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Mohandas, Cynthia

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Impact of West African and African Caribbean ethnicity on progression of early Type 2 Diabetes in South East London and Preliminary findings from South London Diabetes and Ethnicity Phenotyping Study

Dr Cynthia Mohandas

Supervised by: Professor Stephanie Amiel, Dr Louise Goff and Prof Sir George Alberti

A thesis submitted to King's College London in partial fulfilment of the requirement for the degree of Doctor of Medicine

Department of Diabetes and Nutritional Sciences, School of Life Course Sciences, Faculty of Life Sciences & Medicine, King's College London, London, UK

September 2021

Declaration

The data presented in this thesis were collected as part of the South London Diabetes (SOUL-D) and Ethnicity phenotyping (SouL-DeEP) study. The study was divided into two phases, both funded by Diabetes UK (grant numbers: 12/0004473 and 14/0004967) and awarded to Dr Louise Goff, the principal investigator, and co-investigators Professor Stephanie Amiel, Professor Janet Peacock and Professor Margot Umpleby. I declare that my primary responsibilities and contributions to the overall study were centred around:

- Participant recruitment and characterisation
- Study day-to-day management
- Management of participant metabolic visits
- Calculation and preparation of isotope infusions
- Metabolic assessments
- Plasma glucose readings
- Sample processing
- Data entry, cleansing, analyses and interpretations
- Academic presentations and publication

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This thesis would not have been possible without the support of an amazing team of people who constantly supported me all these years and encouraged me to finish this work. I would like to firstly thank my primary supervisor, Prof Stephanie Amiel, who has been so supportive and steadfast throughout the whole process. She first encouraged me to consider research when I was a specialty registrar in King's college Hospital in 2011. I am extremely grateful for the opportunity, I was given to broaden my horizons and be a part of the great team. I would also like to thank my second supervisor, Dr Louise Goff whose knowledge, dedication, focus, academic rigour, and attention to detail are incredible. I am also grateful to Prof Sir George Alberti for his nuggets of wisdom and for being a great mentor. I owe to Toyosi Bello and Meera Ladwa, my fellow researchers on this project who constantly supported me during my writing up phase and offered so much valuable input for this thesis. Toyosi also assisted with sample processing during the metabolic assessments. Thanks to other fellow researchers namely Piya, Yee, Kate and Muna and all the SOUL-D researchers with whom I shared the office space for their friendship and moral support. Sincere thanks to Ines De Abreu, CRF nurse and Bula Wilson (research nurse) without whom it would have been impossible to finish 120 metabolic studies in 18 months and they helped me during all the challenges especially during the clamp studies which sometimes lasted 10-12 hours ; Andrew Pernet (research nurse) also helped me with some metabolic assessments; Fariba Shojaee-Moradie, who not only analysed our C-peptide samples but also helped me during my initial euglycaemic clamps and cheered me up during my research years not to give up.

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Abstract

Background: People of Black African ethnicity are at high risk of Type 2 Diabetes (T2D) as lifestyle changes, as already seen in diaspora environments, are associated with a more aggressive disease onset.

Aims: We investigated the impact of ethnicity on progression of T2D and metabolic regulation.

Methods: 1790 adults with new-onset T2D were recruited and followed for 2 years in South London Diabetes study (SOUL-D). Black West African (BWA), African-Caribbean (AC) and White European (WE) ethnic groups were compared. Insulin secretion was measured in 20 BWA and 20 WE men with T2D duration <5 years, matched for age and BMI, using hyperglycaemic clamps (HC) and mixed meal tolerance tests (MMTT).

Results: BWA and AC were younger ($p<0.001$); had lower waist circumference ($p<0.001$) and higher HbA1c at diagnosis ($p=0.001$). At year 2, HbA1c was not different, but BWA and AC were on more diabetes medications ($p=0.01$). On phenotyping, BWA men had lesser c-peptide responses to IV and oral challenge in the second phase only (HC c-peptide ($p=0.78$), 2nd phase ($p=0.001$) and MMTT ($p=0.002$).2nd phase insulin response to HC ($p=0.01$) was significantly lower in BWA men but not in MMTT($p=0.44$).

Conclusions: BWA ethnicity is associated with younger onset and lesser central obesity at diagnosis of T2D and greater requirement for medication to achieve comparable glycaemic control than WE ethnicity. Exaggerated insulin secretory deficits in early T2D in BWA compared to WE men are compatible with earlier beta cell exhaustion, slightly compensated by possible reduced hepatic insulin clearance to oral glucose challenge. Treatment to preserve insulin secretory capacity may have particular benefit in preventing progression to T2D in BWA populations.

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List of Publications

Original articles

Mohandas C, Bonadonna R, Shojee-Moradie F, Jackson N, Boselli L, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Ethnic differences in insulin secretory function between black African and white European men with early T2D. Diabetes Obes Metab. 2018 Jul;20(7):1678-1687.

Bello O, **Mohandas C**, Shojee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early T2D have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Hakim O, Bonadonna RC, **Mohandas C**, Billoo Z, Sunderland A, Boselli L, Alberti KGMM, 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 T2D. J Clin Endocrinol Metab. 2019 Apr 1;104(4):1201-1210.

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Conference posters and abstracts

Mohandas, C., Ayis, S. A. M., Stahl, D., Chamley, M., Alberti, KGMM., Ismail, K. & Amiel, S. A.Ethnic differences in the clinical progression of early T2D:findings from the South London Diabetes Cohort (SOUL-D) study 11 Mar 2015, Diabetic Medicine. s1 ed. London, Vol. 32. p. 27-28 2 p. A76 (P84)

Mohandas C et al, Impact of West African and African Caribbean ethnicity on progression of early T2D in South East London in ABCD conference October 2015

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Mohandas C et al, Exaggerated insulin secretory deficits in early T2D in men of Black West African ethnicity are not corrected by enhanced incretin responses to oral glucose in Diabetes UK conference March 2015.

Mohandas C et al,Increased incretin release compensates for early insulin secretory deficits in T2D in men of Black West African origin in WCIR,November 2014

Bello. O, Marathe. C.S, **Mohandas. C**, Amiel. S.A, Goff, L.M. Insulin sensitivity indices in Black Africans and White Europeans with T2D: a comparison of fasting, meal tolerance test and Hyperinsulinemic-euglycemic clamp indices. Diabetic medicine. 2017

March;34 (S1 Special Issue: Abstracts of the Diabetes UK Professional Conference 2017, Manchester Central, Manchester, 8–10 March 2017); P160

Bello. O, Ladwa. M, Marathe. C.S, **Mohandas C**, Amiel. S.A, Goff. L.M. The impact of ethnicity on the association between insulin sensitivity and adiposity measures in Black African and White European men with normal glucose tolerance and T2D. Diabetic medicine. 2018 March; 35 (S1 Special Issue: Abstracts of the Diabetes UK Professional Conference 2018); P1

O Bello, M Ladwa, CS Marathe, **C Mohandas**, KMM Alberti, JL Peacock, AM Umpleby, SA Amiel, LM Goff. Ethnic differences in the association between adipose tissue insulin resistance and measures of adiposity. Diabetic medicine. 2019 March; 36 (S1 Special Issue: Abstracts of the Diabetes UK Professional Conference 2019); P63

Presentations

Young researchers meeting,London-February 2014

RANK symposium-July 2014

David Pyke and Peter Watkins Visiting Professor's conference-November 2014

Joint Imperial & KCL DRG meeting-November 2014

Conference abstracts and future oral presentations

RANK symposium, July 2014: Ethnic differences in the development of T2D: a preliminary phenotyping study in people with recent-onset diabetes in SE London.

WCIR-DC conference, November 2014: Increased incretin release compensates for early insulin secretory deficits in T2D in men of Black West African origin.

Diabetes APC, March 2015

1. Ethnic differences in the clinical progression of early T2D: findings from the South London Diabetes Cohort (SOUL-D) study-selected for oral presentation.

2.Exaggerated insulin secretory deficits in early T2D in men of Black West African ethnicity are not corrected by enhanced incretin responses to oral glucose-Selected for YDEF travel grant prize oral presentation.

3. Neither hepatic nor peripheral insulin sensitivity differs between men of Black West African and White European ethnicity with early T2D.

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List of abbreviations:

AA	African-American
AC	African-Caribbean
ACE	Angiotensin converting enzyme
ANOVA	Analysis of Variance
BAM	Black African Men
BMI	Body mass index
BWA	Black West African
CRF	Clinical Research facility
CVD	Cardiovascular Disease
IDF	International Diabetes Federation
ISR	Insulin secretory reserve
IVGTT	Intravenous glucose tolerance test
HDL	High density lipoprotein
LDL	Low density lipoprotein
MetS	Metabolic Syndrome
MRI	Magnetic resonance imaging
MRU	Metabolic research unit
MTT	Meal tolerance test
OGTT	Oral glucose tolerance test
PIS	Participant information sheet
REC	Research Ethics Committee
SouL-D	South London Diabetes study
SouL-DeEP	South London Diabetes Ethnicity Phenotyping
T2D	Type 2 Diabetes
UK	United Kingdom
WE	White European
WEM	White European Men
WEO	White European Origin

CHAPTER 1: INTRODUCTION

1.1 Diabetes overview

T2D Mellitus (T2D) is a metabolic abnormality resulting from insulin resistance and beta cell dysfunction(1–3). Decreased insulin secretion, impaired glucose utilization and reduced hepatic glucose production disrupts the glucose homeostasis and contributes to hyperglycaemia(2,4). The presence of fasting hyperglycaemia which is well documented in diabetic patients subsequently stimulates insulin synthesis. The fasting hyperglycaemia leads to substantial disruption of glucose homeostasis. The beta cell senses this change and produces more insulin in response to the hyperglycaemia. Eventually when the plasma glucose levels continue to be above 200-220 mg/dl, the response of plasma insulin to plasma glucose surge becomes diminished and this is coupled with the deranged release of glucagon from alpha cells in the post prandial period in T2D patients leading to incretin impairment(2,5). Beta cell dysfunction precedes the onset of T2D. In addition to insulin resistance in skeletal muscle, liver and beta cell dysfunction, augmented lipolysis, incretin deficiency, hyperglucagonaemia, accelerated glucose renal reabsorption and insulin resistance in brain contribute to disease progression in T2D(6). Insulin has an array of effects on carbohydrate, lipid and protein metabolism as well as on mRNA transcription and translation(7). Not only obesity but lack of physical activity enhances insulin resistance environment. Fluctuations in glycaemic level leads to changes in plasma insulin levels which directly impacts the plasma free fatty acid (FFA) concentrations. Thus, elevated FFAs were reported in insulin resistant state where compensatory hyperinsulinaemia mechanism has failed. This in turn contributes to development of elevated plasma TG levels in T2D patients(8).Available data reports that elevated plasma FFAs will suppress the uptake of insulin mediated glucose(9).

Diabetes Mellitus remains a predominant cause of morbidity and mortality worldwide and it is estimated that over 6.7 million people aged 20–79 died from diabetes-related causes in 2021(10). Chronic hyperglycaemic exacerbates oxidative stress and inflammatory responses, thus diabetes has profound repercussions for developing vascular complications (11,12). The chief biological component in macrovascular disease is augmented atherosclerosis formation and associated hypercoagulable condition(11). The pathologic process in microvascular disease is complex and the hallmark for development of complications is the chronicity of tissue exposure to elevated blood glucose levels(13). Clinical trials have reported strong relationship between development of microvascular manifestations and the extent of glucose control(14,15).

Diabetic patients are more prone to develop heart failure even in the absence of underlying Coronary Artery Disease (CAD) due to the compromised glucose metabolism(16). The Candesartan in Heart Failure: Assessment of Reduction in Mortality and morbidity (CHARM) programme reported that diabetes mellitus was associated with two fold increase in mortality or cardiovascular event or hospitalisation in heart failure patients on insulin therapy and a 50% higher risk in non-insulin diabetic patients(17).

Several studies have demonstrated ethnicity as one of the risk factors for the development of T2D(18–25). The prevalence of diabetes is higher in the black ethnic groups than the White British population in UK(26).

1.2 Epidemiology and the global burden of T2D

According to the World Health Organization (WHO), diabetes of all types has exponentially increased in the past decades across the globe. The prevalence of diabetes rose from 151 million in 2000 to 463 million in 2019 and it has been estimated to reach an alarming 700 million by 2045 (Fig. 1). Figure 1 explains that the percentage of adults affected by diabetes in 2019 was 4.7% in Africa, 6.3% in Europe, 12.2% in the Middle East and North Africa, 11.1% in North America and the Caribbean, 8.5% in South and Central America, 11.3% Southeast Asia, and 11.4% in Western Pacific region.

Data from the National Diabetes Statistics Report, 2020 conducted in USA revealed that 34.2 million adults were diagnosed to have diabetes and 7.3 million had undiagnosed diabetes. Despite this staggering rate of diabetes prevalence, around 193 million individuals have undiagnosed diabetes. Nearly 120 million people in Southeast Asian and Western Pacific areas were unaware of their disease status due to lack of access to proper healthcare facilities and insidious development of diabetes symptoms and signs.

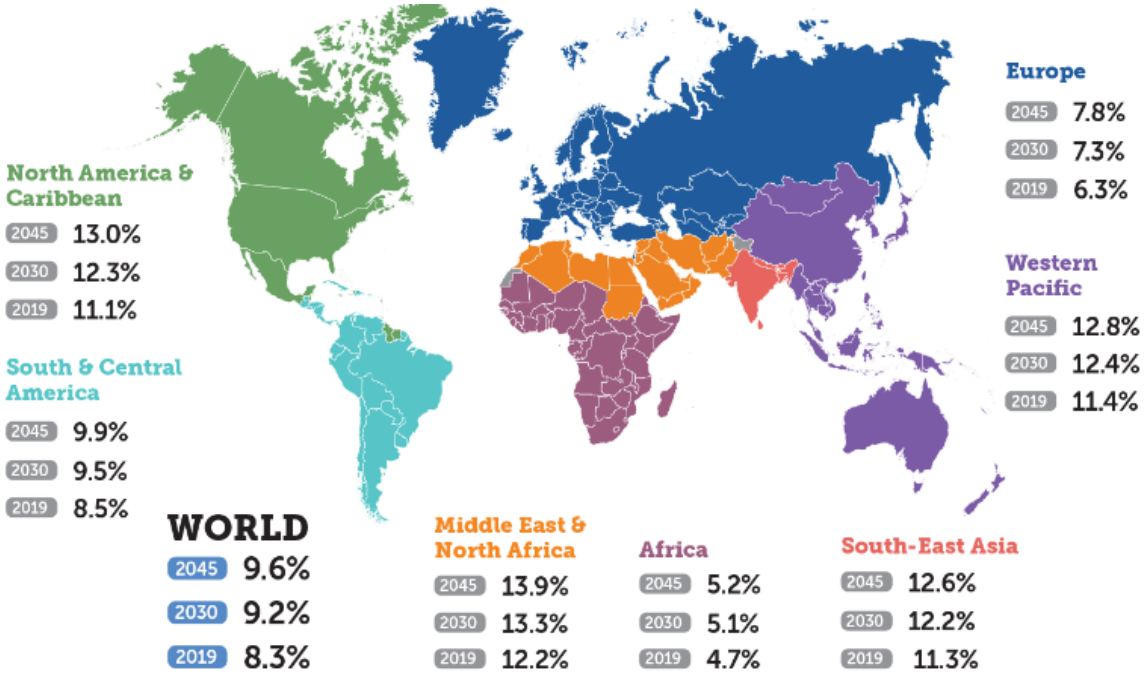


Figure 1: The number of adults (20- 79 years) with diabetes and age-adjusted comparative diabetes prevalence

Source: International Diabetes Federation. IDF Diabetes Atlas, 9th edition. Brussels, Belgium: International Diabetes Federation, 2019.

Prevalence of diabetes is steadily rising in the UK region, from 3.9% in 2003 to a predicted 4.7% by 2030, and the age of onset is gradually decreasing(27). More than 4.9 million people in the UK have diabetes with 13.6 million people are now at increased risk of type 2 diabetes in the UK. 850,000 people are currently living with type 2 diabetes but are yet to be diagnosed. More than 700 people with diabetes die prematurely every week(28)(29). Diabetes is associated with higher morbidity, disability and increases the risk of developing vascular complications from nearly 2 to 11 fold(30–33). Diabetes has been the chief leading cause of non-traumatic lower limb amputation, renal failure, and blindness in working-age groups(34,35) . Individuals with diabetes have astounding rates of high fatality when compared to the general population. Diabetes and its complications have an inverse impact on the life expectancy. Although the prevalence of diabetes in the

UK seems to be lower than the global majority, 10% of the NHS budget has been consumed for management of diabetes and its complications(36,37).

1.2.1 T2D burden in Black population

The adult black African population is disproportionately affected by T2D compared to White Europeans. Diabetes occurs at an early age despite the lower body mass index and upon evaluation, glycaemic control is found to be poorer in black African populations compared to White Europeans. The rate of complications has been reported to be of higher proportion and this observation is not isolated to migrant populations.

The International Diabetes Federation (IDF) has quantified that adults living with diabetes in Africa will increase by 109.6% from 19.8 million in 2013 to 41.5 million in 2035(38). The risk in black African ethnicity is as high as that of Indian Asians in UK. The Southall And Brent Revisited Study (**SABRE**) conducted in the UK, reported that the prevalence of diabetes in first-generation immigrants of African Caribbean Ethnicity aged from 40-70 years was three-fold more than that in White European ethnic (WE) group, equivalent to the incidence found in Indian Asians (34%)(39).

The United Kingdom Prospective Diabetes Study (UKPDS) reported 20 years ago that 30% of the participants had microvascular complications at diagnosis. However, this cohort predominantly consisted of the white population(40). The DRIVE UK study, pooled data from 2 UK diabetes registers (n=57,144) from two multi-ethnic regions, found a higher prevalence of retinopathy in African/Afro-Caribbeans (53%) and South Asians (42%) compared to white Europeans (38%)(41). However, duration and other complications of diabetes were not reported.

Recently, several multi-ethnic cohort studies conducted in UK have identified a higher risk of T2D at lower levels of obesity among ethnic minority groups as compared to the white Europeans(42–44). Modelling of UK Biobank data has demonstrated that the T2D risk in white Europeans was associated with a BMI of 30 kg/m^2 whereas in South Asian groups the T2D risk was present at a BMI of 22 kg/m^2 and a BMI of 26 kg/m^2 in black African-Caribbean groups(43). Data from The Health Improvement Network, a UK longitudinal general practice dataset, demonstrated that 38 % of South Asians and 29 % of black African-Caribbeans with T2D have a BMI below 30 kg/m^2 compared to only 26% of white Europeans. Furthermore, both South Asians and black African-Caribbeans have a significantly higher probability of developing T2D in the normal and overweight BMI categories as compared to white Europeans (42).

In a cross-sectional study among 404,318 individuals conducted in London, it was found that when compared to White ethnicity (5.04%), the crude percentage prevalence of T2D was higher in the Asian (7.69%) followed by Black (5.58%) ethnic groups, while lower in the mixed group (3.42%). After adjusting for differences in age group, sex, and social deprivation, the indigence of T2D was greater in all minority ethnic groups compared with the White groups (OR Asian versus White 2.36, 95% CI 2.26 to 2.47; OR Black versus White 1.65, 95% CI 1.56 to 1.73; OR Mixed/Other versus White 1.17, 95% CI 1.08 to 1.27). The T2D was more predominant in the Asian and Black ethnic groups than the White group(45).

Clinical data shows that a higher proportion of T2D complications were observed in black compared to white communities, which reflects the magnitude of disease curse and the concomitant healthcare burden. Studies from USA suggest that black populations share a

major burden of mortality from stroke, lower limb amputation, end-stage renal disease, diabetic retinopathy and an overall higher incidence of hospitalisation for diabetes-related problems(46). Studies conducted in USA have revealed that the response to conventional prevention program involving weight loss, performed outside of the controlled research environment had diminished results in the Black compared to White communities(47,48). There is paucity of studies in UK on evaluation of the prevention strategy in ethnic population, a recent assessment of the UK Diabetes Prevention Programme has showed notably lower rates of weight loss and slightly lower reduction in HbA1c in individuals belonging to the black community compared to white population(49). Therefore, ethnicity plays a huge role on T2D disease process, and the disparity due to socio-economic and biological factors impacts healthcare system in the UK.

1.3 Pathogenesis of T2D

Traditionally, T2D was described as a result of the insidious loss of the pancreatic beta-cell function, along with impending insulin resistance. A cascade of metabolic abnormalities is triggered in the liver, skeletal muscle, and pancreas with primary defects within adipose tissues(50). Three individual theories have been proposed in the development of T2D. The dysfunctional adipose tissues, and its inability to buffer excess fat, are proposed as primary underlying defects (Fig. 2). The *spillover* theory suggests that excess storage of fatty acids in the visceral compartment due to a reduced capacity of subcutaneous adipocytes to store fatty acids contributes to T2D development (51). The portal theory proposes that hyper- trophic visceral adipocytes are highly lipolytic and have an increased flux of fatty acids as compared to subcutaneous adipocytes. These fatty acids are released into the portal circulation and deposited in the liver, resulting in to ectopic fat accumulation in the liver and hepatic insulin resistance(52). The twin-cycle

hypothesis suggests that increased hepatic fat deposition leads to increased VLDL-TAG export from the liver, which then accumulates in other organs and tissues, particularly the pancreatic beta cells. Consecutively, the pancreatic beta cells failure results in the T2D development(53). It is the accumulation of TAG within these ectopic depots that is believed to play an integral role in the development of T2D by causing metabolic disturbances within the organs/tissues in which it resides, termed as lipotoxicity. Ectopic depots of importance include intrahepatic lipid (IHL), intramyocellular lipid and intrapancreatic lipid (IPL) which contribute to insulin resistance. In response to hepatic and peripheral insulin resistance, compensatory hypersecretion occurs in order to maintain normoglycemia. Eventually, 'β-cell exhaustion' or 'burn-out' occurs whereby the β-cells cannot secrete sufficient insulin, and a hyperglycaemic state develops(50).

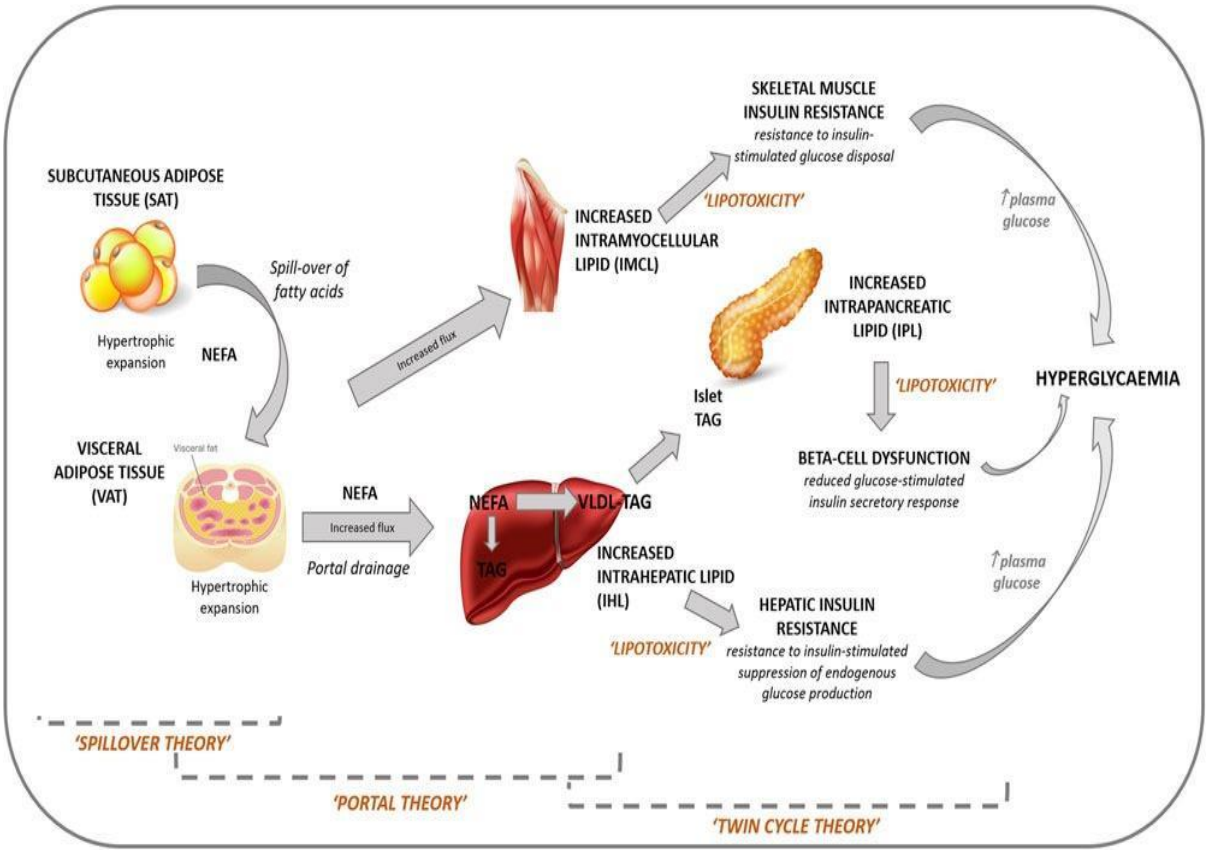


Figure 2: Adipose tissue dysfunction and ectopic fat accumulation in the pathogenesis of T2D(54)***.***

In 2009, the term 'ominous octet' was framed to describe the pathways of eight distinct pathological processes, which results in T2D development (Fig 3). The 'ominous octet' provides an overview of the complexity involved in diabetes pathophysiological events. However currently we understand that T2D pathogenesis is multifactorial and include both genetic and environment elements that affect insulin resistance and lead to loss of pancreatic beta cell function.

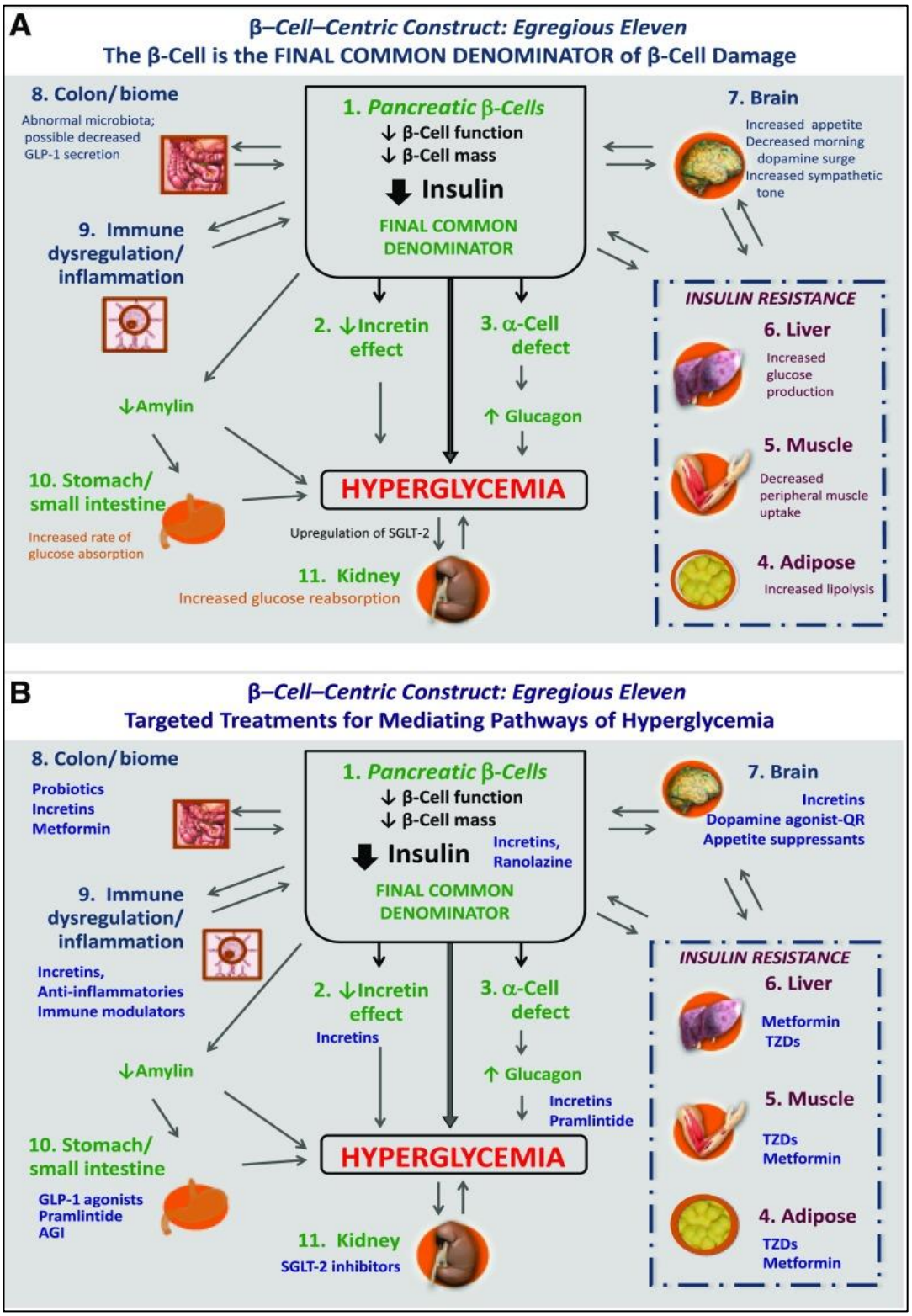


Figure 3: β-Cell–centric construct: the egregious eleven.

