populations. However, subsequent studies showed no differences in IMCL in blacks compared to whites (Ingram et al., 2011, Lawrence et al., 2011, Goedecke et al., 2015, Lee et al., 2013), with one study even reporting lower levels of IMCL in blacks compared to whites (Smith et al., 2010). Furthermore, several studies have reported that IMCL was inversely associated with insulin sensitivity in white populations but not black populations (Ingram et al., 2011, Lawrence et al., 2011, Smith et al., 2010). In contrast, Goedecke *et al.* reported IMCL was inversely associated with insulin sensitivity in black but not white women (Goedecke et al., 2015). Therefore, studies of relationships between IMCL and measures of insulin sensitivity in black populations have shown inconclusive findings. While most studies of IMCL in black populations have been conducted in women or mixed gender populations, currently no studies have been conducted in black *versus* white men.

6.2 Aim

The primary aim of this chapter is to assess and compare ethnic differences in IMCL between WE and BWA men of NGT, IGT and T2D glycaemic states. The secondary aim is to investigate ethnic differences in the relationships between IMCL and skeletal muscle insulin sensitivity.

6.3 Methods

6.3.1 Data acquirement

The data presented in this chapter were acquired from the methods described in chapter 2 (sections 2.3.2, 2.3.2.1, 2.3.4, 2.3.4.1). In brief, IMCL and EMCL were determined by deconvolution of the lipid peaks from a ^{1}H-MRS spectra acquired from a voxel of interest in the soleus muscle. Measures of insulin sensitivity were determined using a hyperinsulinaemic-euglycaemic clamp test with the infusion of stable glucose and glycerol isotopes to determine whole-body insulin sensitivity (M-value), skeletal muscle insulin sensitivity (% change in glucose rate of disappearance [Rd]) and adipose tissue insulin sensitivity (% suppression of lipolysis).

6.3.2 Statistical analysis

The IMCL and EMCL data presented in this chapter for the WE and BWA men of NGT, IGT and T2D groups were compared using a 2-way between-groups ANOVA where ethnicity and glycaemic state were included as independent variables to assess the main effect of each, as well as their interaction (ethnicity*glycaemic state), on the dependent variables (IMCL or EMCL). When significant main effects were present, post-hoc comparisons were undertaken using the Tukey Honestly Significant Difference test to assess which groups differed significantly. The strength of relationships between variables of interest were tested using Pearson's correlation test; partial correlation was used when assessing relationships while adjusting for confounding variables. Ethnic differences in the strength of the relationships between IMCL with measures of insulin sensitivity were examined by fitting a regression with an interaction term for ethnicity with IMCL as the dependent variable.

6.4 Results

6.4.1 Participant characteristics

The clinical characteristics of the WE and BWA men of NGT, IGT and T2D groups are presented in chapter 3, Table 3.2. The two ethnic groups were well matched for age and BMI and showed no significant differences in clinical characteristics except for waist circumference, triglycerides and HbA1c which were significantly lower in the BWA men.

6.4.2 Metabolic characteristics

Whole-body insulin sensitivity (M-value), skeletal muscle insulin sensitivity (% change in glucose Rd) and adipose tissue insulin sensitivity (% suppression of lipolysis) are presented for the WE and BWA men in Chapter 3, Table 3.3. There were no ethnic differences in all the above measures of insulin sensitivity between the WE and BWA men within each glycaemic group as well as the combined glycaemic cohorts.

6.4.3 Intramyocellular lipids

Differences in IMCL and EMCL, tested by ethnicity and glycaemic state, between the WE and BWA men of NGT, IGT and T2D are presented in Table 6.1. There were no ethnic differences in either IMCL or EMCL between the WE and BWA men as indicated by non-significant main effects for ethnicity. However, IMCL and EMCL differed by glycaemic state and were significantly greater in the IGT and T2D states compared to the NGT state. There were no significant ethnicity and glycaemic state interactions for IMCL and EMCL. Individual values of IMCL within each ethnic and glycaemic group are shown in Figure 6.1. There were no ethnic differences in IMCL in the NGT, IGT and T2D states.

Table 6.1: *IMCL and EMCL in the WE and BWA men of NGT, IGT and T2D glycaemic states.*

	NGT		**IGT**		**T2D**				
	WE (n=22)	**BWA (n=18)**	**WE (n=10)**	**BWA (n=10)**	**WE (n=18)**	**BWA (n=19)**	***P* Eth**	***P* Gly**	***P* Eth*Gly**
IMCL (AU)	3.04 (2.43-3.66)	2.97 (2.22-3.71)	4.36 (3.24-5.47)	3.23 (2.00-4.46)	4.23 (3.32-5.15)	5.04 (3.68-6.40)	0.74	**0.001**	0.19
EMCL (AU)	3.23 (2.54-4.15)	3.00 (2.31-3.69)	4.38 (1.84-7.16)	4.15 (1.84-6.23)	4.38 (3.69-5.31)	4.85 (3.46-6.00)	0.77	**0.019**	0.83

Data presented as geometric mean (95% CI) as the data were log transformed to achieve normal distribution. *P* eth: main effect for ethnicity, *P* gly: main effect for glycaemic state, *P* eth*gly: interaction effect for ethnicity*glycaemic state determined using a 2-way between-groups ANOVA.

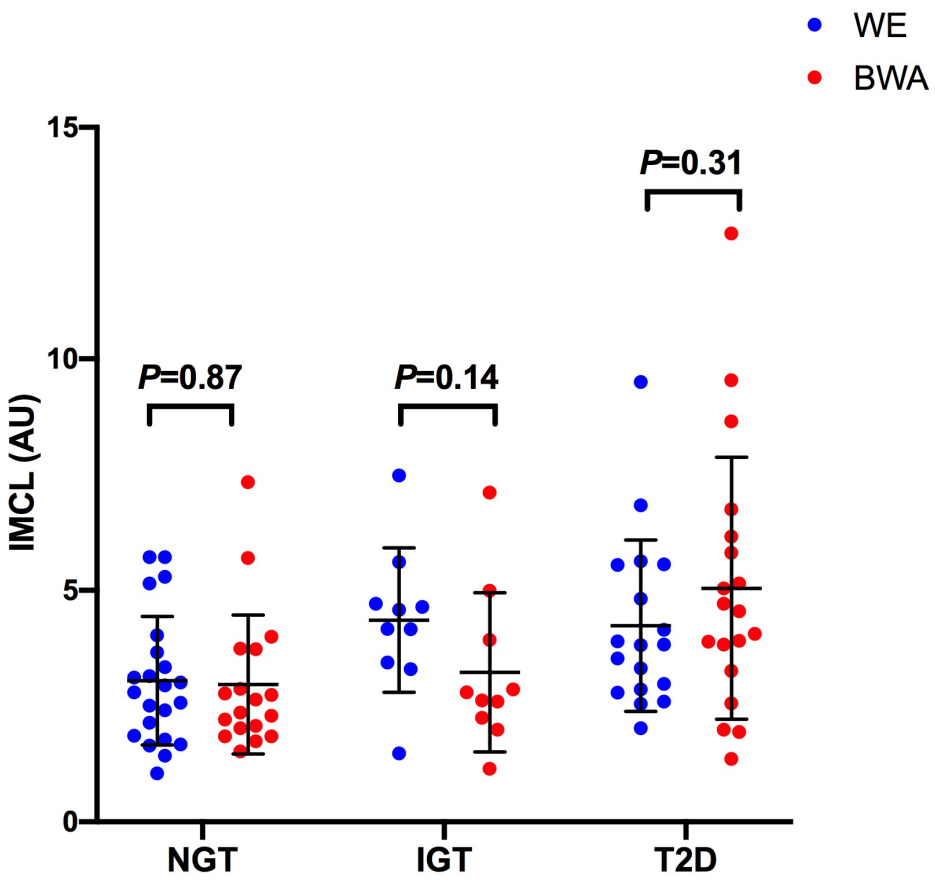

Figure 6.1: *Intramyocellular lipid determined as the ratio of the methylene IMCL peak to water peak from a ^{1}H-MRS spectra in the WE and BWA men of NGT, IGT and T2D groups. Boxplots show median (IQR).*

6.4.4 Relationships between intramyocellular lipids and measures of insulin sensitivity

Relationships between IMCL and measures of insulin sensitivity are presented in Table 6.2 and Figures 6.2, 6.3 and 6.4 (panels A) for the WE and BWA men in all glycaemic cohorts combined. Previous studies have shown with increasing age and BMI, IMCL increases while insulin sensitivity decreases (Nakagawa et al., 2007, Ingram et al., 2011, Krssak et al., 1999). Therefore, to reduce the influence of age and BMI, the above relationships were adjusted for these confounders, Table 6.2.

IMCL was inversely associated with whole-body insulin sensitivity in the WE men while in the BWA men this relationship approached significance. IMCL was inversely

associated with skeletal muscle insulin sensitivity in the WE men but not the BWA men. All significant relationships diminished after adjustments for age and BMI. There were no significant relationships between IMCL and adipose tissue insulin sensitivity in either ethnic group. Analysis of interaction by ethnicity for the relationships between IMCL and measures of insulin sensitivity showed near significant ethnicity interactions for the relationships between IMCL and whole-body insulin sensitivity ($P_{interaction}$=0.081) as well as IMCL and muscle insulin sensitivity ($P_{interaction}$=0.088).

A limitation of conducting correlational analyses in the combined glycaemic cohorts the possible influence of glycaemic state on the relationships between IMCL and insulin sensitivity. To overcome this limitation, relationships between IMCL and measures of insulin sensitivity in the WE and BWA men, shown separately for the NGT and T2D groups, are presented in Table 6.3 and Figures 6.2, 6.3 and 6.4 (panels B). While the resultant correlations were weak, this analysis revealed distinct relationships between the NGT and T2D BWA men which may explain the weak correlations between IMCL and whole-body and muscle insulin sensitivity in the combined glycaemic cohorts (described above). In the NGT BWA men there was no relationship between IMCL and whole-body insulin sensitivity while IMCL and muscle and adipose tissue insulin sensitivity showed positive relationships. However, in the BWA T2D men, the relationships between IMCL and all measures of insulin sensitivity showed negative relationships but did not reach statistical significance. In the WE men, IMCL was significantly inversely associated with whole-body and muscle insulin sensitivity in NGT group, however, no significant relationships were found between IMCL and all measures of insulin sensitivity in the T2D group which had considerably lower sample sizes.

Table 6.2: *Pearson's correlation and partial correlation coefficients showing relationships of IMCL and measures of insulin sensitivity in WE and BWA men of all glycaemic states.*

	WE (n=49)		**BWA (n=44)**	
Intramyocellular lipid	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity	**-0.43**	**0.002**	-0.28	0.070
Adjusted for BMI and age	0.07	0.63	-0.06	0.72
Skeletal muscle insulin sensitivity[a]	**-0.41**	**0.005**	-0.10	0.52
Adjusted for BMI and age	-0.05	0.76	0.09	0.59
Adipose tissue insulin sensitivity[b]	-0.26	0.110	-0.12	0.46
Adjusted for BMI and age	0.24	0.15	-0.12	0.46

[a]n: WE=44, BWA=41; [b]n: WE=39, BWA=40

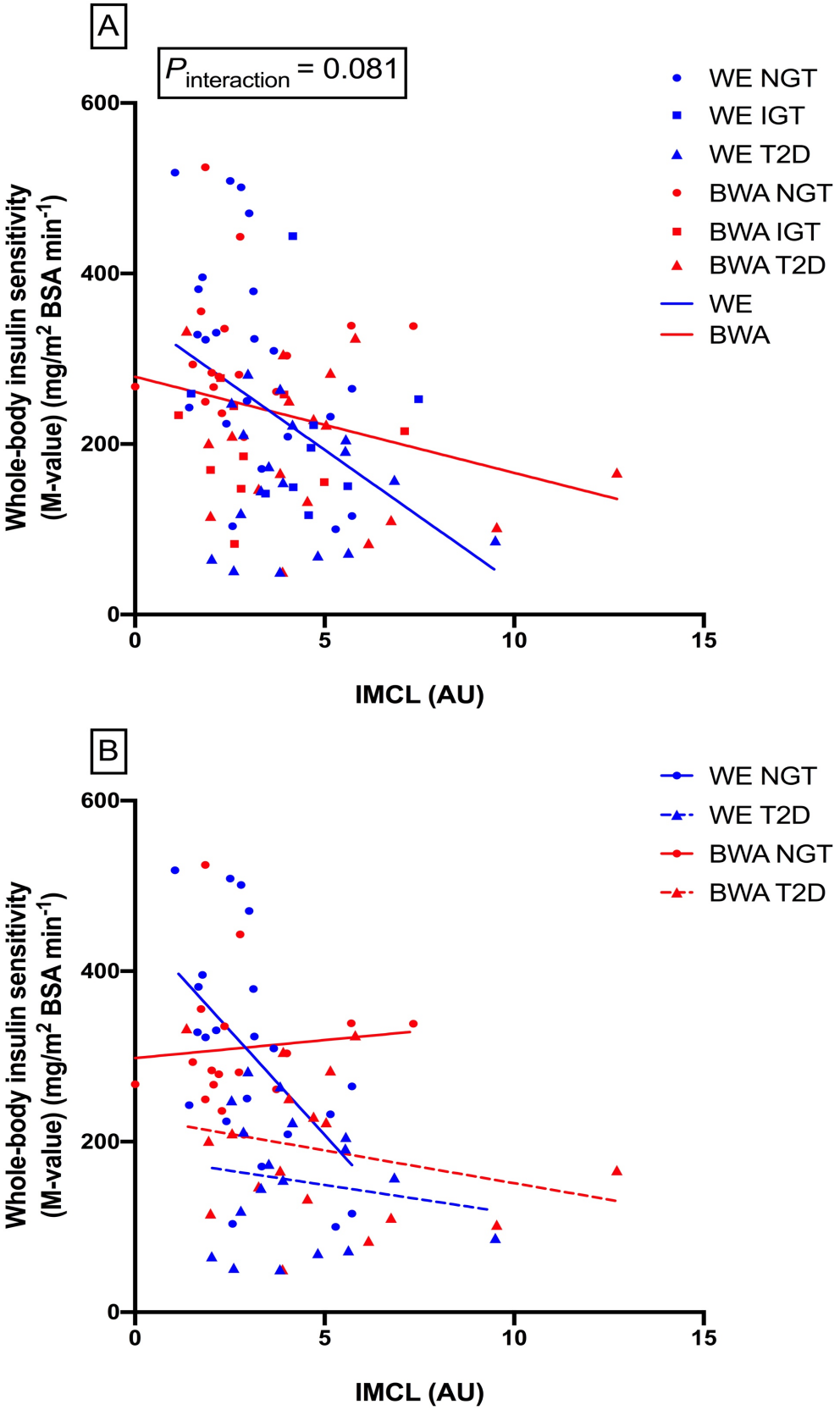

Figure 6.2: *Relationships between IMCL and whole-body insulin sensitivity (M-value) in the WE and BWA men of all glycaemic states (NGT, IGT and T2D), A; and in the NGT and T2D glycaemic states, B.*

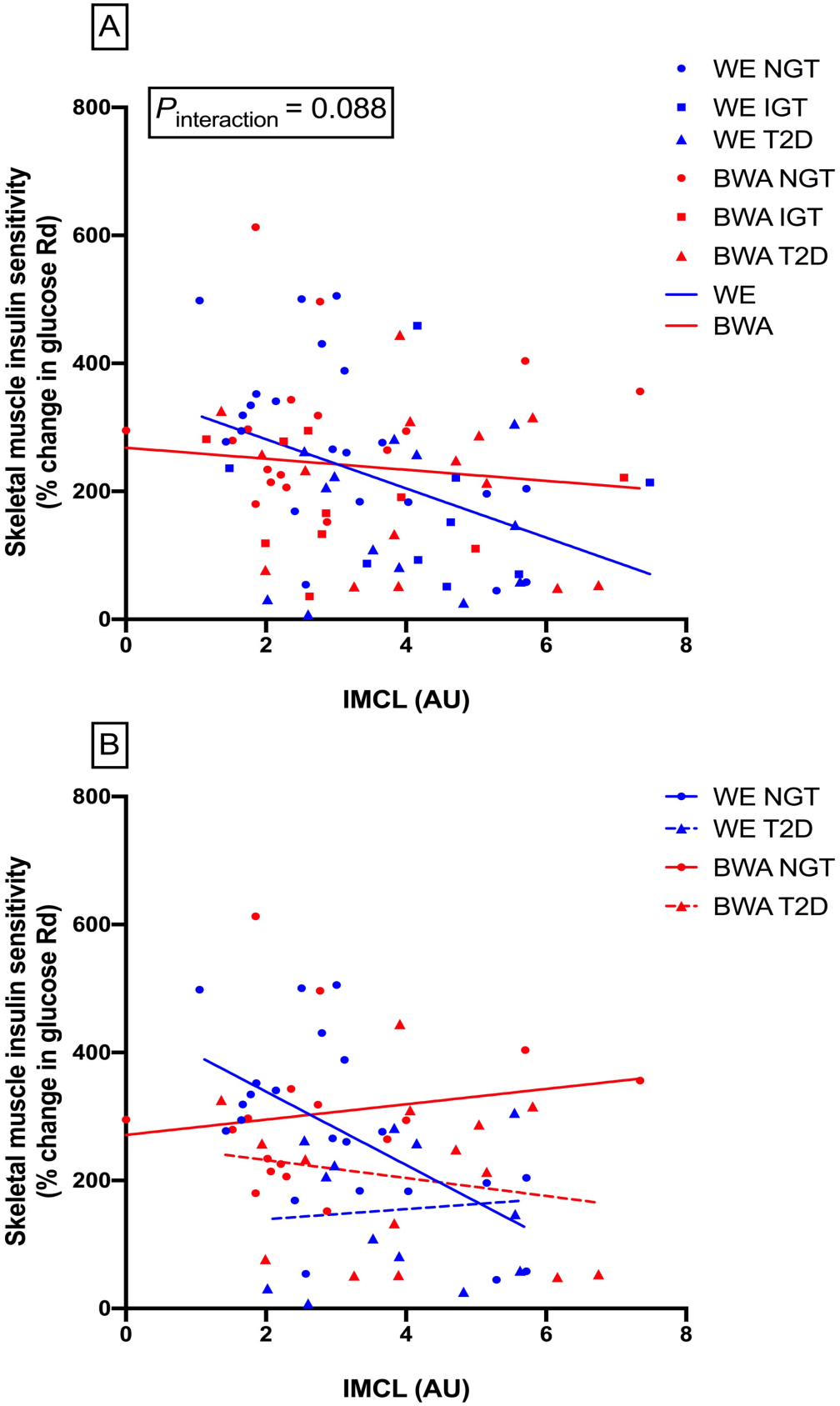

Figure 6.3: *Relationships between IMCL and skeletal muscle insulin sensitivity (% change in glucose Rd from basal) in the WE and BWA men of all glycaemic states (NGT, IGT and T2D), A; and in the NGT and T2D glycaemic states, B.*

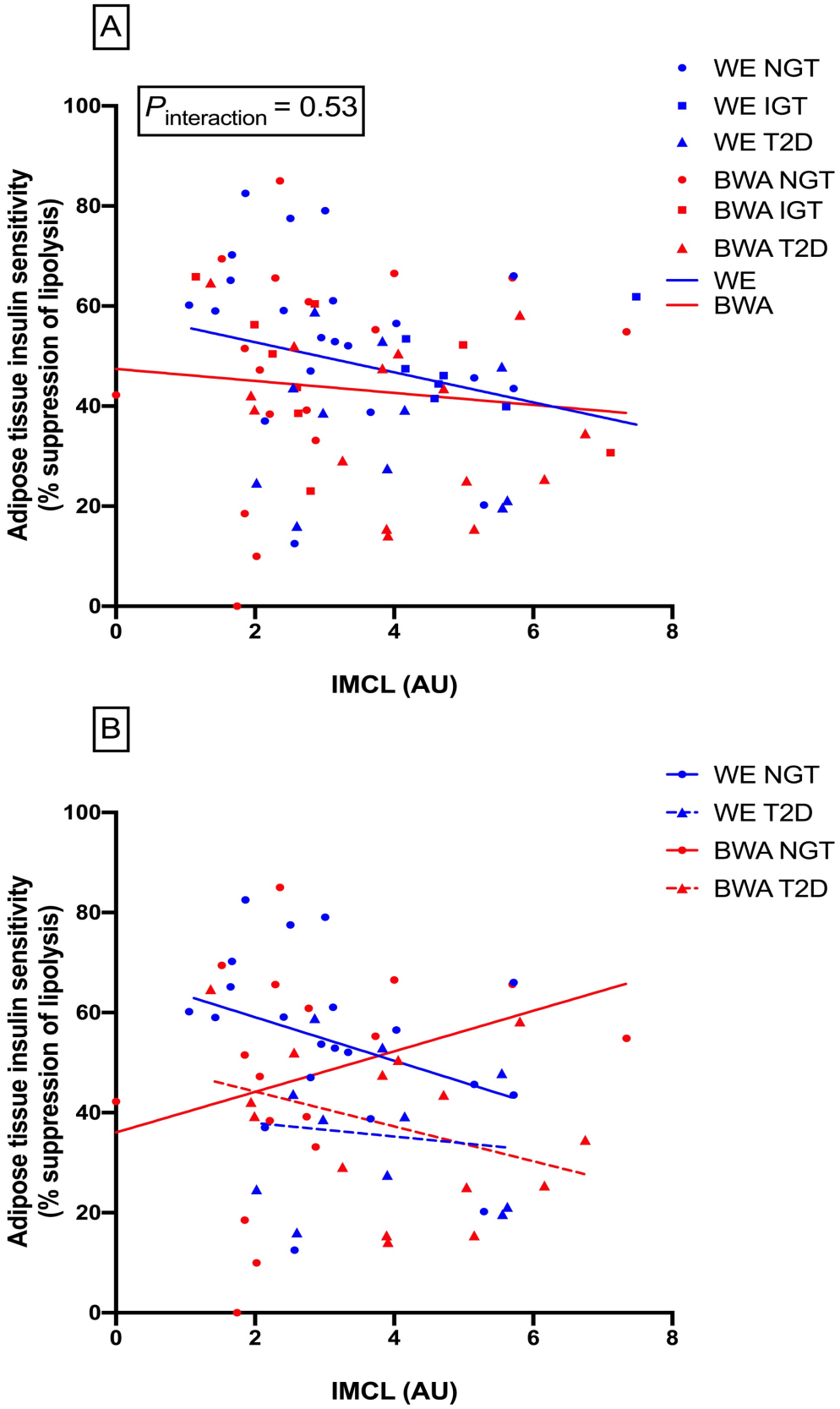

Figure 6.4: *Relationships between IMCL and adipose tissue insulin sensitivity (% suppression of lipolysis) in the WE and BWA men of all glycaemic states (NGT, IGT and T2D), A; and in the NGT and T2D glycaemic states, B.*

Table 6.3: *Pearson's correlation coefficients showing relationships between IMCL and measures of insulin sensitivity in WE and BWA men of NGT and T2D groups.*

	NGT				**T2D**			
	WE		**BWA**		**WE**		**BWA**	
Intramyocellular lipids	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Whole-body insulin sensitivity	**-0.54**	**0.010**	-0.04	0.89	-0.16	0.52	-0.25	0.32
n	**22**		16		18		18	
Skeletal muscle insulin sensitivity	**-0.58**	**0.005**	0.18	0.50	0.09	0.77	-0.18	0.52
n	**22**		16		13		15	
Adipose tissue insulin sensitivity	-0.34	0.13	0.31	0.24	-0.12	0.72	-0.35	0.20
n	21		16		11		15	

6.5 Relationships between measures of regional adiposity and intramyocellular lipids

Relationships between measures of regional adiposity and IMCL are presented in Table 6.4 for the WE and BWA men of all glycaemic cohorts. In the WE and BWA men, IMCL was significantly associated with VAT, and ASAT. While IMCL was associated with IPL in both ethnic groups this relationship was stronger in the WE men than BWA men who showed a trend towards significance. IMCL was associated with IHL in WE but not BWA men.

***Table 6.4**: Pearson's correlation coefficients showing relationships between IMCL and measures of regional adiposity in the WE and BWA men of all glycaemic states.*

	WE (n=49)		**BWA (n=47)**	
Intramyocellular lipids	**r**	***p***	**r**	***p***
VAT	**0.41**	**0.004**	**0.56**	**<0.001**
ASAT	**0.35**	**0.014**	**0.33**	**0.024**
IHL[a]	**0.34**	**0.017**	0.21	0.16
IPL[b]	**0.36**	**0.010**	0.26	0.079

[a]n: BWA=45; [b]n: BWA=46

6.6 Discussion

The purpose of this chapter was to investigate ethnic differences in intramyocellular lipids between WE and BWA men across three glycaemic states: NGT, IGT and T2D. Findings from the present investigation revealed no ethnic differences in IMCL between BWA and WE men in NGT, IGT and T2D states. However, there were ethnic differences in the relationships between IMCL and measures of insulin sensitivity where IMCL was more strongly related to insulin resistance in WE men compared to BWA men.

The lack of ethnic difference in IMCL between the BWA and WE men is consistent with previous findings in adolescents (Liska et al., 2007) and women (Goedecke et al., 2015, Ingram et al., 2011, Lawrence et al., 2011) of black ethnicity compared to their white counterparts.

6.6.1 Intramyocellular lipids and insulin sensitivity

Intramyocellular lipid was inversely associated with whole-body insulin sensitivity in both WE and BWA men, however, this relationship differed by ethnicity (near significant ethnicity interaction) and was more strongly related to whole-body insulin sensitivity in WE men than BWA men. IMCL was inversely associated with muscle insulin sensitivity in WE men but not BWA men, a relationship which also differed by ethnicity (near significant ethnicity interaction). The above findings suggest that IMCL may impact muscle insulin sensitivity to a lesser extent in BWA men compared to WE men. This finding supports two previous studies, conducted in US populations, which reported significant inverse relationships between IMCL and insulin sensitivity in white but not black women (Lawrence et al., 2011) and mixed-gender cohorts (Ingram et al., 2011). However, the opposite was reported in a study conducted in South African women, where IMCL was inversely related to insulin sensitivity in the black but not white women (Goedecke et al., 2015). These contradictions may be explained by the level of overall

adiposity, because the cohorts in the US studies were in the overweight BMI category while the South African study was conducted in women with obesity. It is possible that the harmful effects of IMCL may only occur in black populations with severe obesity or specifically in black women but not black men. Interestingly, it has previously been recognised in large epidemiological studies that the prevalence of T2D in black women is largely explained by their level of adiposity but this was not the case in black men (Brancati et al., 2000, Signorello et al., 2007).

While EMCL has received less attention in research due to its supposed lack of influence on insulin sensitivity, few studies have reported EMCL in black *vs* white populations. In this investigation, EMCL did not differ between the WE and BWA men which was similar to findings previously reported in South African women (Goedecke et al., 2015) and American adolescents (Liska et al., 2007). In contrast to these findings, EMCL was reportedly greater in African American women compared to their white counterparts (Albu et al., 2005, Gallagher et al., 2005, Lawrence et al., 2011) indicating that other factors such as gender may impact EMCL accumulation.

6.6.2 Intramyocellular lipids and adipose tissue insulin sensitivity

The *spillover theory* is the main theory proposed to describe the mechanisms of ectopic fat storage, including IMCL (Brons and Grunnet, 2017). This theory proposes that the dysfunction of adipose tissue, that occurs partly due to its severe expansion, facilitates the storage of excess NEFAs in non-adipose depots (Brons and Grunnet, 2017, Cuthbertson et al., 2017). Decreased insulin sensitivity of the adipose tissue is a prominent feature of dysfunctional adipose tissue, which is also related to ectopic fat storage (Smith and Kahn, 2016). However, the present analysis revealed no relationship between IMCL and adipose tissue insulin sensitivity in either ethnic group, which suggests that the mechanisms of IMCL accumulation may not be directly related to the

dysfunction and insulin resistance of adipose tissue. Other theories that may explain the mechanisms of IMCL accumulation include the reduced oxidation capacity of fatty acids within the muscle cells (Li et al., 2015). This theory proposes that fatty acids within the muscle cells, that are not oxidised due to the lack of energy expenditure, are stored as IMCL. The present study may support this theory due to the lack of association between IMCL and adipose tissue insulin resistance in both ethnic groups; however, further investigations of IMCL and fatty acid oxidative capacity within muscle cells are warranted to elucidate these mechanisms.

6.6.3 Intramyocellular lipids and regional adiposity

To understand the possible drivers of IMCL accumulation, relationships between IMCL and measures of regional fat deposition were assessed, which revealed distinct patterns in each ethnic group. IMCL was associated with VAT, ASAT and IPL in both ethnic groups, however, IMCL was associated with IHL in WE men but not BWA men. This suggests that the increase of SAT and VAT may result in the spillover of NEFAs that subsequently deposit in myocytes, a process which may occur in both WE and BWA men. However, the role of IHL in IMCL accumulation may differ by ethnicity. Since the liver is an important site of lipid metabolism, an increase of IHL may promote the excess release of lipids, that are stored within other organs including the pancreas and muscles. This process may occur to a greater extent in WE men than BWA men. In a similar manner, data presented in Chapter 5 showed IHL was related to IPL in WE men but not BWA. Taken together, it appears that IHL accumulation is less influential in driving ectopic fat deposition in the pancreas and myocytes in BWA men compared to WE men. Furthermore, there is additional evidence, within this study, that suggests lipid output from the liver is lower in BWA men than WE men, indicated by significantly lower TG levels in BWA men.

6.6.4 Strengths and limitations

There are several strengths worth noting, which include the use of ^{1}H-MRS to determine IMCL and EMCL, which allows for the differentiation between lipids stored within and between myocytes. However, a limitation is the difficulty in deconvolution of the lipid peaks when analysing the spectra obtained from participants with obesity due to the greater overlap of lipid peaks in their spectra which makes the separation of the lipid peaks more challenging. Due to this limitation, some of the IMCL or EMCL values may have been exaggerated owing to contamination from neighbouring lipid peaks that have merged together. Another limitation is the assessment of muscle lipids only within the soleus muscle as others have reported varying proportions of IMCL to EMCL in different muscle groups (Boesch et al., 1997, Just Kukurova et al., 2014). Another limitation is the lack of assessment of aerobic capacity levels in the participants as VO_2 max has been shown to be an independent determinant of IMCL (Thamer et al., 2003).

6.7 Conclusion

Though there were no ethnic differences in IMCL accumulation between WE and BWA men, IMCL was more strongly inversely related to measures of peripheral insulin sensitivity in WE than BWA men. This suggests that IMCL may play a more relevant role in the development of T2D in WE men compared to BWA men. Furthermore, the relationships between IMCL and VAT and ASAT in both WE and BWA men suggests that IMCL accumulation may be driven by increasing SAT and VAT accumulation in both ethnic groups. However, the stronger relationships between IHL and IMCL in WE men suggests that excess lipid output by the liver may be a greater driver of IMCL accumulation in WE men compared to BWA men.

Chapter 7: Discussion and conclusions

The main objective of this thesis was to investigate ethnic differences in ectopic fat deposition and their relationships with the metabolic parameters of T2D between white European (WE) and black west African (BWA) men. This was accomplished by the comprehensive analysis of ectopic fat deposition and their relationships with metabolic parameters of T2D across three glycaemic states: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D.

The WE and BWA men were similar in their clinical and metabolic characteristics in the NGT, IGT and T2D groups. Furthermore, both ethnic groups showed similar patterns of deterioration in metabolic parameters in the T2D compared to the NGT states which was evident in the greater level of ectopic fat and a lower level of insulin sensitivity and insulin secretory function in the T2D states. Also similar between the WE and BWA men, was the strong relationships of visceral adipose tissue (VAT) and intrahepatic lipid (IHL) with measures of insulin sensitivity. However, some important ethnic differences include: 1) despite no ethnic differences in overall adiposity, VAT, IHL and intrapancreatic lipid (IPL) were lower in the BWA men compared to the WE men; 2) relationships of IHL and IMCL with metabolic parameters of T2D were weaker in the BWA men compared to the WE men; 3) VAT, IHL and IPL were significantly inversely related to adipose tissue insulin sensitivity in the WE men but not the BWA men; 4) interrelationships between ectopic fat depots were weaker in the BWA men than the WE men. Overall, the evidence presented in this thesis suggests that an increase of ectopic fat deposition that is related to an increase in adiposity is a key component in the progression of T2D in both WE and BWA men. However, there is a lack of evidence to suggest that the greater risk of T2D in BWA men compared to WE men is explained by ectopic fat deposition.

7.1 Ethnic differences in ectopic fat deposition

Previous studies have shown that, at a similar level of overall adiposity, black populations have lower levels of VAT, IHL and IPL compared to their white counterparts (Goedecke et al., 2017, Alderete et al., 2014, Liska et al., 2007, Le et al., 2011, Szczepaniak et al., 2012). The VAT, IHL and IPL data presented in the current analysis of WE and BWA men are consistent with previous reports, which adds to the paradox of greater T2D risk in black populations despite lower VAT, IHL and IPL. The regional fat depots that were investigated include ASAT (dSAT and sSAT), VAT, IHL, IPL and IMCL. There were no ethnic differences in ASAT, dSAT or sSAT, however, VAT, IHL and IPL were lower in BWA men compared to WE men with no differences in IMCL. These ethnic patterns were present within each glycaemic state for all fat depots except for IPL, which was lower in the BWA men of IGT and T2D groups but did not differ by ethnicity in the NGT state.

In both WE and BWA men, VAT, IHL, IPL and IMCL were similarly greater in the IGT and T2D states compared to the NGT state indicted by non-significant ethnicity*glycaemic state interactions. However, the differences in VAT, IHL and IPL levels between the WE and BWA men was greater in the IGT and T2D groups than the NGT groups. Furthermore, relationships between ASAT with IHL, IPL and IMCL were consistently stronger in the WE men than BWA men. This may indicate that with increasing adiposity, the BWA men appear to be less susceptible to ectopic fat storage than WE men. With adipose tissue expansion, the point at which it becomes dysfunctional, and shows a reduced capacity to store triglycerides, varies from one individual to another, and is coined “the personal fat threshold” (Taylor and Holman, 2015). The apparent lower susceptibility of BWA men to ectopic fat storage suggest they have a higher personal fat threshold than WE men; however, other aspects of adipose

tissue function, such as the mechanisms of the breakdown and storage of triglycerides, should be investigated to confirm these speculations.

7.2 Ethnic differences in relationships between regional and ectopic fat depots and metabolic parameters of type 2 diabetes

Studies investigating the role of ectopic fat in the pathophysiology of T2D in black populations have provided inconsistent conclusions. Among studies that specifically investigated relationships between ectopic fat and metabolic parameters of T2D, some suggest ectopic fat is more detrimental in black populations than white populations (Alderete et al., 2013, Goedecke et al., 2015, Szczepaniak et al., 2012) while others suggest it is less detrimental (Ingram et al., 2011, Lawrence et al., 2011, Chung et al., 2019). The evidence presented in this thesis supports the notion that ectopic fat may be less important in the development of T2D in black populations than white populations. Even though several associations were present between ectopic fat depots and metabolic parameters in both WE and BWA men indicating that ectopic fat is an important component of T2D in both ethnic groups, these relationships were consistently stronger in the WE men.