Dysfunction of the β-cells is the final common denominator in DM. *A*: Eleven currently known mediating pathways of hyperglycemia are shown. Many of these contribute to β-cell dysfunction (liver, muscle, adipose tissue [shown in red to depict additional association with IR], brain, colon/biome, and immune dysregulation/inflammation [shown in blue]), and others result from β-cell dysfunction through downstream effects (reduced insulin, decreased incretin effect, α-cell defect, stomach/small intestine via reduced amylin, and kidney [shown in green]). *B*: Current targeted therapies for each of the current mediating pathways of hyperglycaemia. GLP-1, glucagon-like peptide 1; QR, quick release.(55)

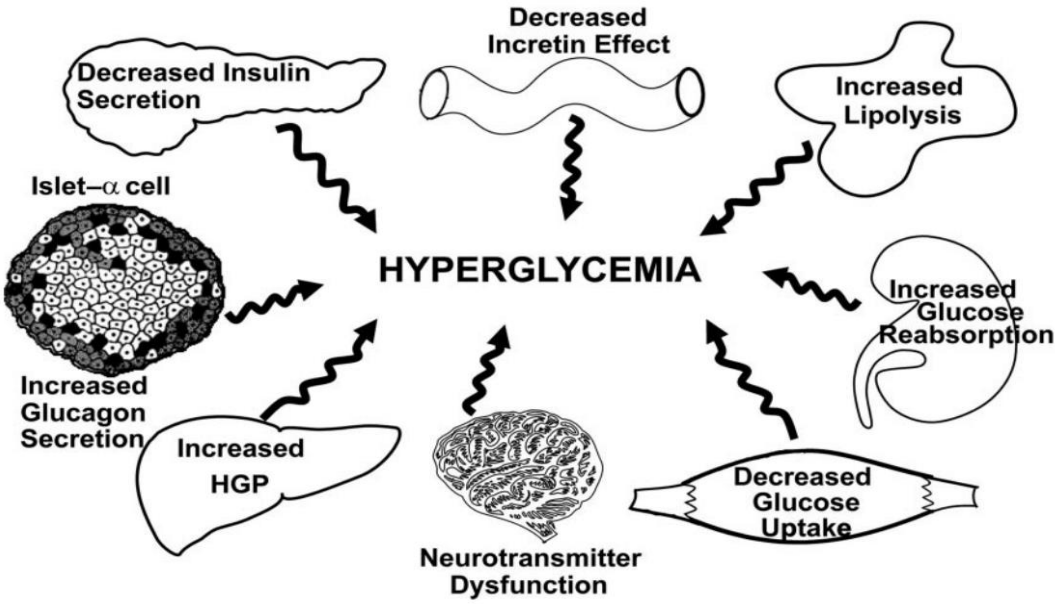


Figure 4: The 'ominous octet' process in T2D.

1.3.1 Abnormalities of Insulin secretion and β-cell defects in T2D

T2D is a heterogeneous disorder caused by a combination of diverse genetic and environmental factors which adversely affect the β-cell function and tissue insulin sensitivity. T2D develops because of progressive degradation in β-cell function coupled with acquired insulin resistance(2)(8)(56). Although Yalow and Berson initially described the findings in 1960, it was not well accepted until 1967 that, the elevated 2-hour post-challenge plasma insulin levels during oral glucose tolerance tests among people with T2D were accompanied by reduced early (30 minutes) insulin responses(57). A recent study on various human models has reported that beta-cell dysfunction was present well before the hyperglycaemia onset, particularly in subjects with impaired glucose tolerance. First-phase insulin response to glucose was decreased at an early stage, thus contributing to postprandial hyperglycaemia. In contrast, late-phase insulin secretion may be preserved for a prolonged term as activated by sustained hyperglycaemia. The United Kingdom Prospective Diabetes Study (UKPDS) suggested that beta-cell

dysfunction commences early (minimum ten years) before hyperglycaemia development (58) .

For several decades, beta-cell function in T2D patients has been the subject of extensive investigation, and tremendous progress has been made during the recent years in understanding the insulin secretion pathology(59). Current studies determined that beta-cell deficit and beta-cell apoptosis were present in patients with T2D(60). Three pathological pathways have been proposed to describe the beta cell dysfunction among subjects with predisposition for T2D development. First, a genetic predisposition may be present among individuals with T2D associated with obesity, as seen observed in certain subjects in the "Maturity-Onset Diabetes of the Youth" (MODY) study (glucokinase gene mutation in MODY 3)(61). Second, in utero malnutrition may result in insufficient beta-cell development and later leading to partial insulin secretory abnormalities. This is known as "thrifty phenotype hypothesis"(62). However, knowledge on the relationship of insulin secretion is unclear compared with the relationships between insulin resistance and metabolic syndromes. Third, an unfavourable metabolic environment may have a harmful influence, primarily through increased glucose levels that may produce glucotoxicity(63,64). Furthermore, a chronic increase in non-esterified fatty acids (NEFA) levels that might cause lipotoxicity, contributing to altered secretion of insulin(65).

A condition with ectopic deposition of triglycerides in pancreatic islets has been reported to contribute to beta-cell dysfunction. Although the lipotoxicity mechanism in the beta-cells remains unclear, it has been reported that the triglycerides accumulation results in higher nitric oxide levels causing oxidative damage and apoptosis of the cells(66).

Finally, defects in insulin signalling pathways combined with insulin resistance in peripheral tissues have been reported to impact the insulin secretion by pancreatic beta cells, suggesting that insulin resistance might be responsible for the beta-cell dysfunction and T2D development(67).

1.3.2 Insulin Resistance in T2D

Insulin Resistance (IR) can be defined as a biological response to subnormal insulin concentrations. It is attributed to many biological actions of insulin in target tissues of the body. T2D is a combination of both insulin resistance and inadequate insulin secretion from the pancreatic beta cells(2) (Figure 2). Although the mechanism by which T2D progresses is not completely understood, insulin resistance is considered to be a prerequisite in its development. Longitudinal studies have found that features of insulin resistance are apparent 10-20 years prior to the onset of T2D(56). Insulin acts on multiple sites and a resistance to its effect can be observed in multiple locations. Insulin resistance in the liver results as a rise in the basal level of hepatic glucose production, through the processes of gluconeogenesis and glycogenolysis(2)(67). In the muscle, insulin resistance affects glucose influx. It is also hypothesized that the plasma free-fatty acid (FFA) concentration mediates insulin resistance, plasma insulin level, and results in glucose intolerance among patients with diabetes. This ultimately contributes to alterations in glucose homeostasis leading to a characteristic hyperglycaemic state. This burdens the pancreatic β-cells to increase their secretion of insulin in order to compensate for compromised insulin sensitivity. The up regulation of β-cell function contributes to the sustenance of normal glucose tolerance (NGT)(68). However, hypersecretion of insulin leads to progressive β-cell failure, insufficient insulin secretion in the face of insulin resistance ultimately gives rise to overt diabetes. Knowledge on insulin actions at

several sites provides a better insight to T2D pathophysiology and effective treatment and prevention strategies.

1.3.2.1 Peripheral insulin sensitivity

The peripheral glucose disposal is described as glucose absorption and metabolism in peripheral tissues such as skeletal muscles and adipose tissue. The peripheral glucose disposal is promoted by insulin through multiple cellular processes that are summarised schematically in Figure 4. In skeletal muscle and adipose tissue, three glucose transporter isoforms are expressed: GLUT1, GLUT3 and GLUT4.

To initiate peripheral glucose disposal, insulin increases glucose transport rate across the cell membranes into peripheral tissue cells through binding and activating the insulin tyrosine kinase cell surface receptor. Upon activation, phosphorylation of insulin receptor substrates commences followed by down streaming of intracellular signalling cascade of substrate phosphorylation and dephosphorylation. This results in transport of intracellular glucose (the GLUT 4 isoform) to the cell membranes. Glucose uptake gets initiated in the cell by GLUT 4 transporters(69). The glucose inside the cell undergoes rapid phosphorylation by a hexokinase enzyme which preserved the glucose in cell for utilisation. The phosphorylated glucose, glucose 6– phosphate, is then reserved as glycogen or gets oxidised through conversion into pyruvate and acetyl-CoA, which later enters the TCA/Krebs cycle in the mitochondria for synthesis of energy.

Insulin further regulates the glucose storage as glycogen. Insulin triggers the glycogen synthase enzyme pathway and also inhibits the glycogen phosphorylase action that breaks down glycogen. This leads to cumulative increase in glycogen, which is then utilized

when blood glucose levels fall(69). Glucose is primarily accumulated in muscles for later use and to a lesser extent in the adipose tissue as glycogen(70,71). Glucose is not stored directly, as glycogen is oxidised for energy along with other metabolic end products which is regulated by insulin through glycolysis involving a series of enzymatic reactions(72). Specifically, insulin initiates the 6-phosphofructokinase an essential enzymatic component in the glycolytic pattern of enzyme reactions(69,73,74). Pyruvate is the final product of glycolysis. In the presence of oxygen (oxidative metabolism), pyruvate gets translocated into the mitochondria and then converted to Acetyl-CoA and converges into the Krebs/TCA cycle for release of energy, CO_2 and H_2O. In the adipose tissue, glucose undergoes glycolysis forming fatty acids which consecutively gets esterified to triglycerides(70–72,75,76).

In the presence of restricted oxygen, the pyruvate reflecting the final stage of glycolysis is transformed to other glucogenic intermediates such as lactate or alanine. This process synthesizes lower energy than oxidative metabolism. The lactate from the muscles enters the liver for transformation to glycogen, and thus lactate is a precursor and sequelae is defined as the Cori cycle(69,72). The conversion of glucose to glycogen by direct method and the indirect conversion of lactate to glycogen constitute the non-oxidative glucose metabolism(77). Few studies using euglycemic insulin clamp have provided data that up to 90% of glucose is preserved as glycogen whereas the rest of the glucose gets oxidised for energy through glycolysis(77–79). A study on quantified glucose disposal following a meal reported that a portion of glucose (33%) into the cell go through direct storage as glycogen and the remaining (67%) encounter glycolysis. This reaction can undergo either an oxidative or a non-oxidative process synthesizing glucogenic intermediates such as

lactate. These non-oxidative products are stored in the liver as glycogen (12%), and the liver utilises these products in glucogenesis process which reforms glucose(79).

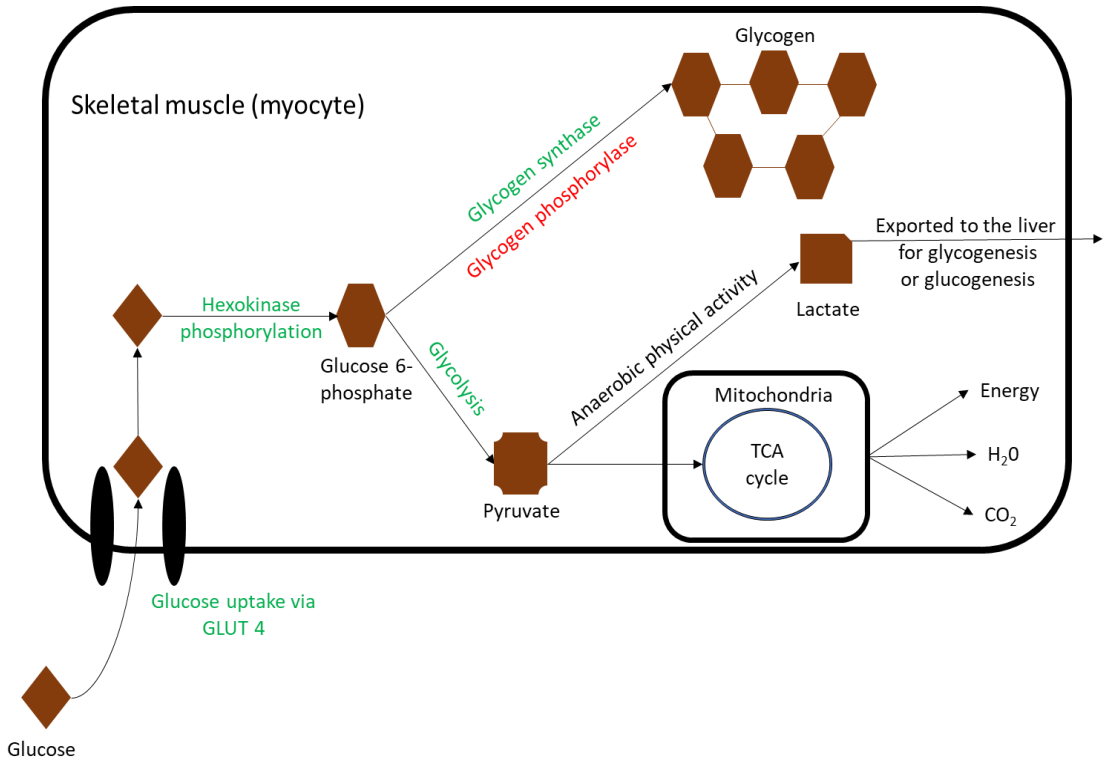


Figure 5: An illustrative diagram representing the process of glucose disposal in skeletal muscle regulated by insulin.

1.3.2.2 Hepatic insulin sensitivity

The liver is a powerhouse for a multitude of metabolic reactions that contribute to glucose homeostasis. The processes include hepatic gluconeogenesis, glycolysis, glycogen synthesis (glycogenesis) and glycogenolysis, which together aids in net hepatic glucose production. Along with other hormones and substrates, insulin affects these processes and facilitates suppression of hepatic glucose production(80). While skeletal muscle is primarily responsive to insulin on stimulation, the liver is sensitive to insulin even at minimal concentrations, including those of the basal/fasting state. The first source of glucose to glucose-requiring organs, such as neural tissues, is via endogenous glucose

production. The organs involved in endogenous glucose synthesis are predominantly hepatic (95%) followed by extrahepatic glucogenic organs (mainly renal; 5%). Hence, liver is an integral part of endogenous glucose production(80–82).

Unlike the muscles, glucose entering into the liver occurs primarily through GLUT 2 glucose transporters and is independent of insulin(83). Glucose that enters the hepatocytes is phosphorylated by glucokinases, rather than hexokinases present in the muscle, which inhibits glucose from being exported by hepatocytes(84). The glucokinases activation is mediated by insulin; therefore, insulin plays a crucial role in retaining glucose in hepatocyte. The glucose is consecutively used as a substrate for glycogen production and for hepatic lipogenesis(76,85,86).

1.4 Ethnic differences in the pathophysiology of T2D

There are phenotypic differences in the presentation of T2D in BWA patients that have implications for their clinical management, but these are not fully understood. While T2D in South Asians is relatively well studied, T2D in BWA population is an emerging science. Insulin resistance increased hepatic glucose production and β-cell dysfunction contribute to the development of impaired glucose tolerance (IGT) and T2D(2). Insulin resistance is associated with an atherogenic lipid profile (87) and other characteristics of the Metabolic Syndrome (MetS) (88), which are thought to explain the high prevalence of cardiovascular disease (CVD) in MetS and T2D (8,89). There is a strong association between central obesity, in the development of insulin resistance and T2D.. However, the process in BWA people may be different from other ethnic groups (90).

In summary BWA have high diabetes risk; possibly more rapid progression of disease; higher risk of stroke and historical evidence of lower risk of MI that may now be changing making it timely to do this project.

1.4.1 Impact of ethnicity on Insulin resistance and Insulin secretory function

Insulin resistance has been reported to be higher among black African-Caribbean populations(91). The association of obesity, specifically central obesity is strongly linked to insulin resistance and T2D development. (92,93). Epidemiological data shows that black communities are diagnosed with T2D at a younger age and have a lower BMI with central adiposity (waist circumference) at diagnosis(42,94,95). Studies conducted on assessment of body composition, showed that greater muscle mass, lower visceral and hepatic fat distribution in black populations contributed to anti-diabetogenic properties(96–99). The progression of T2D increases with age and subsequently accelerating propensity for adiposity(100,101). The black individuals diagnosed with T2D at a younger age and with low BMI suggest that other factors predispose black communities to develop T2D.

Studies conducted on women assessing hepatic and peripheral insulin sensitivity reported either no ethnic differences or reduced sensitivity in Black compared to White populations. The differences might be due to the adiposity status among women(102). Studies on women assessing hepatic insulin sensitivity showed lower gluconeogenesis hence lesser fasting hyperglycaemia in Black as compared to White populations(103). Studies suggest that women have lower insulin sensitivity within black populations than men, which suggests underlying gender-specific pathology(104,105). These findings elucidate ethnic disparities.

Study by Goff *et al*. reported that the Black (west) African Men (BAM) in UK had lower waist circumference than White European Men (WEM) (106). Studies on other ectopic fat deposition in BAM people are limited; however, a study on African Americans (AA) suggests intramyocellular and intrahepatocellular lipids were relatively low(97). There was evidence for higher insulin secretory responses (attributed to compensation for early insulin resistance) in AA people, which may predispose to accelerated β-cell failure with the early development of impaired glucose tolerance (IGT) or fasting glucose (IFG) before the onset of diabetes. This process may be exacerbated in BAM populations. Nevertheless, BAMs reported higher insulin responses compared to white or Latino peers(107,108). Such insulin hypersecretion has recently been recognised in UK-dwelling black African-Caribbeans(106). However, impaired insulin extraction by the liver in response to glucose stimulation may also lead to peripheral hyperinsulinemia. These studies demonstrate that the pathological development of T2D in black African-Caribbean populations is different.

The Prediabetes's Pathobiology in A Biracial Cohort (POPABC) study has reported the strong association of family history and ethnicity. Published data revealed that in the presence of family history, black and white children are susceptible to prediabetes at a similar high rate of 11% per year(109–111).

In a widely accepted paradigm of T2D increased insulin secretion represents a compensation for insulin resistance to maintain normal glucose levels. Hence, it is essential to note that when studies adjust the prevailing insulin sensitivity of black populations, their insulin response to glucose remains more significant than that of white

Europeans, revealing that the phenomenon is not a compensatory response to increased insulin resistance(108,112–114). Therefore, it has been suggested that β-cell function may be enhanced in black population, contributing to their increased risk of T2D by causing early β-cell exhaustion(108).

1.4.2 Impact of Ethnicity on Lipid metabolism

The observation that abdominal/visceral fat is a more sensitive predictor of insulin sensitivity than BMI has led to considerable interest among black populations. Studies of black AA and UK African-Caribbean populations suggest that the pathophysiology of T2D in BAM group is different from other WEM group. WEM developed central obesity, and insulin resistance with an atherogenic lipid profile whereas, black African-Caribbean people developed hypertension and insulin resistance with a cardio-protective lipid profile(91).

Fasting triglycerides (TG) are lower and high-density lipoproteins (HDL) cholesterol levels are higher in black African American men and women than in men and women of White European origin (WEO) (115). These differences occur in children as well as adults(116). In UK, lower fasting TG and higher HDL cholesterol levels have also been observed in African-Caribbean adults(42,91,117). In addition, in a cross-sectional survey among 4,796 children, aged 9-10 years in three UK cities, Black African-Caribbean children had higher HDL-cholesterol and lower TG levels than WEO (118). Studies which have measured postprandial TGs in black Africans are limited in North America, and there have been no studies of postprandial TGs in the UK African-Caribbean population. In North America, postprandial TGs were lower in lean black African American men and women than men and women of WEO (119). In a small study of obese

women, there were no differences between the two ethnic groups whereas no studies have been reported for obese African American men(120). The differences in plasma TGs may be related to the different fat distribution between these two ethnic groups. People of WEO develop central obesity, and this is associated with an atherogenic lipid profile. However, black African American men and women have lower visceral adiposity than white Americans(121). In the same study, abdominal subcutaneous fat was higher in black African American men and women than white men and women. The lower visceral adipose tissue in black African Americans was accompanied by significantly lower apolipoprotein B concentrations and higher plasma HDL cholesterol levels compared with White Americans(122).

Black African men and women also have a reduced prevalence of hepatic steatosis(123). This may also be related to the lower central obesity since visceral fat and hepatic steatosis are positively correlated(124). This correlation suggests significant differences in hepatic TG metabolism between Black African Americans and people of White European Origin. It has been investigated using stable isotope techniques in two recent papers. A Study done by Miller et al investigated TG kinetics in obese African American (AA) and Caucasian women. They found that plasma low - density lipoproteins (VLDL-TG) concentrations were lower in AA women due to a lower total VLDL-TG secretion rate. Lower plasma NEFA concentrations have accompanied this observation. Moreover, their work demonstrated that AA women secrete smaller VLDL particles containing less TG than Caucasian women(125). The Study conducted by Nielsen et al revealed that REE in AA participants were lower, but had similar FFA concentrations and flux than Caucasian participants(126).

Another explanation for the lower postprandial TGs in AA is that there is a lower hepatic uptake of chylomicron remnants after feeding due to increased peripheral hydrolysis of TGs in chylomicrons and subsequent uptake of free fatty acids into adipose tissue. Fasting post heparin lipoprotein lipase (LPL) activity is increased in lean African American men and women compared to people of WEO(119,120). Fasting vastus lateralis skeletal muscle-LPL activity is also higher in sedentary pre-menopausal African American women than non-Hispanic white women(127). Despres et al. also reported that black African American men and women had a higher post heparin LPL and lower hepatic lipase (HL) irrespective of gender. These results showed an HL/LPL ratio twice as high in white Americans as in black Americans(122).

A study of African Americans with insulin resistance showed no insulin resistance effect on post heparin LPL(127). This study contrasts with the insulin resistance of post heparin LPL in people of WEO with Metabolic syndrome(128). It explains the coexistence of insulin resistance with normal triglyceride levels in African Americans, i.e., the insulin sensitivity of lipid metabolism is maintained despite decreased sensitivity of glucose metabolism.

The lower prevalence of hepatic steatosis in men and women of black African Americans also suggests that hepatic TG metabolism differs from men and women of WEO. Hepatic steatosis would be expected to develop when hepatic TG synthesis and TG delivery to the liver exceeds TG export, leading to TG storage. The mechanisms which lead to hepatic steatosis may be related to increased postprandial delivery of remnants to the liver, increased de novo lipogenesis, enhanced free fatty acid delivery from adipose tissue and decreased hepatic fatty acid oxidation. None of these pathways have been measured in

African Americans or in UK, among the African Caribbean population. Increased clearance of TG rich lipoproteins, as described above, would reduce the delivery of remnants, and consequently TGs to the liver. However, the main contributor to TG synthesis is NEFA delivery to the liver. It has been shown that elevated plasma NEFA levels stimulate VLDL production(129). There is some evidence that NEFA release from adipose tissue into the circulation is lower in African Americans and UK African Caribbeans. The UK African Caribbeans with standard glucose tolerance have been shown to have lower NEFA levels than people of WEO(130). African American children have demonstrated to have forty per cent lower lipolysis rates than white American children(131). Although insulin levels were 20% higher in the African American children who would be expected to suppress lipolysis when this was controlled for in multiple regression analysis, this did not explain the lower lipolysis rates. This suggests that Black African Americans have higher insulin sensitivity to lipolysis.

Further evidence from a study conclude that obese African American women who were resistant to insulin's effect on glucose however were sensitive to insulin's anti-lipolytic effects(132). However, in the UK, one study has suggested that NEFA levels may not be lower in African Caribbean men(91). Further studies are needed to clarify whether the rate of lipolysis (as opposed to NEFA levels) is lower in African Caribbean men and whether there is a lower contribution of circulating NEFA to VLDL TG synthesis which may explain the lower TG levels.

1.4.3 Impact of Ethnicity on Cardiovascular Health

There are phenotypic differences in the presentation of T2D in BAM patients that have implications for their clinical management. While T2D in South Asians is relatively well

studied, T2D in BAM people is gaining momentum. Insulin resistance is associated with an atherogenic lipid profile and other characteristics of the Metabolic Syndrome (MetS), which are attributed to the high prevalence of cardiovascular disease (CVD) in MetS and T2D. The role of obesity, specifically central obesity, in the development of insulin resistance and T2D is well established. However, the process in BAM people may be different from other ethnic groups.

BAM are more prone to insulin resistance and studies reveal that hypertension is more prevalent. Atherosclerosis Risk in Communities (ARIC) cohort study reported that Black community have 38% higher risk of ischemic stroke events than the White community(133). Several Cohort studies such as Jackson Heart Study, Atherosclerosis Risk in Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA) and Multi-Ethnic Study of Atherosclerosis (MESA) have reported a higher prevalence of CVD in BAM(134). Ethnicity as a risk factor for CVD was independently studied in MESA study, reported that Black African population in US had a greater risk for health hazards. Goff *et al* showed that in UK BAM, waist circumference was lower than in WEM. Studies on ectopic fat deposition in BAM people are limited but a study in African Americans (AA) suggests intra-myocellular and intra-hepatocellular lipids are relatively low. Interestingly, a report from case series illustrated a higher incidence of Haemoglobin S or C traits in Black Africans linked to Coronary Artery Disease(135). Studies on platelet aggregation demonstrated presence of inherited platelet functioning pathways specific to AA population(136,137). In the UK, the BAM population has cardio-protective lipid profile (138)and lower cardiovascular disease rates (139–141) observed among T2D patients. Existing studies on African Americans are limited.

1.5 Methods for assessment of β-cell functions and insulin sensitivity

Glucose tolerance is primarily an interaction between insulin sensitivity and insulin secretion. The progressive deterioration of these two processes results in glucose intolerance and T2D(142). Insulin sensitivity quantifies the efficiency of insulin to decrease blood glucose concentration by stimulating glucose absorption and reducing its production. The direct method for assessment of insulin sensitivity is the euglycaemic - hyperinsulinaemic glucose clamp and Insulin Suppression test(143). Minimal Model Analysis of Frequently Sampled Intravenous Glucose Tolerance Test, oral Glucose Tolerance Test/Meal Tolerance Test are indirect methods available. When used with stable isotopes, it facilitates hepatic and skeletal muscle insulin sensitivity measurement, thus provides information about the defects in insulin actions. Among Black and White communities, several studies have used the euglycaemic–hyperinsulinaemic clamp method with isotopes to investigate the in vivo tissue-specific insulin resistance. These studies have primarily been conducted on women and high-risk adolescents. In adolescents blacks, peripheral insulin sensitivity is either decreased or similar compared with white populations with no specific reasoning(108,113,144–147). For comparison of hepatic insulin sensitivity, black adolescents had neither had any difference nor any greater sensitivity than white adolescents(113,144–146). Beta-cell function (BCF) assessment is challenging because of the beta-cell response complexities to the secretory stimuli. The secretory response following intravenous glucose is characterised by a first and a second phase and by augmentation and suppression phenomena related to previous glucose exposure. The beta cells usually get accustomed to insulin resistance, thus BCF assessment is mainly dependent on insulin sensitivity. Methods for BCF assessment are homeostasis model assessment (HOMA), oral glucose tolerance test, intravenous glucose

tolerance test ,meal tolerance test, arginine stimulation test and hyperglycaemic tolerance test(142).

1.5.1 Euglycemic hyperinsulinaemic clamp

The euglycemic hyperinsulinaemic clamp is used for the evaluation of insulin sensitivity. DeFronzo et al developed and published the hyperinsulinaemic-euglycemic clamp as a technique for assessing *in vivo* insulin sensitivity(143,148). The clamp method is based on establishing a hyperinsulinaemia condition by giving insulin peripherally and allowing for glucose levelling. Concurrent administration of an exogenous glucose infusion is done to sustain euglycemia in the presence of hyperinsulinaemia. Individual's sensitivity to insulin is measured through the glucose infusion rate needed to sustain euglycaemia during hyperinsulinaemia. This method speculates that the peripheral insulin infusion inhibits the endogenous glucose synthesis thereby making glucose to be the only source is through glucose infusion. The glucose infusion rate is expressed as the M value, which measures cumulative glucose disposal or entire body insulin sensitivity(149). It must be standardised by expressing the infusion rate per metabolic size unit for comparison of M value. It is expressed as fat-free mass (FFM), kg body weight, or body surface area (BSA)(150). Insulin sensitivity can also be denoted as an insulin function by normalising total glucose disposal per unit of insulin(143).

1.5.2 Hyperglycaemic clamp

Hyperglycaemic clamp is used for BCF assessment where in a unit increase in glucose concentration triggers both first- and second-phase secretion. The BCF indices are absolute or incremental (above basal) first- and second-phase insulin responses in a standard glucose profile. First-phase indices are measured as an insulin area in the first 8–10 minutes, whereas second-phase indices are computed as the mean insulin values

after the initial peak declines. BCF indices are also measured using correct insulin secretion which is obtained by using C-peptide deconvolution.

The advantage of the clamp technique is that the insulin sensitivity and BCF indices are unaffected by confounding factors and have an unambiguous physiological functions making it a gold standard method(143). Studies among black and white populations that estimated total body insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp have shown varying results. Studies found decreased whole-body insulin sensitivity in Black women compared to Whites. Among obese black women, the mean differences in total body insulin sensitivity were 40% to 60% lower compared with obese white women, caused by lower non-oxidative glucose disposal(96,151,152). No ethnic differences in total body insulin sensitivity were reported in studies which recruited both genders(153–157). However, a single study among non-diabetic black men reported higher whole-body insulin sensitivity (25%) compared to non-diabetic white men(154). Therefore, the present thesis assesses insulin secretion by hyperglycaemic clamps and meal tolerance tests and insulin sensitivity by euglycemic hyperinsulinaemic glucose clamps using isotope tracers.

1.5.3 Intravenous glucose tolerance test

Bergman et al have developed an indirect method to estimate intravenous glucose tolerance with minimal model analysis for insulin sensitivity. This mathematical model combines two equations with four model parameters(158,159). When altered to include insulin, this method correlates with the glucose clamp method. This model did not generate predictions of insulin sensitivity in cases of insulin secretion without insulin alterations, such as during T2D(149,160).