It is well accepted that VAT is strongly related to T2D (Smith et al., 2012); however, recent research suggests that IHL may be more strongly related to T2D than VAT (Fabbrini et al., 2009, Okamura et al., 2019). A comparison of the relationships between VAT and IHL with whole-body and hepatic insulin sensitivity reveals distinct ethnic patterns. In the WE men, VAT and IHL showed similar strengths in their associations with the measures of insulin sensitivity; however, in BWA men, the associations of VAT were markedly stronger than those of IHL. Additionally, the inverse relationships between VAT and whole-body and hepatic insulin sensitivity persisted after adjustment for age and BMI in the BWA men but not the WE men. On the contrary, the inverse

relationship between IHL and whole-body insulin sensitivity persisted after adjustment for age and BMI in the WE men but not the BWA men. Thus, VAT appears to be more dominant than IHL in its relation to insulin resistance in BWA men while IHL appears to be more dominant in WE men. However, despite the above findings, the high risk of a type 2 statistical error in the relationships involving VAT and IHL in the BWA men must be acknowledged; this type of error may occur when there is no statistically significant correlation present between two variables despite a clear trend and may be explained by the low range of VAT and IHL in the BWA men in comparison to the WE men.

The analysis of relationships between ectopic fat depots and metabolic parameters of T2D in the combined glycaemic cohorts, as well as within the NGT and T2D states, each have their advantages and limitations. Analysis of the combined glycaemic cohorts benefits from greater statistical power; however, relationships may be influenced by glycaemic state of the populations assessed. On the other hand, the individual glycaemic cohorts are more homogenous populations but have considerably lower sample sizes. Despite these limitations, certain trends were particularly evident, which may provide insights into ethnic differences in the role of ectopic fat in T2D. It was particularly noticeable that several relationships between ectopic fat depots and metabolic parameters were stronger in NGT WE men than WE men of combined glycaemic cohorts; however, these relationships were not present in the NGT BWA men but were present in the BWA men of combined glycaemic cohorts. Relationships where this trend occurred include: VAT and M-value, IHL and hepatic insulin sensitivity, IHL and M-value, IHL and insulin clearance, and IMCL and M-value. The lack of significant relationships between ectopic fat depots and metabolic parameters in NGT BWA men may be explained by a low level of adiposity in the NGT groups. Hence, ectopic fat may be more detrimental in black populations during obesity, while in WE men, there appears to be a direct link between

the amount of ectopic fat and a decline in metabolic responses even at lower levels of adiposity.

The greater prevalence of T2D among black populations may be explained by the greater levels of insulin that they exhibit in comparison to their white counterparts, which has been detected from as early as childhood (Kodama et al., 2013, Ku et al., 2000). Prolonged hyperinsulinaemia itself has been proposed to exacerbate insulin resistance, which may be a driver insulin resistance in black individuals (Shanik et al., 2008, Thomas et al., 2019). Indeed, the data presented here showed greater fasting insulin levels and lower insulin clearance in the BWA men of NGT compared to WE men. Over a prolonged period, the greater insulin levels may accelerate the development of insulin resistance in black populations, and may explain their greater risk of T2D despite their lower ectopic fat deposition.

7.3 Ethnic differences in the mechanisms of ectopic fat deposition

There are a number of proposed theories that are thought to explain the mechanisms by which ectopic fat accumulates that are summarised in Figure 7.1 (Goff et al., 2019). The *spillover theory* explains the process by which dysfunctional adipose tissue leads to excess release of NEFAs that deposit in ectopic depots (Snel et al., 2012, Sattar and Gill, 2014)*;* the *portal theory* explains the mechanism by which VAT releases excess NEFAs into the portal vein that deposit in the liver (Item and Konrad, 2012); and the *twin-cycle theory* explains the mechanisms by which excess IHL promotes excess VLDL-TG release by the liver which deposit in the pancreas (Taylor, 2013). The data presented in this thesis suggest ethnic differences exist in the mechanisms of ectopic fat storage. Firstly, ASAT and VAT were related to ectopic fat depots in both WE and BWA men; however, interrelationships between IHL, IPL and IMCL were weaker in the BWA men compared to WE men. Secondly, VAT, IHL and IPL were all inversely related to adipose tissue

insulin sensitivity in WE men but not BWA men, with significant ethnicity interactions present for these relationships. Since, reduced adipose tissue insulin sensitivity is a key feature of dysfunctional adipose tissue, it appears that adipose tissue dysfunction is a lesser determinant of ectopic fat storage in BWA men than WE men.

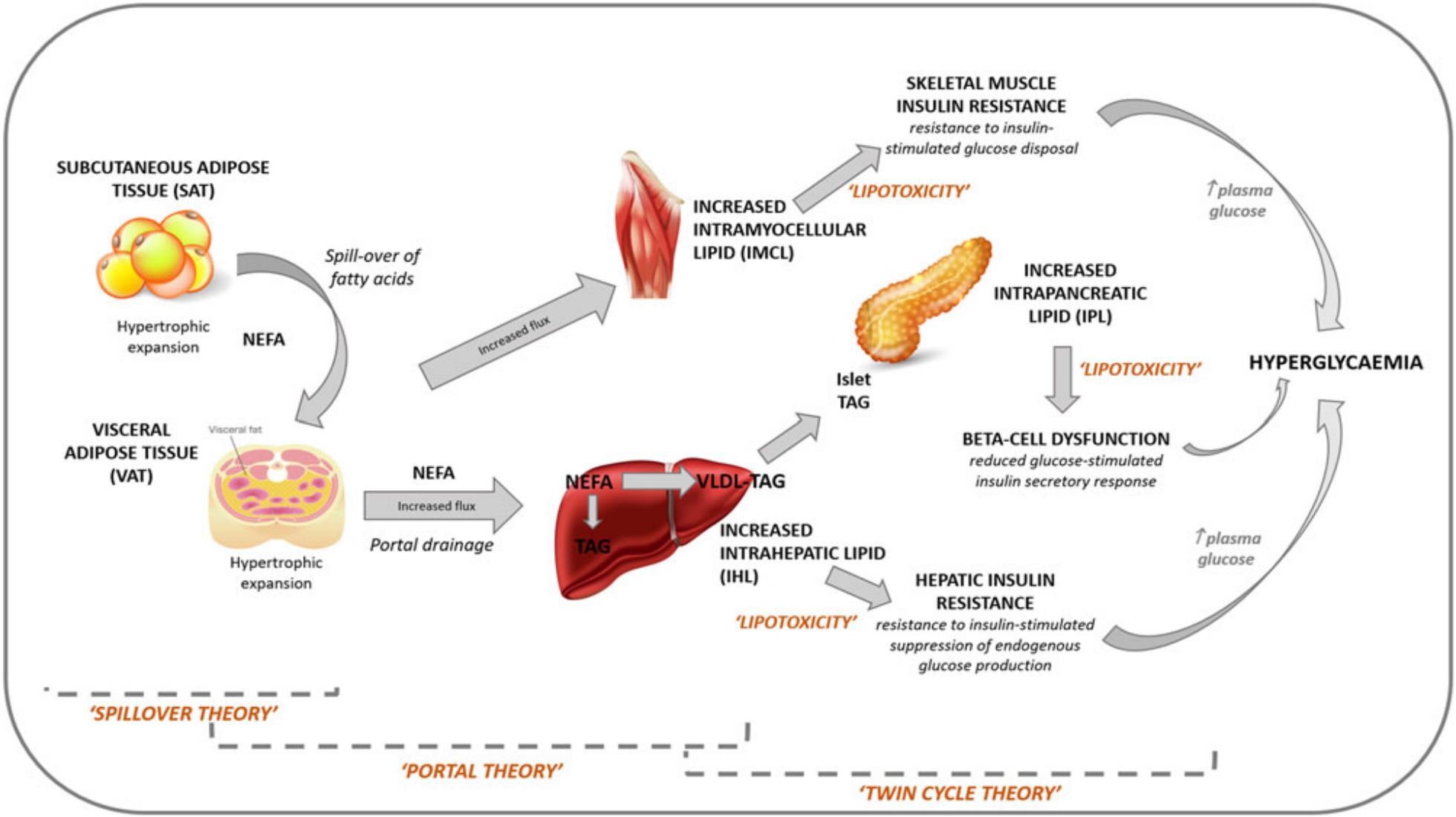

Figure 7.1: *Proposed mechanisms of ectopic fat storage and their role in the pathophysiology of T2D (Goff et al., 2019).*

7.4 Strengths and limitations

This thesis has several strengths worth noting with some limitations to consider. The assessments of ectopic fat depots were conducted using MRI and ^{1}H-MRS, which are currently considered the most precise non-invasive methods for quantifying body composition components. The comprehensive assessment of several metabolic parameters is also another strength which includes the assessment of organ-specific measures of insulin sensitivity via the use of stable glucose and glycerol isotopes during the hyperinsulinaemic-euglycaemic clamp; this is currently considered the gold-standard method to determine organ-specific insulin sensitivity. Furthermore, insulin clearance and ISF were determined using the comprehensive hyperglycaemic clamp with the sophisticated modelling analyses of insulin clearance and parameters of ISF using c-peptide deconvolution models. However, a limitation to both the clamp techniques mentioned above, is their disregard to the physiological responses that may influence insulin sensitivity and beta-cell function. For example, it is well established that an oral glucose ingestion stimulates significantly greater insulin secretion and insulin clearance than a similar concentration of intravenous glucose (Perley and Kipnis, 1967, Shapiro et al., 1987). Therefore, considering ethnic differences in metabolic parameters of T2D under physiological conditions should also be an important focus for future studies.

The analysis of VAT, ASAT, dSAT and sSAT, as well as the three main ectopic fat depots related to T2D, in a single study is another strength of this thesis, since most previous studies of ectopic fat in black populations investigated only one or two fat depots. Furthermore, there are several novel aspects of this thesis that are worth noting, including the assessment of regional IPL and the investigation of relationships between ectopic fat depots and adipose tissue insulin sensitivity; to current knowledge, both these analyses have not previously been conducted in a black population.

This thesis is limited in the study of only men; hence, the findings may not apply to black women who have distinct metabolic functions compared to black men. However, while several previous ethnicity studies in T2D have been conducted in women, there is a relative lack of focus on men making this study of particular importance (Goedecke et al., 2017).

While several trends were evident, the cross-sectional nature of this study limits the speculations of whether ectopic fat causes the decline in metabolic parameters, since correlation does not imply causation. Furthermore, with the strong interlinkage of several parameters related to glucose metabolism, it is particularly difficult to untangle which factors influence others. Therefore, longitudinal studies are highly warranted to aid the understanding of ethnic differences in the role of ectopic fat in T2D.

7.5 Future directions

Ectopic fat is believed to deposit due to the progressive failure of the SAT to store excess energy as triglycerides (Bosy-Westphal et al., 2019, Castro et al., 2014). However, while the evidence presented here suggests that the increase of SAT is accompanied by an increase of ectopic fat in both ethnic groups, there may be some indications that the role of the adipose tissue in ectopic fat accumulation may differ by ethnicity. To expand, in the WE men, ectopic fat appeared to be linked to adipose tissue insulin resistance, a well-known feature of dysfunctional adipose tissue; however, this was not the case in BWA men. Therefore, the study of adipose tissue dysfunction, including ethnic differences in the structure and function of adipose tissue, are strongly proposed for future studies. Furthermore, ethnic differences in factors other than adipose tissue dysfunction may influence ectopic fat storage in black populations that warrant further investigation. Such factors may include genetic susceptibility to insulin resistance, diet, exercise, cellular

oxidative capacity, sex hormones, glucocorticoid hormones and pro-inflammatory cytokines, all of which may differ by ethnicity.

Studies that directly compare the pathophysiology of T2D between men and women of black ethnicity are also highly warranted. Several findings differed between the present study and those previously published that may be explained by gender. Indeed, several epidemiological studies have shown that T2D differs between black men and women, and T2D is driven by adiposity more so in women than men (Signorello et al., 2007, Brancati et al., 2000); however, there is a lack of physiological studies that explain the reasons behind these gender differences.

7.6 Conclusion and final remarks

While the BWA men had lower levels of VAT, IHL and IPL than the WE men, with no ethnic differences in IMCL, the increase of these fat depots was related to the deterioration of several metabolic parameters of T2D in both ethnic groups. Furthermore, VAT, IHL, IPL and IMCL were greater in the T2D and IGT states compared to the NGT state in both WE and BWA men. This indicates that the increase of ectopic fat is an important component of the progression of T2D in both ethnic groups. Albeit, ethnic distinctions occurred in the strengths of the relationships between specific fat depots and relevant metabolic parameters where weaker relationships were commonly reported in the BWA men compared to the WE men. This phenomenon may be explained by the lower range of VAT, IHL and IPL in BWA men but may also suggest that ectopic fat may have lesser lipotoxic effects in BWA men than WE men. Furthermore, the lack of inverse associations between VAT, IHL and IPL with adipose tissue insulin sensitivity suggests that the dysfunction of SAT may be a lesser determinant of ectopic fat storage in BWA men than WE men. Taken together the evidence in this thesis suggests that ectopic fat is related to the progression of T2D in both WE and BWA men but may not explain the greater risk of T2D in black populations and other factors, such as genetic susceptibility to insulin resistance, may be greater determinants. To further elucidate the role of ectopic fat in the development of T2D in black populations, future studies investigating the effects of ectopic fat lowering therapies in a black *vs* white population are highly warranted. In an era approaching personalised medical treatment of T2D, the findings presented in this thesis may inform future treatment and prevention strategies for T2D in black populations. Finally, considering the worldwide increase in the prevalence of T2D in populations of African descent, ethnicity is an area deserving of greater attention in T2D research.

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Appendices

APPENDIX I

DIABETES RESEARCH AND CLINICAL PRACTICE 156 (2019) 107866

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Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity

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ABSTRACT

Aims: We aimed to assess ethnic differences in visceral adipose tissue (VAT), intrahepatic (IHL), intrapancreatic (IPL) and intramyocellular lipids (IMCL) between healthy white European (WE) and black west African (BWA) men.

Methods: 23 WE and 20 BWA men underwent Dixon-magnetic resonance imaging to quantify VAT, IHL and IPL; and proton-magnetic resonance spectroscopy to quantify IMCL. Insulin sensitivity and beta-cell function were determined using homeostasis model assessment (HOMA-2).

Results: BWA men exhibited significantly lower VAT ($P = 0.021$) and IHL ($P = 0.044$) than WE men, but comparable IPL ($P = 0.92$) and IMCL ($P = 0.87$). VAT was associated with IPL in both ethnicities (WE: $P < 0.001$; BWA: $P = 0.001$) but the relationship with IHL differed by ethnicity ($P_{interaction} = 0.018$) and was only significant in WE men (WE: $P < 0.001$; BWA: $P = 0.36$). All ectopic fat depots inversely associated with insulin sensitivity and positively associated with beta-cell function in WE but not BWA men.

Abbreviations: BWA, black west African; BMI, body mass index; dSAT, deep subcutaneous adipose tissue; EMCL, extramyocellular lipid; HbA1c, glycated hemoglobin; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL, intrapancreatic lipid; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; ROI, region of interest; SAT, subcutaneous adipose tissue; sSAT, superficial subcutaneous adipose tissue; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue; WE, white European

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Conclusions: Lower VAT and IHL, and their lack of interrelation, in BWA men suggests ethnic differences exist in the mechanisms of ectopic fat deposition. The lack of association between ectopic fat with insulin sensitivity and beta-cell function in BWA men may indicate a lesser role for ectopic fat in the development of type 2 diabetes mellitus in black populations.

1. Introduction

Ectopic fat, defined as the accumulation of triglycerides in non-adipose tissues, is more strongly related to type 2 diabetes mellitus (T2DM) than measures of whole body adiposity [1,2]. The advancement of magnetic resonance imaging (MRI) technology for the study of regional adiposity, has confirmed that not all fat is equal and the deposition of ectopic fat has a greater association with insulin resistance than subcutaneous depots which are considered metabolically 'safe' fat storage depots [3–5]. Subsequently, it has been well documented that a central distribution of body fat, more specifically deposition of visceral (VAT) and deep subcutaneous adipose tissue (dSAT), is a major determinant of insulin resistance, while peripheral and superficial subcutaneous adipose tissue (sSAT) depositions are associated with favourable metabolic outcomes [6–8].

There are several theories describing the mechanisms of ectopic fat deposition and their role in the pathophysiology of T2DM. The *spillover theory* hypothesises that the limited expandability of SAT results in an overflow of fatty acids to VAT and dSAT [9–11]. The *portal theory* proposes that the highly lipolytic VAT has a greater flux of fatty acids which are released into the portal circulation and become deposited in the liver (intrahepatic lipid, IHL) [12]. The more recent *twin-cycle theory* proposes that increased IHL accumulation leads to increased export of VLDL-triglyceride from the liver, which subsequently deposit in the pancreas (intrapancreatic lipid, IPL) contributing to the beta-cell failure that underlies the development of frank T2DM [13]. There is evidence showing excess ectopic fat deposition is integral to the development of T2DM by causing metabolic disturbances in the organ/tissue in which it resides, termed *lipotoxicity* [14].

The study of ectopic fat in populations of African ancestry has become an area of increasing interest as despite the greater prevalence of T2DM in black populations compared to white populations they typically have lower levels of ectopic fat [15,16]. Potential mechanisms of ectopic fat deposition in black populations are poorly understood due to the lack of studies investigating regional adiposity and all ectopic fat depots related to T2DM in a single study. Therefore, by using gold-standard magnetic resonance methods to determine ectopic fat deposition, we aimed to investigate ethnic differences, between healthy white European and black west African men, in (1) VAT, IHL, IPL and intramyocellular lipids (IMCL), (2) relationships between ectopic fat depots and insulin sensitivity and beta-cell function, and (3) interrelationships between the ectopic fat depots.

2. Materials and methods

The South London Diabetes and Ethnicity (Soul-Deep) 2 study is an observational study which aims to investigate ethnic differences in the pathophysiology of T2DM between white European (WE) and black west African (BWA) men [17]. Participants were recruited by local advertisements in the South London area between April 2016 and May 2018. All participants provided written, informed consent and the study was approved by the London Bridge Research Ethics Committee (reference: 15/LO/1121).

2.1. Participants

Participants were considered eligible if they were male, aged 18–65 years, had a BMI of 20–40 kg/m^2, of WE or BWA ethnicity and normal glucose tolerant determined by a 2-h plasma glucose <7.8 mmol/l during a 75 g oral glucose tolerance test (OGTT). Participants were excluded if they had impaired glucose tolerance or T2DM, contraindications for MRI, liver damage determined by a serum alanine aminotransferase of 2.5-fold above the upper limit of reference range, kidney damage determined by serum creatinine of above 150 μmol/l or were taking medications known to affect the study outcomes. Eligibility was assessed in a screening assessment including (1) full blood count, renal and liver function, HbA1c, fasting lipid profile and sickle cell trait; (2) anthropometric measurements including height, weight, waist circumference, percentage body fat using bioelectrical impedance (Tanita, MC780) and seated blood pressure; (3) a questionnaire to elicit age; self-declared ethnicity of self, parents and grandparents; (4) medical history and current medications; and (5) a 75 g OGTT conducted as previously described to assess glucose tolerance status [18]. The homeostatic model assessment was used to estimate insulin sensitivity (HOMA-S) and beta-cell function (HOMA2-B), which were calculated using the HOMA2 calculator provided by the Diabetes Trial Unit, University of Oxford (https://www.dtu.ox.ac.uk/homacalculator/; accessed 10/08/19).

2.2. Magnetic resonance imaging

Each participant underwent an MRI and magnetic resonance spectroscopy (MRS) scan on a single visit at the Clinical Imaging Department at Guy's Hospital, London. To quantify IHL, IPL, VAT, SAT, dSAT and sSAT each participant underwent a magnetic resonance imaging (MRI) scan on a 1.5 T Siemens Aera scanner. After an overnight fast, participants were

scanned between the neck and the knee in the supine position with body coils placed on the scanned area. From each participant, 384 contiguous, axial T1-weighted gradient-echo images (repetition time:6.77 ms; echo times: 4.77 ms (in-phase) and 2.39 ms (out-of-phase); flip angle: 10°; slice thickness: 3 mm) were acquired, from which fat and water images were produced as part of the Dixon sequence. To reduce motion artefacts, participants were instructed to hold their breath for 15 s while abdominal images were acquired. MRI data was analysed using HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017) by a single analyst who was blinded to clinical data.

2.3. Quantification of visceral and subcutaneous adipose tissue

Areas of VAT, SAT, dSAT and sSAT were determined from an axial MRI image at the L4-5 spinal anatomical position. VAT and SAT areas were highlighted to quantify respective areas. DSAT and sSAT were separated from total SAT by locating the fascia-superficialis that is visible on an MRI image.

2.4. Quantification of intrapancreatic lipid

IPL was measured using a method we have previously described [19]. Briefly, 3 circular regions of interest (ROI) of approximately 1 cm^2 were positioned on each of the head, body and tail regions of the pancreas; ROIs were checked and approved by a consultant radiologist (DC) to confirm correct positioning. In each ROI, pancreatic fat fraction was quantified using the formula: %IPL = (F/(F + W)) * 100, where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image. Mean IPL was calculated as the mean of the head, body and tail IPL.

2.5. Quantification of intrahepatic lipid

IHL was measured using a method we have previously described [20]. Briefly, 4 ROIs were positioned in the liver tissue of 2 MRI images while avoiding blood vessels, bile ducts and obvious artefacts. Hepatic fat fraction was calculated in each ROI by using the formula above and IHL was calculated as the mean of all 8 ROIs.

2.6. Magnetic resonance spectroscopy

Each participant underwent a ^{1}H-MRS scan on a 1.5 T Siemens system to assess IMCL and EMCL of the soleus muscle of the calf. Participants were scanned in the supine position with their right leg placed within an extremity RF coil to obtain images of the soleus muscle in which a voxel ($1.3 \times 1.3 \times 3.0\,mm^3$) was positioned while avoiding gross marbling. Localised proton spectra were acquired using a PRESS sequence (TR 2000 ms; TE 30 ms) to obtain two spectra: a water-suppressed lipid spectra and a lipid-suppressed water spectra. The water resonance was set to 4.7 ppm, the IMCL resonance was set to 1.3 ppm and the EMCL resonance was set to 1.5 ppm. MRS spectra were analysed on the Java-Based Magnetic Resonance User Interface (jMRUI) software with the inclusion of prior knowledge to assist in identification of the 4 lipid peaks, methyl and methylene IMCL and EMCL peaks [21]. IMCL and EMCL were expressed in arbitrary units as the ratio of the methylene IMCL or EMCL peaks to internal water.

2.7. Statistics

The Soul-Deep study included 20 per group to allow a difference of one standard deviation to be detected with power 90% and 2-sided significance for the primary outcome variable (beta-cell insulin secretory function). Non-normally distributed variables were log transformed to achieve a normal distribution. Significance of ethnic differences in variables was tested using an independent samples t-test. Comparison of proportions was conducted using Fisher's exact test. The strength of relationships between fat depots of interest were assessed using Pearson's correlation. The ethnic differences in relationships between fat depots of interest were examined by fitting a regression between the pairs fat depots with an interaction term for ethnicity. All statistical analyses were performed using SPSS 25.0. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Participant characteristics

The clinical characteristics of the 20 BWA and 23 WE normal glucose tolerant men are presented in Table 1. There were no significant ethnic differences in age, weight, BMI, waist circumference, HbA1c, blood pressure and cholesterol levels. Metabolic parameters including fasting insulin, fasting-glucose, 2-h glucose, HOMA2-S and HOMA2-B also showed no differences by ethnicity. Fasting triglycerides were significantly lower in the BWA men ($p = 0.005$).

3.2. Visceral and subcutaneous adipose tissue deposition

Data from the analysis of abdominal adipose tissue at the L4-5 position are presented in Fig. 1 and Table 2. There were no significant ethnic differences in abdominal SAT between the two ethnic groups ($p = 0.74$). However, VAT was significantly lower in the BWA men compared to the WE men ($p = 0.021$), and the ratio of VAT to SAT was also significantly lower in the BWA men ($p = 0.038$). There were no significant ethnic differences in dSAT ($p = 0.78$), sSAT ($p = 0.93$) or the ratio of dSAT to sSAT ($p = 0.24$).

3.3. Intrahepatic lipid, intrapancreatic lipid and intramyocelluar lipid deposition

The IHL, IPL and IMCL data are presented in Fig. 2. The BWA men exhibited significantly lower IHL compared to the WE men ($p = 0.044$). Non-alcoholic fatty liver disease (NAFLD), defined as a liver fat level above 5% determined by Dixon-MRI [22], was present in 10% (2/20) of the BWA men compared to 35% (8/23) in the WE men ($p = 0.08$). We found no significant ethnic differences in IPL ($p = 0.92$); further investigation of regional pancreatic fat deposition showed no significant ethnic differences in IPL in the head ($p = 0.86$), body ($p = 0.12$) and

Table 1 – Clinical characteristics of the BWA and WE men.

	BWA (n = 20)	WE (n = 23)	Mean difference (95% CI)	P
Age (years)	32 (12)	36 (14)	3.6 (−4.5 to 11.6)	0.38
Weight (kg)	85.0 (13.4)	86.5 (16.5)	1.5 (−7.8 to 10.8)	0.74
BMI (kg/m^2)	27.0 (3.4)	26.5 (4.5)	−0.42 (−2.9 to 2.1)	0.74
BMI normal/overweight/obese (n)	6/11/3	11/9/3	–	–
Whole-body fat (%)	20.9 (4.9)	20.9 (6.8)	−0.08 (−3.8 to 3.6)	0.97
Waist circumference (cm)	88.2 (8.9)	93.8 (14.6)	5.6 (−2.0 to 13.2)	0.14
Fasting Insulin (pmol/l)	59.1 (30.9)	64.4 (39.9)	5.30 (−16.9 to 27.5)	0.63
Fasting glucose (mmol/l)	5.14 (0.47)	5.20 (0.39)	0.06 (−0.21 to 0.32)	0.67
2-h glucose (mmol/l)	5.22 (1.13)	5.09 (1.26)	−0.13 (−0.87 to 0.61)	0.72
HOMA2-S	108.0 (45.7)	113.8 (61.2)	5.80 (−27.2 to 38.8)	0.73
HOMA2-B	93.1 (28.4)	97.5 (37.3)	4.40 (−16.3 to 25.1)	0.67
HbA1c IFCC (mmol/mol)	37.5 (5.2)	35.9 (2.9)	−1.6 (−4.3 to 1.0)	0.22
HbA1c DCCT (%)	5.58 (0.47)	5.44 (0.24)	−0.14 (−0.38 to 0.10)	0.25
Systolic blood pressure (mm/Hg)	122.3 (13.0)	121.9 (9.1)	−0.39 (−7.2 to 6.5)	0.91
Diastolic blood pressure (mm/Hg)	71.7 (12.0)	71.1 (8.2)	−0.52 (−6.8 to 5.8)	0.87
Total cholesterol (mmol/l)	4.36 (1.03)	4.76 (1.05)	0.40 (−0.24 to 1.04)	0.21
LDL cholesterol (mmol/l)	2.71 (0.81)	2.99 (0.82)	0.28 (−0.23 to 0.78)	0.27
HDL cholesterol (mmol/l)	1.32 (0.45)	1.27 (0.31)	−0.05 (−0.29 to 0.19)	0.67
Triglycerides (mmol/l)	0.72 (0.25)	1.10 (0.56)	0.39 (0.12–0.65)	**0.005**

Data presented as mean (SD). Differences between the two ethnic groups were determined using independent samples *t*-tests.
Abbreviations: BMI, body mass index; BWA, black west African; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; WE, white European.

tail ($p = 0.22$) regions of the pancreas, Table 2. MRS data analysis of muscle lipids showed no significant ethnic differences in IMCL ($p = 0.76$) or EMCL ($p = 0.69$), Table 2.

3.4. *Interrelationships between the ectopic fat depots*

There were ethnic differences in the strength and significance of associations between ectopic fat depots, Fig. 2 and Table 3. VAT was significantly associated with IHL in WE but not BWA men, with a significant ethnicity interaction ($P_{interaction} = 0.018$). VAT was significantly associated with IPL and IMCL in both ethnic groups. IHL was significantly associated with IPL and IMCL in WE men but not BWA men, furthermore, IPL was significantly associated with IMCL in WE men but not BWA men; there were no significant ethnicity interactions for these relationships (all $P_{interaction} > 0.05$). SAT was significantly associated with VAT and IPL in both ethnic groups, however, the associations between SAT with IHL and IMCL were significant only in WE; a significant ethnicity interaction was present only for the relationship between SAT and IHL ($P_{interaction} = 0.022$).

3.5. *Relationships between ectopic fat depots and metabolic parameters*

Relationships between ectopic fat depots and HOMA2-S and HOMA2-B are presented by ethnicity in Table 4. VAT, IHL, IPL and IMCL were all significantly inversely associated with HOMA2-S and associated with HOMA2-B in WE men but not BWA men.

4. Discussion

In this study, we have comprehensively assessed ectopic fat deposition in the liver, pancreas and skeletal muscle as well as visceral fat storage in a cohort of well-matched normal glucose tolerant black west African and white European men. We have found lower visceral and hepatic fat in BWA men compared to WE men but no ethnic differences in pancreatic and muscle lipids. While there were strong interrelationships between the ectopic fat depots in the WE men, this was not the case in the BWA men in whom we found no associations between IHL and either VAT or IPL. Additionally, all ectopic fat depots were strongly related to beta-cell function and inversely related to insulin sensitivity in WE men but not BWA men. To our knowledge this is the first study to undertake an ethnic comparison of the four principle ectopic fat depots related to T2DM in a black *vs* white population.

Our findings of lower VAT and IHL in the BWA men are consistent with earlier studies and provide further evidence for lower VAT and IHL in people of black ethnicity despite their apparently paradoxical increased risk for T2DM [15,16,23,24,33]. Our data showing a strong association between VAT and IHL in WE men provides evidence to support the portal vein theory which states that VAT is the main driver of IHL, however, this may not be the case in BWA men as we found no association between VAT and IHL in the BWA men. D'Adamo *et al.* previously reported similar findings in adolescents where IHL increased with increasing VAT in whites but not blacks [25]. The ethnic differences in the relationship between VAT and IHL may be explained by ethnic differences in dietary nutrient intake which has been suggested to influence IHL deposition [26,27]. The associations between SAT and IHL were similar to those of VAT where SAT was associated with IHL in WE but not BWA men. Since both VAT and SAT were not associated with IHL in the BWA men, it appears that the mechanisms of IHL deposition in black populations may differ from white populations and appear to be independent of VAT and SAT deposition.

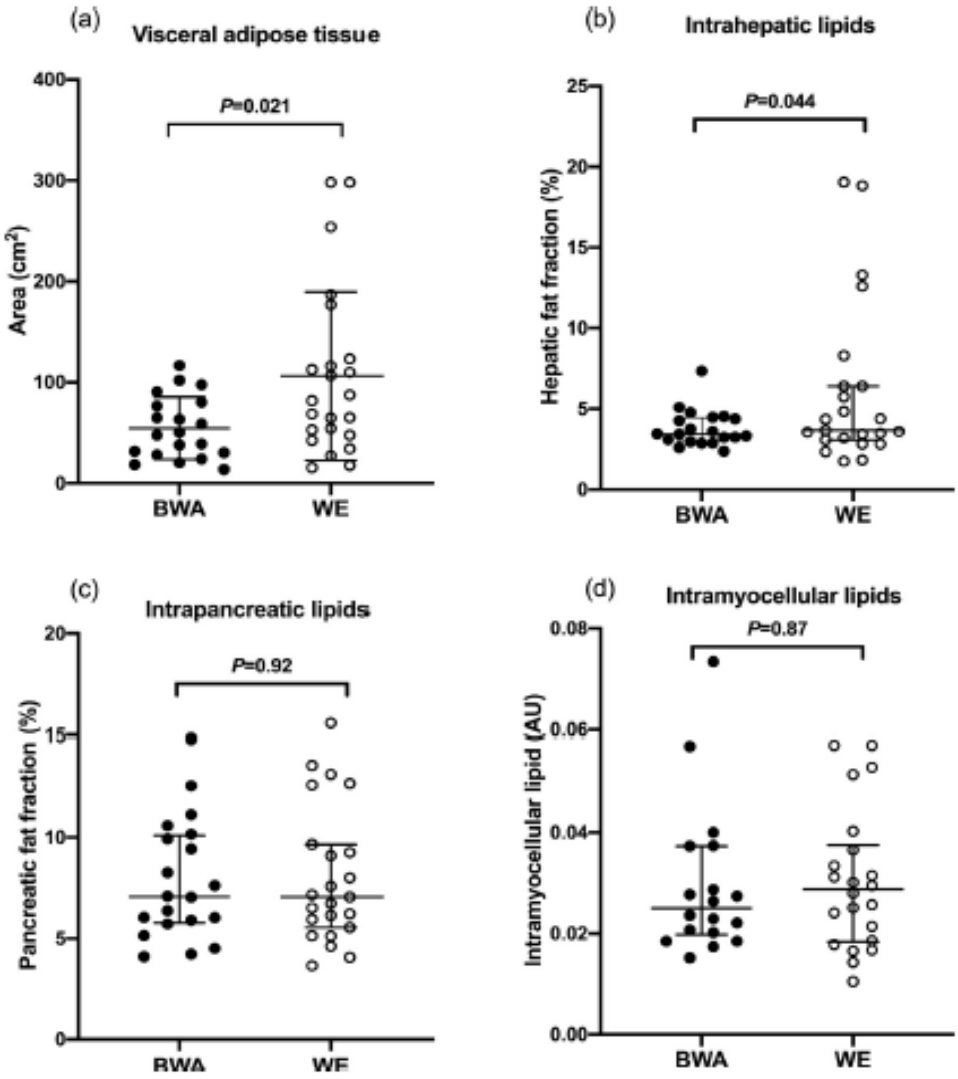

Fig. 1 – Ectopic fat deposition in white European (WE) and black west African (BWA) men, visceral adipose tissue, (a); hepatic fat fraction, (b); pancreatic fat fraction, (c); intramyocellular lipid, (d). Box and whisker plots show the median and IQ range. Ethnic differences in visceral adipose tissue and hepatic fat fraction ware determined using an independent samples t-test.

It has been suggested that the deposition of visceral and ectopic fat occurs because of a reduced capacity of SAT to expand, leading to a "spillover" of free fatty acids from the SAT to ectopic depots [1,9,11]. Our findings in WE men supports this theory as we reported strong interrelationships between all the ectopic depots in WE men, however, in BWA men other processes may contribute to ectopic fat storage as we reported weaker interrelationships between the depots, except for VAT with IPL and IMCL. Furthermore, our data suggests that organ lipotoxicity and insulin resistance due to ectopic fat storage occurs simultaneously within the muscles and liver resulting from dysfunctional SAT; this process may occur to a greater extent in WE men than BWA men. Supporting the twin-cycle hypothesis that explains IPL deposition, we showed that IHL was associated with IPL in WE men, however, this relationship was not significant in the BWA men indicating that this hypothesis may not occur or occurs to a lesser extent in black individuals.

Our data suggest that black individuals have a reduced susceptibility to store VAT as our BWA subjects had similar SAT but lower VAT compared to whites; similar findings have also been reported in black adolescents and adults [25,28] and we have found the same differences to be present in early T2DM [19]. Our finding of no ethnic differences in dSAT and sSAT in men is matched by previously reported data in black *versus* white men [29]. However, in obese populations, blacks have shown greater sSAT deposition with no differences in dSAT compared to their white counterparts indicating a preferential deposition of excess fat in the favourable sSAT depot in black populations with increasing whole-body adiposity [30,31].

We have previously shown that IPL is lower in BWA men compared to WE men with early T2DM [19], however, we found no ethnic differences in IPL in this study of normal glucose tolerant men. This disparity may indicate that WE men have a greater susceptibility to store triglycerides in

Table 2 – Fat deposition in the BWA and WE men.