The intravenous glucose tolerance test (IVGTT) measures glucose, insulin and C-peptide after a glucose bolus injection. Information on the b-cell responses and insulin-mediated glucose disposal are determined from first-phase (acute) insulin secretion and second-phase (more prolonged) insulin secretion(158,161). Several studies have extensively used the IVGTT method in black and white population, and prominent insulin resistance have been reported in black participants(162–164). In 1993, the first study was conducted among black and white participants using the IVGTT with minimal model analysis had reported no ethnic difference in insulin sensitivity in average glucose tolerant obese women(165). Another large study using IVGTT found about 35 to 52% lower insulin sensitivity in Black participants than white participants(166,167). 'The Insulin Resistance Atherosclerosis Study' (IRAS), study has used the IVGTT with minimal modelling and reported 29% to 41% lower insulin sensitivity in black participants than whites without T2D and no ethnic difference was observed in T2D patients(168,169). When participants were pooled in the IRAS study, insulin sensitivity was lower in black participants compared to white participants(169). Since then, multiple studies on patients without T2D have reported higher insulin resistance in black subjects compared to the whites(170–175). However, among patients with T2D less pronounced differences in insulin sensitivity were observed, elucidating early impairments were initiated even before progressing to T2D(125,162). Several studies have reported increased insulin sensitivity in healthy black population compared to white adolescents due to early defect of insulin sensitivity(113,176,177). However, it is unclear as some studies have reported no differences in the IVGTT measurements(125,127,165,172).

1.5.4 Mixed meal tolerance test

The **Mixed meal tolerance test (**MMTT) monitors the physiological responses more closely during an individual's routine life compared to OGTT, MMTT, and IVGTT. OGTT and MMTT were formulated for evaluation of insulin secretory function under more physiological environment. During the MMTT, as with the OGTT, the full incretin hormonal effect and the bodily responses to a load of mixed nutrients absorbed from the gut at differing rates after an oral ingestion of nutrients(178,179). Although MMTT has greater physiological significance it is more difficult to standardise and cumbersome to administer.

The OGTT captures the complex relationships between glucose levels, insulin action, insulin secretory response, and hepatic insulin extraction. The OGTT measures the amplitude and accuracy of first-phase (acute) and second-phase insulin secretion rates coupled with the oral glucose after the load(180). The first-phase reactions occur within the first 30 min after oral glucose administration. Simultaneously, the second-phase reactions occur around 30-120 (or 30-180) min after the oral glucose load. The advantage of OGTT included is the simple administration in clinical practices. The OGTT has a greater physiological significance than the IVGTT as it measures full incretin hormone effects triggered by oral ingestion(181,182).

1.5.5 Fasting measurements

Under fasting conditions two methods have been used in population studies for determining the glucose and insulin balance relationships:

i) Insulin/proinsulin ratio: The insulin/proinsulin ratio reflects BCF. It is a representative marker of inappropriate intracellular processing of the pro-hormone to insulin(178,179).

ii) The homeostasis model assessment (HOMA): The United Kingdom Prospective Diabetes Study (UKPDS) for the first time used HOMA to evaluate the long-term effectiveness of several treatments among patients with T2D. HOMA model describes glucose-insulin homeostasis and is calculated using steady-state blood concentrations of insulin and fasting glucose levels to determine the target-tissue sensitivity to insulin and β-cell deficiency. HOMA was developed in 1985 and is determined from combination of beta cell function (HOMA-%B) and insulin sensitivity (HOMA-%S)(183).

1.5.6 Comparative evaluation of assessment methods

Estimation of insulin sensitivity based on representative estimates has shown to be associated with derived clamp insulin sensitivity measures in black adults(184,185). However, a study by Pisprasert et al showed that in African Americans and European Americans groups had same insulin sensitivity as measured from the clamp while surrogate estimates showed African Americans to be more insulin resistant(153). This reflects more pronounced hyperinsulinemia, which is typically reported in black communities(108,113,186). Hyperinsulinaemia may lead to an overestimation of insulin resistance from the mathematical modelling of glucose and insulin dynamics. This modelling is the basis for the surrogate indices(187). These studies suggest that using

surrogate estimates for measuring insulin sensitivity or resistance in black populations can be inconsistent which requires to be evaluated(153,188). Pisprasert et al suggested that the prevailing hyperinsulinemia in black communities have an impact on insulin sensitivity measured using IVGTT with minimal model analysis(153). The minimal model analysis is also based on mathematical modelling of glucose and insulin concentrations. No studies have compared the IVGTT with minimal modelling to the clamp in black participants.

The IVGTT is an established test for first phase insulin secretion(56). The hyperglycaemic glucose clamp assesses both the first and the second phase insulin secretion and it is considered as the gold standard for the assessment of insulin secretion. Therefore, the present thesis assessed the insulin secretion by hyperglycaemic clamps and mixed meal tolerance tests.

Understanding health disparities in BAM is important in health research. Most studies report that BAM have poor outcomes when compared to WEM. Diabetes is a major contributor of morbidity and disability worldwide. The proportion of T2D is higher than the T1D. Studies on Black African ethnic population as a risk factor for development of T2D are limited. This study explores the association of Black African ethnicity and T2D Mellitus.

1.5.7 Black ethnicity and T2D Summary

To conclude, the narratives in the literature have implicated black populations have a more aggressive disease onset. Early and intensified hyperinsulinemia in pre-diabetes may represent greater insulin secretion, which may predispose to earlier beta-cell exhaustion. Black populations have greater insulin resistance compared to white populations, the studies which assess and compare whole-body and tissue-specific insulin sensitivity from the clamp and/or isotopic tracers produce conflicting findings and have primarily been conducted in women without T2D.

1.6 Aims

This thesis aims to study the impact of black African and African-Caribbean (AC) ethnicity on the early progression of T2D and associated cardiovascular risk factors in a diaspora population. The other aim is to study the ethnic differences in insulin secretion between BAM and WEM with T2D.

1.6.1 Objectives

1. To study the impact of BWA and AC ethnicity on progression of early T2D from the SOUL-D study
2. To test the hypothesis that BAM will have significantly greater deficits in insulin secretion compared to WEM by the time they manifest T2D from the SouL-DeEP (South London Diabetes Ethnicity Phenotyping) study

CHAPTER 2: MATERIAL AND METHODS

2.1. SOUL-D Study Methods

2.1.1 Introduction

This chapter details the rationale for study design and describes the principal methods and procedures for my studies presented in this thesis.

2.1.2 Study design

This study is a sub-group analysis of patient data collected in the SOUL-D study. It is a prospective cohort study looking at the association between depression and diabetes outcomes at 2 years in people with newly diagnosed T2D.

2.1.3 Ethical approval

Ethical approval was granted by the King's College Hospital Research Ethics Committee (reference 08/H0808/1) and by Lambeth, Southwark and Lewisham Primary Care Trusts (reference RDLSLB 410) and all participants gave informed consent.

2.1.4 Setting and sampling frame

The database included patient data from 96 GP practices that had consented to be a part of the SOUL-D study. These GP practices were from the South East London boroughs of Lambeth, Southwark, Lewisham and Bromley, serving a multi-ethnic and socioeconomically diverse population of 1.25 million. The study identified 2,406 patients with a diagnosis of diabetes in the last 6 months. From this population, the study recruited 1,790 patients who had given informed consent. Data collected at baseline and patients were then followed up after 1 and 2 years (Figure 6).

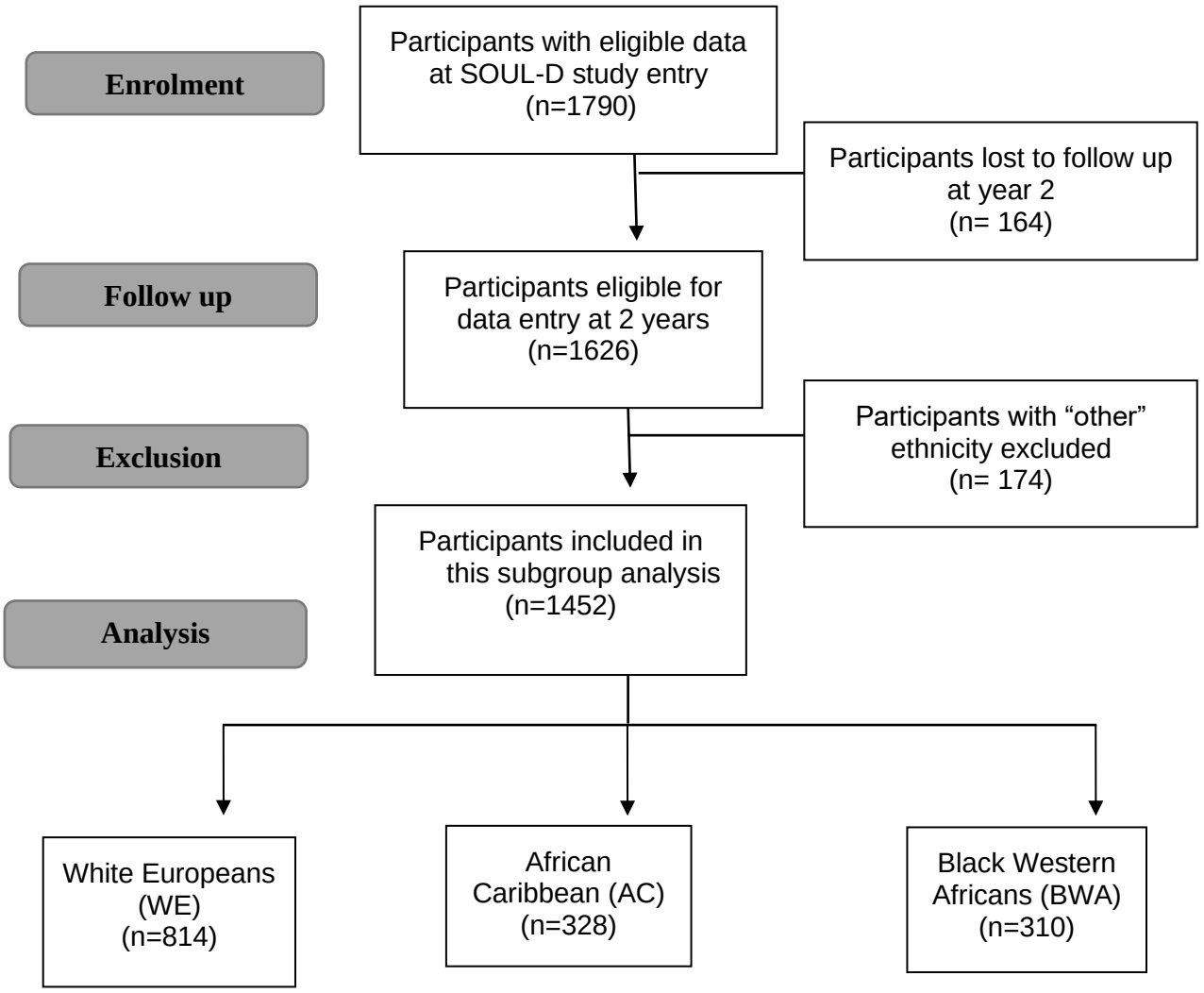


Figure 6: Flow diagram on recruitment of participants for SOUL-D study

2.1.5 Study population, case definition and study criteria

Diagnosis of T2D was made by the GPs, based on local guidelines that recommended use of the clinical criteria recommended by World Health Organization (WHO) as at the time of starting the study(189).When the WHO revised its criteria to allow for diagnosis by glycated haemoglobin (HbA1c) alone, this criterion was also accepted(190). All participants recruited to the study had a clear diagnosis of T2D confirmed and had several serial HbA1c measurements done by primary care at least 3 to 6 monthly. We had access to their diagnostic HbA1c on all our participants recruited for our study. So had a confirmed diagnosis and it did not affect our subject selection. Recent diagnosis was defined as 6 months (or less) duration. Table 1 shows the inclusion and exclusion criteria for the SOUL-D study.

Table 1: The inclusion and exclusion criteria for the SOUL-D study

Inclusion	**Exclusion**
• Males and females • Age > 18 years • Diagnosis of T2D by the WHO criteria • Recruited within 6 months of diagnosis of T2D	• Patients with dementia • Patients with terminal illness • Other types of diabetes, such as gestational diabetes • Temporary residents outside catchment area • Severe end-stage diabetes complications defined as registered blind, on renal dialysis or above knee amputation (this group are usually in receipt of intensive medical care)

2.1.6 Sample selection and method of recruitment for SOUL-D study

GP practices were invited to participate by the Primary Care Trust Diabetes Champion, clinical leads, managers, the Primary Care Research Network (PCRN) and the research team. The practices' electronic diabetes registers were screened for all current cases of diabetes (no more than 6 months duration) at enrolment of the practice and every 6 months during 2 years of recruitment. The lead GP (for diabetes) sent letters to potentially eligible patients describing the study and inviting participation. Consenting patients were invited to meet a SOUL-D researcher, usually at the patients' own practices, to complete baseline clinical data collection and complete the study questionnaires. At that visit, they were asked to undertake an additional fasting blood test. The researcher also accessed each participant's medical notes to obtain data collected at diagnosis of diabetes, i.e. before any treatment had been initiated, and to confirm patient histories with regard to symptoms at presentation and past history of macrovascular events and other therapies.

Invitations to repeat the visit were made at one and two years. We aimed to align visits with patients' routine diabetes screening since much of the data (apart from questionnaires) was the same as that collected for Quality and Outcomes Framework.

Each data collection visit took approximately 60 minutes, with an extra attendance to the GP practice or other local phlebotomy service for the fasting blood sample. If the participants had not been fasting at the time of the data collection, the SOUL-D researchers were not able to take blood.

Blood samples were delivered to one of the receiving hospital pathology departments, where they were centrifuged, analysed, aliquoted, and then frozen on the same day, using

routine pathology collection services. The local pathology departments forwarded study samples to the central laboratory at King's College Hospital NHS Foundation Trust (Viapath, London UK).

2.1.7 Measures

Baseline data were collected by a research assistant who administered a standardised data-collection schedule which included medical history, self-reported questionnaires, current prescribed medications, and physical examination. The main socio-demographic data collected were age (years) sex; and self-reported ethnicity based on 2001 UK census methods and migration (1^{st} or 2^{nd} generation) status; body mass index (BMI) (kg/m^2) at diagnosis were uploaded from the QOF database where possible. Researchers independently measured weight and waist circumference of the participants. Medication status (presence or absence of oral hypoglycemic agents and insulin) at recruitment was recorded from patient history, confirmed from the participants' medical records. Dates of diabetes diagnosis and HbA1c at diagnosis were recorded from medical records. HbA1c (%) at diagnosis was measured in one of 3 local laboratories according to IFCC methods (aligned with the DCCT) based on HPLC (Premier 9210 analyser, supplied by Menarini, Italy) and then quantified during capillary electrophoresis or electron spray ionization mass spectrometry all were DCCT standardised. Thereafter HbA1c at recruitment, one and two year follow up was measured at the King's College Hospital laboratory, latterly Viapath, using the Primus Ultra 2 analyzer (Primus Corporation, Kansas City, MO) only. The assay methods used wereTrinity Biotech Ultra 2 boronate affinity chromatography (coefficient variations (CV%) 0.82%, 0.91%, and 0.46% for normal, intermediate, and high HbA1c values based on 20 assays with the same runtime), the Trinity Biotech Premier Hb9210 analyser, also a boronate affinity chromatography-based high

performance liquid chromatography system (CV% 1.62%, 1.59%, and 1.68%for low, medium, and high values, resp.), and TOSOHG7 ion exchange with imprecision CV% less than or equal to 1.2%. For all three laboratories, the CV% was well below the recommended upper limit of 2% CV and there were no changes in the methodologies between 2008 and 2013. Percentage (%) values were converted to mmol/mol by subtracting 2.15 and then multiplying by 10.929(191).

The anti-hypertensive medications and lipid-lowering medications along with diuretics and aspirin use were recorded at baseline, year 1 and year 2. The table 2 below displays the anti-hypertensive and lipid-lowering medications that were recorded.

Table 2: List of Medications of Participants in SOUL-D record

ANTI-HYPERTENSIVES	DIURETICS	LIPID-LOWERING AGENTS
Amlodipine	Bendroflumethiazide	Atorvastatin
Ramipril	Furosemide	Simvastatin
Doxazosin	Spironolactone	Bezafibrate
Felodipine	Hydrochlorothiazide	Fenofibrate
Labetolol	Other	Colestyramine
Atenolol		Ezetimibe
Other		Other

2.1.8 Methods for sub-study

Data were extracted from the SOUL-D database for sub-group analysis. The variables that were extracted were demographical data including age, gender, ethnicity, and duration of T2D, height, weight, BMI and waist circumference.

Initially ethnicity was divided into WE, black and other ethnicity. The 'Other' category consisted of Chinese, Asian or Mixed ethnicities. For my study, black ethnicity was sub-divided as BWA and AC based on the country of origin, according to the United Nations classification of BWA countries (192) (Table 3). Those that were neither BWA nor AC were then re-allocated to the 'Other' category.

Table 3: BWA Countries

S NO	COUNTRIES	S NO	COUNTRIES
1	Benin	9	Liberia
2	Cape Verde	10	Mauritania
3	Ghana	11	Nigeria
4	Guinea Bissau	12	Sierra Leone
5	Burkina Faso	13	Mali
6	Gambia	14	Niger
7	Guinea	15	Senegal
8	Ivory Coast	16	Togo

2.1.9 Data analyses

Data were initially edited in Microsoft Excel version 14.4.8. The required variables were then uploaded into SPSS version 22. Box-plots were created for each continuous variable in SPSS and any outliers were noted. These outliers were then crosschecked against the original patient records from the SOUL-D files and corrected or left blank.

2.1.10 Statistical analyses

Statistical analysis was performed using SPSS version 22. Histograms were created for each variable to assess skewness and kurtosis, and Kolmogorov-Smirnov test was used to assess normality of the distribution of values. Descriptive analysis was carried out with data presented as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the variable means of the three ethnic groups in this study with a post-hoc Tukey's test to determine where differences occurred. This technique was used to analyse continuous variables such as BMI, waist circumference. Variance between groups, which is due to the independent variable, was compared to variance within each group, which is believed to be due to chance. An F ratio was calculated, which represents the variance between groups divided by the variance within the groups. A large F ratio indicates that there is more variability between the groups (caused by the independent variable) than there is within each group. A significant F test indicates that the null hypothesis may be rejected, however, it does not tell us which of the three groups differ. To determine this, a post-hoc Tukey's test was used, which revealed the significance in multiple comparisons. This test has a tight control over type 1 errors (rejection of null hypothesis when it is true). An alpha level of 0.01 was used to minimise the possibility of type 1 errors.

Chi-square test was used to examine the association between ethnicity and categorical variables, namely gender and medications. This tests whether the two variables are independent. If the significance value is less than 0.01, then the hypothesis that the variables are independent can be rejected and it can be confidently said that the variables are related. Similar to the ANOVA, this test is unable to give an indication of where the significance. We used a Poisson regression model (StataCorpLLC2013) to investigate the associations between ethnicity and medication used, examining the data in its longitudinal form using a random effect Poisson model.

2.2 SouL-DeEP Study Methods

The data analysed in this thesis have been collected as part of a cross-sectional observational study, the SouL-DeEP study(193) This project was able to assess and compare metabolic functions and body composition parameters related to T2D in BAM and WEM with T2D. The primary aim of the SouL-DeEP study was to investigate the hypothesis that early pronounced peripheral insulin resistance in black men, leads to an early and exaggerated compensatory insulin secretion, that ultimately fails and drives the development of early-onset T2D in the BAM population. The study was powered to detect an ethnic difference in insulin secretory function; secondary outcomes focused on quantifying tissue-specific insulin sensitivity and fat depots to generate an all-encompassing view of potential ethnic differences in the pathophysiology of T2D between BAM and WEM. A schematic for the overall SouL-DeEP study design is shown in Figure 7.

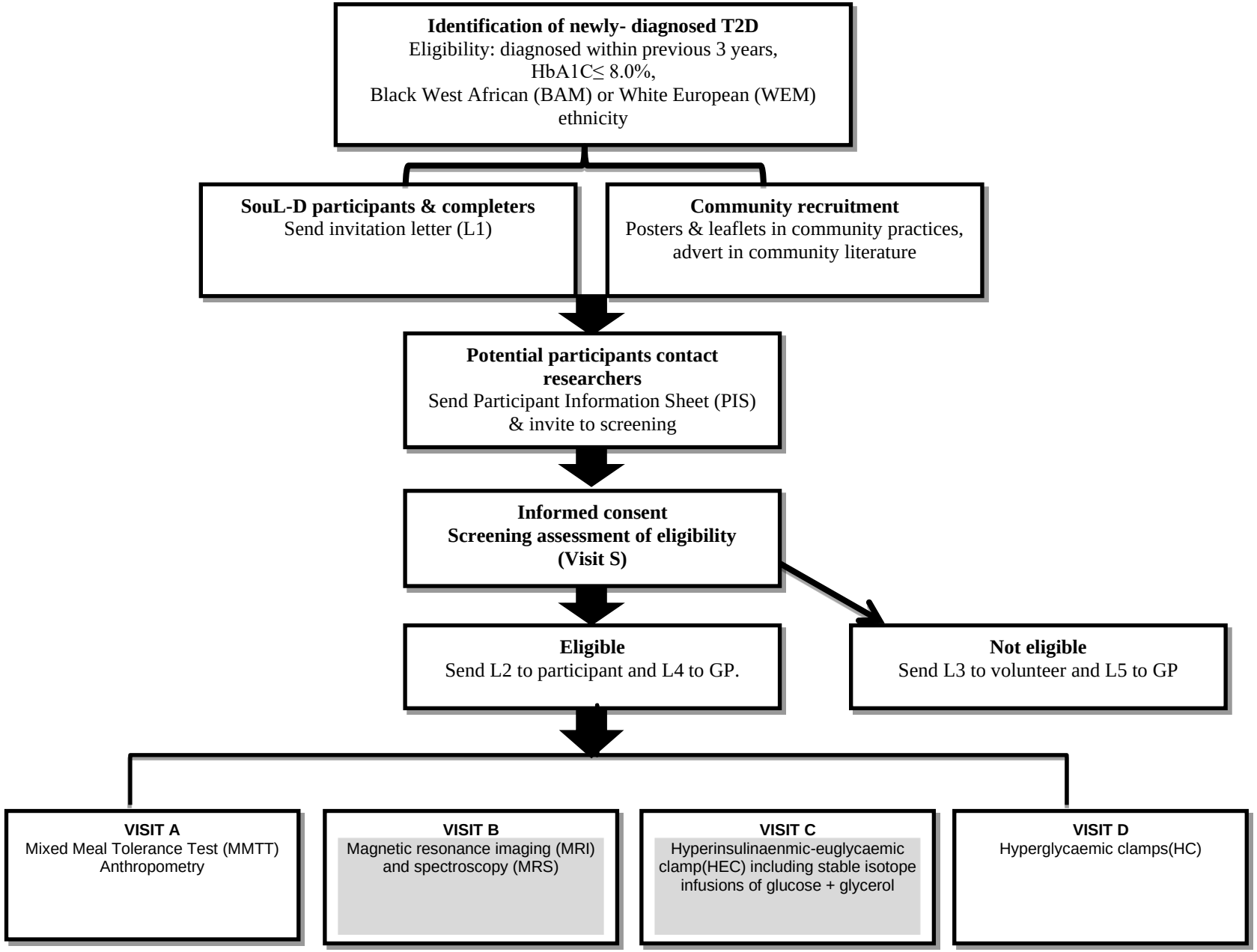


Figure 7: SouL-DeEP Study Flow Chart

Only the data from unshaded boxes Visit A and Visit D are included and discussed in this thesis.

2.2.1 Study Design:

This is a single time-point observational comparison of β-cell insulin secretory function, whole body insulin sensitivity, hepatic and peripheral insulin sensitivity of glucose metabolism, insulin sensitivity of lipolysis and intrahepatocellular, intrapancreatic and intramyocellular lipid storage in males of black West African and white European origin, aged 18-65 years, with newly-diagnosed T2D (within 5 years of diagnosis). I have presented mainly the β-cell insulin secretory function in this thesis. This was part of a larger programme of work that looked at insulin sensitivity and insulin secretion. As mentioned in the declaration section, I conducted all the metabolic assessments including the hyperinsulinemic-euglycemic clamps for assessment of hepatic, skeletal muscle, and adipose tissue insulin sensitivity on all participants, as well as the preliminary data analyses. So I have included the data published in the relevant sections and referenced, but not the entire dataset, which was also used in Dr Bello's thesis.

2.2.2 Regulatory approvals:

Regulatory approvals were obtained from Research Ethics Committee (REC)-London Bridge REC (12/LO/1859); and Research and Development Committee-KCH (KCH13-066) Research and Development Committee-GSTT (RJ113/N085).

2.2.3 Study population:

A total of 40 men with recent-onset (within 5 years of diagnosis) T2D aged between 25 and 65 years were included in the study. Study participants were 20 men with 4 grandparents belonging to BAM ethnicity and 20 men with 4 white European grandparents. BAM and WEM who had completed SOUL-D with a diagnosis of diabetes within 2.5 years were recruited from local primary care practices and from the databases

of the local community-based diabetes eye screening programme (DECS). Participants in similar age group (± 5 yrs) and BMI (± 3 kg/m2) range were chosen. As WEM were more likely to have lesser waist circumference (WC), lighter men in the BAM cohort were selected in order to avoid weight related difference in insulin sensitivity for glucose metabolism. Matching for BMI was performed to determine the differential evolution of insulin secretion and to identify the differences in insulin sensitivity between glucose and lipid metabolism. The study was restricted to men to avoid gender related differences in metabolism as T2D in BWA women was associated with obesity, which was a strong confounding factor.

2.2.4 Selection criteria

2.2.4.1 Inclusion Criteria

The following inclusion and exclusion criteria are listed below:

- 18-65 years of age
- BMI of 25-40 kg/m^2
- BWA or WE) (self-declared and confirmed by grandparental origins)
- T2D diagnosed (according to WHO criteria) within 5 years
- T2D treated with nothing more than lifestyle +/- Metformin*
- HbA1c of ≤8.0%
- Able to provide informed consent

*Metformin was stopped 7 days before any procedure.

2.2.4.2 Exclusion Criteria

Subjects were excluded from participation in the study if any of the following conditions exist:

- Thiazolidinedione treatment
- Insulin treatment
- Oral steroids treatment
- Beta-blockers treatment
- Drugs or conditions thought by the investigators to have significant impact on the study protocol or outcomes.
- Serum creatinine of >150 mmol/l
- Serum alanine transaminase level >2.5-fold above the upper limit of the reference range
- Contraindications for MRI
- Positive auto-antibodies for anti-insulin, anti-GAD or anti-IA2
- Sickle cell disease (trait permitted)
- Unwillingness/inability to follow the protocol

2.3 SOUL DEEP Study Protocol

All selected participants underwent a complete screening assessment. After obtaining the willingness from the participants, they were allocated anonymised study ID codes to maintain confidentiality. The anonymised codes of the participants were used on study documents. Eligible participants then completed the assessment visits detailed below.

2.3.1 Screening

Prior to the screening visit the participants were provided with a copy of the participant information sheet. The investigator began the screening visit by explaining the requirements of the study and offering to answer any questions. The participants were asked to give formal written consent in the presence of the researcher.

A screening assessment included:

- Age
- Self-declared ethnicity of self, parents and grandparents
- Date of diabetes diagnosis
- Relevant past medical history & co-morbidities
- Current medications
- Contraindications for MRI (e.g. metal implants/prostheses, claustrophobia, pace maker)
- Body weight
- Height
- Waist circumference, as the mid-point between the lowest rib and the iliac crest.
- Seated blood pressure assessed with an automated sphygmomanometer, using the average of 3 measurements.

- Blood sample was taken for assessment of electrolytes and creatinine (renal function), liver function tests, full blood count and sickle cell trait, full lipid profile, fasting glucose, HbA1c and auto-antibodies for anti-insulin, anti-GAD and anti-IA2.

The duration of screening assessment was about 30 minutes. The participants were offered a light meal after the screening appointment to avoid ill effects of prolonged fasting. The screening results were sent through post (L2) the selected participants whereas the screening results (L3) of the non-eligible participants were sent to them and their results were informed to their GP. Following screening, a GP letter (L4 (eligible) and L5 (not eligible)) were written to inform the GP of the nature of the study and the participants screening results (anthropometrics, blood pressure and biochemistry), a copy of the PIS was included in the letter.

2.3.2 Assessment Visits

Assessment visits were scheduled in random order. Although there were 4 assessment visits in the study, I have included data from only 2 assessment visits which were mixed meal tolerance tests and hyperglycaemic clamps for which I carried out the metabolic assessments and analysed the data.

Participants were informed to undertake the following instructions in preparation for All assessment visits:

- To refrain from strenuous exercise and physical activity in the 48 hours preceding the visit.
- To avoid alcohol consumption in the 24 hours preceding the visit.
- To consume a standardised diet the day prior to the assessment visits. The diet was matched to the participant's daily energy requirements and which provided

~50% of calories from carbohydrates (30-35% fat, 15-20% protein) which were spreaded evenly throughout the day, ensuring that no more than 30% of the daily carbohydrate load was consumed in the evening meal.

- To refrain from eating or drinking anything other than water after 10 pm on the evening before the visit or on the morning of the visit.

2.3.2.1 Assessment of incretins and post prandial Beta cell function – Mixed Meal Tolerance Test (MMTT)

The MMTT was performed in the Metabolic Research Unit (MRU), Franklin-Wilkins Building, King's College London. The meal tolerance test consisted of the consumption of a specified volume, based on body weight, of Ensure Plus milkshake drink (Abbott Nutrition, UK) in a 5 minute period. Upon arrival at the Metabolic Research Unit participants had the meal tolerance test procedure explained to them in brief. Seated blood pressure was assessed using an automated sphygmomanometer with a series of three measurements, each 2 minutes apart, were recorded. A cannula was inserted into the antecubital fossa into the non-dominant arm using standard aseptic techniques. Two fasting samples 10 minutes (-10, 0 minutes) apart were drawn and cannula flushed with 0.9% NaCl solution. The participants consumed the standardised test drink (Ensure Plus). The volume of drink was calculated based on 6cals (4mls) per kg body weight. The drink was consumed within a 5-minute period and no other liquids or foods were consumed for the duration of the MMTT. Blood samples were taken at 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 minutes for assessment of glucose, C-peptide and insulin. GLP-1 and GIP were assessed at -10, 0, 30, 60, and 120. At time points -10, 0, 30, 60 and 120 the blood sample volume was 10ml and at all other time points the volume was 7.5 ml. The total volume of blood collected was 110 ml. At the end of the meal tolerance

test, the intravenous cannula was being removed and the participants were provided with a light lunch meal.