	BWA (n = 20)	WE (n = 23)	Mean difference/Ratio of geometric mean (95% CI)	P
Subcutaneous adipose tissue (SAT), L4-5 (cm^2)*	181.9 (136.1–242.9)	193.2 (149.7–249.3)	1.06 (0.73–1.54)	0.74
VAT: SAT, L4-5	0.30 (0.21)	0.47 (0.29)	0.17 (0.01–0.32)	**0.038**
Deep SAT, L4-5 (cm^2)*	102.6 (72.9–144.6)	109.8 (75.7–159.3)	1.07 (0.65–1.76)	0.78
Superficial SAT, L4-5 (cm^2)*	76.6 (60.3–97.3)	75.5 (61.6–92.6)	0.87 (0.73–1.33)	0.93
Deep SAT: superficial SAT, L4-5	1.42 (0.49)	1.68 (0.84)	0.26 (−0.17 to 0.69)	0.24
EMCL (AU)[a]	0.031 (0.015)	0.033 (0.018)	0.0027 (−0.008 to 0.013)	0.61
PFF head (%)*	5.61 (4.40–7.14)	5.46 (4.41–6.76)	0.97 (0.71–1.33)	0.86
PFF body (%)*	6.33 (5.24–7.65)	6.24 (5.11–7.62)	0.99 (0.75–1.29)	0.92
PFF tail (%)*	7.25 (5.92–8.89)	7.56 (6.31–9.04)	1.04 (0.80–1.35)	0.75

Data presented as mean (SD) or geometric mean (95% CI) for log transformed data (*). Differences between the two ethnic groups were determined using independent samples t-tests.

Abbreviations: BWA, black west African; CI, confidence interval; EMCL, extramyocellular lipid; HFF, hepatic fat fraction; IMCL, intramyocellular lipid; PFF, pancreatic fat fraction; WE, white European.

[a] N for WE = 22 and BWA = 18.

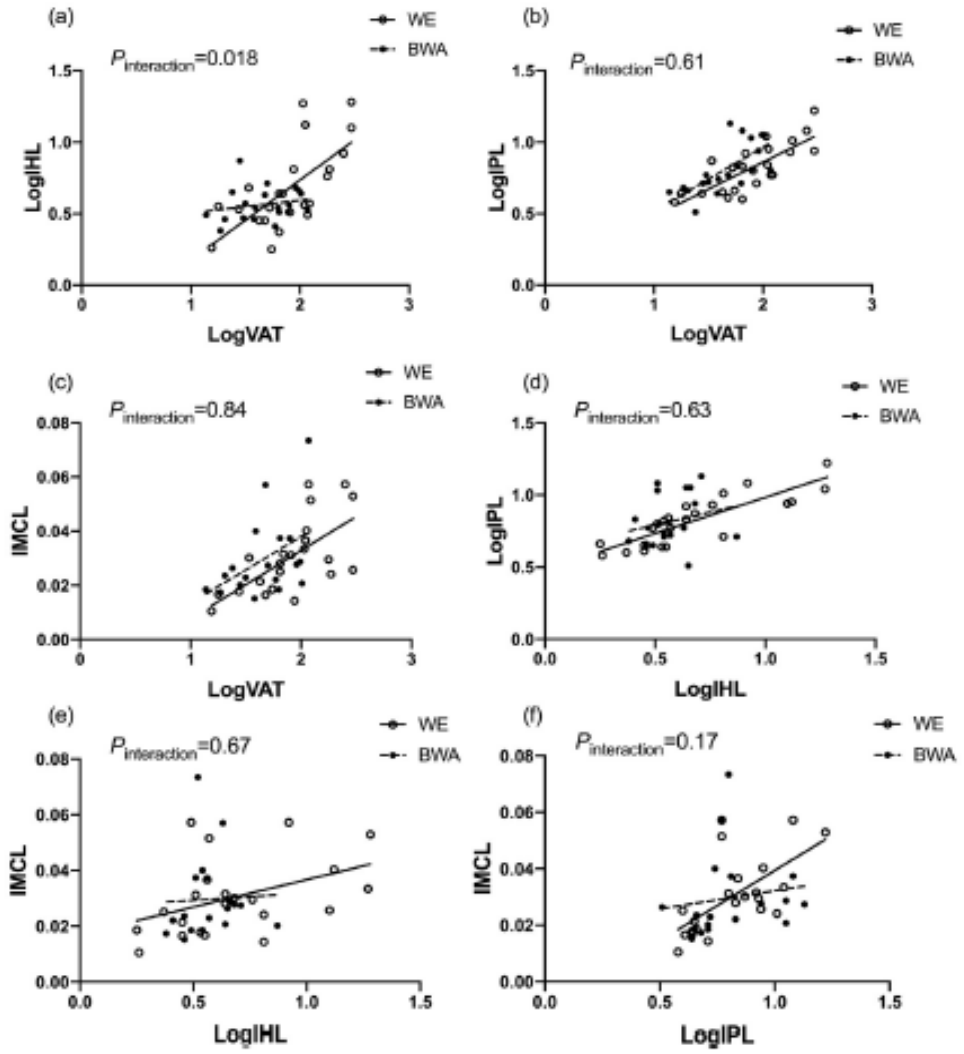

Fig. 2 – Relationships between ectopic fat depots in men of white European (WE) and black west African (BWA) ethnicity. Relationships between visceral adipose tissue (VAT) and intrahepatic lipids (IHL), (a); VAT and intrapancreatic lipids (IPL), (b); VAT and intramyocellular lipids (IMCL), (c); IHL and IPL, (d); IHL and IMCL, (e); and IPL and IMCL, (f).

Table 3 – Correlations between measures of regional adiposity and ectopic fat depots in WE and BWA men.

		SAT		VAT		IHL		IPL	
		WE	BWA	WE	BWA	WE	BWA	WE	BWA
VAT	r	**0.80**	**0.59**	–	–				
	p	**<0.001**	**0.006**	–	–				
IHL	r	**0.55**	0.15	**0.67**	0.22	–	–		
	p	**0.006**	0.53	**<0.001**	0.36	–	–		
IPL	r	**0.71**	**0.59**	**0.78**	**0.69**	**0.85**	0.24	–	–
	p	**<0.001**	**0.006**	**<0.001**	**0.001**	**<0.001**	0.31	–	–
IMCL	r	**0.65**	0.15	**0.65**	0.45	0.42	0.05	**0.63**	0.15
	p	**0.001**	0.54	**0.001**	0.059	0.051	0.86	**0.002**	0.55

Correlation coefficients determined using Pearson's correlation. SAT, VAT, IHL, and IPL were log transformed to achieve a normal distribution. N = 20 BWA and 23 WE for all correlations except for IMCL where n = 18 BWA and 22 WE.

VAT and SAT determined from an abdominal magnetic resonance image at the L4/5 position; IHL and IPL determined by magnetic resonance imaging; IMCL determined by magnetic resonance spectroscopy.

Abbreviations: BWA, black west African; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL, intrapancreatic lipid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WE, white European.

the pancreas during the development of T2DM. Our findings are supported by an investigation of IPL in black *versus* white/Hispanic women by Lingvay *et al.* who found IPL was significantly lower in black women with T2DM compared to white/Hispanic women but no ethnic differences in IPL were found in the non-diabetic lean or obese women [32]. In our data, VAT was associated with IPL in both WE and BWA men, however, VAT was associated with IHL only in the WE men indicating that the pancreas may be more susceptible to lipid accumulation compared the liver in BWA men.

The paradox of greater prevalence of T2DM but lower or similar ectopic fat in black populations compared to white populations indicates that black individuals may be more sensitive to lipotoxicity or that other factors are driving their greater risk of T2DM. Interestingly, whilst we found significant relationships between all measures of ectopic fat and insulin sensitivity (inverse correlations) and beta-cell function in WE men, these were not found in the BWA men, who had comparable insulin sensitivity and beta-cell function. These findings suggest that lipotoxicity by ectopic fat may play a lesser role in the development of T2DM in black populations compared to white populations.

Our report of lower IHL in BWA men compared to WE men but no ethnic differences in IPL may indicate that pancreatic lipotoxicity contributes more so to the pathophysiology of T2DM compared to hepatic lipotoxicity in black populations. Toledo-Corral previously showed that IPL predicted prediabetes status in black adolescents but IHL did not [34]. However, further investigations of relationships between IHL and IPL with metabolic parameters are required to confirm these speculations.

Several studies using data from large cohorts have shown a lower prevalence of NAFLD in black populations [35]. Here we have reported a lower level of NAFLD in the BWA men compared to WE men which was similar to our findings in men with T2DM [20]. An increase of IHL has been linked with the upregulation of hepatic lipogenic processes resulting in hyperlipidaemia [36]. The lower IHL reported in blacks may explain the commonly reported favourable lipid levels in black populations compared with their white counterparts [37,38].

Our finding of no ethnic differences in IMCL supports previous studies conducted in adults and adolescents [30,39]. In studies investigating the relationships between IMCL and metabolic function, Ingram *et al.* showed IMCL was inversely associated with muscle insulin sensitivity in white adults but not black adults which indicates that IMCL deposition may play a less important role in insulin resistance in black populations [39]. Furthermore, studies comparing ectopic fat deposition between white and black groups have been studied more extensively in adolescents and female populations with a lack of studies being conducted in black men [15,16,40].

Our study has several strengths worth noting, including the analysis of VAT, IHL and IPL using MRI, which is considered to be the gold-standard non-invasive method to quantify these ectopic fat depots. Our two ethnic groups were well matched for age and BMI, furthermore, we determined metabolic status using an OGTT to include only normal glucose tolerant individuals. Another strength is the inclusion of only men in this study as there is consistent evidence that the pathophysiology of T2DM differs between men and women in both white and black ethnic groups [41]. Furthermore, studies comparing ectopic fat deposition between white and black groups have been studied more extensively in adolescents and female populations with a lack of studies being conducted in black men [15,16,40].

The limitations of this study include the lack of whole body SAT analysis using MRI. Although we have reported percentage whole body fat using bio-electric impedance, this method, which estimates body fat through the use of regression equations, does not account for ethnic variations in body composition [42,43]. Our study was conducted on a small sample size, although, is comparable in size to other studies conducted on ectopic fat in a black *versus* white population. Our study lacks data on markers of adipose tissue dysfunction such as pro-inflammatory cytokines which may differ by ethnicity as others have shown [44,45]; hence, investigating these markers may provide insight into the ethnic differences in ectopic fat deposition and is suggested for future studies. Another limitation is that the present study is restricted to a cohort of men with normal glucose tolerance and who may not be destined to develop T2DM. We propose

Table 4 – Correlations between ectopic fat depots and fasting measures of insulin sensitivity and beta-cell function in WE and BWA men.

		HOMA2-S		HOMA2-B	
		WE	BWA	WE	BWA
VAT	r	**−0.57**	−0.37	**0.68**	0.07
	p	**0.003**	0.11	**0.001**	0.77
IHL	r	**−0.66**	−0.27	**0.73**	0.34
	p	**0.001**	0.25	**<0.001**	0.14
IPL	r	**−0.57**	−0.11	**0.66**	−0.19
	p	**0.004**	0.64	**0.001**	0.41
IMCL	r	**−0.62**	−0.06	**0.61**	−0.14
	p	**0.002**	0.82	**0.003**	0.58

Correlation coefficients determined using Pearson's correlation. VAT, IHL, IPL and IMCL were log transformed to achieve a normal distribution. N = 20 BWA and 23 WE for all correlations except for IMCL where n = 18 BWA and 22 WE.

VAT and SAT determined from an abdominal magnetic resonance image at the L4/5 position; IHL and IPL determined by magnetic resonance imaging; IMCL determined by magnetic resonance spectroscopy.

Abbreviations: BWA, black west African; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL, intrapancreatic lipid; VAT, visceral adipose tissue; WE, white European.

further work must be done to investigate relationships between ectopic fat depots and metabolic parameters in each ethnic group.

In conclusion, despite the greater risk of T2DM in black populations, the BWA men exhibited lower levels of visceral adipose tissue and hepatic lipids compared to WE men. Additionally, the lack of association between ectopic fat depots and insulin resistance and beta-cell function in BWA men, which were present in WE men, may indicate that ectopic fat deposition plays a lesser role in the pathophysiology of T2DM in black men compared to white men. Furthermore, the lack of significant interrelationships between ectopic fat depots in BWA men compared to WE men indicates that current theories that describe the mechanisms of ectopic fat deposition may not apply to black populations, which warrants further investigation.

Author contributions

L.M.G. formulated the research question and designed the study, supervised data collection and interpretation. S.A.A. formulated the research question and designed the study. J.L.P. formulated the research question, designed the study, and provided statistical advice. A.M.U. formulated the research question and designed the study. O.B. supervised data collection and performed the metabolic assessments. M.L. supervised data collection and performed the metabolic assessments. H.S. conducted MRI data analysis. D.C. conducted MRI data analysis. E.S. conducted MRI data analysis. G.C.E. coordinated MRI data acquisition. O.H. undertook data analysis and interpretation and drafted the manuscript. All authors contributed to the intellectual content and reviewed the final version of the submitted manuscript.

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Louise Goff is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis.

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Declaration of Competing Interest

The authors declare that there is no duality associated with this manuscript.

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APPENDIX II

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BRIEF REPORT

WILEY

Ethnic differences in intrahepatic lipid and its association with hepatic insulin sensitivity and insulin clearance between men of black and white ethnicity with early type 2 diabetes

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Abstract

Intrahepatic lipid (IHL) is linked with reduced hepatic insulin sensitivity and insulin clearance. Despite their high risk for type 2 diabetes (T2D), there have been limited investigations of these relationships in black populations. We investigated these relationships in 18 white European (WE) and 18 black West African (BWA) men with T2D <5 years. They underwent magnetic resonance imaging to quantify IHL, a hyperinsulinemic euglycaemic clamp with [6,6 $^2H_2$] glucose infusion to assess hepatic insulin sensitivity and a hyperglycaemic clamp to assess insulin clearance. BWA men had lower IHL than WE men (3.7 [5.3] vs 6.6 [10.6]%, $P = 0.03$). IHL was inversely associated with basal hepatic insulin sensitivity in WE but not BWA men (BWA: $r = -0.01$, $P = 0.96$; WE: $r = -0.72$, $P = 0.006$) with a significant interaction by ethnicity ($P_{interaction} = 0.05$); however, IHL was not associated with % suppression of endogenous glucose production by insulin in either ethnicity. IHL showed a trend to an association with insulin clearance in BWA only (BWA: $r = -0.42$, $P = 0.09$; WE: $r = -0.14$, $P = 0.58$). The lack of association between IHL and hepatic insulin sensitivity in BWA men indicates IHL may play a lesser detrimental role in T2D in BWA men.

KEYWORDS
African, ethnicity, hepatic fat, insulin clearance, insulin sensitivity, lipotoxicity

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Peer Review
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1 | INTRODUCTION

Black populations are disproportionately affected by type 2 diabetes (T2D), with 2–3 times greater prevalence compared with white populations[1] despite typically having lower intrahepatic lipids (IHL) and visceral adipose tissue (VAT).[1,2] IHL is usually elevated in individuals with T2D and is inversely associated with both hepatic insulin sensitivity and insulin clearance.[3] Consistently, insulin clearance has been shown to be lower in black populations compared with white populations.[4] However, investigations of ethnic differences in hepatic insulin sensitivity have shown inconsistent findings.[5-7] We have previously reported similar hepatic insulin sensitivity but lower insulin clearance in black West African (BWA) compared with white European (WE) men.[8,9] Despite the literature reporting ethnic differences in IHL, hepatic insulin sensitivity and insulin clearance between black and white populations, these have not previously been investigated in a single study to understand their relationships and how ethnicity impacts on these in the development of T2D in black populations. Therefore, our aim was to investigate ethnic differences in IHL and its relationship with hepatic insulin sensitivity and insulin clearance in BWA and WE men with early T2D.

2 | METHODS

This investigation was conducted as part of the South London Diabetes and Ethnicity Phenotyping study (Soul-Deep).[10] Data on metabolic variables for 92% of the present cohort have been previously reported[8,9]; the present analyses relate to the whole cohort in whom relevant data were available. Participant recruitment and data collection took place from April 2013 to January 2015. The study was approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided written informed consent.

2.1 | Participants

Participants were recruited from primary care practices in London and deemed eligible to participate if they (a) were aged 18–65 years, (b) had a body mass index (BMI) of 25–40 kg m^{-2}, (c) self-reported WE or BWA ethnicity, (d) had a diagnosis of T2D (<5 years), and (e) were treated with lifestyle and/or metformin only. Further details of eligibility criteria are published in the protocol.[10] Participants attended all assessments after an overnight fast. If on metformin, participants were instructed to cease taking it for 7 days prior to each visit. Physical activity was measured as hours per day of moderate intensity activity using accelerometry watches worn for 4 consecutive days (MotionWatch 8.0, CamTech, Boerne, Texas).