2.3.2.2 Assessment of pancreatic insulin secretory function by hyperglycaemic clamps

The participants were admitted to the Clinical Research Facility at 8am having fasted from 22:00 hours the previous evening. They were asked to refrain from eating or drinking anything other than water. The participants were interviewed if they had followed all the instructions in preparation for the assessment visit and consumed a standardised diet the day prior to their visit. Participant weight without shoes and with light clothing, in kilograms was recorded. The height was also checked using a wall-mounted stadiometer for calculation of BSA of the participant. Calculation of the body surface area of the participant using the Mostellar formula was done which determined the priming dose glucose infusion rates (Table 4). A 20G (pink) cannula was inserted retrograde into the dorsum of the hand for blood sampling and a 3-way tap with extension was attached. Then 0.9 % NaCl infusion was attached to the blood sampling cannula and allowed infusion slowly via a standard giving set and pump for maintenance of the patency of the cannula. The hand was secured with the blood sampling cannula in the heated hand-warming unit and achieved arterialized venous blood. Baseline blood samples at time points -20, -10 and 0 minutes were taken from the cannula in the dorsum of the hand. The 3 way tap was closed to the saline and “dead space blood” of approximately 2.5ml. The saline was then allowed for infusion again. An 18G (green) cannula was inserted into the antecubital fossa vein for 20% dextrose infusion and a 3-way tap was attached and the cannula was flushed with 0.9% NaCl. 1 500ml bag of 20% glucose for infusion via IVAC was prepared. The pump and line were primed and then attached to the antecubital fossa cannula. At 0 minutes, after drawing the final baseline

blood sample the priming dose of 20% dextrose was commenced (Table 4). Blood samples were consecutively drawn every 2 minutes until 10 minutes and then every 5 minutes, throughout for the assessment of plasma glucose, using the YSI (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). The rate of infusion of 20% glucose (GIR) was adjusted in order to maintain plasma glucose at basal + 6.9 mmol/l throughout the 120 minute period. In addition to the 5-minute blood glucose samples, 7ml blood sample at 2,4,6,8,10,15,20,30,40,50,60,75,90,105,120 minutes were taken for the assessment of insulin, c-peptide and glucose. At 120 minutes, all the measurements were taken and the 20% glucose infusion was stopped. The participants were given a light meal. Blood glucose was checked again after 10 minutes. If the blood glucose was stable, then all cannulae were removed. Once the patients felt stable, they were allowed to leave. They were advised to avoid heavy exercise for 24 hours.

Table 4: Glucose prime during hyperglycaemic clamp

Time (in minutes)	**Glucose infusion rate, mg/m^2 Surface area/min**
0-1	1,768
1-2	1,428
2-3	1,156
3-4	918
4-5	782
5-6	646
6-7	544
7-8	442
8-9	408
9-10	374
10-11	340
11-12	306
12-13	272
13-14	238

2.3.3 Metabolite Assays

2.3.3.1 Glucose

Plasma glucose concentration during the screening visit was measured at the bedside on an automated glucose analyser followed by centrifugation at room temperature for 15 seconds (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). This method utilises a biosensor containing an immobilised enzyme membrane which oxidises the glucose from the sample into gluconolactone and hydrogen peroxide. The hydrogen peroxide is then oxidised, and the resulting electron flow is linearly proportional to the hydrogen peroxide and therefore the glucose concentration. Inter and intra coefficients of variation were <2%. For all other metabolites, samples were collected in BD vacutainers, centrifuged at 4°C at 3000rpm for 10 minutes to extract the plasma, which was stored at -80° for analysis in batches.

Samples were collected in plain vacutainers and allowed to clot for 20 minutes before centrifugation to extract the serum for insulin concentration analyses. This serum was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare, Camberley, UK). This required the sample to be incubated with two insulin specific monoclonal mouse antibodies; one was labelled with an acridinium ester, and the other was covalently coupled to a paramagnetic particle. This created a two-site sandwich immunoassay with insulin being the protein of interest. Following an incubation period, a magnetic field was applied which held the insulin sandwich structure together in a solid phase on the sample reaction cuvette. Any components not bound to the magnetic field remained in the liquid phase and were removed. The cuvette was then washed with deionised water before a hydrogen peroxide reagent was added to begin the light emission reaction with the acridinium ester. The light

emission intensity was measured on a luminometer as relative light units which has a directly proportional relationship to the insulin concentration given in international units per litre (IU/L). The intra-assay coefficient of variation was between 3.2 – 4.6% and the inter-assay coefficient of variation was between 2.6 – 5.9%.

Measurement of the screening blood samples (for triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol) was conducted in the central laboratory at King's College Hospital from SST vacutainers. For HbA1c, samples were collected in EDTA vacutainers and measured by boronate affinity and high-performance liquid chromatography (Premier Hb9210 analyser, Trinity Biotech, Jamestown, NY, USA). Glycated haemoglobins have a glucose moiety bound by a ketoamine bond which creates a diol group. Bonorate affinity chromatography involves the binding of a bonorate to this diol group and the glycated haemoglobin is quantified. The intra-assay coefficient of variation was between 0.72 – 1.26% and the inter-assay coefficient of variation was between 1.28 – 1.62%.

2.4.1 Hormone Assays

2.4.1.1 1nsulin (CENTAUR XP)

Principle of the Assay

Insulin reagent was supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, Gu16 8QD. The Siemens Advia Centaur assay was a two-site sandwich immunoassay using direct chemiluminometric technology using constant amounts of two antibodies. Sample was incubated with two insulin-specific antibodies. The first was in the Lite reagent, which was a monoclonal mouse anti-insulin antibody labelled with acridinium ester. The second antibody in the

solid phase was a monoclonal mouse anti-insulin antibody, which is covalently coupled to paramagnetic particles. Insulin forms a sandwich between the two antibodies. After the incubation, a magnetic field was applied causing the solid phase (including the sandwich) to be held at the site of the reaction cuvette while the liquid phase is aspirated. The cuvette was then washed with deionised water. Acid reagent (containing hydrogen peroxide) was then added to the cuvette to begin the light emission reaction with the acridinium ester. The cuvette was then moved to the luminometer and base reagent was added to enhance the light reaction. Light intensity was measured immediately and converted to relative light units. These have a direct proportional relationship with the insulin concentration.

Technical Data

Sample requirements

The specimen was serum from a plain or gel separator vacutainer. The usual precautions for vein puncture were observed. Serum was placed in the freezer at -20°C until assayed. To assay the sample in duplicate a minimum of 50 μL was required.

Table 5: Intra-assay precision of Insulin

Sample	1	2	3
N	20	20	20
Mean (mIU/L)	14.68	45.72	124.51
% CV	4.6	3.2	3.3

Table 6***: Inter-assay precision of Insulin***

Sample	1	2	3
N	20	20	20
Mean (mIU/L)	14.68	45.72	124.51
% CV	5.9	2.6	4.8

Sensitivity: The minimum detectable concentration was 0.5 mIU/L.

Linearity: Serum concentrations was up to 300 mIU/L.

Reference range: The current quoted reference range for insulin was 4.0 - 26 mIU/L

Standardization: The ADVIA Centaur Insulin assay standardization to World Health Organization (WHO) 1st IRP 66/304 assigned values for calibrators were traceable to this standardization.

2.4.1.2 Glucagon like Peptide – 1 (Total) GLP-1

Explanation of the Test

GLP-1 is constituted from 37 amino acid residues, but its active forms, GLP-1(7-36) amide and GLP-1(7-37) are found in both pancreas and intestine. In the hypothalamus, GLP-1(7-36) amide occupies 55-94% of immunoreactive GLP-1, while in the ileum, its population is 27-73%, while only a minute amount is observed in the pancreas. GLP-1 is mainly secreted in the form of GLP-1(7-36) amide.

GLP-1 is considered to be one of the incretins together with GIP. It was reported that basal level of plasma amidated GLP-1 was 4.1 pmol/l, and that it increased to 15.4 pmol/l at 10 minutes after the administration of 1g/kg of glucose to the stomach. GLP-1 enhances glucose-dependent insulin secretion, inhibits stomach movement and acid secretion, inhibits glucagon secretion, stimulates somatostatin secretion, lowers appetite, induces the intestinal epithelial growth, influences LH, TSH, CRH, oxytocin, vasopressin section in the pituitary gland, enhances glucose disposal in the peripheral tissue independent of insulin, and induces pancreatic islets growth including beta cell proliferation.

Another incretin, GIP, strongly enhances GLP-1 secretion. GLP-1 secretion from the lower intestine (ileum) may be caused by cholinergic impulse and stimulation of peptidergic mediators and not by the direct stimulation by food.

Principle of the Assay

GLP-1 Elisa kit is distributed by Millipore Corporation, 290 Concord Road, Billerica, MA 01821 USA. This kit was for non-radioactive quantification of Glucagon-Like Peptide-1 [i.e. GLP-1 (7-36 amide) and GLP-1 (9-36)] in plasma and other biological

media. The GLP-1 sequence is highly conserved between the species, with no sequence variation occurring in all mammals.

This assay is based, sequentially, on: 1) capture of total GLP-1 from the sample by a polyclonal antibody, immobilised in the wells of a microwell plate, 2) washing to remove unbound materials, 3) binding of an biotinylated anti- GLP-1 monoclonal antibody to the captured molecules, 4) washing off unbound conjugate, and 5) conjugation of horseradish peroxidase to the immobilized biotinylated antibodies 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity was measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured GLP-1 Total in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of GLP-1.

Technical Data

Sample requirements

For plasma collection, blood was collected in Vacutainer® EDTA-plasma tubes with DPPIV inhibitor. At 4°C and1000 xg for 10 minutes in refrigerated centrifuge or place tubes on ice are centrifuged within one hour. Ideally 50µL of DPPIV Inhibitor was added to a 4mL EDTA tube prior to the blood being added. Specimens were stored at 4^{o}C if they were tested within 3 hours of collection. For longer storage, specimens were stored at ≤-20^{o}C. Multiple (>3) freeze/thaw cycles were avoided. To analyse the sample in duplicate at least 100 µL of plasma was required.

Table 7: Intra-assay precision of GLP-1

	Level 1	Level 2
N	8	8
Mean (pmol/L)	32	216
CV%	1	2

Table 8: Inter-assay precision of GLP-1

	Level 1	Level 2
N	8	8
Mean (pmol/L)	39	220
CV%	12	10

Linearity-Any results above 1000 pmol/L were reported as >1000 pmol/L. If necessary, the samples were diluted using assay buffer and were repeated in the next batch.

Sensitivity-The minimal detectable GLP-1 concentration was 1.5 pmol/L

2.4.1.3 Human Gastric Inhibitory Polypeptide (GIP)

Explanation of The Test

Gastric Inhibitory Polypeptide (GIP) is a 43 amino acid peptide structurally related to glucagon and secretin and is found in the mucosa of upper intestine produced by K-Cells. GIP was originally detected as a factor inhibiting the secretion of gastric acid and gastrin secretion. Its major action has now been determined to be a potent stimulant of B-Cells to release insulin and is also known as Glucose-Dependent Insulinotropic Peptide.

Principle of the Assay

GIP Elisa kit is distributed by Millipore Corporation, 290 Concord Road, Billerica, MA 01821 USA. This kit is for non-radioactive quantification of human GIP in human serum, plasma, tissue extract and cell culture samples. This kit has 100% cross reactivity to human GIP (1-42) and GIP (3-42).

This assay is a sandwich ELISA based sequentially on: 1) capture of human GIP molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-GIP monoclonal antibodies, 2) wash away of unbound materials from samples, 3) binding of a second biotinylated anti-GIP polyclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) incubation of streptavidin-Horseradish peroxidase conjugate to bind to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5' tetramethyl benzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in

absorbency is directly proportional to the amount of captured human GIP in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human GIP.

Technical Data

Sample requirements

Although DPPIV inhibitor is not required to be added to serum/plasma samples for measurement of total GIP, it is recommended that DPPIV inhibitor should be added during sample collection so that the samples may be used in the future for the measurement of (1-42) GIP.

Patient should be fasting 10 - 12 hours prior to collection. Antacid medications or medications that affect intestinal motility or Insulin secretion should be discontinued, if possible, for at least 48 hours prior to collection.

The specimen should be serum from a plain or gel separator vacutainer. The usual precautions for vein puncture should be observed. Plasma collected from EDTA tubes can also be used.

Blood samples should be spun at 4°C and serum/plasma aliquoted into a 2mL tube and placed in the freezer at -20°C until assayed. For long term storage keep at -70°C. Avoid repeat freeze-thaw cycles.

To assay the sample in duplicate a minimum of 40 µL is required. However, the sample tube should contain at least a volume of 100 µL to account for the dead volume and dilution.

Table 9: Intra-assay precision of GIP

	Level 1	Level 2	Level 3	Level 4
N	6	6	6	6
Mean (ng/L)	15	21	185	279
CV%	6.7	7.3	8.8	3.0

Table 10: Inter-assay precision of GIP

	Level 1	Level 2	Level 3	Level 4
N	6	6	6	6
Mean (ng/L)	26	50	134	166
CV%	6.1	3.3	2.3	1.8

Linearity: Any results above 2250 ng/L was reported. The samples were diluted when required using Assay Buffer and repeated in the next batch.

Sensitivity: The minimal detectable GIP concentration was 4.2 ng/L

Reference range: The reference range for GIP was 75 – 325 ng/L

2.4.1.4 C-peptide

Explanation of the Test

C-peptide levels reflect pancreatic β-cell reserve and have been regarded a better measure of this than insulin. Meire 2009EMD Millipore's Human C-Peptide radioimmunoassay kit was for the quantitative determination of Human C-Peptide in serum, plasma, and other tissue culture media. It is a completely homologous assay since the antibody was raised against purified Human C-Peptide and both the tracer and the standard are prepared with Human C-Peptide.

Principles of the Procedure

In radioimmunoassay, a fixed concentration of labelled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabelled antigen is added to this system, there is competition between labelled tracer and unlabelled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to the antibody will decrease as the concentration of unlabelled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A calibration or standard curve is set up with increasing concentrations of standard unlabelled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labelled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

Technical Data

The EMD Millipore Human C-Peptide assay utilizes ^{125}I-labeled Human C-Peptide and a Human C- Peptide antiserum to determine the level of C-Peptide in serum, plasma or tissue culture media by the double antibody/PEG technique.

Sample requirements

A maximum of 100 µL per assay tube of serum or plasma was used. Care was taken when using heparin as an anticoagulant, since an excess will provide falsely high values. Human C-Peptide was protected from proteolysis during assay procedures and sample storage.

Trasylol (Aprotinin) at a concentration of 500 KIU per mL of serum or plasma was added to samples to protect from proteolysis. For unprotected samples there was a loss of approximately 25% HCP after 3 months storage at ≤ -20°C. No loss was observed when unprotected sample were stored at ≤ -70°C for up to 12 months. Specimens were stored at 4°C if they will be tested within 24 hours of collection. For longer storage, specimens were stored at ≤ -20°C. Samples with gross haemolysis or lipemia were avoided.

Intra- and inter assay precision

The intra assay (between assays) CV was 4.2% and inter assay (within) was 6.4% for c-peptide.

Sensitivity: The lowest level of C-Peptide was 0.065 ng/mL + 2 SD when a 100 µL sample size was used.

Linearity: Aliquots of pooled human serum containing varying concentrations of human C-Peptide were analyzed in the 100 µL, 75 µL, 50 µL and 25 µL, volumes. Dilution factors of 1, 1.33, 2 and 4 representing 100 µL, 75 µL, 50 µL and 25 µL, respectively, were applied in calculating observed concentrations.

Reference range**:** Fasting C-peptide range 0.5-1.5 ng/ml

2.5 Statistical Methods

Sample size calculation: The primary outcome, upon which our study is powered, is insulin secretory reserve. As it is a novel study there are no directly comparable data to use; however, the reported differences in insulin secretion between patients with T2D and non-diabetic subjects were large (≥ one standard deviation) (43-45). A 2-group comparison with 20 per group was performed to detect a difference of 1.0 standard deviation with power 90% and significance level 5%: we recruited approximately 23 persons per group, to get 20 to completion, allowing for a 10% drop-out. This sample size had the power to detect differences in lipolysis and insulin sensitivity data (46-48).

2.5.1 Statistical analysis

All datasets were tested for normality (Shapiro-Wilks test) and non-normally distributed variables were transformed (log 10). Normally distributed variables were reported as means and standard deviations and proportions. Log normal data were transformed back to give geometric mean and 95% CI for the ratio of the geometric mean.

Demographic data were described using descriptive statistics. The independent sample t-test was used to compare the differences between the two ethnic groups. Area under the curve and incremental area under the curve were calculated for glucose, insulin,C-peptide,GLP1 and GIP from all studies. Further exploratory analyses using multiple regression was performed for potential anthropometric, biomedical and clinical confounding factors. Skewed data was transformed and presented as unadjusted and adjusted means, confidence intervals, and regression coefficients. The analyses were performed in SPSS software, version 24 (IBM Analytics).

CHAPTER 3: SOUL-D RESULTS

3.1 Introduction

The fastest growth in prevalence of T2D worldwide over the next twenty years is expected to occur in Africa, where the IDF predicts an expansion of 143%(194) .People of Black African ethnicity are at high risk of T2D as lifestyle changes, as already seen in diaspora environments. In the United States, the risk for T2D in people of Black African ethnicity is double that of non-Hispanic white populations .(195) In the UK's SABRE cohort, the prevalence of T2D was three-fold higher in first-generation immigrants of AC ethnicity aged 40 to 70 years than in people of WE ethnicity and equivalent to that in the Indian Asian ethnic group, previously considered to be at highest risk in the UK. (196) The picture is also seen in youth, with higher prevalence and more rapid increase in incidence in T2D in black compared to white youth. (197) .In the present analysis of the SOUL-D data, we have investigated the impact of black ethnicity on progression of glycaemic control over the first two years after diagnosis of T2D in a primary care system with equality of access in relation to early treatment regimens, with additional analysis of other cardiovascular risk factors in diabetes, namely hypertension and lipid profiles.

3.2 Aim

To study the impact of BWA and AC ethnicity on the early progression of T2D and associated cardiovascular risk factors in a diaspora population.

3.3 Methods

A sub-group analysis of data collected prospectively in the SOUL-D cohort study was undertaken. The methods of recruitment and data collection have been published.(198) In brief, people with new-onset diabetes were identified from review of primary care practice registers and consenting eligible participants enrolled in data collection within six months of diagnosis and at 12 and 24 months thereafter. Ninety-six urban practices from the South East London Boroughs of Lambeth, Southwark, Lewisham and Bromley participated in the study. Ethical approval was granted by the King's College Hospital Research Ethics Committee (reference 08/H0808/1) and by Lambeth, Southwark, and Lewisham Primary Care Trusts (reference RDLSLB 410) and all participants gave informed consent.

The ethnicity of participants in the SOUL-D study was self-declared, using the methodology of the 2001 UK census, (199) which includes the options "Black and Black British", subdivided into African, Caribbean and Other" and "Mixed" including "White and Black African" and "White and Black Caribbean". Participants were also asked to name their country of birth. For the present analysis, we included three groups: people who declared themselves as having white European (WE), Black West African (BWA) or African-Caribbean (AC) origin, defining West Africa according to the United Nations classification (which lists 16 countries: Benin, Burkina Faso, Cape Verde, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Mali, Mauritian, Niger, Nigeria, Senegal, Sierra Leone and Togo. (192)

Data were collected from baseline (recruitment), 12 and 24-month visits. The socio-demographical parameters included were age, sex, and self-reported ethnicity. The

anthropometric measurements recorded were height, weight, body mass index (BMI), waist circumference. HbA1c and other data at diagnosis were taken from clinical records. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded by the research team at the time of recruitment and fasting blood samples taken for HbA1c and lipid profile (including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density (LDL) cholesterol) (HPLC using Premier 9210 analysers, supplied by Menarini, Italy, all DCCT standardised, and the Siemens Advia 2400 analysers respectively). All measures were repeated at year one and year two.

Prescriptions of any oral hypoglycaemic agents, anti-hypertensives, lipid-lowering medications, diuretics, or aspirin as reported by the participants were recorded and checked against their medication records at recruitment, year 1 and 2.

Anti-hypertensive agents were grouped according to class: beta-blockers, alpha-blockers, calcium channel blockers (CCB), angiotensin-converting-enzyme (ACE) inhibitors, diuretics.

3.3.1 Statistical Analyses:

Participants were included only if they had data for all three-time points for HbA1c, blood pressure, and lipid profile. Statistical analysis was performed using SPSS version 22. One-way analysis of variance (ANOVA) was used to compare variables between the three ethnic groups, with a post-hoc Tukey's test to determine where differences occurred. Chi-square testing was used to examine the association between ethnicity and categorical variables, namely gender and medications, with a post hoc chi square test. Boxplots were created for each continuous variable in SPSS and any outliers were noted. These outliers were then crosschecked against the original patient records from the SOUL-D files and

corrected or left blank. We used poison regression model (StataCorpLLC2013) to investigate the associations between ethnicity and medication used. The data was examined in its longitudinal form using a random effect Poisson model.

3.4 Results

Of the 1790 participants in the SOUL-D database, 164 were lost to follow up at the end of two years and 174 were excluded because of reporting ethnicity other than those under consideration in this study. Of the remaining 1452 members, 310 (17.3%) reported themselves as BWA, 328 (18.3%) as AC, and 814 (45.5%) as WE (Figure 6,8 and 9).

3.4.1 Baseline characteristics

Assessment of the baseline characteristics (Table 11) showed time since diagnosis of diabetes at recruitment was not significantly different between the groups. The BWA and AC cohorts were significantly younger ($p<0.001$; WE>BWA, AC) than WE. There were fewer males in both black groups. There was no significant difference in BMI; although, waist circumference was significantly lower in the BWA and AC cohort compared to the WE.

HbA1c at diagnosis and recruitment was higher in BWA and AC. (WE < BWA and AC, $p < 0.001$). This was on no diabetes medication for the diagnostic HbA1c, but higher rates of diabetes medications in the black groups by the time of recruitment, (59.7% and 67.1% vs 47% on one or more agent, BWA, AC and WE respectively, $p <0.001$; 11.3%, 10.1% and 7.6% on two drugs). Fasting triglycerides were lower in the BWA than in either AC or WE, despite higher rates of prescription of lipid lowering drugs, all statins, in WE

(Table 2). Total cholesterol was not different, but LDL and HDL cholesterol were lowest in WE.

There were no significant differences in measured blood pressure between the groups, and no significant difference in numbers of people prescribed antihypertensive agents, although there were some variations in the nature of the drug prescribed by ethnic group (Table 2) and more AC were prescribed >2 anti-hypertensives, compared to the other groups (16.4% vs. 10.0% in BWA and 10.5% in WE, $p<0.05$).

3.4.2 Progression over two years

BMI did not change significantly from baseline in any of the three groups. The waist circumference had increased in all groups however, with the largest increase in the WE, in whom it remained significantly higher than in the other groups (WE > BWA, AC $p<0.01$) (Table 11).

At two years, HbA1c was not different between groups (Table 11). However, the black participants were being prescribed more diabetes medications (Figure 10), with progressive increase to year 2. These differences were already present at year one (Table 13,14). Insulin secretagogues and insulin sensitisers were commonly prescribed ($p<0.001$) in black groups.

Systolic blood pressure fell during the study, with no significant differences in systolic or diastolic blood pressure at one or two years between groups. The proportion of people on antihypertensive medication was not different between groups at either one or two years, but at year one more people in the AC group were on more than two antihypertensive

medications (13.7% vs 8.7% BWA and 10.8% WE, $p < 0.05$) (Table S2) and by year two there were more than three times the proportion of people on more than two medications in both BWA and AC ethnicity compared to WE ethnicity (25.3, 24.6 vs. 7.9% respectively, $p<0.05$).

Total cholesterol fell throughout the study in all three groups, however there was no significant difference between the groups (Table 11). Triglyceride levels remained unchanged in all groups, with the BWA and AC continuing to display significantly lower levels than the WE ($p<0.001$). LDL-cholesterol had fallen in all three groups, with higher values in the two black groups at one year, ($p < 0.001$ AC vs WE, and $p< 0.01$ BWA vs WE) (Table 13) but no significant difference between groups at two years. HDL-cholesterol was not different between the groups at one and two years. This was achieved in association with an overall increase in the proportion of participants taking lipid lowering agents, mostly statins, such that the percentages on treatment did not differ if analysed by "lipid lowering therapy" or "statins" (Table 14). A significant difference between the three groups was observed, with a higher percentage of WE on lipid-lowering agents at both one and two years ($p<0.001$) (Table 12 &14).

Using the Poisson regression model, we studied the association between ethnicity and medication. After adjusting for HbA1c, BMI, age and gender, both BWA (IRR 1.19,95%CI 1.06-1.33) and AC (IRR 1.23,95% CI 1.1-1.38) ethnicity groups were strongly associated with increased number of medications from baseline to year 2. The adjustment made little difference, suggesting that differences were primarily due to ethnicity rather than the factors we have considered. So black ethnicity is an independent predictor of drug use, WE use less, and it is significant.

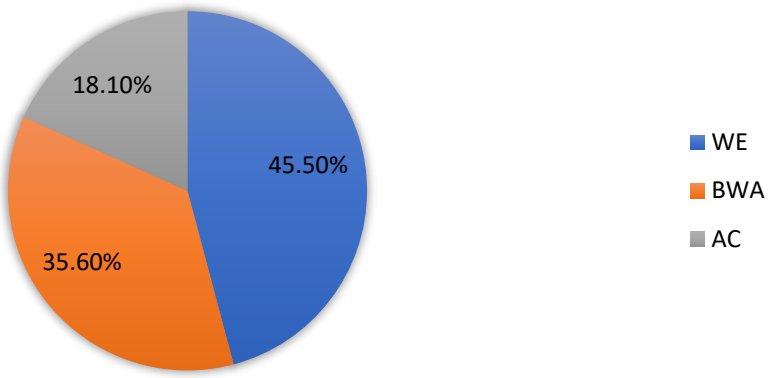


Figure 8: Initial breakdown of ethnicity in the SOUL-D study

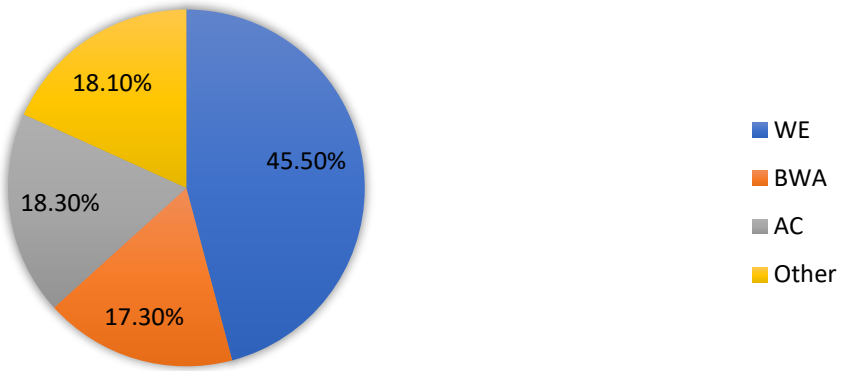


Figure 9: Further breakdown of ethnicity in the SOUL-D study, based on the origin of country

In total, there were 1,452 patients for analysis, which consisted of 814 WE, 328 AC and 310 BWA individuals. The diagram below illustrates the study population selection process.

Table 11: Demographic data and clinical characteristics of study population at baseline and two years' post diagnosis

Characteristic	BWA	AC	WE	*P		BWA	AC	WE	**P
	Baseline					Two years			
n (%)	310 (21)	328 (23)	814 (56)			310 (21)	328 (23)	814 (56)	
Age (years)	50.6±10.4	56.8±10.8	59.7±9.4	<0.001 [a]					
Duration of diabetes (months)	4.1±2.1	4.3±2.1	4.4±2.1	NS					
Gender (% male)	55.5	34.5	61.7	<0.001 [a]					
BMI (kg/m^2)	31.7±5.8	32.6±6.7	32.5±6.8	NS		31.4±5.2	32.5±6.9	32.4±6.5	NS
Waist circumference (cm)	103.1±12.1	104.0±15.6	107.1±14.9	<0.001 [b]		104.0±11.8	104.6±14.8	108.7±14.6	<0.01[e]
HbA1c (%)	8.6±2.5 (diagnostic)	8.1±2.4	7.6±1.9	<0.001 [d]		6.9±1.3	6.8±1.2	6.9±1.1	NS
	7.3±1.6 (baseline)	7.0±1.5	6.8±1.3	<0.001 [d]					
Total cholesterol (mmol/l)	4.4±1.0	4.7±1.1	4.6±1.1	NS		4.3±0.9	4.4±1.1	4.4±1.1	NS
Triglyceride (mmol/l)	1.1±0.6	1.2±0.6	2.0±1.4	<0.001 [a]		1.1±0.6	1.2±0.6	1.9±1.4	<0.001[a]
LDL-cholesterol (mmol/l)	2.7±0.9	2.8±0.9	2.5±0.9	<0.001 [c]		2.5±0.8	2.6±1.0	2.4±0.8	NS
HDL-cholesterol (mmol/l)	1.2±0.3	1.3±0.3	1.2±0.3	<0.001 [d]		1.3±0.3	1.3±0.3	1.2±0.4	NS
Systolic blood pressure (mmHg)	138.4±18.3	136.8±18.0	137.0±17.5	NS		132.1±14.9	134.0±15.7	135.7±15.5	NS
Diastolic BP (mmHg)	84.2±10.5	84.0±11.1	82.9±10.9	NS		81.2±10.0	82.4±12.3	79.8±10.4	NS

Values are mean ± standard deviation

*p values post hoc adjusted for multiple comparisons with Tukey's.

** p values are post hoc adjusted for multiple comparisons with standardised residuals

AC: African Caribbean; BMI: body mass index; BP: blood pressure; BWA: Black West African; HBA1C: glycated hemoglobin; HDL: high density lipoproteins; LDL: low density lipoproteins; NS: not significant; TC: total cholesterol; TG: triglycerides; WE: White European.

[a] WE > BWA and AC, $p < 0.001$

[b] WE > BWA, $p < 0.001$; WE > AC, $p < 0.01$

[c] WE < BWA, $p < 0.01$; WE < AC, $p < 0.001$

[d] WE < BWA and AC, $p < 0.001$

[e] WE > BWA and AC, p<0.

Table 12: Diabetes, blood pressure and lipid-lowering medications in study population at baseline and two years' post-diagnosis

Medications *at baseline*	BWA	AC	WE	**P* value		BWA	AC	WE	**P* value
	Baseline					Year two post-diagnosis			
n (%)	310 (21)	328 (23)	814 (56)			310 (21)	328 (23)	814 (56)	
Oral hypoglycaemic agents	178(58.4)	197(60.1)	382(47.5)	<0.001[a]		156(77.6)	153(70.8)	343(58.2)	<0.001[a]
Metformin	168(56.9)	187(58.8)	352(45.7)	<0.001[a]		152(61.0)	146(58.4)	316(47.3)	<0.001[a]
Sulphonylurea	35(21.5)	38(22.4)	84(16.6)	NS		54(35.1)	41(26.1)	96(20.7)	<0.001[a]
Lipid-lowering drugs	150(48.9)	174(53.0)	530(65.4)	<0.001[b]		150(48.9)	174(53.0)	530(65.4)	<0.001[b]
Statins	149(48.5)	168(53.0)	515(65.4)	<0.001[b]		149(48.5)	168(53.0)	515(65.4)	<0.001[b]
Anti-hypertensives	175(57.0)	194(59.1)	447(55.2)	NS		175(57.0)	194(59.1)	447(55.2)	NS
Beta-blockers	11(7.8)	15(10.2)	61(14.5)	NS		11(7.8)	15(10.2)	61(14.5)	NS
Alpha-blockers	12(8.5)	26(16.6)	29(7.5)	<0.01[c]		12(8.5)	26(16.6)	29(7.5)	<0.01[c]
Calcium channel blockers	113(46.5)	108(45.2)	146(28.9)	<0.001[d]		113(46.5)	108(45.2)	146(28.9)	<0.001[g]
ACE inhibitors	52(28.7)	50(27.6)	193(35.1)	NS		52(28.7)	50(27.6)	193(35.1)	NS
Diuretics	67(21.8)	87(26.5)	145(17.9)	<0.01[e]		67(21.8)	87(26.5)	145(17.9)	<0.01[h]
Aspirin	45(17.7)	71(24.4)	191(28.3)	<0.01[f]		45(17.7)	71(24.4)	191(28.3)	<0.01[f]

AC: African Caribbean; ACE: angiotensin converting enzyme; BWA: Black West African; NS: not significant; WE: White European;

* p values are post hoc adjusted for multiple comparisons with standardised residuals

[a] WE < AC and BWA, $p < 0.001$

[b] WE > AC and BWA, $p < 0.001$

[c] AC > BWA and WE, $p < 0.01$

[d] WE < BWA and AC, $p < 0.001$

[e] WE < AC and BWA, $p < 0.01$

[f] WE > AC and BWA, $p < 0.01$

[g] BWA and AC > WE, $p < 0.001$

[h] BWA and AC > WE, $p < 0.01$

Table 13: Clinical characteristics of recruited cohort at year 1

Characteristic	BWA	AC	WE	*P value
n (%)	310 (21)	329 (23)	814 (56)	
BMI (kg/m^2)	31.6±6.0	32.6±6.5	32.3±6.3	NS
waist circumference (cm)	102.7±11.9	104.2±13.5	107.5±15.5	<0.001[b]
Total cholesterol (mmol/l)	4.2±0.9	4.6±1.1	4.5±1.0	NS
Triglyceride (mmol/l)	1.1±0.6	1.2±0.8	2.0±1.5	<0.001[a]
LDL-cholesterol (mmol/l)	2.6±0.8	2.7±0.9	2.4±0.8	<0.001[c]
HDL-cholesterol (mmol/l)	1.3±0.3	1.3±0.4	1.2±0.4	<0.05[d]
Systolic BP (mmHg)	133.9±18.8	135.0±16.1	137.0±17.3	NS
Diastolic BP (mmHg)	82.4±11.1	82.7±11.1	81.8±10.9	NS

AC: African Caribbean; BMI: body mass index; BP: blood pressure; BWA: Black West Africans; HBA1C: glycated haemoglobin; HDL: high density lipoproteins; LDL: low density lipoproteins; TC: total cholesterol; TGL: triglycerides; WE: White Europeans. Data are mean ± standard deviation

*All p values are post hoc adjusted for multiple comparisons with Tukey's.

NS: nonsignificant

[a] WE>BWA, AC; p<0.001

[b] WE>BWA; p<0.001

[c] AC, BWA >WE; p<0.001

[d] AC, BWA>WE; p<0.05

Table 14: Medications prescribed to the recruited cohort at year 1

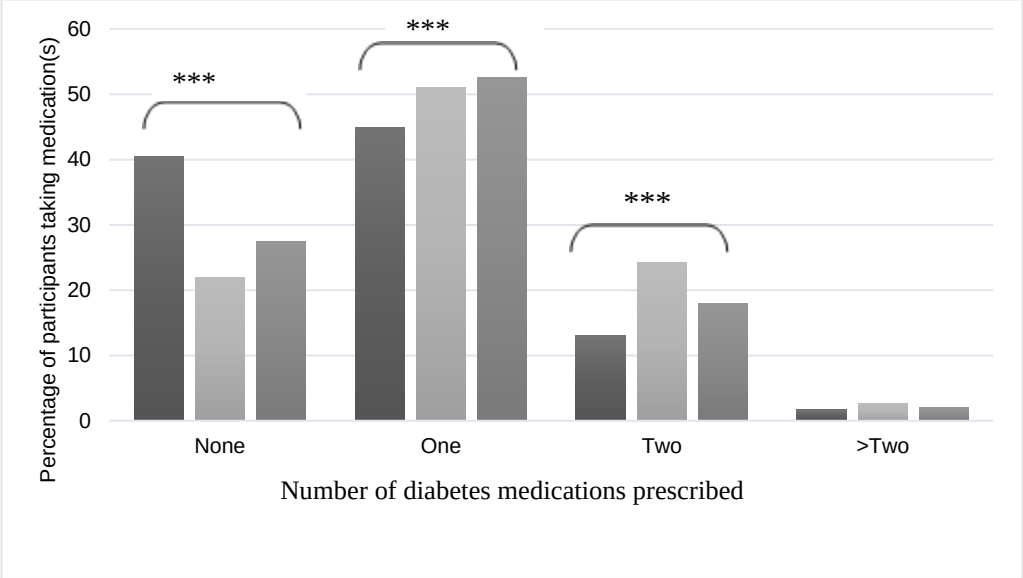
Medications at year 1	BWA (n=310)	AC (n=328)	WE (n=814)	*P value
Oral hypoglycaemic agents	181(70.7)	189(68)	392(55.2)	<0.001[a]
Metformin	178(63)	180(61.4)	366(50)	<0.001[a]
Sulphonyl-urea	46(30.7)	41(26.6)	100(21.5)	NS
Lipid-lowering drugs	136(53.3)	169(60.8)	512(72.1)	<0.001[a]
Statins	131(47.1)	165(56.3)	492(66.2)	<0.001[a]
Anti-hypertensive	155(60.8)	181(65.1)	432(60.8)	NS
Beta-blocker	12(8.6)	12(9.1)	54(14.1)	NS
Alpha-blockers	12(8.6)	23(16.1)	33(9.1)	NS
Calcium channel blockers	101(44.1)	101(45.9)	143(30.4)	<0.001[b]
ACE inhibitors	53(29.3)	59(33.0)	188(36.4)	NS
Diuretics	65(25.9)	70(25.4)	147(21.0)	NS
Aspirin	36(14.8)	55(20.9)	175(26.8)	<0.001[a]

AC: African Caribbean; ACE: Angiotensin Converting Enzyme; BWA: Black West Africans; WE: White Europeans; Data are numbers of participants (percent)

*All p values are post hoc adjusted for multiple comparisons with Tukey's

[a] WE>BWA, AC, p<0.001

[b] WE<BWA, AC, p<0.001



p<0.001

AC: African Caribbean; BWA: Black West African; WE: White European.

Dark grey bars indicate % of WE; light grey bars indicate % of BWA and mid-grey bars indicate % of AC for each number of prescriptions. *** = p<0.001

Figure 10: Medications taken by the study cohort at 2 years.

3.5 Discussion

We have prospectively examined the impact of BWA and AC ethnicity on progression of T2D and associated cardiovascular risk factors over the first two years after diabetes diagnosis in an urban population in the UK, comparing their data to those from people of WE ancestry diagnosed in the same time frame in the same primary health care system. The novel finding of our research is that our black participants achieved similar, and by current criteria, acceptable, glycaemic outcomes at two years as their white European origin peers but associated with a greater amount of medication prescribed. We noted a similar pattern with hypertension. We found lower fasting triglycerides in the two black populations at baseline, but this apparently cardioprotective lipid profile persisted only in the BWA participants, being lost in the AC over time.

Both our black populations had younger age at diagnosis of diabetes (nine years for the BWA and three years for the AC) and lower waist circumference, although BMI was not different between groups. These features have been reported in other studies of British black populations (42,106,196) as in autochthonous African populations (200) and are consistent with a more aggressive disease onset. We believe this is the first study then prospectively to examine disease progression in a single health care setting.

The apparent requirement for greater medication to achieve glycaemic treatment targets in the black participants of the SOUL-D cohort is open to several interpretations. The most important possibility is that this is evidence of a more aggressive disease onset. T2D in the UK Black population is less well studied than in other ethnic minorities but there is at least one US study in which African Americans had poorer glycaemic control than their white compatriots (201–204).We have reported evidence for a greater insulin

secretory deficit in early T2D in males of BWA ethnicity, compared to a WE peer group, (205) a finding reflected in a recent systematic review of the literature, which noted that findings in normoglycemic states are however highly variable. (206) The increasing need for medication in the present study, where health care is free at the point of delivery, is consistent with a more rapid progression of insulin lack. It is noteworthy that within two years of diagnosis, the increased burden of medication is at least delivering a similar HbA1c between ethnic groups.

Another possibility, which needs urgent further investigation, is that the dysglycaemia of the black groups is responding differently to the medications used sequentially in treatment protocols for newly diagnosed T2D. This should be addressed at least for African Americans by emerging data from the current GRADE study, which is exploring responses to first line diabetes medications in a large American cohort.(207) The local protocol for initial management of T2D in our area at the time of the study recommended lifestyle modification with metformin as the first line diabetes medication, followed by an insulin secretagogue if HbA1c targets were not being met. While more rapid progression to the addition of the insulin secretagogue fits with a more rapid deterioration in beta cell function, it might also relate to a failure of impact of insulin sensitization by metformin in people of BWA and AC ethnic origin. Metformin prescription at baseline was lower in the two black groups, and this persisted at year one, so it is conceivable an opportunity was missed. By the second year, this difference had been reversed, with slightly lower rates of prescription in the WE. The reasons for slow utilisation of metformin in the black populations, and its possible impact on future glycaemic outcomes, need to be explored.

A third possibility relates to possible ethnic differences in HbA1c. We previously reported that HbA1c was higher at diagnosis of T2D in black participants in the SOUL-D cohort and here we report that this was driven by both the BWA and AC participants. (198) Higher HbA1c in black populations with diabetes in the US has been reported and interpreted as poorer control and implicated in higher rates of complications. (201) However, there is also evidence that HbA1c may be higher in black ethnicity, driven by higher HbA1c for any degree of dysglycaemia by glucose tolerance test criteria in black people. (208) There have been suggestions that this may relate to differences in glycation, (209) or red cell life-span, (210) and this may be expected to result in more medication being used to drive levels to the same target. In the Ford study, HbA1c was not higher in blacks for normoglycaemia, and using the relevant diagnostic HbA1c criteria for diabetes gave a 6-fold increase in false positive diagnoses. In a black population in Durban, HbA1c had a high degree of specificity and sensitivity in diagnosing diabetes by glucose criteria, with an optimal cut off *lower* than that currently recommended worldwide (≥ 42 mmol/mol, or 6% vs ≥ 48 mmol/mol, or 6.5%) and correlated well with known diabetes risk factors, (211) although the data suggests a slightly greater discrepancy in prevalence of diabetes diagnosed by HbA1c vs glucose methods with age. In view of epidemiological evidence that diabetes complications happen at similar HbA1c concentrations in both black and white populations, (212) there is no current justification for adjusting the target, and the greater therapeutic burden needs to be addressed.

Medications were recorded by interview with participants and checked against clinical records. We did not formally assess participant adherence with prescribed medication in the SOUL-D cohort. While there are data to suggest an impact of ethnicity, (213,214) as well as other factors including multi-morbidity/polypharmacy, convenience, age, support

(215) on adherence to medication, the evidence that HbA1c was reasonably well-controlled by year two and the differential in HbA1c seen at diagnosis had resolved, does not suggest that there was a systematic failure to take prescribed medication in any of our participant groups, although we cannot rule this out and an increased number of prescriptions may have made that more of an issue in the black groups. (215)

Finally, we should consider whether the black population is being diagnosed at a later stage of their diabetes as an explanation for both the higher HbA1c at diagnosis, and the need for more medication to achieve target values. Although we cannot rule this out, all three groups had equal access to health care, which was free at point of delivery. It is possible that cultural differences may influence access, however, similar percentages of people being asymptomatic at diagnosis in all the study cohorts makes this an unlikely explanation for our findings. A recent UK database study of a predominantly white population with established diagnoses of T2D (mean duration around six years) recently reported that apparent equivalence of management of diabetes at diagnosis across racial groups, although it did provide data suggesting escalation of therapy was slower in Black (and South Asian) patients thereafter. (216) We did not formally test for the therapeutic inertia to which this finding was ascribed but the equivalence of outcome for greater prescription does not suggest this was occurring in our population. The SOUL-D population was nearly 40% of black ethnicity, compared to the 2.1% in the database study. It remains possible that when our population reaches the diabetes duration of the Mathur study, outcomes may be different.

With regard to other cardiovascular risk factors in people with T2D, we noted that our BWA participants displayed the highest blood pressure and the AC participants were

more likely to be on more than two anti-hypertensives at baseline. Rather in line with the diabetes data, by year 2 the BWA and the AC had achieved the lowest BP but were three times more likely to be taking more than two anti-hypertensive agents for BP control compared to WE.

In terms of cholesterol, WE had higher triglyceride and slightly lower total and LDL cholesterol at baseline, than the other groups, but by two years, there were no differences between groups in total, LDL or HDL cholesterol, although the higher triglycerides persisted, despite being most likely to be on lipid-lowering therapy. Previous studies have consistently suggested that the UK's AC population display a cardio-protective lipid profile. (138)Results from this study are at variance with these findings, although the data may be confounded by different proportions of statin use. Although in need of updating, there is evidence to suggest lower risk of coronary artery disease and higher risk of stroke in UK black populations. (139,140,217)The paradox of presence of a more favourable lipid profile despite higher cardiovascular disease risk in the black population has been reported in South Africa (218) and greater use of statins may be important in reducing cardiovascular risk in all groups in the present study. (219)

There are further studies exploring ethnic differences in development of early complications which could be due to differences in adherence to lifestyle, diet composition, micronutrients, food security, lack of exercise, activity thermogenesis and stress and interesting data to support Black ethnicity as an independent predictor of medication use. Dibato et al (220) evaluated the temporal patterns of cardiometabolic multimorbidity (CM) and depression in White Caucasians (WCs) and African Americans (AAs) with early-onset type 2 diabetes and their impact on long-term atherosclerotic

cardiovascular disease (ASCVD). AAs have higher cardiovascular risk compared with WCs, particularly in early-onset T2D. CM and depression at diabetes diagnosis have been increasing over the past two decades in both ethnic groups. Jadawji et al (221) conducted a systematic review and meta-analysis of published observational evidence to assess the difference in the prevalence and progression of diabetic nephropathy, and the development of end-stage renal disease (ESRD) in people from three different ethnic groups with T2D. The results of this review did not show a significant link between ethnicity (South Asian, white European and African Caribbean) and the prevalence of microalbuminuria. However, the IRR for ESRD in African Caribbean compared with white European participants was significantly higher. Further research is needed to explore the potential non-albuminuric pathways of progression to ESRD. Buscemi et al (222) showed that the U.S. Black mortality rate was 2.21 times higher than the white rate. This city-level data is important to inform more targeted local policy interventions and programming to promote health equity, particularly within cities with the greatest inequities. Ferdianand et al (223) studied the effects of empagliflozin and showed that in blacks with T2D, empagliflozin reduced glycohemoglobin, body weight, and BP. The effect of empagliflozin on BP increased from 12 to 24 weeks, suggesting a full antihypertensive effect takes ≥6 months to be fully realized. At week 24, the placebo-subtracted BP effect was similar to standard antihypertensive monotherapies, suggesting that empagliflozin may be beneficial for this high-risk population. Mathur et al's(216) cohort study conducted in adults with T2D in the UK found limited evidence of systematic ethnic inequalities in identification of T2D and management of cardio-metabolic risk around the time of initial diagnosis. Findings from this study may be illustrative of a wider trend of shrinking inequalities in diabetes care. Future work examining the extent to which ethnic differences are explained by genetic factors and

whether ethnic disparities manifest later in the care pathway, for example, in relation to long-term risk factor control as suggested here, will be necessary to understand how patterns of ethnic disparities in risk factor control and long-term outcomes are evolving in the UK.

In conclusion, our data show that health care services in London are escalating treatment prescription more rapidly in the black participants and by so doing, achieving equitable control of HbA1c and blood pressure but at the expense of more medication. It is possible that treatment options may fail for readily with increasing diabetes duration in the BWA and AC populations and further studies are urgently required to understand the underlying pathologies driving the observations in order better to tailor medications to slow the rate of progression of diabetes optimally in all three groups.

CHAPTER 4: SOUL DEEP RESULTS-

ASSESSMENT OF INSULIN

SECRETION BY MMTT

4.1 Introduction

In the UK and elsewhere, adults of Black African ancestry are disproportionately affected by T2D compared with White Europeans(224); it occurs at a younger age and develops at a lower body mass index (198,225). T2D develops as a result of insulin resistance, both peripheral and hepatic, resulting in reduced glucose uptake and failure to suppress endogenous glucose production, respectively. Initially this failure of insulin action is adequately compensated for by an increased beta-cell insulin secretory response which eventually fails and hyperglycaemia and frank T2D ensues(226) . The development of T2D in populations of African ancestry appears distinct from other ethnic groups; insulin resistance has been extensively reported(107),however this is often in the absence of central adiposity(227) and without the characteristic lipid profile normally associated with insulin resistance (228). Notably a marked hyperinsulinaemic response to glucose has been consistently reported in pre-diabetic adolescents and adults of Black African ethnicity(113) which may predispose to an earlier beta-cell exhaustion however there have been no studies of beta-cell function in T2D in Black African populations.

We hypothesised that BAM would have greater exhaustion of insulin secretory reserve in early but established T2D. We compared insulin secretory responses to both oral stimulation and intravenous glucose between BAM and WEM with early (<5 years) T2D, matched for age and BMI.

4.2 Aim

The purpose of this study was to test the hypothesis that BAM will have significantly greater deficits in insulin secretion compared to WEM by the time they manifest T2D.

4.3 Methods

4.3.1 Participants

This is already described in detail in Chapter 2.2.3 and 2.2.4). Men of Black West African or White European ethnicity (self-declared and confirmed by parental/grandparental birthplace), aged 18-65 years, with a BMI 25-40 kg/m^2, a documented diagnosis of T2D within 5 years, treated with lifestyle advice ± metformin, and HbA1c ≤63·9 mmol/mol were recruited through the South London Diabetes Study and screening of patient databases in South London General Practices. Participants were deemed ineligible if any of the following conditions existed: thiazolidinedione or insulin treatment, chronic oral steroid treatment, beta-blocker treatment, serum creatinine >150 mmol/l, serum alanine transaminase level >2.5-fold above the upper limit of the reference range, positive auto-antibodies for anti-insulin, anti-GAD or anti-A2, sickle cell disease (trait permitted), medications believed to affect the study's outcome measures, or unwillingness to follow the protocol.

In most, but not all, studies, antihypertensive treatment with angiotensin converting enzyme inhibitors (ACE inhibitors) improves insulin sensitivity, whereas beta-blockers decrease insulin sensitivity. In summary, treatment with metoprolol decreased insulin sensitivity with metabolic syndrome, whereas nebivolol lacked detrimental metabolic effects(229,230). Large clinical trials are needed to compare effects of nebivolol and the β(1) receptor antagonist metoprolol on clinical outcomes in patients with hypertension and the metabolic syndrome. ACE inhibitors are established in the treatment of hypertension and heart failure; both conditions are complicated by resistance to insulin-mediated glucose disposal(231). There have been conflicting reports about the effects of ACE inhibitors on insulin sensitivity and glycaemic control. A number of studies, both

with captopril and with enalapril, have shown small increases in insulin sensitivity, and there is evidence that this is due to enhanced glucose uptake into skeletal muscle(229). The interpretation of these studies, however, is often compromised by poor trial design, lack of full placebo data, various indirect measurements of insulin sensitivity, and heterogeneous patient populations in whom the biochemical mechanisms of insulin resistance (and drug responses) may not be the same. Overall, there probably is a modest class effect of ACE inhibitors that enhances insulin-mediated glucose disposal; the mechanism of this effect is likely to be a combination of increased muscle blood flow, local renin-angiotensin system blockade, and elevated kinin levels. Drug-induced hyperglycaemia and diabetes is a global issue. Glycaemic adverse events occur more frequently with thiazide diuretics and with certain beta-blocking agents than with calcium-channel blockers and inhibitors of the renin-angiotensin system. Among the anti-infectives, severe life-threatening events have been reported with fluoroquinolones(232), especially when high doses are used. Steroid diabetes is more frequently associated with high doses of glucocorticoids. During screening, we were careful to avoid participants on any drugs with clear evidence affecting the insulin secretion and insulin sensitivity measures.

All participants completed a comprehensive medical screening visit before study entry and BAM were matched with WEM for age (± 5 years) and BMI (± 3 kg/m^2). Recruitment and data collection took place between April 2013 and January 2015.

4.3.2 Procedures

Participants underwent 3 metabolic assessments in random order, separated by ≥7 days. Prior to each assessment participants ceased taking metformin for 7 days, avoided strenuous activity for 48 hours, refrained from alcohol for 24 hours, and consumed a standardised diet the day prior. In this Chapter, MMTT is discussed in detail.

Mixed meal tolerance test assessment of insulin and incretin secretion.(Chapter 2.3.1.1)

A 3-hr MMTT was conducted using a liquid milkshake (Ensure Plus, Abbott Nutrition, UK), providing 6 kcals/kg body weight. Plasma glucose and serum insulin, c-peptide, GLP-1 and GIP were assessed.

Analyses of samples.

Plasma glucose concentration was measured by automated glucose analyser (Yellow Spring Instruments, Ohio, USA). Serum insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK). Serum c-peptide was measured by a radioimmunoassay (Millipore Ltd, Hertfordshire, UK). GLP-1 and GIP (total) concentrations were measured by fluorescent ELISA methods (EGLP-35K and EZHGIP-54K, Merck Millipore, UK).

Calculations.

The area, and incremental area, under the curve (AUC & iAUC) were calculated, using the trapezoidal rule, for insulin, c-peptide, glucose, GLP-1 and GIP. First and second phase insulin secretion (HC) were quantified using c-peptide iAUC for time period 0-10 and 10-120-minutes, respectively.

4.4 Statistics

The primary outcome was insulin secretory function. Our sample size calculation was informed by available published data that showed large differences in insulin response at around one standard deviation or more. We estimated that a 2-group comparison with 20 per group would detect a difference of 1·0 standard deviations with 90% power and a significance level of 5%.

Normally distributed data are expressed as mean ± standard deviation, and log-normal data were back transformed to give geometric mean and 95% CI. Differences between ethnic groups were determined using the independent samples t-test. Analysis of covariance was used to test for differences by ethnicity whilst adjusting for the impact of BMI. Analyses were performed using SPSS, version 22 (IBM Analytics, NY).

4.5 Demographics

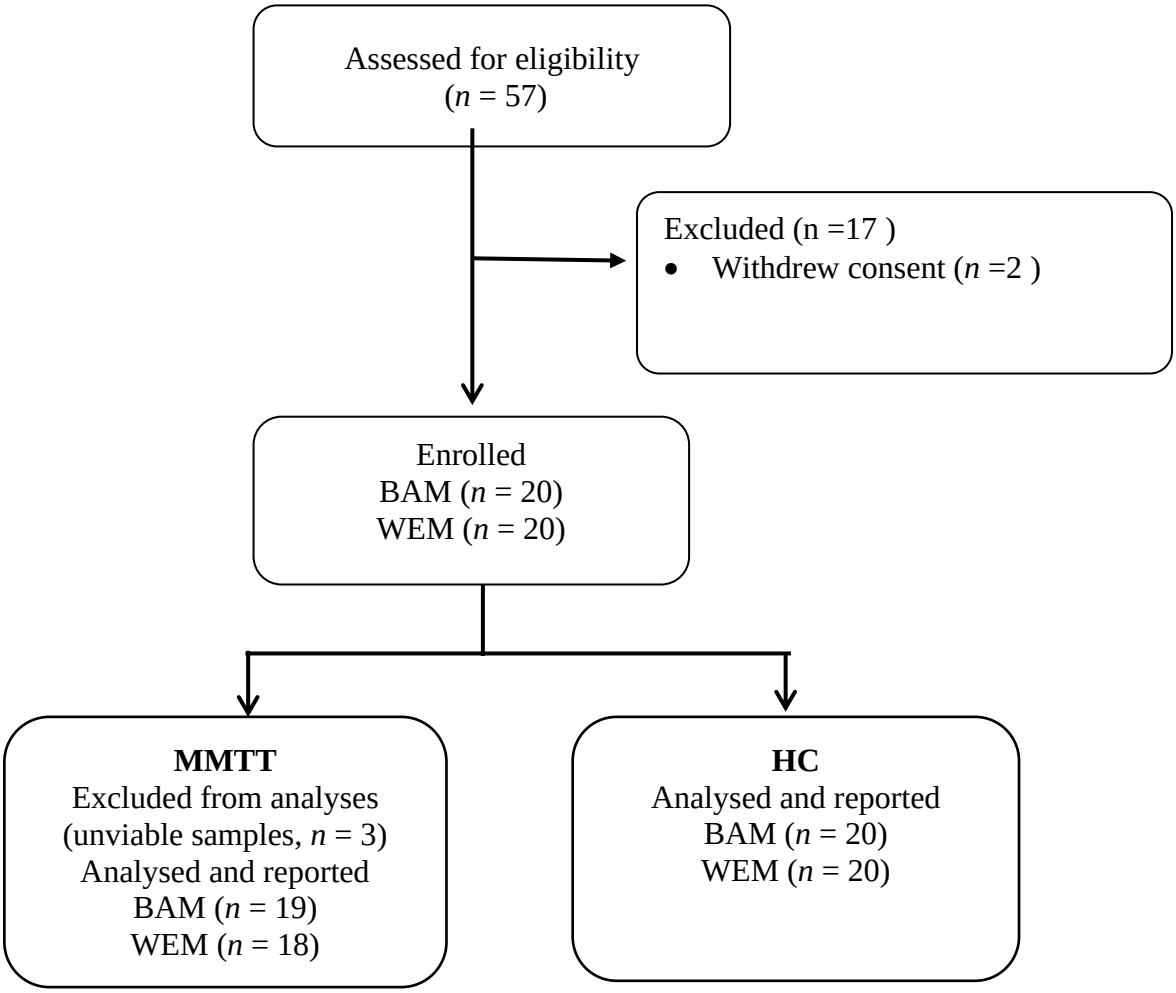


Figure 11: Study flow chart

A total of 57 participants were assessed for eligibility, 42 of whom were considered eligible. 2 participants withdrew from the study thus data were collected and analysed on 20 BAM and 20 WEM; 3 participants had incomplete MMTT data, 1 incomplete HEC data.

4.6 Results

4.6.1 Clinical characteristics

Forty participants, 20 BAM and 20 WEM, were recruited. By design, there were no significant ethnic differences in age, BMI, duration of diabetes, HbA1c, or mode of management (Table 15). Waist circumference, and plasma triglycerides were significantly lower ($p<0{\cdot}05$), in BAM ($p<0{\cdot}05$).