2.2 | Magnetic resonance imaging

A Dixon-based magnetic resonance imaging (MRI) sequence was used on a 1.5 Tesla Siemens scanner to obtain images for the quantification of IHL and VAT. Participants were scanned lying supine on a spine RF coil with body phased array RF coils placed over the chest, abdomen and pelvis. While abdominal images were acquired, participants were instructed to complete three 17-second breath-holds. From each participant, contiguous, axial T1-weighted gradient-echo images (repetition time: 6.77 ms; echo times: 4.77 ms [in-phase], 2.39 ms [out-of-phase]; flip angle: 10°) each with a slice thickness of 3 mm were acquired, from which water and fat images were produced. Images were analyzed using HOROS v.1.1.7 (www.horosproject.org). IHL was measured by selecting two abdominal MRI images representing the superior and inferior parts of the liver. Four circular regions of interest (ROIs) in identical positions were placed within the liver tissue of each pair of water and fat images (Figure S1). ROIs were positioned to include the posterior, anterior, medial and lateral sections of the liver. ROI areas ranged from 20 to 30 cm^2, intending to cover as large an area of liver as possible while avoiding blood vessels, bile ducts and artefacts. Using the formula: % IHL = (F/[F + W])*100, where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image, the hepatic fat fraction was calculated in each ROI and IHL was calculated as the mean of all eight ROIs. Total abdominal VAT and body subcutaneous adipose tissue (SAT) (neck to knee, excluding arms) was determined using an automated MRI analysis technique (Klarismo Ltd, London, UK) as previously described.[11]

2.3 | Clamp assessments

Whole-body insulin sensitivity (M-value) during the high dose insulin infusion (40 mU m^{-2} body surface area [BSA] min^{-1}), hepatic insulin sensitivity (% suppression of endogenous glucose production [EGP] during the low dose insulin infusion [10 mU m^{-2} BSA min^{-1}]) and basal hepatic insulin sensitivity index were measured using a two-step hyperinsulinemic euglycaemic clamp with the infusion of [6,6 $^2H_2$] glucose,

according to previously described methodology.[9] The basal hepatic insulin sensitivity index was calculated as the reciprocal of the product of basal EGP rate (mmol/BSA min^{-1}) and fasting insulin concentration (pmol L^{-1}). To assess and model insulin clearance, each participant underwent a hyperglycaemic clamp, described in detail elsewhere.[8]

2.4 | Statistical analysis

Ethnic differences were determined using independent samples t-test for normally distributed variables or a Mann–Whitney test for variables that could not be log-transformed to normal. ANCOVA was used, with VAT, BMI and age as separate covariates, to investigate ethnic differences in IHL and VAT. Correlations were assessed using Pearson's correlation; partial correlation was used to investigate associations while adjusting for VAT, BMI and age. Significance of an interaction by ethnicity was assessed using multiple regression with ethnicity*logIHL used as an interaction term. Analyses were conducted with SPSS version 25.0; $P \leq 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Participant characteristics

The 18 BWA and 18 WE men were well matched for age and BMI (Table 1). The BWA men had significantly lower IHL and total VAT mass (Table 1, Figure S2). After adjustment for BMI, the ethnic differences in VAT remained significant ($P = 0.008$) but not for IHL ($P = 0.18$). After adjustment for VAT, there were no ethnic differences in IHL (WE: 6.07 [SE 1.16] vs BWA: 5.56 [SE 1.16]%, $P = 0.70$). Non-alcoholic fatty liver disease (NAFLD), defined as liver fat above 5% determined by Dixon-MRI,[12] was present in 33% of BWA men compared with 67% of WE men ($P = 0.047$).

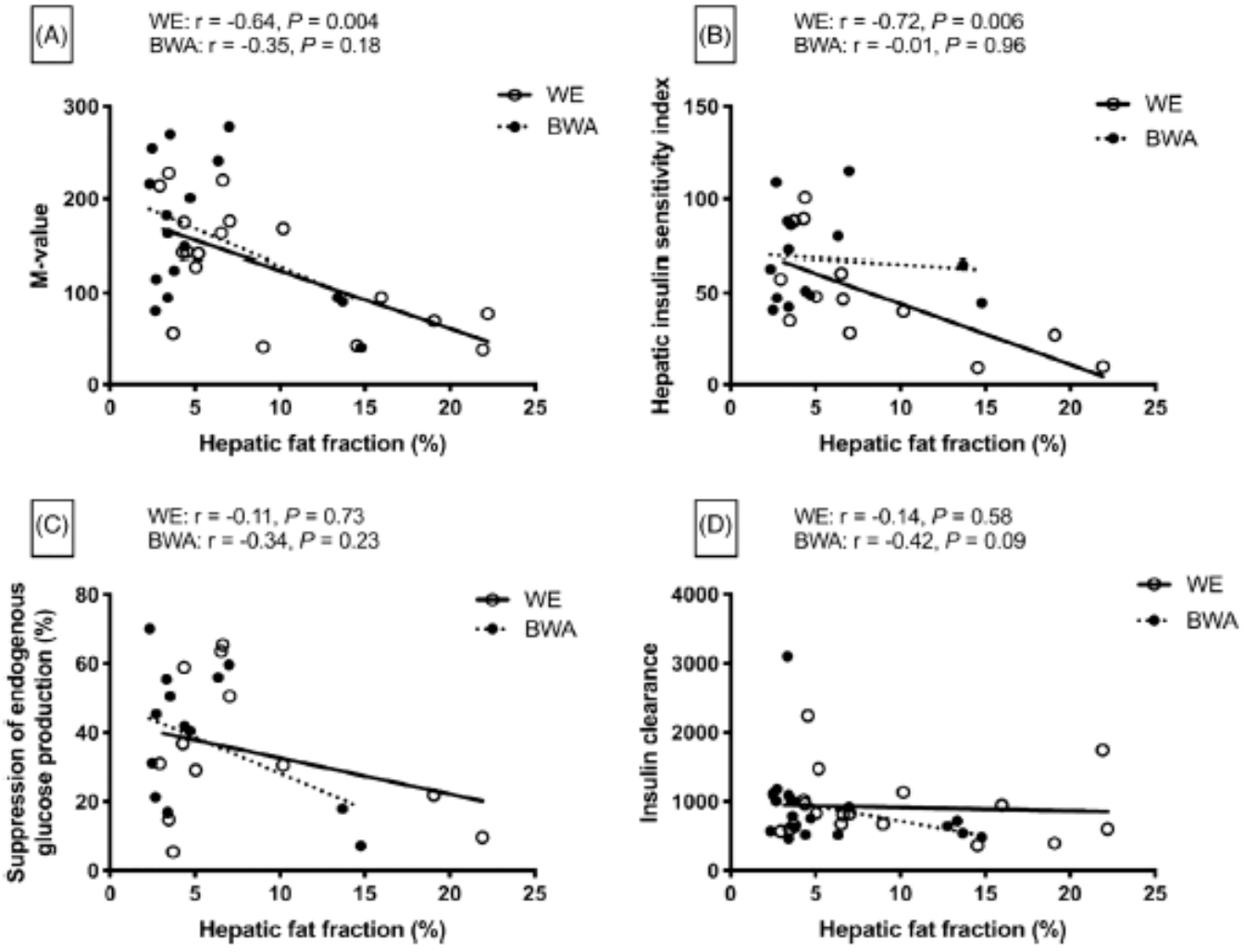

FIGURE 1 Relationships between hepatic fat fraction and A, hepatic insulin sensitivity index (basal) ([mmol m^{-2} body surface area min pmol $L]^{-1}$), B, suppression of hepatic glucose production (%), C, whole-body insulin sensitivity (M-value) (mg m^{-2} body surface area min^{-1}), and D, insulin clearance (mL m^{-2} body surface area min^{-1}) in white European (WE) and black West African (BWA) men. Relationships between hepatic insulin clearance and E, suppression of endogenous glucose production, and F, hepatic insulin sensitivity index (basal) in WE and BWA men. Black circles with dotted line = BWA men; white circles with solid line = WE men

TABLE 1 Clinical and metabolic characteristics of black West African and white European men

	BWA (n = 18)	WE (n = 18)	P
Age (years)†	54.9 (9.3)	58.5 (6.3)	0.67
Body weight (kg)	92.3 ± 12.3	99.8 ± 16.7	0.14
BMI (kg/m^2)	29.8 ± 3.5	31.5 ± 4.1	0.18
Waist circumference (cm)	104.9 ± 10.2	111.9 ± 13.0	0.08
SAT (neck to knee) (kg)*‡	12.6 (10.5–15.2)	14.6 (12.2–17.6)	0.24
VAT, total (kg)[b]	3.99 ± 1.54	6.09 ± 2.46	**0.006**
IHL (%)†	3.7 (5.3)	6.6 (10.6)	**0.03**
Diabetes duration (years)†	3.0 (2.2)	3.0 (1.3)	0.42
Statin use§	10/18	16/18	**0.026**
Fasting glucose (mmol L^{-1})	6.63 ± 0.67	6.88 ± 1.38	0.50
HbA1c (%)	6.67 ± 0.68	6.64 ± 0.70	0.90
ALT‡ (IU L^{-1})	26.7 (21.8–32.5)	31.2 (25.7–37.7)	0.24
Systolic BP (mm Hg)	136.7 ± 13.8	130.9 ± 14.2	0.22
Diastolic BP (mm Hg)†	89.0 (8.7)	83.0 (12.5)	0.06
Total cholesterol (mmol L^{-1})	4.11 ± 0.73	4.27 ± 0.70	0.50
LDL-cholesterol (mmol L^{-1})	2.32 ± 0.56	2.28 ± 0.66	0.85
HDL-cholesterol (mmol L^{-1})	1.18 ± 0.38	1.19 ± 0.25	0.92
Triglyceride (mmol L^{-1})†	1.05 (0.70)	1.60 (1.25)	**0.03**
Moderate activity time (h/d)[c]	2.1 ± 0.66	1.9 ± 0.90	0.74
Metabolic characteristics			
M-value (mg m^{-2} BSA min^{-1})[d]	162.0 ± 75.0	128.5 ± 63.7	0.17
Hepatic basal insulin sensitivity index ([mmol m^{-2} BSA min pmol L]$^{-1}$)*	68.0 ± 24.6	49.1 ± 29.4	0.09
Suppression of endogenous glucose production (%)*	37.9 ± 19.5	34.7 ± 20.7	0.70
Average insulin clearance (mL m^{-2} BSA min^{-1})†	732.8 (505.7)	814.6 (450.2)	0.61

Note: Data presented as mean ± SD or geometric mean (95% CI) for log-transformed data (‡) or median (interquartile range) for non-variable data (†) or number of participants for ordinal data (§). *P*-values determined using independent samples t-tests for normally distributed data, Mann–Whitney test for non-variable data or chi-squared test for ordinal data. Bold font was used to highlight findings with a statistically significant probability. N for [a]WE = 17, BWA = 16; [b]WE = 17, BWA = 17; [c]WE = 10, BWA = 7; [d]WE = 18, BWA = 16; [e]WE = 12, BWA = 14.

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; BSA, body surface area; BWA, black West African; HDL, high density lipoprotein; IHL, intrahepatic lipid; LDL, low density lipoprotein; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WE, white European.

3.2 | Metabolic characteristics

There were no ethnic differences in whole-body insulin sensitivity (M-value) or hepatic insulin sensitivity, expressed as % suppression of EGP during the low-dose insulin infusion (Table 1), consistent with earlier findings we reported from a smaller sample from this cohort.[9] However, there was a trend towards higher basal hepatic insulin sensitivity index in the BWA men (Table 1). Insulin clearance was not different between BWA and WE men (Table 1), which was again consistent with our earlier report.[8]

3.3 | Relationships between IHL and insulin sensitivity

Relationships between IHL and the measures of insulin sensitivity are presented in Figure 1A-C. The inverse associations between IHL and both whole-body insulin sensitivity (M-value) and basal hepatic insulin sensitivity reached statistical significance only in WE men. In multiple regression analysis a significant ethnicity interaction was found in the relationship between IHL and basal hepatic insulin sensitivity ($P_{interaction}$ = 0.05); no other significant ethnicity interactions were found. There were no changes in the associations after adjustment for VAT, BMI or age, except for the relationship between IHL and M-value, which reduced in significance in WE men after adjustment for BMI (*P* = 0.13) (Table S1).

3.4 | Relationships between IHL and insulin clearance

Relationships between IHL and insulin clearance are presented in Figure 1D. IHL was inversely associated with insulin clearance, which neared significance in BWA but not WE men; partial correlation adjusting for VAT reduced the significance of this relationship (BWA:

r = −0.41, P = 0.11; WE: r = −0.29, P = 0.27); no significant ethnicity interaction was found ($P_{interaction}$ = 0.40).

4 | DISCUSSION

In this study of WE and BWA men with early T2D, we investigated ethnic differences in hepatic fat and its relationship with hepatic insulin sensitivity and insulin clearance. Consistent with published data,[13] BWA men had lower IHL and VAT. We found additional ethnic differences in relationships between IHL and hepatic insulin sensitivity and insulin clearance, whereby in WE men IHL was inversely related to basal hepatic insulin sensitivity and whole-body insulin sensitivity, which was not the case in BWA men. In BWA men we found a trend towards an inverse relationship between IHL and insulin clearance, which was not found in the WE men. Our findings suggest that IHL is implicated in the metabolic derangements of the liver in T2D differently according to ethnicity. To our knowledge, this is the first study to investigate relationships between IHL and insulin clearance in a black population; the trend towards an inverse relationship in BWA but not WE men suggests that the reduction of insulin clearance may be modulated differently depending on ethnicity.

Despite relationships between IHL and whole-body insulin resistance being commonly reported, the mechanisms that link the two are less well understood. Current investigations show an excess of liver fat leads to an accumulation of lipid intermediates causing hepatic mitochondrial dysfunction, inflammation and increased very low density lipoprotein - triacylglycerol production which may result in hepatic and systemic insulin resistance.[14] Our finding of IHL being inversely associated with basal hepatic insulin sensitivity and whole-body insulin sensitivity, which reached significance in WE but not BWA men, may indicate the above detrimental effects of lipid intermediates occurring to a greater extent in WE men.

There was no relationship between IHL and suppression of EGP in either ethnic group. This could indicate a decreased effect of IHL on hepatic insulin sensitivity in the insulin-stimulated state compared with the basal state in WE men. To our knowledge only one other study has investigated the relationship between IHL and hepatic insulin sensitivity using the hyperinsulinemic euglycaemic clamp with infusion of isotopically labelled glucose[6]; the authors found that IHL was associated with hepatic insulin sensitivity in obese black South African women but not in obese white South African women, which contradicts our findings. There are several potential explanations for this, such as glycaemic state; our study included participants with T2D whereas the South African women were normal glucose-tolerant. The disparities may also be a result of gender differences, as there is consistent evidence showing that the phenotype of T2D differs by gender within populations of African descent.[15]

The presence of NAFLD was comparable with that reported in other large multiethnic cohorts[16] and was significantly lower in the BWA men. One of the main theories that explains how IHL accumulates is the portal theory, which states that excess VAT releases free fatty acids directly into the portal vein, subsequently depositing as IHL.[17] Our study may support the portal theory as after adjustment for VAT, IHL no longer differed by ethnicity, suggesting that the lower IHL in BWA men may be driven by lower VAT. Indeed, ethnic differences in the mechanisms of SAT expansion may explain the differences we found in VAT, as others have suggested[18]; however, we did not directly measure adipogenesis in our study, which may be an indication for further research.

The strengths of this study include the use of the rigorous hyperinsulinemic euglycaemic clamp method combined with the infusion of [6,6 $^2H_2$] glucose to determine both whole-body and hepatic insulin sensitivity. However, our study is not without its limitations. Our sample size is small; in these secondary analyses we may not have sufficient power to reliably detect ethnic differences. Our measurement of insulin clearance does not differentiate hepatic from extrahepatic insulin clearance; rather it is a measure of whole-body insulin clearance. However, it has been shown that ~80% of endogenous insulin is degraded in the liver.[3] Our WE men had greater statin use, which may have resulted in lower hepatic fat accumulation and reduced the ethnic discrepancies because of the lipid-lowering effects of statins. Another limitation is studying only men with T2D; however, previous studies have mostly focused on women, because of the greater prevalence of T2D in black women compared with men. Our study redresses this.

In conclusion, our study shows ethnic differences in the relationships between IHL and metabolic variables of the liver. The lack of inverse association between IHL and basal hepatic insulin sensitivity in the BWA men, found in the WE men, suggests that fasting hepatic insulin resistance occurs independently of IHL in BWA men. However, the reduction of insulin clearance may be influenced by IHL more so in black men with T2D compared with white men.

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Louise Goff is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis.

CONFLICT OF INTEREST

The authors declare that there is no duality associated with this manuscript.

AUTHOR CONTRIBUTIONS

L.M.G. formulated the research question and designed the study, supervised data collection and interpretation, and performed the minimal modelling analysis. S.A.A. formulated the research question and designed the study, and supervised data collection and interpretation. J.L.P. formulated the research question, designed the study, and provided statistical advice. A.M.U. formulated the research question and designed the study. K.G.M.M.A. supervised data collection and interpretation. C.M. coordinated the study and data acquisition, and performed the metabolic assessments. O.B. undertook data acquisition and analysis. G.C.E. coordinated MRI data acquisition. B.W. and H.S. undertook MRI data analysis. F.S.M. and N.J. undertook data acquisition. R.C.B. and L.B. performed the modelling analysis. O.H. undertook data analysis, statistical analysis and drafted the manuscript. All authors contributed to the intellectual content and reviewed the final version of the submitted manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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APPENDIX III

CLINICAL RESEARCH ARTICLE

Associations Between Pancreatic Lipids and β-Cell Function in Black African and White European Men With Type 2 Diabetes

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Context: Intrapancreatic lipid (IPL) has been linked to β-cell dysfunction. Black populations disproportionately develop type 2 diabetes (T2D) and show distinctions in β-cell function compared with white populations.

Objective: We quantified IPL in white European (WE) and black West African (BWA) men with early T2D and investigated the relationships between IPL and β-cell insulin secretory function (ISF).

Design, Setting, and Participants: We performed a cross-sectional assessment of 18 WE and 19 BWA middle-age men with early T2D as part of the South London Diabetes and Ethnicity Phenotyping study.

Main Outcome Measures: The participants underwent Dixon MRI to determine IPL in the pancreatic head, body, and tail and subcutaneous and visceral adipose tissue volumes. Modeled first- and second-phase ISFs were comprehensively determined using C-peptide measurements during a 3-hour meal tolerance test and a 2-hour hyperglycemic clamp test.

Results: The WE men had greater mean IPL levels compared with BWA men ($P = 0.029$), mainly owing to greater IPL levels in the pancreatic head ($P = 0.009$). The mean IPL level was inversely associated with orally stimulated first-phase ISF in WE but not BWA men (WE, $r = -0.554$, $P = 0.026$; BWA, $r = -0.183$, $P = 0.468$). No association was found with orally stimulated second-phase ISF in either WE or BWA men. No associations were found between the mean IPL level and intravenously stimulated ISF.