Table 15: Clinical characteristics of Black African and White European participants

	BAM (n = 20)	**WEM (n = 20)**	***P***
Age (years)	54·4 ± 7·7	56·2 ± 6·3	0·42
Weight (kg)	92·4 ± 11·8	101·9 ± 17·2	0·05
Height (cm)	175·5 ± 7·2	177·4 ± 5·5	0·35
BMI (kg/m^2)	30·0 ± 3·5	32·3 ± 4·5	0·09
Waist circumference (cm)	104·9 ± 9·7	113·2 ± 12·9	**0·03**
Duration of diabetes (years)	2·7 ± 1·3	3·1 ± 1·0	0·21
HbA1c (%)	6·8 ± 0·7	6·7 ± 0·7	0·76
HbA1c (mmol/mol)	50·1 ± 7·7	49·4 ± 8·0	0·76
Systolic blood pressure (mm Hg)	137·5 ± 13·7	130·9 ± 13·7	0·13
Diastolic blood pressure (mm Hg)	86·1 ± 7·4	82·2 ± 9·0	0·15
Total cholesterol (mmol/l)	4·1 ± 0·7	4·4 ± 0·8	0·22
LDL-cholesterol (mmol/l)	2·3 ± 0·5	2·3 ± 0·7	0·90
HDL-cholesterol (mmol/l)	1·2 ± 0·4	1·2 ± 0·2	0·76
Triglyceride (mmol/l)	1·3 ± 0·7	1·8 ± 0·9	**0·04**
Treated with metformin (%)	75	55	0·06

Data are arithmetic mean ± standard deviation. Differences between ethnic groups tested using independent samples t-test. BMI, body mass index; HbA1c, glycated haemoglobin; HDL, high density lipoprotein (-cholesterol); LDL, low density lipoprotein (-cholesterol).

4.7 Assessment of incretins and post prandial β-cell function-Meal tolerance test

4.7.1 Beta-cell insulin secretory function

Fasting concentrations of c-peptide were significantly lower in BAM (Table 16 and Figure 14).). The glucose response to the oral challenge (MMTT) was not different between groups (Figure 12). The insulin iAUC to the meal was similar between the ethnic groups (Table 16 and Figure 13).

4.7.2 Incretin responses

Fasting and post-prandial concentrations of GIP, but not GLP-1, were significantly higher in BAM during the MMTT (Table 16 and Figures 15 and 16)

Table 16: Meal tolerance test assessment of insulin secretory function in Black African and White European participants

Meal tolerance test	**BAM (n = 19)**	**WEM (n = 18)**	***P*^{a}**	***P*^{b}**
Fasting glucose (mmol/l)	7·45 ± 1·35	7·52 ± 1·62	0·99	0·83
Glucose iAUC (mmol/l min^{-1})	539·3 ± 315·4	567·1 ± 253·9	0·77	0·78
Fasting insulin (pmol/l)	84·8 (68·8, 104·6)	109·1 (81·6, 146·0)	0·15	0·28
Insulin iAUC (nmol/l min^{-1})	50·1 (37·1, 67·7)	58·1 (44·7, 75·5)	0·44	0·78
Fasting c-peptide (nmol/l)	0·574 (0·483, 0·681)	0·846 (0·708, 1·012)	**0·002**	**0·006**
c-peptide iAUC (nmol/l min^{-1})	63·4 ± 19·1	90·6 ± 58·5	**0·002**	**0·003**
Fasting GLP-1 (pmol/l)	13·43 ± 10·09	11·22 ± 6·13	0·42	0·32
GLP-1 iAUC (pmol/l min^{-1})	842·6 (564·3, 1258·1)	813·2 (594·7, 1112·0)	0·70	0·61
Fasting GIP (ng/l)	44·29 ± 24·66	30·43 ± 13·69	**0·04**	0·07
GIP iAUC (µg/l min^{-1})	47·0 ± 16·9	36·5 ± 14·9	**0·05**	**0·03**

Data are mean ± SD or geometric mean (95% CI) for log-normal data. Positively skewed data transformed (log^{10}) prior to statistical testing. *P*^{a}: differences between ethnic groups tested using independent samples t-test, *P*^{b}: differences between ethnic groups, adjusted for BMI, tested using analysis of covariance. iAUC, incremental area under the curve, calculated using the trapezoidal rule.

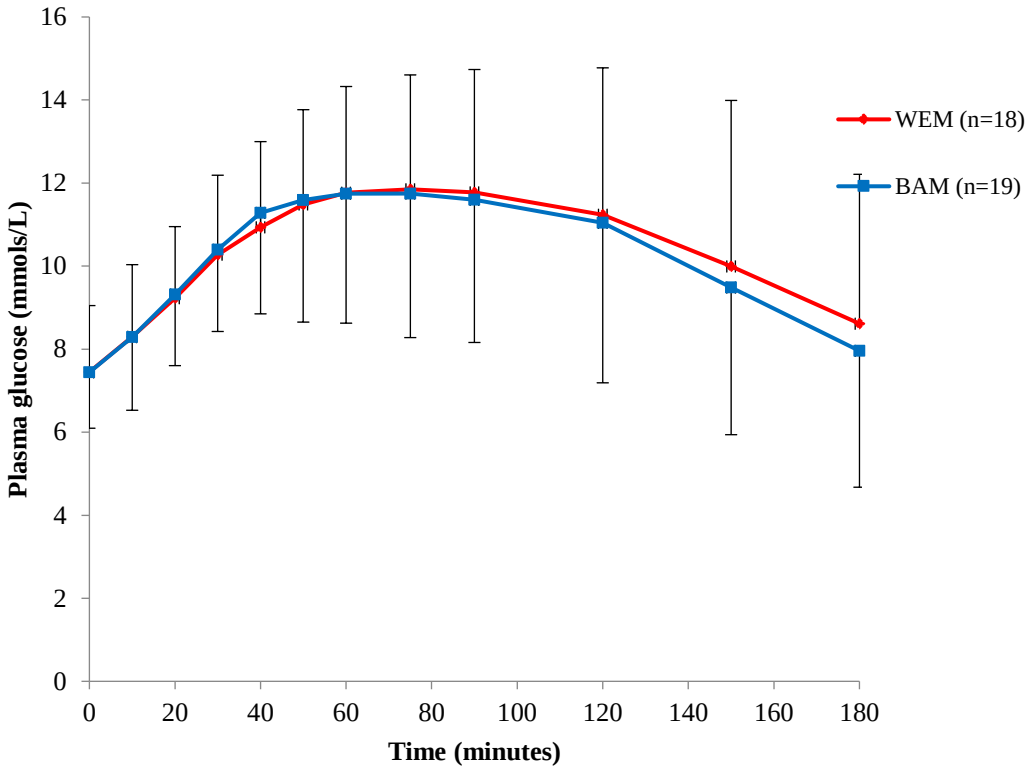


Figure 12: Plasma glucose response to a mixed meal tolerance test in Black African and White European participants

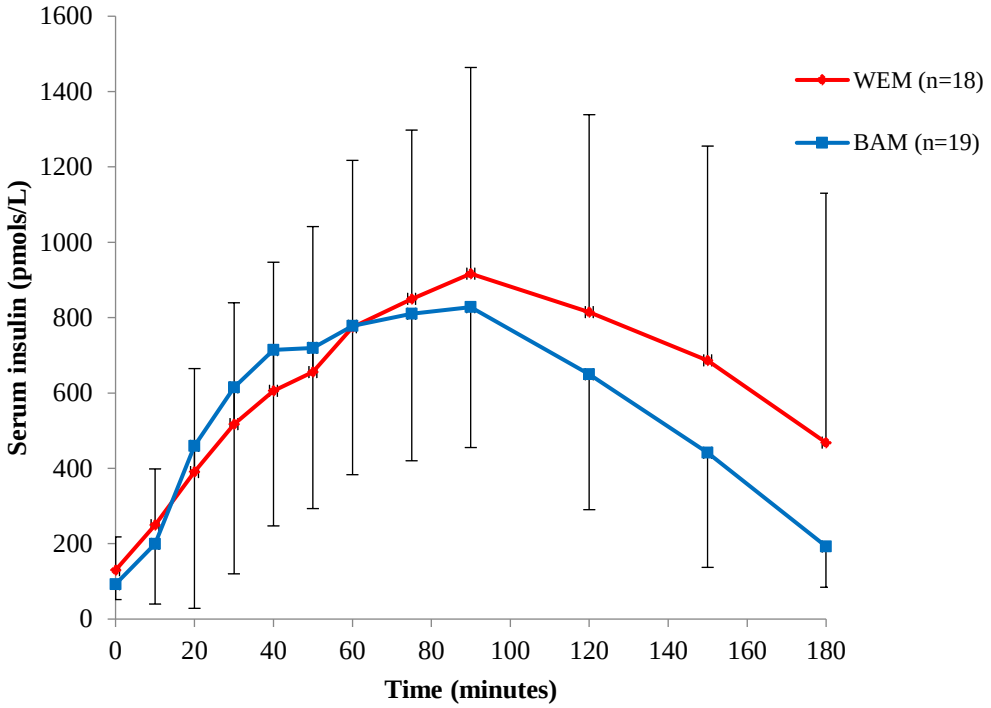


Figure 13: Plasma serum insulin response to a mixed meal tolerance test in Black African and White European participants

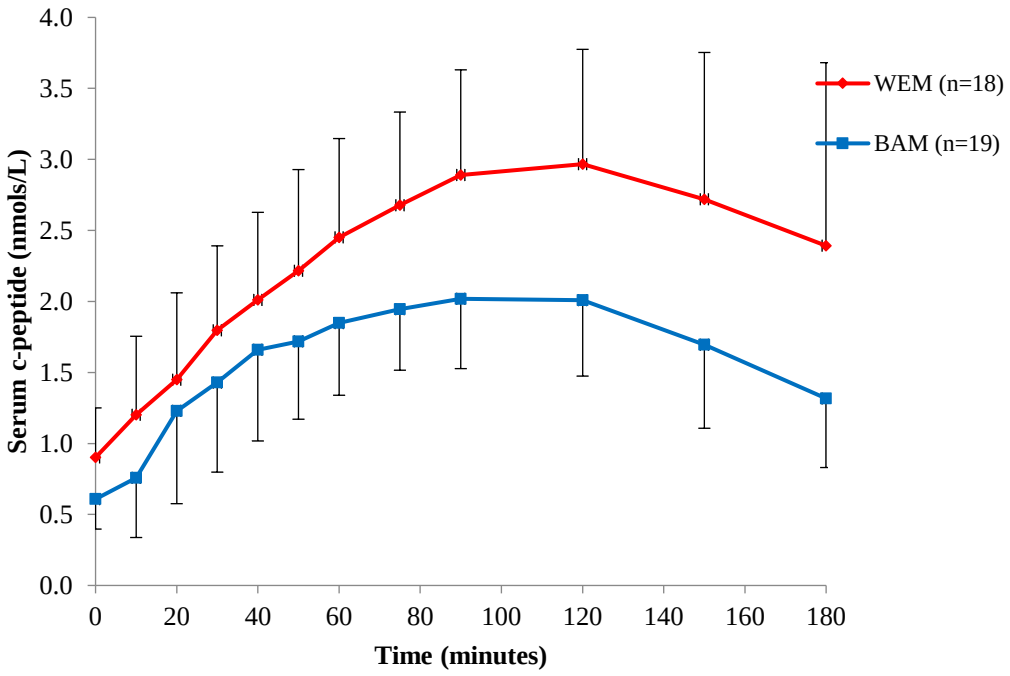


Figure 14: Plasma c-peptide response to a mixed meal tolerance test in Black African and White European participants

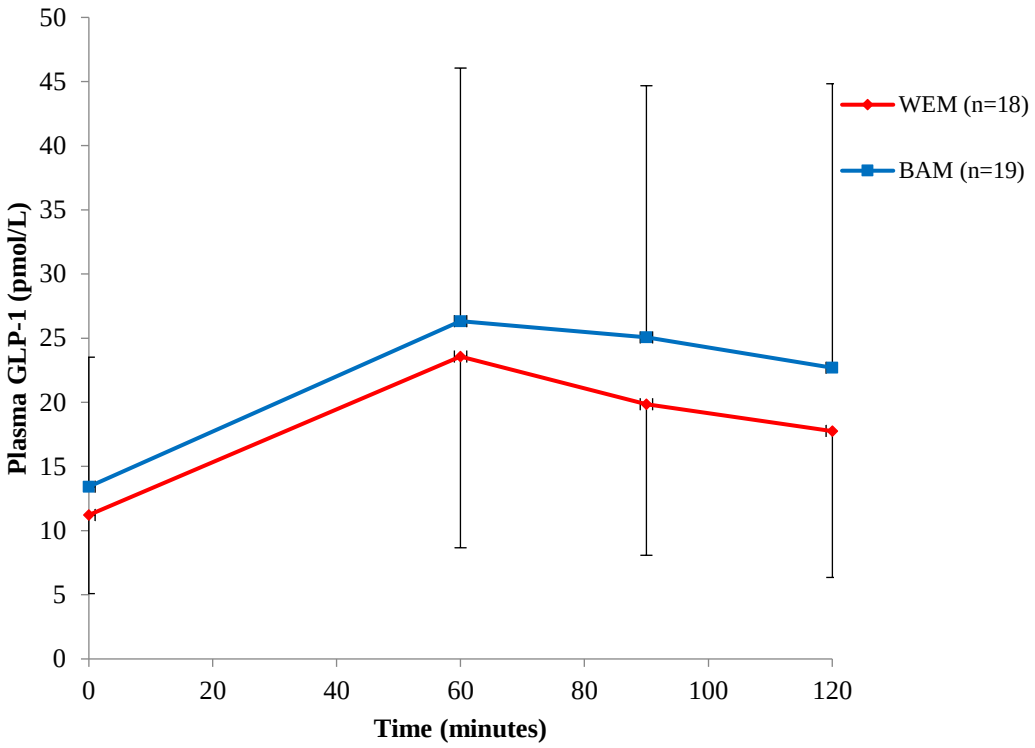


Figure 15: Plasma GLP-1 response in the mixed meal tolerance test amongst Black African and White European participants

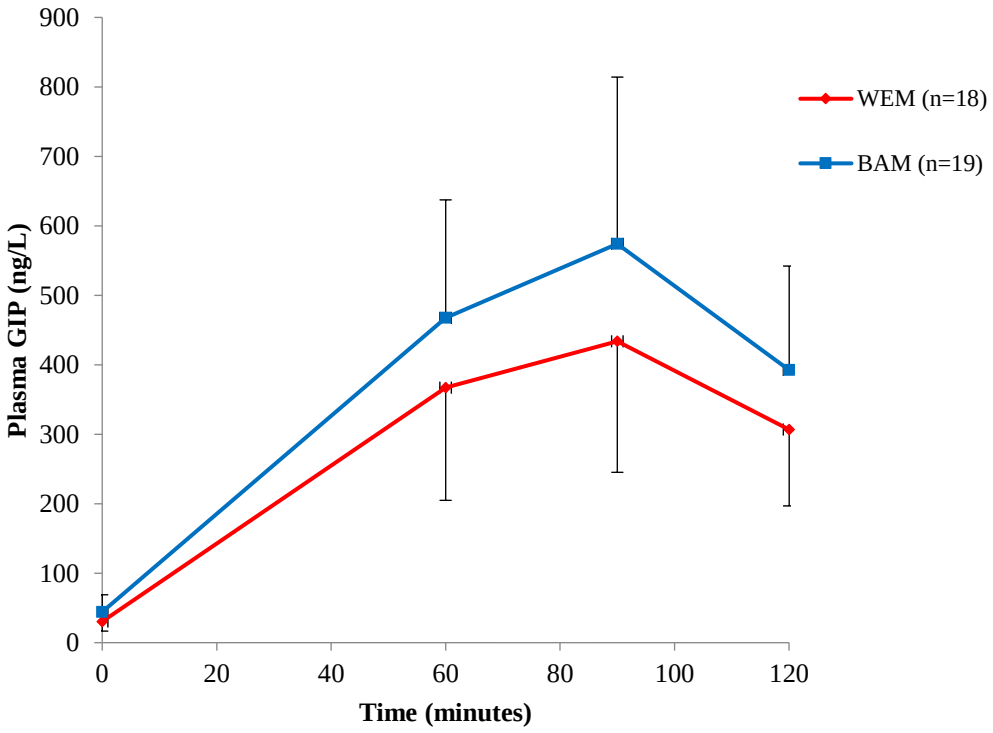


Figure 16: Plasma GIP response in the mixed meal tolerance test amongst Black African and White European participants

4.8 Discussion

Our primary hypothesis was that compared to WE ethnicity, men of BWA ethnicity with early T2D will have greater insulin secretory deficit. Our data support our primary hypothesis that compared to men of WE ethnicity, men of BWA ethnicity had similar glucose profiles after a MMTT with reduced c-peptide response and relatively preserved peripheral insulin concentrations; there was a trend towards increased GIP response in BWA men which was lost when adjusted for BMI and metformin use. Thus there is greater insulin secretory deficit but possible reduced hepatic insulin clearance in BWA men.

I have included the insulin clearance data from my publication to better understand my thesis. I am the first author of this publication, and my contribution is clearly explained in the declaration section. The insulin clearance data and minimal modelling analyses were analysed by co-authors Bonadonna et al(233). There were no ethnic differences in average insulin clearance during the intravenous challenge (BAM 897.6 (699.0-1152.4) versus WEM 830.8 (637.2-1082.9) mL/m^2 BSA/min) [mean difference 1.08, 95% CI 0.76,-1.55]; (P= 0.663). In response to oral glucose, the average clearance appeared lower in BAM, but this difference was not statistically significant (BAM 506.2 vs WEM 630.1 mL/m^2 BSA/min [mean difference −123.9, 95% CI −270.5, 22.6]; P = 0.095). When average clearance was plotted against average insulin concentration of each test, however, a hyperbolic relationship was apparent with a clear, significant difference between the groups, implying that in BAM average insulin clearance was lower at any average insulin concentration achieved during meal/clamp tests(233).

The implication of Figure 17 is that, at the same total insulin output during an intravenous or an oral challenge, BAM achieve higher insulin curves, which may compensate for reduced β-cell secretion and contribute to peripheral insulin levels.

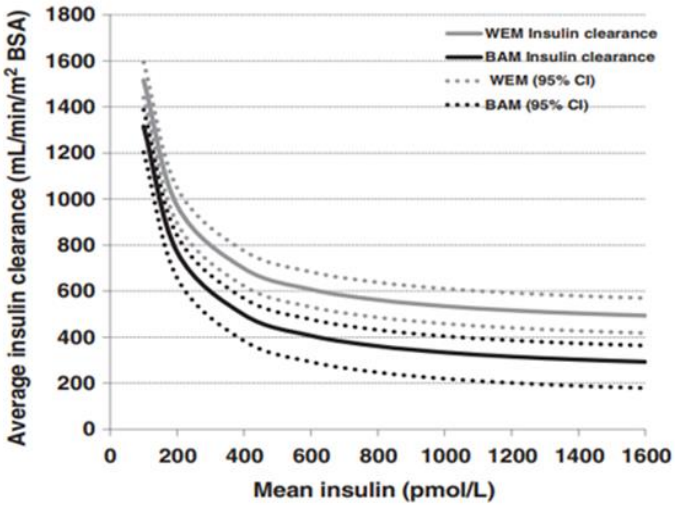


FIGURE 4 Relationship between average insulin clearance and average insulin concentration during the hyperglycaemic clamp and the mixed meal test black African men (BAM) and white European men (WEM). Average insulin clearance (mL/min/m^2 BSA):
BAM = 224 + (109 151/average insulin concentration),
:5 + (109 151/average insulin concentration)

Figure 17: Insulin clearance between BAM and WEM(233)

Studies of beta-cell function are inherently difficult because of complex interplay of the key variables, but initial studies in AA children and adolescents, used intravenous glucose tolerance test (IVGTT), showed lower insulin sensitivity and glucose effectiveness but higher insulin responses compared to White or Latino peers(107,108,234) . Although data from studies of AA have been important in providing information in our study design, there are several factors limiting the impact and reliability of these data as the studies have been small and the methodologies lacked rigor and/ or relied on surrogate measures of key variables. The populations studied have been diverse and not always well controlled. African-American populations are markedly different from UK BWA populations: for example there is a greater level of morbid obesity in AA and a greater

risk of myocardial infarction (235), the cause of which is not fully understood but likely has both genetic and environmental components and perhaps related to the morbid obesity; extrapolating those results to our UK BWA population is incorrect as they still have a lower incidence of MI compared to WE(236–238). We utilised gold standard methodologies. IVGTT utilises a supraphysiological acute glucose challenge, producing an insulin response not seen after oral glucose, which will vary according to the degree of insulin resistance(239) and is an established test for first phase insulin secretion . People with T2D may lack first phase secretion (240)but still respond to a meal tolerance test. The graded glucose infusion test provides a robust evaluation of the β-cell dose-response to a controlled plasma glucose rise but does not assess first phase secretion (239). Goff et al demonstrated reduced insulin sensitivity and more pronounced hyperinsulinaemia in the RISCK study (106).But in that study, hyperinsulinaemia was observed in female AC participants with prediabetes and those results cannot be directly compared to our BWA males with early T2D. We have restricted the study to men to avoid gender related differences in metabolism as T2D in BWA women is strongly associated with obesity and we do not want this to be a confounder (241).We restricted the study to one gender because the intensive protocols for the optimal study of human metabolism *in vivo* limit the numbers of participants, so it was desirable to minimise the biological variance of the sample. Gender impacts body habitus and the relationship between body habitus and metabolism. We then chose men because of the stronger association between obesity and diabetes in women(242), obesity was not the focus of our investigation, and the phenotype of T2D in black populations is gender-specific(243). Correcting mathematically for BMI and waist circumference does not necessarily remove the biomedical impact of obesity, and the M value in the hyperglycaemic clamp will be influenced by endogenous insulin secretion. M value reflects whole body glucose uptake

and although this is largely muscle driven, the adipose tissue does play a role. Because of the strong association between adiposity and female sex in BWA women in particular, and needing to conduct this study in one gender only, we focussed on men in this study. It would of course be of interest to repeat the study in women. A recent paper by Succurro et al. described this in non-diabetic first-degree relatives of people with T2DM. It showed sex-related differences in whole-body insulin sensitivity and insulin secretion in a group of Caucasian subjects with varying degrees of glucose tolerance. This study suggested that deterioration of glucose homeostasis in women is associated with a greater fat accumulation and worsening in insulin sensitivity as compared with men.

African-American studies also demonstrated a reduction in the amount of insulin extracted by the liver in response to glucose stimulation(234,244) leading to an alternative compensatory response to insulin resistance that raises peripheral insulin levels without a need to increase insulin secretion and may conserve beta-cell function.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are important determinants of postprandial insulin secretion(245,246). Our data showed an enhanced GIP secretion in BWA but demonstrated no significant differences or associations between the two groups.

SOUL-D study reported that people of AC and BWA ethnicity, with newly diagnosed T2D in South London lower waist circumference and a more favourable lipid profile than those of WE origin(247). Our data also revealed lower waist circumference and lower triglycerides when compared to WE men. However, the rest of the lipid profile did not show any significant difference between the two groups despite no differences in lipid lowering agents between the two groups. We matched for age and BMI as WE men are

reported to have higher waist circumference (238,247). Matching for WC, we probably would have recruited lighter men in the BWA cohort and might have defined a weight related difference in insulin sensitivity for glucose metabolism. Because we were interested in a differential evolution of insulin secretion and a difference in insulin sensitivity between glucose and lipid metabolism, we matched for BMI.

Based on these data, in early T2D, BWA men have greater insulin secretory deficits but reduced hepatic insulin clearance compared to WE men. Early treatment to support insulin secretion may be important to reduce progression of T2D in BWA people. Further understanding the progression of T2D between BWA and WE population will also better assist in outreach and prevention program.

CHAPTER 5: SouL-DeEP

ASSESSMENT OF INSULIN SECRETION BY HYPERGLYCAEMIC CLAMPS

5.1 Introduction

In Chapter 1.3.1 and 4.1, a detailed introduction on insulin secretion and impact of black ethnicity on insulin secretion is reviewed. In this chapter data from assessment of insulin secretory function in response to intravenous glucose stimulation is presented. The measurement of insulin secretory capacity is complex. Techniques based on the measurement of circulating insulin concentrations only partially reflect insulin secretion and fail to account for hepatic insulin clearance. C-peptide measurement overcomes this and reflects more precisely true pancreatic insulin secretion. The IVGTT is the most commonly used method, but it is often restricted to assessing only first phase insulin secretion.The Hyperglycaemic clamp is a more rigorous method that distinguishes first and second phase insulin secretion.

5.2 Aim

Accordingly, we evaluated and compared beta-cell insulin secretory response to hyperglycaemia stimulated via intravenous glucose infusion (using a 2-h hyperglycaemic clamp; HC) between BAM and WEM with recent-onset T2D, matched for age and body mass.

We tested the hypothesis that BAM with early T2D would have greater insulin secretory deficits compared to WEM.

5.3 Methods

5.3.1 Participants (Chapter 2.2.3 ,2.2.4 and 4.2.1)

Men of Black West African or White European ethnicity (self-declared and confirmed by parental/grandparental birthplace) aged 18-65 years, with a BMI 25-40 kg/m^2, a documented diagnosis of T2D within 5 years, treated with lifestyle advice ± metformin, and HbA1c ≤63·9 mmol/mol were recruited through the South London Diabetes Study and screening of patient databases in South London General Practices. Participants were deemed ineligible if any of the following conditions existed: thiazolidinedione or insulin treatment, chronic oral steroid treatment, beta-blocker treatment, serum creatinine >150 mmol/l, serum alanine transaminase level >2.5-fold above the upper limit of the reference range, positive auto-antibodies for anti-insulin, anti-GAD or anti-A2, sickle cell disease (trait permitted), medications believed to affect the study's outcome measures, or unwillingness to follow the protocol. All participants completed a comprehensive medical screening visit before study entry and BAM were matched with WEM for age (± 5 years) and BMI (± 3 kg/m^2). Recruitment and data collection took place between April 2013 and January 2015.

5.3.2 Procedures

Participants underwent 3 metabolic assessments in random order, separated by ≥7 days. Prior to each assessment participants ceased taking metformin for 7 days, avoided strenuous activity for 48 hours, refrained from alcohol for 24 hours, and consumed a standardised diet the day prior.

5.3.2.1 Hyperglycemic clamp assessment of insulin secretory function.

(Chapter 2.3.2.1)

A 2-hr HC was conducted. A 15-min priming regimen, based on body surface area, was used to rapidly increase the plasma glucose concentration to 6·9 mmol/l above fasting, which was subsequently maintained for a further 105-mins by adjustment of the glucose infusion rate. Plasma glucose, and serum insulin and c-peptide were assessed.

5.4 Analyses of samples.

Plasma glucose concentration was measured by automated glucose analyser (Yellow Spring Instruments, Ohio, USA). Serum insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK). Serum c-peptide was measured by a radioimmunoassay (Millipore Ltd, Hertfordshire, UK).

5.4.1 Calculations

The area, and incremental area, under the curve (AUC & iAUC) were calculated, using the trapezoidal rule, for insulin, c-peptide and glucose. First and second phase insulin secretion (HC) were quantified using c-peptide iAUC for time period 0-10 and 10-120-minutes, respectively.

5.5 Statistics

The primary outcome was insulin secretory function. Our sample size calculation was informed by available published data that showed large differences in insulin response at around one standard deviation or more. We estimated that a 2-group comparison with 20 per group would detect a difference of 1·0 standard deviations with 90% power and a significance level of 5%.

Normally-distributed data are expressed as mean ± standard deviation, and log-normal data were back transformed to give geometric mean and 95% CI. Differences between ethnic groups were determined using the independent samples t-test. Analysis of covariance was used to test for differences by ethnicity whilst adjusting for the impact of BMI. Pearson correlation coefficient was used to examine the relationship between adiposity/fat distribution and insulin sensitivity. Analyses were performed using SPSS, version 22 (IBM Analytics, NY).

5.6 Results

5.6.1 Clinical characteristics

Forty participants, 20 BAM and 20 WEM, were recruited, mean age 55.3 ± 7.0 years, mean BMI 31.1 ± 4.2 kg/m^2. The participants had been diagnosed with diabetes for 2.9 ± 1.1 years, had a mean HbA1c of 49.9 ± 7.8 mmol/mol, and 65% of participants were treated with metformin and the remainder were treated with lifestyle management alone. There were no ethnic differences in age, duration of diabetes, HbA1c, mode of management or other clinical characteristics apart from waist circumference and plasma triglycerides, which were both significantly lower (p<0.05) in BAM, body weight and BMI were lower in BAM but did not reach significance (p=0.05 and p=0.08, respectively).

5.7 Assessment of pancreatic insulin secretory function by hyperglycaemic clamps

5.7.1 Beta-cell insulin secretory function

Beta-cell insulin secretory function was principally assessed by HC, which induces hyperglycaemia (6.9 mmol/l above fasting) via intravenous infusion of glucose and enables first and second phase insulin secretion to be distinguished and evaluated. Fasting concentrations of c-peptide were significantly lower in BAM than WEM and fasting insulin concentrations were of borderline significance, although after adjusting for BMI fasting insulin was no longer different between ethnic groups (Table 17). The insulin and c-peptide responses to hyperglycaemia in the HC are shown in Figure 18,19 and Table 17. Due to basal differences, the incremental increase in insulin and c-peptide was calculated for the first phase (0-10 mins) and second phase (10-120 mins) response. Neither the first phase insulin or c-peptide response was different between the ethnic groups (Table 17) however BAM experienced significantly reduced insulin and c-peptide responses in the second phase (Table 17)

Table 17 Hyperglycaemic clamp assessment of insulin secretory function in Black African and White European participants

Hyperglycaemic clamp	**BAM (n = 20)**	**WEM (n = 20)**	***P*[a]**	***P*[b]**
Fasting glucose (mmol/l)	7·51 ± 1·63	7·29 ± 1·20	0·64	0·37
Fasting insulin (pmol/l)	68·5 (52·7, 89·1)	103·8 (73·9, 145·7)	**0·05**	0·27
Insulin iAUC 0-10 mins (pmol/l min^{-1})	77·6 (21·3, 283·4)	116·2 (40·5, 333·7)	0·61	0·64
Insulin iAUC 10-120 mins (nmol/l min^{-1})	11·7 (1·8, 7·5)	23·8 (17·1, 33·0)	**0·01**	**0·02**
Fasting c-peptide (nmol/l)	0·578 ± 0·188	0·946 ± 0·354	**<0·001**	**0·001**
C-peptide iAUC 0-10 mins (nmol/l min^{-1})	0·362 (0·089, 0·704)	0·593 (0·228, 1·066)	0·35	0·33
C-peptide iAUC 10-120 mins (nmol/l min^{-1})	54·5 (39·0, 76·0)	104·8 (84·6, 129·7)	0·001	**0·001**
M value (mg/m^2 BSA min^{-1})	156·1 (136·7, 178·2)	175·9 (159·7, 193·8)	0·16	**0·03**
SI (mL/ m^2 BSA min^{-1})/pmol/l)	0·349 (0·252, 0·453)	0·218 (0·155, 0·284)	**0·03**	0·10

Data are mean ± SD or geometric mean (95% CI) for log-normal data. Positively skewed data were transformed (log^{10}) prior to statistical testing. *P*[a]: differences between ethnic groups tested using independent samples t-test, *P*[b]: differences between ethnic groups, adjusted for BMI, tested using analysis of covariance.BSA, body surface area; iAUC, incremental area under the curve, calculated using the trapezoidal rule; M, glucose disposal in final 60 minutes of the clamp; SI, insulin sensitivity. iAUC 0 – 10 mins represents first phase, iAUC 10 – 120 mins represents second phase.