Conclusions: The IPL levels were lower in BWA than WE men with early T2D, and the lack of inverse association with first-phase ISF in BWA men indicates that IPL might be a less important determinant of the development of T2D in BWA than in WE men. (***J Clin Endocrinol Metab*** **104: 1201–1210, 2019)**

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Abbreviations: BWA, black West African; IAUC, incremental area under the curve; IHL, intrahepatic lipid; IPL, intrapancreatic lipid; IPL_{HEAD}, intrapancreatic lipid of pancreatic head; IPL_{BODY}, intrapancreatic lipid of pancreatic body; IPL_{MEAN}, mean intrapancreatic lipid; IPL_{TAIL}, intrapancreatic lipid of pancreatic tail; ISF, insulin secretory function; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WE, white European; σ^1, first-phase insulin secretory function; σ^2, second-phase insulin secretory function.

Type 2 diabetes (T2D) is more prevalent and develops at a younger age among populations of black compared with white European (WE) ethnicity (1, 2). The pathophysiological processes of T2D are well documented and include insulin resistance, ectopic fat deposition, and pancreatic β-cell dysfunction (3). The role of pancreatic lipid accumulation in the development of T2D has been receiving increasing attention. Through the process of lipotoxicity, intrapancreatic lipid (IPL) is believed to cause β-cell damage (4, 5) through the release of lipid intermediates and free fatty acids, which interfere with cellular signaling and cause β-cell apoptosis (6). *In vivo* studies have shown consistently that IPL is inversely associated with insulin secretory function (ISF), specifically, the first-phase response (4, 7). Similar to visceral adipose tissue (VAT) and intrahepatic lipid (IHL), IPL has been found to be elevated in individuals with T2D (8, 9). Also, in studies investigating reversal of T2D, mobilization of IPL appears to be a key component for achieving normalization of glycemia (10).

Increasing evidence has shown distinctions in the pathophysiology of T2D in populations of African ancestry. Typically, lower levels of VAT and IHL have been reported compared with those in white populations (11). Additionally, β-cell dysfunction will be more evident. A greater insulin response to glucose stimulation has been consistently reported among healthy and prediabetic black populations compared with those of white ethnicity (12, 13). Also, in the diabetic state, black men might have lower insulin secretion compared with white men in response to both oral and intravenous glucose (14). To date, investigation of the effect of black ethnicity on IPL levels and its relationship to the metabolic abnormalities of T2D has been limited. However, given the lesser deposition of VAT and greater β-cell dysfunction typically observed in black populations, it is reasonable to hypothesize that IPL, and its role in the pathophysiology of T2D, might differ by ethnicity. A small number of studies of healthy adults and adolescents have reported lower IPL levels among black populations compared with other ethnic groups (7, 15, 16). Also, in a comparison of healthy and prediabetic adolescents of black and Hispanic ethnicity, the IPL level was found to be the strongest predictor of prediabetes in the black population but not the Hispanic population (17). Investigations of the effect of IPLs on β-cell function in black populations have shown inconsistent findings. Although no relationship was found between IPL levels and insulin secretory function in adolescents, studies of healthy adults have shown that IPL is more strongly associated with β-cell function in black populations compared with other ethnic groups (7, 15). To date, studies of IPL and β-cell function in black populations have been limited to healthy cohorts with limited development of the metabolic abnormalities of T2D. Furthermore, these studies have assessed only the first-phase insulin secretory response. Also, only indirect assessments of insulin secretion have been performed, which have limited utility in black populations because they do not account for hepatic insulin clearance, for which ethnic differences are well established (18). We recently reported deficits in second-phase insulin secretory function through comprehensive modeling of C-peptide, present in black African but not WE men with early T2D (14). The aim of the present study was to assess IPLs and investigate the relationship with first- and second-phase ISF, assessed comprehensively using C-peptide modeling, to explore the hypothesis that men of black (West) African (BWA) ethnicity with early T2D will have lower IPL levels than those of WE ethnicity.

Materials and Methods

The present investigation was conducted as a part of the South London Diabetes and Ethnicity Phenotyping study (Soul-Deep), details of which have been previously reported (19). The present study was conducted at King's College Hospital and Guy's Hospital, London, and approved by the London Bridge National Research Ethics Committee (approval no. 12/LO/1859). Also, all participants provided written informed consent. Recruitment and data collection were performed from April 2013 to January 2015.

Participants

Potential participants were identified through primary care practices in South London. Men of WE or BWA ethnicity were recruited. Ethnicity was self-declared and confirmed through grandparent birthplace, where the countries included were North West European and West African countries, defined by the United Nations Statistics Division. The participants also provided information on their birthplace. Eligibility was confirmed in a screening visit, and participants were considered eligible if they had met the following criteria: age, 18 to 65 years; body mass index, 20 to 40 kg/m^2; a recent diagnosis of T2D (<5 years before starting the study); using only lifestyle alone or metformin to manage T2D. The exclusion criteria included the use of thiazolidinedione, insulin, oral steroids, β-blockers, or other medication that can affect the study outcome; a contraindication for MRI, such as metal implants; kidney or liver damage identified by a serum creatinine of >150 mmol/L or serum alanine transaminase level increased >2.5-fold greater than the upper limit of the reference range.

Procedures

The participants attended 3 assessment visits in random order within a maximum period of 6 months. Each participant underwent MRI for assessment of IPL, a 2-hour hyperglycemic clamp, and a mixed meal tolerance test for assessment of ISF through the measurement and mathematical modeling of C-peptide. For each assessment, the participants presented in a fasted state, having refrained from eating or drinking anything

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other than water from 10 PM the night before. The participants were instructed to refrain from strenuous exercise and physical activity in the 48 hours preceding the visit, refrain from consuming alcohol in the 24 hours preceding the visit, and to consume a standardized diet the day before (~50% of calories from carbohydrates, evenly spread throughout the day, with no >30% of daily carbohydrates consumed in the evening meal). The participants taking metformin were instructed to cease taking it for 7 days before each visit.

MRI fat quantification

A Dixon-based MRI sequence was used on a 1.5 Tesla Siemens scanner to acquire images for the assessment of IPL. With the participant lying supine, images were obtained from the neck to the knee (excluding the arms), with coils placed on the abdominal region. During acquisition of the abdominal images, on instruction by the radiographer, the participants undertook three 15-second breath holds to reduce the occurrence of motion artifacts. For each participant, 320 contiguous, 3-mm slice thickness, T1-weighted transverse spin-echo images [repetition time, 6.77 ms; echo time, 4.77 ms (in-phase), 2.39 ms (out-of-phase), flip angle, 10°] were obtained.

Intrapancreatic fat was determined by analysis of MRI scans using HOROS, version 1.1.7, software (available at: www.horosproject.org; accessed December 21, 2017) by locating one or more axial images with the largest area of the head, body, and tail of the pancreas and extracting the corresponding fat and water images. On each of the fat and water images, one circular region of interest of 1 cm^2 was drawn on each of the head, body, and tail of the pancreas (Fig. 1). A region of 1 cm^2 was used as recommended by a recent review of MRI methods used to determine IPL, because it ensures the regions are within the border of the pancreas and avoids inclusion of VAT and the splenic vein (9). Using the following formula, %IPL = [F/(F+W)] × 100, where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image, the pancreatic fat fraction was calculated in each region, termed the IPL_{HEAD}, IPL_{BODY}, and IPL_{TAIL}. The IPL_{MEAN} was calculated as the average of the head, body and tail regions. Owing to the subjective nature of locating the regions of interest on the head, body and tail of the pancreas, IPL quantification was conducted by two independent investigators with a statistically significant correlation reported ($r = 0.62$; $P < 0.001$) and an interobserver coefficient of variation of 14%.

The total abdominal VAT mass and subcutaneous adipose tissue (SAT) mass from the neck to the knee region (excluding the arms) were quantified using an automated analysis method (Klarismo Ltd., London, United Kingdom). For VAT mass quantification, each image in the abdominal region was analyzed for the VAT area, and the area was multiplied by the slice thickness of 3 mm to determine the volume of VAT. For SAT mass quantification, all MRI scans acquired were analyzed for the SAT area, which was multiplied by the slice thickness of 3 mm to determine the volume of SAT. The volume of VAT and SAT were converted from cubic milliliters to liters and then converted to kilograms by multiplying by 0.9 kg/L (the density of fat) (20).

Insulin secretory function during hyperglycemic clamp

A 2-hour hyperglycemic clamp was conducted for assessment of ISF (21). After collection of three basal blood samples, a

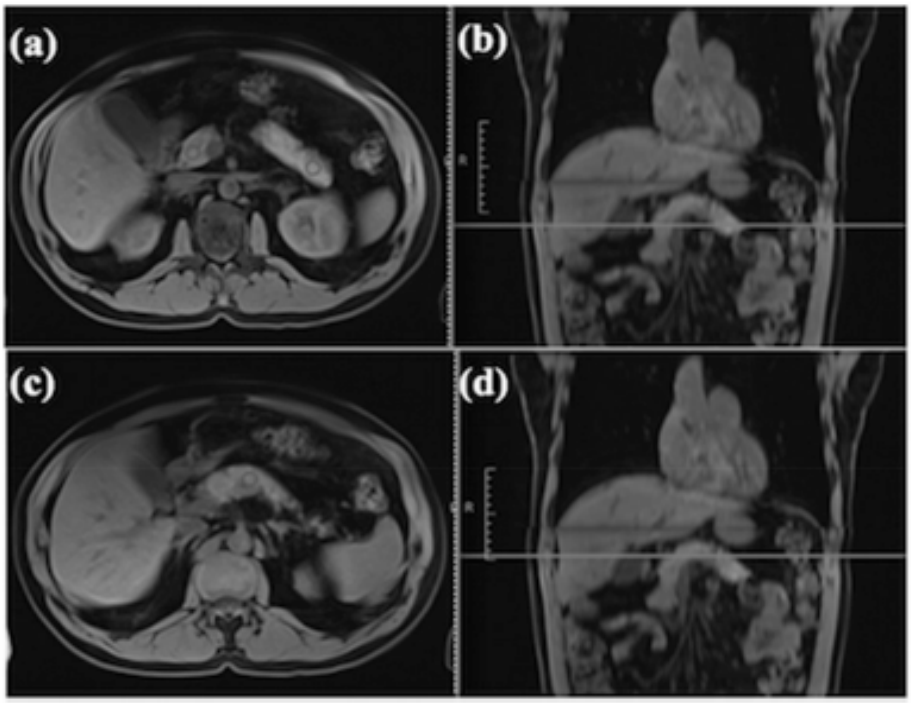

Figure 1. Selection of positioning of the circular regions of interest in the pancreas head, body, and tail to quantify IPLs. (a) Circular regions of interest of 1 cm^2 drawn on the head and tail of the pancreas on an axial abdominal MRI image. (b) Coronal MRI image, with the horizontal line depicting the position of the axial image (a). (c) Circular region of interest of 1 cm^2 drawn on the body of the pancreas on an axial abdominal MRI image. (d) Coronal MRI image with the horizontal line depicting the position of the axial image (c).

20% glucose infusion was administered to achieve a hyperglycemic state of 6.9 mmol/L above basal for a period of 2 hours. The blood samples were collected at −20, −10, 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes to measure the plasma glucose and serum insulin and C-peptide.

Insulin secretory function during a meal tolerance test

A 3-hour mixed meal tolerance test was conducted to assess ISF under physiological conditions. After an overnight fast, participants consumed a liquid milkshake (Ensure Plus; Abbott Nutrition, Berkshire, UK) providing 6 kcal/kg body weight, containing carbohydrates, protein, and fat. Blood samples were taken at −10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 minutes to measure plasma glucose and serum insulin and C-peptide.

Calculations

The incremental area under the curve (iAUC) was calculated, using the trapezoidal rule, for insulin and C-peptide responses to each challenge. To calculate an index of first- and second-phase insulin secretion in the hyperglycemic clamp, we used the iAUC for C-peptide over 0 to 10 minutes for the first phase and 10 to 120 minutes for the second phase, in analogy to DeFronzo *et al*. (21).

Model-based measurement of β-cell function

The glucose, insulin, and C-peptide curves during the hyperglycemic clamp and meal tolerance test were modeled using methods previously described (22–24) and SAAM-II, version 1.2, software (SAAM Institute, Seattle, WA). The main outputs of the model are glucose sensitivity of first-phase secretion (σ^1), expressed as the amount of insulin secreted in response to a rate of increase in glucose of 1 mmol/L between time 0 and 1 minute of the study: $(pmol \cdot m^{-2}BSA)/(mmol \cdot l^{-1} \cdot \min^{-1})$; glucose sensitivity of second-phase secretion (σ^2), expressed as the steady-state insulin secretion rate in response to a step increase in glucose of 1 mmol/L above baseline, in $(pmol \cdot \min^{-1} \cdot m^{-2}BSA)/(mmol \cdot l^{-1})$

Biochemical analyses

We measured plasma glucose using an automated glucose analyzer (2300 STAT Glucose Analyzer; Yellow Spring Instruments, Yellow Springs, OH). Serum insulin was determined by immunoassay using chemiluminescent technology (ADVIA Centaur System; Siemens Health Care, Ltd., Camberly, UK), where the interassay and intra-assay coefficients of variation ≤5.9% and 4.6%, respectively. Serum C-peptide was determined by radioimmunoassay (Millipore Ltd., Hertfordshire, UK).

Statistical analysis

The variables that were positively skewed were log-transformed to give a normal distribution. The statistical significance of differences in the variables of interest between the ethnic groups were tested using an independent samples *t* test for normal data or the Mann-Whitney *U* test for data that could not be transformed to normal. To investigate ethnic differences in IPL, we initially conducted independent samples *t* tests. We then used analysis of covariance, adjusting for VAT, to determine whether differences in IPL were independent of the VAT. The associations between IPL and parameters of ISF were explored using Pearson correlation. Partial correlation was used to investigate these associations with adjustment for VAT. To investigate the ethnic differences in the distribution of IPL among the head, body, and tail of the pancreas, a mixed between-within subjects ANOVA was performed. SPSS, version 24.0 (IBM Corp., Armonk, NY) was used for all statistical analyses, and $P < 0.05$ was considered to indicate statistical significance.

Results

Participant characteristics

We studied data from 19 BWA and 18 WE men (Fig. 2). The BWA men were first-generation West African migrants (born in Nigeria, n = 11; Ghana, n = 5; Sierra Leone, n = 2, Ivory Coast, n = 1). The characteristics of the two ethnic groups are presented in Table 1. The groups were well-matched for age, weight, and body mass index, with no substantial differences in T2D duration, fasting glucose, glycated hemoglobin, blood pressure, and liver function, as represented by alanine aminotransferase and measures of cholesterol (Table 1). Metformin use was not different between the two ethnic groups ($P = 0.248$), with 56% of the WE and 74% of the BWA men receiving treatment with metformin. The waist circumference was greater, although the difference was not statistically significant, in the WE men. The fasting triglyceride concentrations were significantly greater in the WE men. Analysis of the MRI data from the whole abdominal cavity showed that the weight of the abdominal VAT was significantly greater in the WE men. However, no ethnic difference were found in the SAT measured between the neck and knee (excluding the arms; Table 1).

Insulin secretory function

The C-peptide responses to the intravenous (hyperglycemic clamp) and oral (meal tolerance test) stimulations are presented in Table 2. Basal C-peptide was significantly lower in the BWA than in the WE men, although no ethnic differences were found in basal insulin (Table 2). The C-peptide iAUC during the meal test was significantly lower among the BWA men. This was also the case with the hyperglycemic clamp, specifically in the second phase (Table 2). The C-peptide data were modeled with the glucose curves to provide an estimate of the first- and second-phase glucose sensitivity of the β cells (σ^1 and σ^2, respectively). The modeled data showed similar findings of lower second-phase insulin secretion, although the results failed to reach statistical significance.

Pancreatic fat analysis

The mean IPL and the IPL of the head, body, and tail regions are shown in Fig. 3. The BWA men exhibited a significantly lower IPL_{MEAN} than the WE men (WE, 10.08% ± 2.46% *vs* BWA, 8.22% ± 2.51%; $P = 0.029$), which was driven by the significantly lower IPL_{HEAD} in the BWA men (WE, 9.66% ± 3.14% *vs* BWA, 7.03% ± 2.65%; $P = 0.009$). After adjustment for VAT, the

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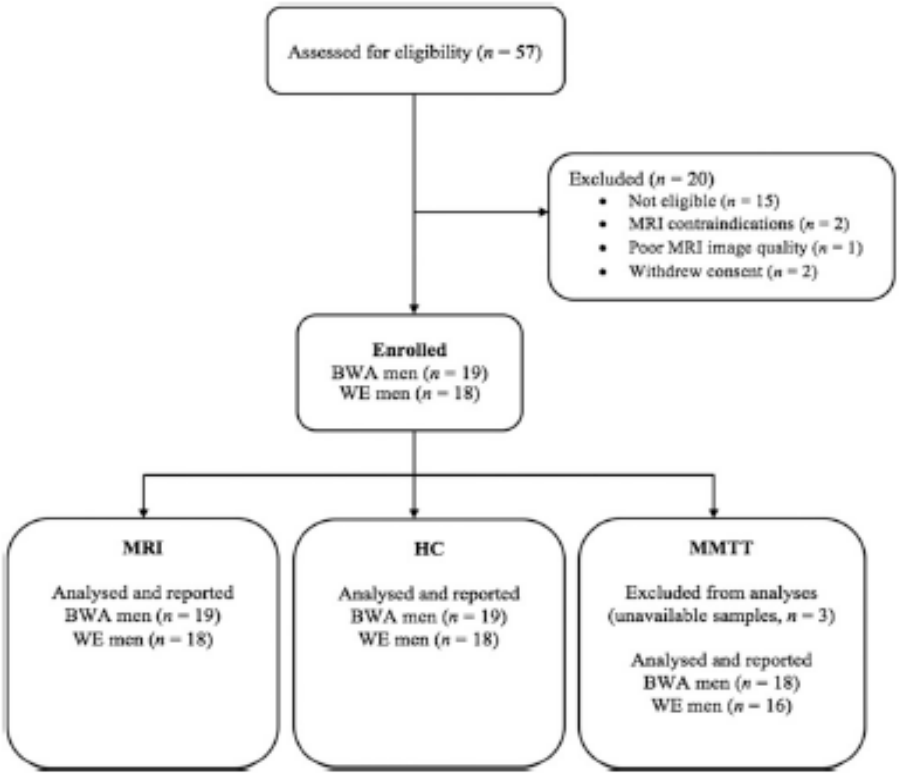

Figure 2. Study flowchart. Of the 57 participants initially assessed for eligibility, 19 BWA and 18 WE men were enrolled in the present study, and 20 participants were exduded, of whom 15 were not eligible, 2 had contraindications for MRI, 1 had poor MRI image quality, and 2 participants had withdrawn consent. HC, hyperglycemic clamp; MMTT, mixed meal tolerance test.