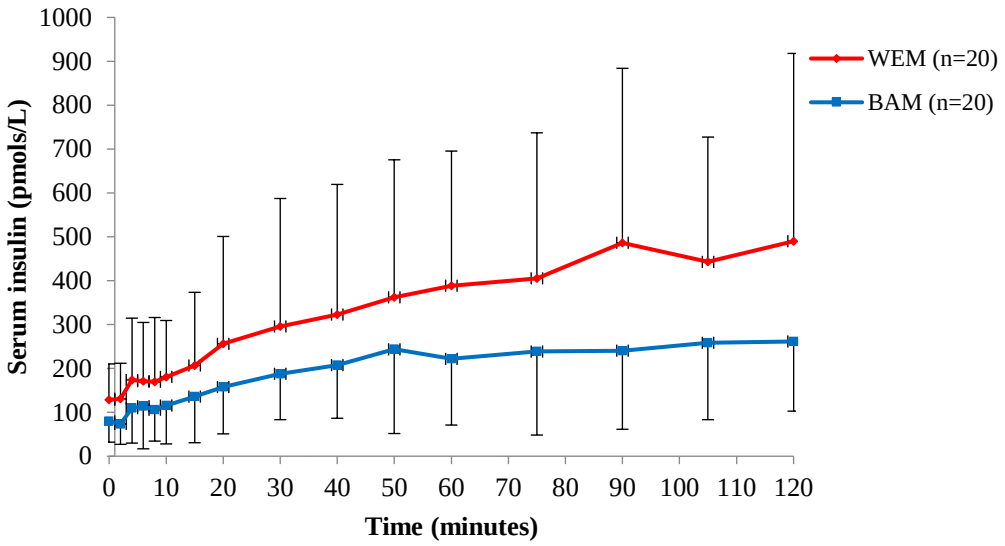


Figure 18: Serum insulin responses to intravenous glucose in the hyperglycaemic clamp in Black African and White European participants

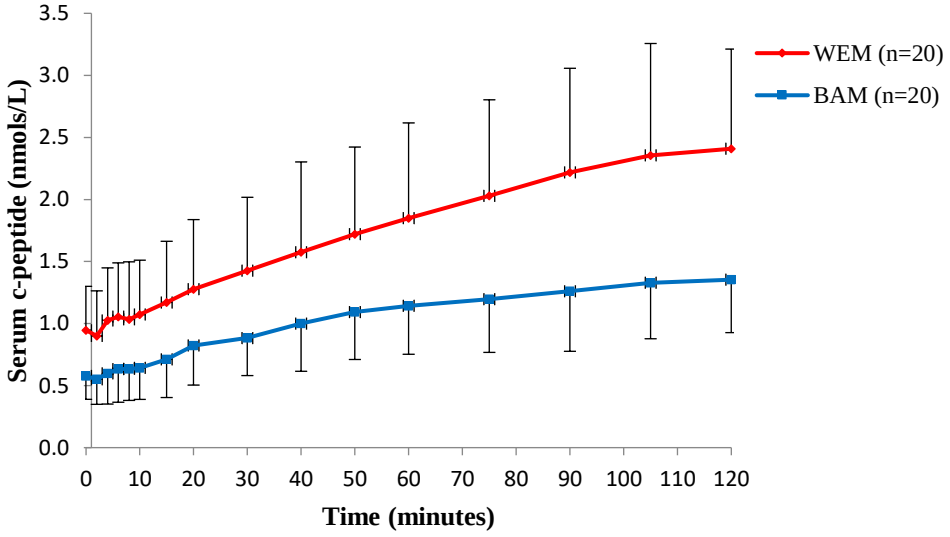


Figure 19: Serum c-peptide responses to intravenous glucose in the hyperglycaemic clamp in Black African and White European participants

5.8 Discussion

This study demonstrates differences in the metabolic processes involved in glucose dysregulation in men of Black African ethnicity with recently diagnosed T2D compared to White Europeans. Our ethnic groups, who had the same duration of diagnosed diabetes, HbA1c, fasting glucose and clinical management, exhibited almost identical glucose responses to a meal challenge (Table 16 & Figure 12 in Chapter 4.) yet we found significantly lower insulin secretory function in response to both intravenous and oral stimuli, assessed through the measurement of c-peptide, in BAM. Notably the insulin response in the HC was significantly reduced in BAM however there were no differences when the MMTT was used to invoke hyperglycaemia via the gut. This was explained by significantly reduced insulin clearance in BAM only in response to the oral challenge, and perhaps related to the significantly greater GIP response to the meal that BAM experienced.

It has been extensively reported that populations of African ancestry exhibit hyperinsulinemia compared to White Europeans (186,242,248).Hyperinsulinemia is understood to be a compensatory mechanism that occurs in response to heightening insulin resistance, and is an indication of augmented beta-cell insulin secretion, which eventually fails resulting in insulin deficits and hyperglycaemia. That these pathophysiological processes occur in African populations has been evidenced by studies of African-Americans and native Ghanaians and shown that, as in other populations, insulin secretion is blunted in patients with T2D compared to ethnically matched normal glucose tolerant controls (249,250) . We had hypothesised that early and intensified hyperinsulinaemia would lead to a relatively early failure of beta-cell secretory capacity and greater insulin deficits in early T2D in BAM compared to WEM.

The measurement of beta-cell insulin secretory capacity is inherently complex and should focus on assessment of c-peptide to account for first pass hepatic insulin clearance. We studied stimulated beta-cell function comprehensively; the HC enabled us to measure and distinguish first and second phase insulin secretion, whilst the MMTT assessed the physiological response of the beta-cells to nutrients, and incretin hormones. Our results show significantly reduced second phase insulin secretion amongst BAM compared to WEM. Second phase secretion is quantitatively very important in the maintenance of glucose homeostasis, given that it can be sustained in response to prolonged hyperglycaemia (251). Our data do not permit an exploration of the mechanisms for the deficient second phase secretion, which is thought to represent release of insulin from storage granules. It is well established that insulin secretion is triggered by glucose entering the beta-cells via GLUT2, followed by a cascade of coupling events. Whilst the first phase response can be triggered by potassium and non-nutrient secretagogues, only glucose and fuel secretagogues can yield a sustained second phase response. Our study is the first to describe ethnic specific incretin responses in T2D and leads us to hypothesise that amongst BAM higher GIP may promote greater reductions in insulin clearance in response to hyperglycaemia, which results in maintenance of peripheral insulin concentrations, and that these mechanisms provide some compensation for the significantly lower insulin secretory capacity of the beta-cells.

In the present study, BAM exhibited significantly higher postprandial GIP concentrations, which may have contributed to the non-significant trend for lower average insulin clearance that was observed(233). The effect of GIP on insulin clearance is unclear; some authors have demonstrated an insulin clearance-reducing effect of GIP(252), whilst others have shown no effect(253,254). Some of the conflict in these findings may have occurred

because insulin clearance appears to adapt to insulin resistance and glucose intolerance, a potential mechanism by which β-cell function is preserved in the progression to T2D(255). There has been very little investigation of incretin hormones within black populations and, in the few studies that exist, the focus has been on the role of incretins in the upregulation of insulin secretion; Michaliszyn et al (256) reported no difference in GLP-1 and or GIP levels. We modelled the impact of the mixed meal, including, but not limited to, the effect of the incretin response on insulin secretory function ("meal effect"), but detected no ethnic differences(233). Michaliszyn et al (256) modelled the "potentiation factor," which describes the modulation of the relationship between glucose concentration and insulin secretion and comprises several mechanisms, including the release of endogenous incretin hormones. In contrast to our data, they found no differences in incretin concentrations in response to an oral glucose challenge, but reported a significantly higher early potentiation factor in black participants(256). Our data suggest that, by the time diabetes develops, BAM may have no greater β-cell response to GIP than WEM, but that their higher GIP response may cause lower average insulin clearance in response to hyperglycaemia, which results in maintenance of peripheral insulin concentrations, and that these mechanisms provide some compensation for the significantly lower insulin secretory capacity of the β cells(233).

GIP appears to offer an additional survival benefit by not only stimulating intestinal glucose transport and maximally releasing insulin to facilitate nutrient storage but also by its insulin-mimetic properties, including enhanced uptake of glucose by adipocytes(257). This physiological redundancy offered by insulin and GIP ensured the survival of organisms during times when food was scarce, as food is no longer scarce, at least in the

West, this survival advantage appears to have contributed to the current obesity epidemic (258).

There was no data collected on birth weight or more detailed lifestyle data but agree that would have been interesting to see whether adverse early life factors mediate the effects of beta-cell function and can be included in future studies. It is well established that low birth weight is associated with the development of non-communicable chronic diseases in later life(259,260). However, the association of birth weight with type 2 diabetes is "J" or "U" shaped, i.e. the prevalence of diabetes is increased in individuals at both extremes of birth weight. The mechanisms underlying this relationship are not clear. However, both beta-cell dysfunction (261)and insulin resistance (262)in childhood and adulthood may occur at the extremes of birth weight. Faster growth in the first 6 months of life is associated with higher serum adiponectin in later life. So it implies that low growth in early infancy may lead to low adiponectin levels with associated insulin resistance and glucose intolerance in later life(263). Finally, there may be an interaction with young adults whose mothers had lower socio-economic status during pregnancy have more adverse outcomes associated with low birth size compared to those from higher status(264).

In 1955, Hugh-Jones In described an unusual variant which he called "J-type" diabetes – "J" standing for Jamaica. In more recent times, there have been other acronyms for J-type such as atypical diabetes, phasic insulin-dependent diabetes, ketosis-prone diabetes, type 3 diabetes, ketosis-prone type 2 diabetes and Flatbush diabetes. This variant was associated with insulin resistance, phasic dependency of insulin and a lean phenotype. It most resembles type 1B diabetes in the WHO classification. It is more common in non-white populations, and marginal nutritional status may play a role in some persons.

Patients with AKPD have periods in which they are insulin-requiring resulting in ketosis, especially during metabolic stresses, e.g. infections (265).At other times, their need for insulin decreases and a good glycaemic control can be achieved with only lifestyle modification and/or oral antidiabetic agents. It is conceivable that they may have reduced beta-cell mass and glucose-stimulated insulin secretion due to malnutrition in early life similar to the marasmic child (266). APKD may be a heterogeneous collection of different phenotypes with different degrees of impaired beta-cell function and autoimmunity (i.e. anti-GAD 65 and anti-IA-2 antibodies) (265) and some persons who have less autoimmunity, i.e. antibody negative but with preserved beta-cell reserve, demonstrate a clinical course more in keeping with type 2 diabetes despite having periods of ketosis (267). Persons with positive autoantibodies tend to eventually need insulin therapy, while persons with preserved beta-cell function may have periods of insulin independence (265). A few candidate genes have been examined to explain this variant of diabetes, but no genome-wide association studies have been done to date. This interesting condition which we observe in Black adult population in the UK too needs further exploration in future studies as we did not include such patients in our studies.

A limitation of our work is that we have not investigated the cellular mechanisms that underlie our ethnic differences in metabolic function; additionally, we have only captured the metabolic phenotype of T2D, therefore we cannot allude to the mechanisms by which hyperglycaemia progresses and how this may be ethnically distinct. However, a major strength of our work is that we have studied T2D in well-matched participant groups; our patients developed T2D at the same age, had the same duration of diagnosis and level of glycemic control, and exhibited the same glucose response to a meal challenge. We are therefore confident that we have recognised novel ethnic distinctions in T2D

pathophysiology which may have important clinical implications. Our data suggest that the principal abnormality of T2D in BAM is loss of beta-cell insulin secretory function, whereas in WEM the opposite is true and beta-cell function is relatively preserved. the mechanisms in BAM that drive beta-cell dysfunction are not clear, potentially BAM may have lower beta-cell mass or a steeper slope of decline in beta-cell function as diabetes develops. In light of these findings, it may be pertinent to consider therapeutic strategies that augment these physiological processes; BAM may achieve greater clinical benefit from therapeutic agents that support beta-cell function such as the incretin therapies, whilst WEM may be better supported by insulin sensitising agents. In conclusion we have recognised in this study that hyperglycaemia in BAM is affected more strongly by deficits in beta-cell function, and that the incretin hormones may play a damage-limitation role in maintaining peripheral insulin concentrations by reducing insulin clearance.

CHAPTER 6: CONCLUDING DISCUSSION

6.1 Introduction

In this chapter, the findings from both SOUL-D and preliminary findings from SouL-DeEP studies will be discussed cohesively to draw a conclusion. The interpretation and evaluation of the findings will be presented in relation to the aims and hypotheses of the thesis, together with the implications for future research and clinical practice.

6.2 Discussion

6.2.1 Summary of findings-SOUL-D

Compared to WE

- BWA and AC were younger at diabetes diagnosis
- BWA had lower waist circumference
- HbA1c at diagnosis and at recruitment was higher
- HbA1c at 2 years was not different
- Black patients were more likely to be on more diabetes medications at year 2
- Black ethnicity was strongly associated with increased number of medication from baseline to year 2

6.2.2 Summary of Findings SouL-DeEP

Compared to men of WE ethnicity, men of BWA ethnicity:

- Significantly reduced insulin and C-peptide responses in the second phase of HC
- Similar glucose profiles after a MMTT with
    - Reduced C-peptide response
    - Relatively preserved peripheral insulin concentrations
    - Enhanced fasting and post-prandial GIP concentrations

This study demonstrates differences in the metabolic processes involved in glucose dysregulation in men of Black African ethnicity with recently-diagnosed T2D compared to White Europeans. Our ethnic groups, who had the same duration of diagnosed diabetes, HbA1c, fasting glucose and clinical management, exhibited almost identical glucose responses to a meal challenge and we found significantly lower insulin secretory function in response to both intravenous and oral stimuli, assessed through the measurement of c-peptide, in BAM. Notably the insulin response in the HC was significantly reduced in BAM however there were no differences when the MMTT was used to invoke hyperglycaemia via the gut. This was explained by significantly reduced insulin clearance in BAM only in response to the oral challenge, and perhaps related to the significantly greater GIP response to the meal that BAM experienced.

It has been extensively reported that populations of African ancestry exhibit hyperinsulinaemia compared to White Europeans(242,248,268) .Hyperinsulinaemia is understood to be a compensatory mechanism that occurs in response to heightening insulin resistance, and is an indication of augmented beta-cell insulin secretion, which eventually fails resulting in insulin deficits and hyperglycaemia. That these pathophysiological processes occur in African populations has been evidenced by studies of African-Americans and native Ghanaians and shown that, as in other populations, insulin secretion is blunted in patients with T2D(249,250). We had hypothesised that early and intensified hyperinsulinaemia would lead to a relatively early failure of beta-cell secretory capacity and greater insulin deficits in early T2D in BAM compared to WEM.

In contrast to our findings, studies mostly in children and youth showed relative insulin hypersecretion and hyperinsulinaemia in the BA. However, the children and adolescents with T2D in these studies are characterised by morbid obesity(269–271). Morbid obesity may account for the insulin hypersecretion seen in young BA populations (272). It may also be the case that insulin dynamics in BA alter during their life course; one may postulate that they exhibit hypersecretion of insulin during their youth, before developing deficits in later adult life. The UK Prospective Diabetes Study Group (UKPDS), in a study of over 5000 subjects with newly diagnosed T2D, found more severely impaired beta cell function as evidenced by lower HOMA%B in African Caribbeans compared with UK Caucasians. Outcome measures determined by insulin responses (such as AIR, CIR and disposition index) are generally higher in BA subjects with T2D(273) , while outcome measures determined by C-peptide responses are lower (274,275). Recently, a large cohort of African Americans with T2D was found to have higher beta cell insulin secretion in response to the OGTT, despite greater insulin sensitivity, compared with white Americans (207) suggesting that primary insulin hypersecretion in BA persists into the development of T2D. Therefore, there is conflicting evidence within a small literature base.The discrepancies may relate to the populations under study.There is evidence that hyperinsulinaemia is associated with African American (as opposed to indigenous African) origin(276), female sex in BA(104) and with increasing BMI. Therefore, male sex, geographic origin and relatively low adiposity may contribute to the distinctive findings of the SouL-DeEP BWA population.

6.2.3 Comparing findings from the hyperglycaemic clamp and the mixed meal tolerance test

We utilised gold standard methodologies. IVGTT utilises a supraphysiological acute glucose challenge, producing an insulin response not seen after oral glucose, which will vary according to the degree of insulin resistance (239) and is an established test for first phase insulin secretion . People with T2D may lack first phase secretion(240) but still respond to a meal tolerance test. The graded glucose infusion test provides a robust evaluation of the β-cell dose-response to a controlled plasma glucose rise but does not assess first phase secretion (239). We have restricted the study to men to avoid gender related differences in metabolism as T2D in BWA women is strongly associated with obesity(241).The measurement of beta-cell insulin secretory capacity is inherently complex and should focus on assessment of c-peptide to account for first pass hepatic insulin clearance. We studied stimulated beta-cell function comprehensively; the HC enabled us to measure and distinguish first and second phase insulin secretion, whilst the MMTT assessed the physiological response of the beta-cells to nutrients, and incretin hormones. Our results show significantly reduced second phase insulin secretion amongst BAM compared to WEM. Second phase secretion is quantitatively very important in the maintenance of glucose homeostasis, given that it can be sustained in response to prolonged hyperglycaemia (251). Our data do not permit an exploration of the mechanisms for the deficient second phase secretion, which is thought to represent release of insulin from storage granules. It is well established that insulin secretion is triggered by glucose entering the beta-cells via GLUT2, followed by a cascade of coupling events. Whilst the first phase response can be triggered by potassium and non-nutrient secretagogues, only glucose and fuel secretagogues can yield a sustained second phase response.

There were no ethnic differences in mean fasting or clamped glucose,insulin iAUC or first-phase C-peptide .Fasting c-peptide was lower in BWA with a trend for second-phase insulin iAUC that did not achieve statistical significance.During the MMTT the two ethnic groups showed same glucose response but mean C-peptide iAUC was significantly lower in BWA.The MMTT insulin iAUC was not significantly different between the two ethnic groups.

Diverging findings from IV and oral studies in BA populations have been noted in other studies. The absence of hyperinsulinaemia in response to oral glucose compared with intravenous glucose has been recognised in obese African American adolescents (277); in another study of African Americans with pre-diabetes, while insulin and C-peptide responses to oral glucose trended to lower than white Americans, their acute insulin response to intravenous glucose was (nonsignificantly) higher (274).

The reasons for these ethnic-specific findings dependent on the route of carbohydrate administration are unknown. The enteral route involves gut and neural signals, predominantly the incretinergic system, which do not come into play during intravenous glucose-stimulated beta cell secretion.

Our protocol allowed us to assess the incretin effect in our subjects by measuring insulin secretory responses to both intravenous and oral challenges. As we did not match the glucose concentrations achieved with the two challenges and the oral challenge included other nutrients, we could not measure the effect itself but we were able to measure Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), important determinants of postprandial insulin secretion(245,246). The data

indicate a trend towards higher postprandial GIP levels in the BA men. GIP is known to have insulinotropic and anti-apoptotic effects in the beta cell (278) and higher levels might therefore plausibly be associated with greater beta cell insulin secretion, which is in opposition to the findings made here. There are few data on ethnic differences in incretin levels in BA and WE populations and the findings from the small number of studies are mixed. Two studies in African American youths found lower postprandial GLP-1 responses compared with their European American peers (279,280) with the former also finding no ethnic difference in GIP levels - while two other studies found, in contrast, that African American adults had higher GLP-1 responses after oral glucose compared with white Americans (281,282) . Another study in youth found no ethnic difference in either GLP1 or GIP responses(256) .Therefore, there is no available literature to corroborate the findings of elevated postprandial GIP in the BWA men in SouL-DeEP.

Some of the variation in the literature may be explained by method of assessment e.g. enzyme-linked immunosorbent assay (ELISA) versus radio-immunoassay and the determination of total versus intact incretin levels; in SouL-DeEP, total incretin levels were measured and therefore the measurements may not be wholly representative of biologically-active incretin concentrations. Our study is the first to describe ethnic specific incretin responses in T2D and leads us to hypothesise that amongst BAM higher GIP may promote greater reductions in insulin clearance in response to hyperglycaemia, which results in maintenance of peripheral insulin concentrations, and that these mechanisms provide some compensation for the significantly lower insulin secretory capacity of the beta-cells. However further studies are needed to confirm the above preliminary findings.

The preservation of insulin responses to intravenous glucose challenge relative to C-peptide in our BWA group suggests reduced insulin clearance in the BWA and is compatible with data from studies in African Americans (234,268). Our data do not allow us to determine whether this is as a result of earlier hypersecretion of insulin or a compensatory response to insulin resistance that raises peripheral insulin levels without a need to increase insulin secretion and may conserve beta-cell function.

However, Ladwa et al investigated relationships between insulin clearance, insulin secretion, hepatic fat accumulation and insulin sensitivity in black African (BA) and white European (WE) men with normal glucose tolerance with the same protocol, matched for age and body mass index. While normally glucose-tolerant BA men have similar insulin secretory responses to their WE counterparts, they have markedly lower insulin clearance, which does not appear to be explained by either insulin resistance or hepatic fat accumulation. Low insulin clearance may be the primary mechanism of hyperinsulinaemia in populations of African origin(283).

Measurements of insulin secretion in vivo cannot be fully interpreted without information on insulin sensitivity. In the additional work undertaken by our team and included in my publication and Dr Bello's thesis, insulin sensitivity was measured during a two-step hyperinsulinaemic-euglycaemic clamp using tracer infusions of stable isotopes to measure glucose kinetics and lipolysis, was performed on all participants by myself and further analyses carried out by Dr Bello et al and I am the second author in that publication (284). There were no significant differences between the M value (glucose infusion rate/insulin concentration, a measure of whole body insulin sensitivity); rate of tissue glucose uptake (Rd, from the glucose tracer data); rate of endogenous glucose production (Ra from tracer data) or lipolysis (glycerol Ra from tracer data). I reproduce a summary table from the publication in Table 18 for better understanding of my work. I can conclude that the differences in insulin secretory responses between the groups presented here are not confounded by differences in insulin sensitivity. It is in contrast to the existing literature showing insulin resistance in pre-diabetes.

Based on our data, we can conclude that in early T2D, BWA men have greater insulin secretory deficits but reduced hepatic insulin clearance compared to WE men. Early treatment to support insulin secretion may be important to reduce progression of T2D in BWA people. Further understanding the progression of T2D between BWA and WE population will also better assist in outreach and prevention program.

Table 18: Two-stage hyperinsulinaemic -euglycaemic clamp assessment of insulin sensitivity in BAM and WEM with type 2 diabetes

Measurement	Basal				Hyperinsulinaemic–euglycaemic clamp			
	BAM *n* = 15	**WEM *n* = 12**	**Mean difference or ratio of the geometric mean (95% CI) (BAM − WEM) (95% CI)**	***p***	**BAM *n* = 18**	**WEM *n* = 15**	**Mean difference (BAM − WEM) (95% CI)**	***p***
Glucose disposal rate (M; mg kg^{-1} min^{-1})	–	–	–	–	4.52 (2.07)	4.00 (1.70)	0.52 (−0.82, 1.89)	0.44
Peripheral glucose utilisation (R_d; μmol kg^{-1} min^{-1})	–	–	–	–	26.8 (10.4)	24.2 (8.5)	2.60 (−4.22, 9.41)	0.44
Endogenous glucose production (R_a; μmol kg^{-1} min^{-1})	8.82 (1.49)	9.25 (1.66)	−0.43 (−1.69, 0.81)	0.48	5.76 (1.73)	6.50 (2.34)	−0.74 (−2.18, 0.71)	0.31
Lipolysis (glycerol R_a; μmol kg^{-1} min^{-1})	1.51 (1.31, 1.75)	1.82 (1.55, 2.15)	0.83 (0.67, 1.02)	0.08	1.06 (0.47)	1.18 (0.33)[a]	−0.12 (−0.43, 0.19)	0.43

Data expressed as mean (SD) for normally distributed data and geometric mean (95% CI) for skewed data
M values and glucose R_d assessments were derived from the high-dose insulin infusion (40 mU m^{-2} BSA min^{-1}), glucose and glycerol R_a assessments were derived from the low-dose insulin infusion (10 mU m^{-2} BSA min^{-1}) of the hyperinsulinaemic–euglycaemic clamp and at baseline

[a]WEM sample size = 13.*p* values were generated using an independent sample Student's *t* test to compare BAM and WEM

6.2.4 Relationship between SouL-DeEP and SOUL-D study

We have prospectively examined the impact of Black West African(BWA) and African-Caribbean (AC)ethnicity on progression of T2D and associated cardiovascular risk factors over the two years after diabetes diagnosis in a British urban population in the UK, comparing their data to those from people of white European ancestry diagnosed in the same time frame in the same primary health care system. The novel finding of our research is that our black participants achieved similar, and by current criteria acceptable, glycaemic outcomes as their white European-origin peers but associated with a greater amount of medication prescribed.

Both our black populations had younger age at diagnosis of diabetes and lower waist circumference, although BMI was not different between groups. These observations are in keeping with data from other studies of British black populations(42,106,196) and autochthonous African populations(200). We believe this is the first study prospectively to examine disease progression in a single health care setting.

The apparent requirement for greater medication to achieve glycaemic treatment targets in the black participants of the SOUL-D cohort is open to several interpretations. One possibility is that this is evidence of a more aggressive disease progression from onset of the diabetes. We have reported a greater insulin secretory deficit in early T2D in males of BWA, compared to WE, ethnicity in this thesis. The increasing need for medication in the present study, where health care is free at the point of delivery, is consistent with a more rapid progression of insulin deficiency.

Our two groups were matched for BMI, age and short diabetes duration. A possible contributor to poorer insulin secretory function may be a longer duration of diabetes in the BWA group but the subjects were recruited in a community that has open access to nationally funded health care and was implementing a screening programme for cardiovascular risk. Participants were identified either from a research study that had specifically recruited people with a new diagnosis of T2D(285) or from the local eye screening programme to which all people with newly-diagnosed diabetes were referred. As diabetes is detected on average 10 years earlier in the Black members of this population(285), the matching of age and diabetes duration may have detected BWA participants with a more slowly evolving diabetes than the norm but this would have played against our finding a greater insulin secretory deficit. It is important to acknowledge that SouL-DeEP was a cross-sectional rather than longitudinal study and that this has implications for the interpretation of these findings. In common with all cross-sectional studies of T2D, as the condition is largely asymptomatic on presentation, the true duration of the disease is difficult to establish. It raises the possibility that, in the T2D cohort, the BA men may have been intolerant to glucose for a longer duration than their age-matched WE counterparts. Large cohort studies have demonstrated a chronic and progressive decline in beta cell function (around 5% per year) with a longer duration of diabetes (286) and residual beta cell function exhibits a close inverse association with disease duration(287,288). Whether rates of deterioration differ by ethnicity is unknown. The inference is that the BA men with T2D may exhibit greater beta cell dysfunction than the WE men of matched age due to a longer duration of their diabetes. While this must always be taken into consideration in any interpretation of these data, in this study the majority of the men with T2D were identified at an asymptomatic stage through an early screening programme and there were no significant ethnic differences in their fasting

plasma glucose, HbA1c or anti-diabetic therapy. This suggests that they were diagnosed at a similar stage in the natural history of their diabetes.

Diabetes control as reflected by HbA1c was similar in the two groups, and although numerically more of the BWA group were taking metformin, this was stopped at least one week before any metabolic study and there were no significant differences in the fasting blood glucose levels.

The lower waist circumference of the BWA men compared to the WE men of equivalent BMI has been previously described (289). It argues for a lower intra-abdominal fat mass, which may be associated with enhanced hepatic insulin sensitivity in this group and also to the lower triglyceride values we and others have reported. Had we matched our participants for waist circumference, we would have anticipated recruiting men of lower BMI in the BWA cohort and defined a weight-related difference in insulin sensitivity for glucose metabolism.

The terms “Black” and “White” do not describe a genetically homogenous group in either case. We deliberately restricted our Black population to people with four grandparents from the countries that make up geo-political West Africa and our White volunteers to people with four white European grandparents. The British BWA population will be different from both the US African American and the UK Caribbean population in the pre-diabetic studies referenced in this thesis, but they do share important features such as the very high risk for T2D in westernised societies (and increasingly in Africa); the high risk for hypertension and stroke and, at least until recently, the relatively low risk for myocardial infarction attributed to a so-called cardioprotective lipid

profile(236,238,290). While hypertension, like T2D, is associated with insulin resistance, the apparent absence of insulin resistance once diabetes has been diagnosed in our participants and the role of the impaired insulin secretory response merits further investigation, especially given the greater level of morbid obesity, and most recently even of myocardial infarction in diabetes in African Americans (235).

With regard to other cardiovascular risk factors in people with T2D, our BWA participants displayed the highest blood pressure and the AC participants were more likely to be on more than two anti-hypertensives at baseline. Rather in line with the diabetes data, by year two the BWA and the AC had achieved the lowest blood pressures but were three times more likely to be prescribed more than two anti-hypertensive agents for blood pressure control compared to WE.