IPL_{MEAN} showed no statistically significant ethnic differences (WE, 9.60% ± 0.65% *vs* BWA, 8.60% ± 0.63%; $P = 0.305$), and the ethnic difference in IPL_{HEAD} had decreased in statistical significance (WE, 9.04% ± 0.68% *vs* BWA, 7.18% ± 0.66%; $P = 0.074$). We investigated regional IPL depositions in the head, body, and tail regions within and between each ethnic group using mixed between-within subjects ANOVA. We found statistically significant differences in IPL among the pancreatic sections in the BWA men (Wilks lambda, 0.791; $P = 0.019$), with a substantial main effect for ethnicity ($P = 0.029$). No statistically significant differences were found in the distribution of IPL between the two ethnic groups ($P = 0.474$).

Relationships between IPL and ISF

The associations between IPL and measures of insulin secretory function are listed in Table 3. The IPL_{MEAN} was significantly and inversely associated with the meal test first-phase insulin secretion (σ^1) in the WE men. However, the relationship, although negative, was not statistically significant in the BWA men (WE, $r = -0.55$, $P = 0.026$; BWA, $r = -0.18$, $P = 0.468$; Fig. 4). The association was specifically with IPL_{HEAD} in the WE men ($P = 0.023$; Table 3). No evidence was found for a linear relationship between the IPL_{MEAN} and the meal test second-phase insulin secretion (σ^2) in either ethnic group (WE, $r = 0.06$, $P = 0.813$; BWA, $r = 0.05$, $P = 0.856$). No statistically significant associations were found between IPL_{MEAN} and intravenously stimulated insulin secretion (σ^1 and σ^2) in either ethnic group (Table 3). However, analysis of region-specific associations with intravenously stimulated insulin secretion (σ^1 and σ^2; Table 3) showed inverse associations between IPL_{TAIL} with both σ^1 ($P = 0.092$) and σ^2 ($P = 0.074$), which had neared statistical significance in the WE men but not the BWA men (σ^1, $P = 0.18$; σ^2, $P = 0.26$). No changes were found in the statistically significance of the relationships between IPL (mean and region specific) and ISF after adjusting for VAT (data not shown).

Discussion

In our comparison of BWA and WE men with early T2D, we found ethnic differences in the deposition of pancreatic fat and its association with β-cell function. The men of BWA ethnicity exhibited lower IPL compared with the WE men, predominantly owing to the lower IPL deposition in the head of the pancreas. Furthermore, we recognized ethnic distinctions in the relationship between IPL and ISF, such that IPL is inversely associated with ISF only in WE men, leading us to speculate that IPL might

Table 1. Clinical Characteristics of BWA and WE Men

Characteristic	BWA (n = 19)	WE (n = 18)	*P* Value[a]
Age, y[b]	54 (12)	59 (6)	0.51
Weight, kg	92.6 ± 12.1	99.8 ± 16.7	0.14
BMI, kg/m^2	30.0 ± 3.6	31.5 ± 4.1	0.24
Waist circumference, cm	105.0 ± 9.9	111.9 ± 13.0	0.08
VAT, total, kg[c,d]	3.7 (3.1–4.5)	5.6 (4.6–7.0)	0.003*
SAT, kg[c,f]	11.5 (9.6–13.6)	13.2 (10.9–15.8)	0.25
Diabetes duration, years[b]	3.0 (2.0)	3.0 (1.3)	0.34
Fasting glucose, mmol/L	6.56 ± 0.73	6.88 ± 1.33	0.38
HbA1c, %	6.71 ± 0.67	6.64 ± 0.70	0.79
HbA1c, mmol/mol	49.8 ± 7.5	49.1 ± 7.6	0.79
ALT, IU/L[c]	27.3 (22.5–33.1)	31.2 (25.7–37.7)	0.31
Systolic BP, mm Hg	136.6 ± 13.5	130.9 ± 14.2	0.22
Diastolic BP, mm Hg	85.8 ± 7.6	82.6 ± 9.5	0.25
Total cholesterol, mmol/L	4.09 ± 0.72	4.27 ± 0.70	0.44
LDL cholesterol, mmol/L	2.32 ± 0.54	2.28 ± 0.66	0.87
HDL cholesterol, mmol/L	1.17 ± 0.37	1.19 ± 0.25	0.81
Triglycerides, mmol/L[b]	1.10 (0.60)	1.60 (1.25)	0.03*

Abbreviations: ALT, alanine aminotransferase; BP, blood pressure; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] *P* values determined using independent samples *t* tests for normally distributed data or the Mann-Whitney *U* test.

[b] Data presented as median (interquartile range) for nonparametric data.

[c] Data presented as mean ± SD or geometric mean (95% CI) for log transformed data.

[d] WE, n = 17; BWA, n = 18.

[e] Statistically significant.

[f] WE, n = 17; BWA, n = 17.

be a less important determinant of the development of T2D in BWA than in WE men.

The accumulation of IPL and other depots of ectopic fat has been thought to occur owing to reduced expandability of SAT during energy surplus and prolonged release of free fatty acids from SAT, due to adipocyte insulin resistance, which subsequently deposits as ectopic fat (8, 25). The interrelated nature of ectopic fat depots has been demonstrated by Lê *et al.* (15), who showed a correlation among VAT, IHL, and IPL in a multiethnic cohort. Because it has been well established that VAT and IHL are lower in black populations (26, 27), we hypothesized that the IPL would also be lower. In line with previous studies, our BWA men exhibited lower VAT compared with the WE men. We found that IPL no longer differed by ethnicity after adjusting for VAT. This suggests that the lower IPL in the BWA men is driven by lower VAT, indicating a central role of VAT in determining IPL deposition. Our findings extend the results from previous T2D studies of healthy individuals, which reported lower IPL among black groups compared with white and Hispanic ethnic groups (7, 15). Furthermore, we have provided data on differences in regional deposition of IPL according to ethnicity. We found the greater IPL deposition in WE men to be specific to the head of the pancreas. This is important because the development of T2D has been shown to be specifically associated with loss of β-cell mass from the pancreatic head (28). Also, several studies have shown links between lipotoxicity and β-cell apoptosis (6, 29). During the progression from normal glucose tolerance to T2D, the loss of first-phase ISF is understood to be the most critical dysfunction of β cells (30). Additionally, the detrimental effects of IPL have consistently been shown to relate to first-phase ISF (4, 31). Our protocol enabled us to differentiate first- and second-phase insulin secretory function and to investigate, for the first time, to the best of our knowledge, ethnic differences in the relationship between these and IPL. Thus, our findings suggest that β-cell lipotoxicity might be a less important determinant of ISF in BWA than in WE men. This is consistent with findings from a recent investigation of prepubertal youth of black and white ethnicity (32) that reported greater declines in β-cell function in white youth in response to a lipid infusion, suggesting greater susceptibility of the β cells to acute lipotoxicity in white youth compared with black youth. However, it should be noted that IPL was not measured in that study. To date, studies of IPL in black populations have been limited to first-phase ISF, expressed as the "acute insulin response" measured using the intravenous glucose tolerance test (7, 15, 16). In contrast, our findings indicated the potential importance of also assessing second-phase ISF. Our results showing that BWA men have lower second-phase ISF, which we have previously explored in more detail (14), indicate that a decrease in second-phase ISF might have a more prominent etiological role in β-cell dysfunction in black populations but is not related to IPL.

In contrast to our findings, Szczepaniak *et al.* (7) reported that IPL was associated with the intravenous glucose tolerance test "acute insulin response" in both normal glucose-tolerant white and black ethnic groups. The acute insulin response is considered comparable to the first-phase response of the hyperglycemic clamp but in this case, only insulin was measured. In our study, we have quantified ISF through the measurement of C-peptide, which provides a more accurate estimation of β-cell function than measurement of insulin alone. This is especially important when studying ethnic comparisons of β-cell function, as it has been extensively reported that black populations exhibit different insulin responses to glucose compared with other ethnic groups and that this response results from a combination of altered insulin secretion and hepatic insulin clearance. To date, many ethnic comparison studies have been limited to the measurement of insulin.

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Table 2. Metabolic Parameters of β-Cell Function in BWA and WE Men

Parameter	BWA (n = 19)	WE (n = 18)	*P* Value[a]
Basal insulin, pmol/L[b,c]	84.0 (67.3–104.8)	110.1 (79.2–153.0)	0.15
Basal c-peptide, nmol/L[c,d]	0.57 (0.31)	0.84 (0.33)	0.006[e]
Meal tolerance test results[c]			
C-peptide iAUC, nmol/L/min	63.3 ± 19.6	91.0 ± 30.1	0.003[e]
σ^1, (pmol/m^2 BSA)/(mmol/L/min)	1460 ± 1161	1155 ± 678	0.36
σ^2, (pmol/min/m^2 BSA)/mmol/L)[b]	63.0 (42.8–92.7)	69.6 (51.7–93.7)	0.67
Hyperglycemic clamp results			
C-peptide iAUC 0–10 min, nmol/L/min[d]	0.18 (0.46)	0.28 (1.74)	0.35
C-peptide iAUC 10–120 min, nmol/L/min[b]	55.7 (39.3–78.9)	108.3 (86.0–136)	0.002[e]
σ^1, (pmol/m^2 BSA)/(mmol/L/min)[d]	20.3 (118.1)	25.6 (126.0)	0.90
σ^2, (pmol/min/m^2 BSA)/mmol/L)[d]	8.2 (15.3)	16.1 (26.0)	0.08

Abbreviation: BSA, body surface area.

[a]*P* values determined using independent samples *t* tests for normally distributed data or the Mann-Whitney *U* test.

[b]Data presented as mean ± SD or geometric mean (95% CI) for log transformed data.

[c]WE, n = 16; BWA, n = 18.

[d]Data presented as median (interquartile range) for nonparametric data.

We found ethnic differences in the regional distribution of IPL within the pancreas such that BWA men had greater IPL in the tail compared with the head of the pancreas. In contrast, in the WE men, we found no apparent regional variation in IPL deposition. However, when we studied the region-specific relationships between IPL and ISF, we found, in the WE men, an inverse relationship, which neared statistical significance, between IPL in the tail with both intravenously stimulated first-phase and second-phase ISF, which was not seen in the BWA men. Studies conducted in humans have shown regional variation in the distribution of β cells within the pancreas, concluding that the tail of the pancreas has a more than twofold greater density of β cells compared with the head and body (28). The inverse relationship we observed, albeit of borderline statistical significance, between IPL in the tail and ISF in WE men might indicate greater β-cell lipotoxicity in WE men and, in turn, reduced β-cell function in the tail of the pancreas, an association not seen in the BWA men. We propose further work should be conducted to investigate ethnic differences in the role of regional IPL on region-specific β cells within the pancreas.

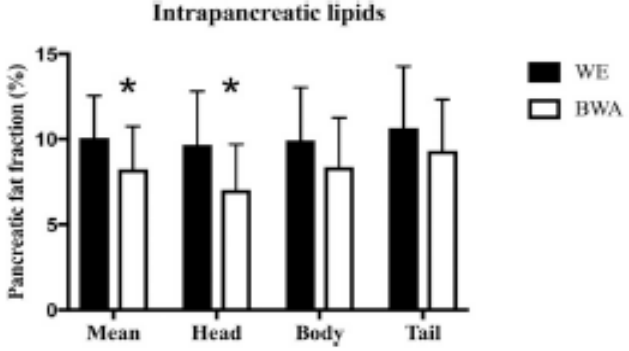

Figure 3. Mean IPLs and IPLs of the head, body, and tail stratified by ethnicity. Data presented as mean ± SD. *Statistically significant $P < 0.05$ determined using an independent samples *t* test between WE and BWA men.

To the best of our knowledge, our study design, using both the hyperglycemic clamp and mixed meal tolerance test, enabled us to compare, for the first time, in a single study, distinctions in the associations between IPL and ISF in response to orally *vs* intravenously stimulated glycemia. Our findings of substantial associations only between IPL and orally stimulated ISF help to explain previous contradictory results between studies that used oral *vs* intravenous methods (31, 33). These findings suggest an interaction among incretin hormones, IPL, and ISF. Recent studies have shown a link between β-cell lipotoxicity and a reduced incretin effect, in which increasing concentrations of free fatty acids were associated with a downregulation of the GLP-1 receptor in a mouse model (34). Our results might suggest that IPL negatively affects incretin signaling in β cells and further hinder an insulin secretory response to a meal in the WE men but not in the BWA men. This ethnic difference can be explained by differences in incretin levels, which have previously differed between black and white populations, with some studies reporting greater incretin levels in blacks (14, 35, 36) and others have reported lower (37). Further investigations are needed to understand the ethnic differences in the relationships between incretins and IPL.

Our study had several strengths, including the measurement of C-peptide for the assessment of insulin secretion and the use of two methods to comprehensively measure insulin secretory function, distinguish first- and second-phase secretion, and determine the role of incretin hormones. Another strength was our investigation of regional variation of IPL in the head, body, and tail of the pancreas and how this differs between and within each

Table 3. Pearson Correlation Coefficients Between IPL and Metabolic Measures of Insulin Secretory Function

	Meal Test σ^{1a}		Meal test σ^{2a}		Hyperglycemic Clamp σ^{1b}		Hyperglycemic Clamp σ^{2b}	
Variable	BWA	WE	BWA	WE	BWA	WE	BWA	WE
IPL_{MEAN}	−0.18	−0.55[c]	−0.16	0.05	−0.29	−0.24	−0.14	−0.36
IPL_{HEAD}	−0.04	−0.56[c]	−0.09	0.13	−0.30	−0.01	−0.32	−0.27
IPL_{BODY}	−0.21	−0.24	−0.10	0.24	−0.22	−0.16	−0.08	−0.10
IPL_{TAIL}	−0.33	−0.36	−0.32	−0.16	−0.32	−0.41	−0.27	−0.43

[a]WE, n = 16; BWA, n = 18.

[b]WE, n = 19; BWA, n = 18.

[c]$P < 0.05$.

ethnic group. Our study also benefited from MRI analysis of pancreatic fat, which has been suggested to be superior to magnetic resonance spectroscopy for IPL analysis owing to the irregular size and morphology of the pancreas, especially in diabetic populations (9, 38, 39). However, our study also had limitations. Small regions of interest were used in the MRI analysis of IPL to reduce contamination with VAT, as recommended by investigators of the methods used to quantify IPL using Dixon-MRI (9, 40), although we could not guarantee that VAT contamination did not occur owing to poor participant compliance with the breath holds. Also, we could not determine whether the mean IPL represents the total IPL, because we did not measure the volume of the pancreas, which has been reported to be 33% less in individuals with early T2D compared with healthy controls (41). Our study was deliberately limited to studying men, because consistent evidence has shown sex differences in the pathophysiology of T2D in African populations (42, 43), limiting the generalizability of our findings. However, despite the greater prevalence of T2D in black women compared with black men (1), our data are valuable owing to the lack of studies of men in this field. Furthermore, our study focused on BWA ethnicity and all our participants were first-generation migrants (born in countries of West Africa). Thus, when comparing our study to previous works, differences might be present between black populations residing in the United Kingdom and those residing in the United States or other regions in terms of lifestyle behaviors, socioeconomic factors, and access to health care that could influence the development of T2D. We could not determine a causal relationship between β-cell function and IPL accumulation owing to the cross-sectional nature of the present study. Our study was also conducted on a small sample size although, despite this, the size was comparable to that in other studies of IPL and ISF. An investigation of normal glucose-tolerant and impaired glucose-tolerant groups of both WE and BWA ethnicity would enable us to gain an understanding of the effect of IPL accumulation on β-cell function during the progression of T2D.

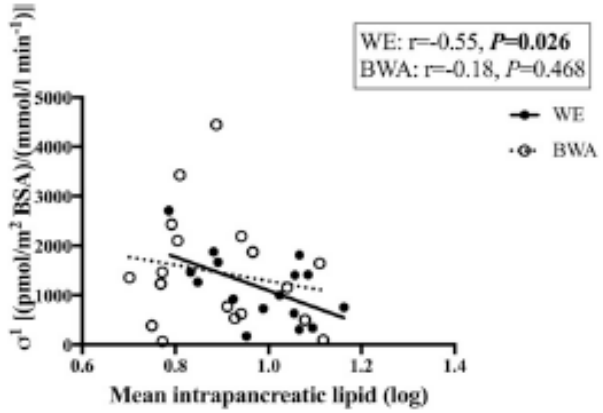

Figure 4. Relationships between log mean IPL and orally stimulated first-phase insulin secretory function, σ^1 [(pmol/m^2 BSA)/(mmol/L/min)] in WE and BWA men. White circles indicate BWA men; black circles, WE men; dashed line, BWA men; solid line, WE men. Mean intrapancreatic fat was calculated as the mean of the pancreatic fat fraction of the head, body, and tail of the pancreas quantified using a Dixon-based sequence MRI method. BSA, body surface area.

In conclusion, our results have demonstrated ethnic differences in the deposition of pancreatic fat and its association with β-cell function. Our findings of lower IPL among BWA men with T2D suggest that the lipotoxicity in the pancreas might be less dominant in the pathogenesis of T2D in BWA than in WE men. Furthermore, ethnic distinctions in the relationship between IPL and ISF such that it relates to insulin secretory function in WE men but not in BWA men suggest that IPL might be a lesser determinant of β-cell dysfunction in BWA men with early T2D.

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