Despite higher triglyceride and slightly lower total and LDL cholesterol at baseline in WE, by two years, there were no between-group differences in total, LDL or HDL cholesterol. Higher triglycerides persisted, despite WE being most likely to be prescribed lipid-lowering therapy. Previous studies have suggested that the UK's AC population display a cardio-protective lipid profile (138). Our results are at variance with these, although our data may be confounded by different statin use. There is previous evidence to suggest lower risk of coronary artery disease and higher risk of stroke in UK black populations (139,140,217).The paradox of a more favourable lipid profile despite higher cardiovascular disease risk in the black population has been reported in South Africa (218). Greater use of statins may be important in reducing cardiovascular risk in all groups in the present study(219).

6.3 Strengths and limitations

A limitation of our work is that we have not investigated the cellular mechanisms that underlie our ethnic differences in metabolic function; additionally, we have only captured the metabolic phenotype of T2D, therefore we cannot allude to the mechanisms by which hyperglycaemia progresses and how this may be ethnically distinct. However, a major strength of our work is that we have studied T2D in well-matched participant groups; our patients developed T2D at the same age, had the same duration of diagnosis and level of glycaemic control, and exhibited the same glucose response to a meal challenge. We are therefore confident that we have recognised novel ethnic distinctions in T2D pathophysiology which may have important clinical implications. Our data suggest that the principal abnormality of T2D in BAM is loss of beta-cell insulin secretory function, whereas in WEM the opposite is true and beta-cell function is relatively preserved. the mechanisms in BAM that drive beta-cell dysfunction are not clear, potentially BAM may have lower beta-cell mass or a steeper slope of decline in beta-cell function as diabetes develops.

6.3.1 Study design and population

The participants recruited to the study displayed the typical expected characteristics of their ethnic origin, implying that recruitment achieved a cohort representative of the population from which the sample was drawn. The ethnic groups were well-matched. A particular strength was that multiple parameters of interest were measured in each participant, so as to be able to examine the relationships between them. However, as a cross-sectional study, while associations between variables of interest could be examined, the causal direction of relationships could not be established. The aims of the study were to examine metabolic phenotypes and therefore genetic data were not collected. This

meant that the independent effect of African and European ethnicities on the study outcomes could not be assessed. It also meant that the categorisation of study participants into each ethnic group depended upon self-identification, although all efforts were made to limit ethnic heterogeneity within ethnic groups by d that participants declared four grandparents and both parents from strictly defined geographical regions of West Africa and Northern Europe, respectively. The number of participants in the study was comparable to similar studies (291,292), but did not allow sufficient power for some of the outcome measures which have been discussed in this thesis. Finally, as only West African men were studied, the findings may not be generalisable to other BA populations such as children, adolescents, and women; but as a little studied group, this also gave the study novelty.

6.3.2 Methodological techniques

6.3.2.1 SouL-DeEP

The use of gold standard metabolic techniques was a clear strength of this study.

Both the methods used to measure beta cell function in this study have their own distinct advantages and limitations. During the meal test, the effect of varying glucose levels and the input of incretins and other gut signals interfere with the analysis of beta cell responses, whereas during the clamp, a known dose of glucose is delivered directly to the beta cell and insulin secretion can be assessed in the absence of the dynamic interaction between varying glucose and insulin levels. The hyperglycaemic clamp is therefore a precise and highly reproducible study which allows clear separation of first and second phase responses. On the other hand, the clamp does not examine beta cell responses over a physiological range of blood glucose concentrations and does not include the incretin effects which would play a role in beta cell responses in the day-to-day life of the

participant. The use of both the clamp and meal test in combination allowed the examination of two different aspects of beta cell function and overcomes the limitations of either test individually. Had only a single technique been used, assumptions would have been made and only part of the picture appreciated. Carbohydrate metabolism and the development of T2D are highly complex processes with many players. Although several of the most important physiological variables were measured in this study, this was not exhaustive; for example, the study did not examine gastric emptying time, glucose effectiveness and other gut signals. Furthermore, while information from participants was collected regarding postcode of residence, employment status, smoking status, diet and physical activity, this was not analysed as part of this thesis and therefore does not form part of the discussion here. Therefore, the effects of important environmental variables, such as diet and socioeconomic status, were not evaluated. Finally, analysis and interpretation of some aspects of the data was hampered by the limited literature base. There are very few metabolic studies in West African men and some areas, such as the molecular mechanisms and regulation of insulin clearance, are poorly understood. This entails a degree of speculation in the discussion; however, it does provide a springboard from which to generate further hypotheses for future exploration. Also this was the pilot study followed by detailed modelling and analyses of the insulin sensitivity data in T2D by Goff et al and Bello et al. Ladwa et al has investigated relationships between insulin clearance, insulin secretion, hepatic fat accumulation and insulin sensitivity in BAM and WEM with NGT leading to better understanding of T2D in black Africans.

6.3.2.2 SOUL-D study

One major drawback of the SOUL-D study was the start date of anti-hypertensive and lipid-lowering medications were not recorded. Therefore, there is uncertainty whether there were any patients on pre-existing anti-hypertensives or cholesterol lowering medications. However, most patients were initiated on medications at baseline to control risk factors post-diabetes diagnosis, and any statistical effect of pre-existing medications would be minimal. The doses of the anti-hypertensive and lipid-lowering medications were not taken into account in this study, which may potentially have an influence on the results seen. It may be that there is no statistically significant difference in the numbers of individuals on certain medications, though it may be that one ethnicity requires higher doses compared to the other ethnicity. This is a potential stepping-stone for new studies to compare the dose of medications required to achieve BP or lipid control in different ethnicities.

6.4 Clinical implications

In light of these findings, it may be pertinent to consider therapeutic strategies that augment these physiological processes; BAM may achieve greater clinical benefit from therapeutic agents that support beta-cell function such as the incretin therapies, whilst WEM may be better supported by insulin sensitising agents. In conclusion we have recognised in this study that hyperglycaemia in BAM is affected more strongly by deficits in beta-cell function, and that the incretin hormones may play a damage-limitation role in maintaining peripheral insulin concentrations by reducing insulin clearance.

While the study findings do not lead to direct changes to clinical practice, they do form the basis of hypotheses which may be tested in future interventional studies. Therapies directed towards protection of the beta cell should be investigated. For example, while numerous studies report that early, short-term intensive insulin treatment in patients with a new diagnosis of T2D may lead to better long-term glycaemic control(293–295) ,the effects of ethnicity on this phenomenon have not been examined. We may hypothesise that in BA populations, their susceptible beta cells might entail that early resolution of glucotoxicity and the opportunity for the beta cells to rest and recover would be particularly beneficial to their long-term outlook. Indeed, this would be supported by the finding that in African Americans with T2D, intensive insulin therapy achieves greater HbA1c improvement for the same insulin increment compared with other ethnic groups(296) .

Therefore, the research question might be: in BA with a new diagnosis of (non-ketosis prone) T2D, does early short-term aggressive treatment of glucose levels with insulin, followed by standard treatment, lead to better outcomes than standard treatment alone?

Sufficient improvement in surrogate markers such as HbA1c and BMI, followed by hard endpoints such as complication rates, would have the potential to alter our standard management of T2D in this ethnic group.

6.5 Future directions

Findings from this study have highlighted the changing phenotypes of the UK Black African population. Previous studies have failed to separate African ethnicity into specific countries of origin, and in doing so in this study, we have shown that differences exist between BWA and AC ethnicity with regards to cardiovascular risk factors. Of particular interest is the mixed lipid profile observed in the AC cohort of patients. Whether this cohort of patients has lost the cardio-protective lipid profile observed in previous studies due to longer duration of westernisation (residence in the UK) compared to BWA, remains to be explored. Longer follow up of these patients could reveal whether this AC cohort of patients continue to deteriorate in terms of cardiovascular risk factors, following the trends that are currently being seen in the US AA population. Further studies should also be conducted looking in particular at the BWA communities, as there is limited literature surrounding these individuals at present and whether these individuals will also show any deterioration in the cardio-protective lipid profile in the future. Lastly, the prevalence of microvascular and macrovascular complications in the SOUL-D cohort of patients can also be explored and associations made with current risk factors that have been explored in this study.

6.6 Conclusion

In conclusion, our SOUL-D data showed that in early T2D, primary care services in London are escalating treatment prescription more rapidly in black patients and thereby achieving equitable control of HbA1c and blood pressure at the expense of more medication. It is possible that treatment options may fail more readily with increasing diabetes duration in BWA and AC populations and further studies are urgently required to understand the underlying pathologies driving the observations in order better to tailor medications to slow the rate of progression of diabetes optimally in all three ethnic groups. Our preliminary SouL-DeEP data presented in this thesis has helped to understand that observation better as in early T2D, BAM have greater insulin secretory deficits compared to WEM. Future longitudinal and intervention studies in combination with genetic analyses, are needed for a better understanding of the pathophysiology of T2D in black Africans. That will play a pivotal role in effective prevention and management strategies.

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Appendices

Appendix A (publication)

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WILEY

ORIGINAL ARTICLE

Ethnic differences in insulin secretory function between black African and white European men with early type 2 diabetes

Cynthia Mohandas MD[1] | Riccardo Bonadonna MD[2] | Fariba Shojee-Moradie PhD[3] | Nicola Jackson PhD[3] | Linda Boselli PhD[4] | K. George M. M. Alberti PhD[1] | Janet L. Peacock PhD[5] | A. Margot Umpleby PhD[3] | Stephanie A. Amiel MD[1] | Louise M. Goff PhD[1]

[1]Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK
[2]Department of Medicine and Surgery, University of Parma and Azienda Ospedaliera Universitaria di Parma, Parma, Italy
[3]Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
[4]Division of Endocrinology and Metabolic Disease, University of Verona School of Medicine, Verona, Italy
[5]School of Population Health and Environmental Sciences, King's College London, London, UK

Correspondence
Louise M. Goff PhD, Diabetes Research Group, Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Guy's Campus, Henriette Raphael Building, Room 2.29, London SE1 1UL, UK.
Email: louise.goff@kcl.ac.uk

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Aim: To test the hypothesis that men of black (West) African ethnicity (black African men [BAM]) with early type 2 diabetes (T2D) would have greater insulin secretory deficits compared with white European men (WEM), following prediabetic hypersecretion.

Methods: In 19 BAM and 15 WEM, matched for age, body mass index and duration of diabetes, we assessed and modelled insulin secretory responses to hyperglycaemia stimulated intravenously (hyperglycaemic clamp) and orally (meal tolerance test).

Results: With similar post-challenge glucose responses, BAM had lower second-phase C-peptide responses to intravenous glucose (BAM 70.6 vs WEM 115.1 nmol/L/min [ratio of geometric mean 0.55, 95% confidence interval (CI) 0.37, 0.83]; $P = .006$) and to oral glucose (BAM 65.4 vs WEM 88.5 nmol/L/min [mean difference −23.2, 95% CI −40.0, −6.3]; $P = .009$). Peripheral insulin response in BAM to oral glucose was preserved (BAM 47.4 vs WEM 59.4 nmol/L/min [ratio of geometric mean 0.89, 95% CI 0.59, 1.35]; $P = .566$), with relative reductions in insulin clearance (BAM 506.2 vs WEM 630.1 mL/m^2 BSA/min [mean difference −123.9, 95% CI −270.5, 22.6]; $P = .095$), associated with enhanced incretin responses (gastric inhibitory polypeptide incremental area under the curve: BAM 46.8 vs WEM 33.9 μg/L/min [mean difference 12.9, 95% CI 2.1, 23.7]; $P = .021$).

Conclusions: In early T2D, BAM had significantly lower insulin secretory responses to intravenous and oral stimulation than WEM. Lower insulin clearance, potentially driven by increased incretin responses, may act to preserve peripheral insulin concentrations. Tailoring early management strategies to reflect distinct ethnic-specific pathophysiology may improve outcomes in this high-risk population.

KEYWORDS
β cell, African, ethnicity, insulin secretion, type 2 diabetes

1 | INTRODUCTION

Populations of African ancestry are disproportionately affected by type 2 diabetes (T2D)[1]; it develops at a younger age[2] and at a lower body mass[3] in black people than in white people.

The main pathophysiological processes of insulin secretory failure and insulin resistance that underlie T2D are well documented,[4] but differences in the pathogenesis based on ethnicity are increasingly recognized. There is a growing amount of literature examining metabolism in black populations without diabetes, with studies in African-American children and adolescents without diabetes describing marked hyperinsulinaemia compared with other ethnicities,[5–11] and extensive reports that black populations, both indigenous[12] and diasporic,[12–19] exhibit a hyperinsulinaemic response to glucose. Conventionally, hyperinsulinaemia is understood to occur in response to heightening insulin resistance, but this does not fully explain the response in black populations.[6,9,11] Studies in children measuring C-peptide have described a combination of increased insulin secretion

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and reduced hepatic insulin clearance.[7,9] Studies in healthy and prediabetic adults have shown lower rates of insulin clearance,[13,15,19] but heterogeneity in the populations has made independence from insulin resistance and body weight/composition differences difficult to ascertain. If intensified hyperinsulinaemia represents greater insulin secretion, it may predispose to earlier β-cell exhaustion in the development of T2D. To date, no studies have undertaken comparisons of β-cell function in black African and white European populations with recent-onset T2D. As this may be the time people first present to health services, this is an important phase to understand.

The measurement of insulin secretory capacity is complex. Techniques based on the measurement of circulating insulin concentrations only partially reflect insulin secretion and fail to account for hepatic insulin clearance. Measurement of C-peptide overcomes this and reflects more precisely true pancreatic insulin secretion. The intravenous glucose tolerance test is the most commonly used method, but it is often restricted to assessing only first-phase secretion. The hyperglycaemic clamp is a more rigorous method that distinguishes first- and second-phase secretion; however, it does not account for the role of incretin hormones, which can be assessed by a meal tolerance test.

The aim of the present study was to assess insulin secretory function comprehensively, in response to both intravenous and oral stimulation, in order to explore the hypothesis that men of black (West) African ethnicity (black African men [BAM]) will have significantly greater insulin secretory deficits than white European men (WEM) by the time they manifest T2D.

2 | PARTICIPANTS AND METHODS

The study was conducted at the Clinical Research Facility, King's College London, UK and was approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided informed consent. Recruitment and data collection took place in April 2013 to January 2015.

2.1 | Participants

Participants were BAM and WEM (ethnicity self-declared, confirmed by grandparental birthplace), aged 18 to 65 years, with a body mass index (BMI) 25 to 35 kg/m^2, a documented diagnosis of T2D within 5 years, treated with lifestyle advice ± metformin, and a glycated haemoglobin (HbA1c) concentration ≤ 63.9 mmol/mol (<8%), and were recruited from South London General Practices taking part in an early detection T2D screening programme.[20] Participants were deemed ineligible if they: were treated with other diabetes medications, chronic oral steroids or β-blockers; had a serum creatinine concentration > 150 mmol/L; had a serum alanine transaminase level > 2.5 times above the upper limit of the reference range; had positive auto-antibodies for anti-insulin, anti-glutamic acid decarboxylase or anti-A2; had sickle cell disease (trait permitted); or were taking medications believed to affect the outcome measures. Participants completed a medical screening before study entry. BAM were matched with WEM for age (±5 years) and BMI (±3 kg/m^2).

2.2 | Study design

Assessment visits were completed in random order and separated by a minimum of 7 days. For each assessment, participants arrived having refrained from eating or drinking anything other than water from 10:00 PM the night before. Participants were instructed to refrain from strenuous exercise and physical activity in the preceding 48 hours and from alcohol in the preceding 24 hours, and to consume a standardized diet the day before (~50% of calories from carbohydrate, evenly spread throughout the day, with no more than 30% of daily carbohydrate consumed in the evening meal). Participants on metformin were instructed to cease taking it for 7 days prior to the visit.

2.2.1 | Hyperglycaemic clamp assessment of first- and second-phase insulin secretory function

A 2-hour hyperglycaemic clamp test was conducted.[21] Participants were weighed in light clothing and their body surface area (BSA) calculated.[22] An antecubital fossa vein was cannulated for administration of intravenous glucose; a second cannula was inserted retrogradely into the dorsum of the hand, and placed in a warming unit, to achieve arterialized venous blood samples. Three fasting samples (−20, −10 and 0 minutes) were collected before starting the glucose infusion (20% glucose) at time 0 minutes; a priming regimen, based on BSA,[21] was used for the first 15 minutes to increase rapidly the plasma glucose to 6.9 mmol/L above fasting. The glucose infusion rate was then adjusted to maintain plasma glucose at 6.9 mmol/L above fasting for a further 105 minutes. Blood sampling occurred every 2 minutes for the first 10 minutes and every 5 minutes thereafter to inform adjustment of the glucose infusion rate for "clamping" the plasma glucose. Blood samples were drawn at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes for the assessment of plasma glucose, serum insulin and C-peptide.

2.2.2 | Mixed meal tolerance test assessment of insulin and incretin secretion

A 3-hour meal tolerance test was conducted using a liquid milkshake (Ensure Plus, Abbott Nutrition, Maidenhead, UK), providing 6 kcal/kg body weight. An antecubital fossa vein was cannulated for blood sampling. After the collection of fasting samples at time −10 and 0 minutes the participants consumed the drink within 5 minutes. Blood was collected at 10, 20, 30, 40, 50, 60, 75, 90, 120, 150 and 180 minutes for the assessment of glucose, non-esterified fatty acids (NEFA), insulin and C-peptide, and for glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) at 30, 60 and 120 minutes.

2.2.3 | Magnetic resonance imaging assessment of visceral fat deposition

Visceral fat and skeletal muscle mass were assessed using magnetic resonance imaging, in a 1.5-T Siemens scanner. Participants lay supine and a single T1-weighted axial image, of 3-mm thickness, was acquired at the L4-L5 region of the abdomen and the thighs (20 cm below the neck of the femur), using a 2-point variant Dixon imaging

protocol. The area of visceral fat and volume of skeletal muscle was quantified using Osirix image processing software, version 6.0.2 (Pixemo, Switzerland).

2.2.4 | Analyses of samples and calculations

We measured plasma glucose by automated glucose analyser (Yellow Spring Instruments, Yellow Springs, Ohio); serum insulin by immunoassay using chemiluminescent technology (ADVIA Centaur System; Siemens Healthcare Ltd, Camberly, UK); serum C-peptide by radioimmunoassay (Millipore Ltd, Watford, UK); plasma NEFA by enzymatic colorimetric assay (Wako Diagnostics, Richmond, Virginia) on an automated clinical chemistry analyser (ILab 650; Instrument Laboratories, Holliston, Massachusetts); and GLP-1 and GIP (total) by fluorescent ELISA methods (EGLP-35K and EZHGIP-54K, Merck Millipore, UK).

The area under the curve (AUC) and incremental AUC (iAUC) were calculated, using the trapezoidal rule, for insulin, C-peptide, glucose, NEFA, GLP-1 and GIP. To calculate an index of first- and second-phase insulin secretion in the hyperglycaemic clamp we measured the iAUC for C-peptide over 0 to 10 minutes for first-phase, and 10 to 120 minutes for second-phase insulin secretion, similarly to DeFronzo et al.[21]

Model-based measurement of β-cell function: the glucose, insulin and C-peptide curves during the hyperglycaemic clamp and meal tolerance test were modelled using methods previously described[23–25] (SAAM-II 1.2 software; SAAM Institute, Seattle, Washington). The main outputs of the hyperglycaemic clamp 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, in (pmol/m^2 BSA)/(mmol/L/min), 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/min/m^2)/(mmol/L). Modelling of the glucose and C-peptide curves of the meal test enables an estimation of the equivalent of first-phase insulin secretion (σ^1), whereas second-phase insulin secretion is assessed and presented through the stimulus response curve of the insulin secretion rates at 4, 5.5, 8, 11 and 15 mmol/L of glucose. The variable σ^2, as defined above, is the slope of the rising branch of the curve relating plasma glucose concentration to insulin secretion rate.

In both the hyperglycaemic clamp and meal test, average insulin clearance was computed according to the following formula (derivation and correct interpretation are presented in the File S1):

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

in which 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 insulin concentration at the end of the study, I_{Basal} is insulin concentration at the beginning of the study, and MRT_{Ins} is the mean residence time of insulin, which was assumed to be 27 minutes as reported in Navalesi et al.[26]

The reconstructions of β-cell function during the hyperglycaemic clamp and the meal tolerance test were combined to enable modelling of the effect of incretins on insulin secretion: the "meal effect." This was done by taking the β-cell reconstructed from the hyperglycaemic clamp and challenging it, in an in silico experiment, with the plasma glucose curve of the meal test, thus computing the time course and the total amount of insulin secretion rate; this is the in silico equivalent of infusing in vivo intravenous glucose to mimic the glucose curve seen during the meal tolerance test. The effect of the meal on β-cell insulin secretion can be measured by comparing the total insulin secretion of the meal in vivo with that of the in silico simulation of intravenous glucose infusion to mimic the glucose curve elicited by the meal test:

$$Meal\ effect = \frac{AUC\ ISR_{Meal} - AUC\ ISR_{Intravenous}}{AUC\ ISR_{Meal}}$$

Further details of the computation of the "meal effect" are provided in the File S1-S7.

2.3 | Statistics

All datasets were tested for normality (Shapiro–Wilks test) and non-normally distributed variables were transformed (log 10) for analysis. Normally distributed data are expressed as mean ± SD, and log-normal data were back transformed to give geometric mean and 95% CI for the ratio of the geometric mean. Differences between ethnic groups were determined by independent samples *t* test using the raw data where they were normally distributed or logarithmic-transformed data where not. *P* values ≤.05 were considered statistically significant. Note that for the data analysed on the natural scale, the null value is 0 and so where $P < .05$, the 95% CI will exclude 0, but for the data analysed on the log scale and back-transformed to give the ratio of geometric means, the null value is 1 and so where $P < .05$, the 95% CI will exclude 1. The relationship between average insulin clearance and average insulin concentration was analysed by linear and non-linear regression analysis, as described in the File S1. Analyses were performed using SPSS software, version 24 (IBM Analytics).

3 | RESULTS

A total of 34 participants, 19 BAM and 15 WEM, were studied. Their mean (SD) age was 54.7 9 (7.4) years and BMI 29.7 (2.7) kg/m^2. The participants had been diagnosed with diabetes for 2.9 (1.1) years, the mean HbA1c was 49.3 (7.6) mmol/mol; 65% of participants were treated with metformin and the remainder with lifestyle management alone. By design, there were no significant ethnic differences in age, BMI, duration of diabetes, HbA1c, or management (Table 1). Mean visceral fat was significantly lower, and skeletal muscle area significantly higher in BAM (Table 1). The BAM were first-generation West African migrants (born in Nigeria, *n* = 11; Ghana, *n* = 5; Sierra Leone, *n* = 2; Ivory Coast, *n* = 1).

3.1 | β-Cell insulin secretory function

In the hyperglycaemic clamp there were no ethnic differences in mean fasting (Table 2) or "clamped" glucose (BAM 14.4 ± 1.28 vs WEM 14.8 ± 1.68 mmol/L; $P = .45$). Fasting C-peptide was lower in BAM (Table 2). There were no significant ethnic differences in first-phase C-peptide or insulin iAUC. Second-phase C-peptide secretion

TABLE 1 Clinical characteristics of black African and white European participants

	BAM (n = 19)	WEM (n = 15)	Mean difference (95% CI)	P
Age (y)	54.1 (7.7)	55.5 (7.1)	−1.3 (−6.6-3.9)	.602
Weight (kg)	90.6 (9.2)	94.2 (11.6)	−3.6 (−10.8-3.7)	.326
Height (cm)	175.4 (7.4)	176.8 (5.8)	−1.4 (−6.1-3.4)	.561
BMI (kg/m^2)	29.5 (2.6)	30.1 (2.7)	−0.62 (−2.5-1.3)	.510
Waist circumference (cm)	103.7 (8.2)	107.5 (8.8)	−3.86 (−9.8-2.1)	.194
Visceral fat area[a] (cm^2)	130.8 (54.1)	189.0 (75.7)	−58.2 (−104.2 to −12.2)	**.015**
Thigh skeletal muscle area[a] (cm^2)	434.2 (49.6)	379.2 (57.2)	55.0 (17.0-93.0)	**.006**
Duration of diabetes (y)	2.8 (1.2)	2.9 (1.0)	−0.09 (−0.88-0.69)	.815
Fasting glucose (mmol/L)	6.67 (0.97)	6.81 (1.37)	−0.14 (−0.95-0.68)	.732
HbA1c (mmol/mol)	49.9 (7.7)	48.6 (7.8)	1.26 (−4.15-6.74)	.631
HbA1c (%)	6.7 (0.68)	6.6 (0.72)	0.11 (−0.38-0.60)	.650
Systolic blood pressure (mm Hg)	137.3 (14.1)	131.8 (13.9)	5.5 (−3.3-15.4)	.262
Diastolic blood pressure (mm Hg)	85.6 (7.4)	82.9 (10.1)	2.7 (−3.4-8.8)	.376
Total cholesterol (mmol/L)	4.12 (0.70)	4.30 (0.72)	−0.18 (−0.68-0.32)	.470
LDL cholesterol (mmol/L)	2.34 (0.53)	2.29 (0.70)	0.06 (−0.37-0.48)	.794
HDL cholesterol (mmol/L)	1.17 (0.38)	1.24 (0.24)	−0.07 (−0.29-0.16)	.557
Triacylglycerol (mmol/L)	1.32 (0.75)	1.70 (0.71)	−0.38 (−0.89-0.14)	.143
Metformin use (%)	74	53		.09

Abbreviations: BAM, black African men; BMI, body mass index; CI, confidence interval; HbA1c, glycated haemoglobin; WEM, white European men. Data are arithmetic mean (SD). Differences between ethnic groups tested using independent samples t test. Bold font was used to highlight findings with a statistically significant probability.

[a] Data obtained for 14 WEM and 19 BAM.

(iAUC) was significantly lower in BAM, with a trend for second-phase insulin iAUC that did not achieve statistical significance (Figure 1 and Table 2). The modelled glucose sensitivity of the β-cell (σ^1 and σ^2) showed similar trends.

During the meal tolerance test the two ethnic groups exhibited the same glucose response; however, mean C-peptide iAUC was significantly lower in BAM. The meal insulin iAUC was not significantly different between ethnic groups (Table 3 and Figure 2). The modelled data from the meal tolerance test showed no significant ethnic differences in first-phase insulin secretory function (Table 3), but second-phase secretory function was lower in BAM (P = .01). The insulin secretion rate was lower amongst BAM at 4 mmol/L (P = .019) and 5.5 mmol/L (P = .02). This difference was lost at higher glucose concentrations of 8 mmol/L (P = .112), 11 mmol/L (P = .199) and 15 mmol/L (P = .247; Figure 3).

3.2 | Insulin clearance

There were no ethnic differences in average insulin clearance during the intravenous challenge (hyperglycaemic clamp; Table 2). In response to oral glucose the average clearance appeared lower in BAM, but this difference was not statistically significant (Table 3). When average clearance was plotted against average insulin concentration of each test, however, a hyperbolic relationship was apparent

TABLE 2 Hyperglycaemic clamp assessment of insulin secretory function in black African and white European participants

	BAM (n = 19)	WEM (n = 15)	Mean difference/ratio of geometric mean (95% CI)	P
Fasting glucose (mmol/L)	7.39 (1.59)	7.20 (1.12)	0.19 (−0.80-1.18)	.699
Fasting insulin* (pmol/L)	66.6 (50.8-87.4)	84.0 (57.3-123.3)	0.81 (0.51-1.23)	.290
Insulin iAUC 0 to 10 min* (pmol/L/min)	103.8 (28.5-378.1)	75.0 (13.8-408.5)	0.77 (0.13-4.48)	.764
Insulin iAUC 10 to 120 min* (pmol/L/min)	14 454 (8430-24 786)	21 999 (13 636-35 498)	0.57 (0.32-1.02)	.060
Fasting C-peptide (nmol/L)	0.576 (0.193)	0.837 (0.299)	−0.261 (−0.433-−0.089)	**.004**
C-peptide iAUC 0 to 10 min* (nmol/L/min)	0.697 (0.131-1.546)	1.227 (0.273-2.897)	0.98 (0.20-4.86)	.984
C-peptide iAUC 10 to 120 min* (nmol/L/min)	70.6 (52.5-94.8)	115.1 (84.8-156.3)	0.55 (0.37-0.83)	**.006**
σ^{1*} ((pmol/m^2 BSA)/(mmol/L/min))	65.6 (27.0-159.2)	95.3 (42.5-213.8)	0.69 (0.21-2.20)	.507
σ^{2*} ((pmol/min m^2 BSA)/mmol/L))	6.8 (4.1-11.4)	12.4 (7.2-21.6)	0.55 (0.26-1.14)	.105
M value (mg/m^2 BSA/min)	167.2 (38.4)	185.4 (37.0)	−18.2 (−44.7-8.4)	.173
Average insulin clearance* (mL/m^2 BSA/min)	897.6 (699.0-1152.4)	830.8 (637.2-1082.9)	1.08 (0.76-1.55)	.663

Abbreviations: σ^1, glucose sensitivity of β cell during first-phase insulin secretion; σ^2, glucose sensitivity of β cell during second-phase insulin secretion; BAM, black African men; BMI, body mass index; BSA, body surface area; CI, confidence interval; iAUC, incremental area under the curve, calculated using the trapezoidal rule; M, glucose disposal in final 60 minutes of the clamp; WEM, white European men. iAUC 0 to 10 min represents first phase, iAUC 10 to 120 min represents second phase. Data are mean (SD) or geometric mean (95% CI) for log-normal data*. Positively skewed data were transformed ($\log^{10}$) prior to statistical testing. Differences between ethnic groups tested using independent samples t test. Bold font was used to highlight findings with a statistically significant probability.

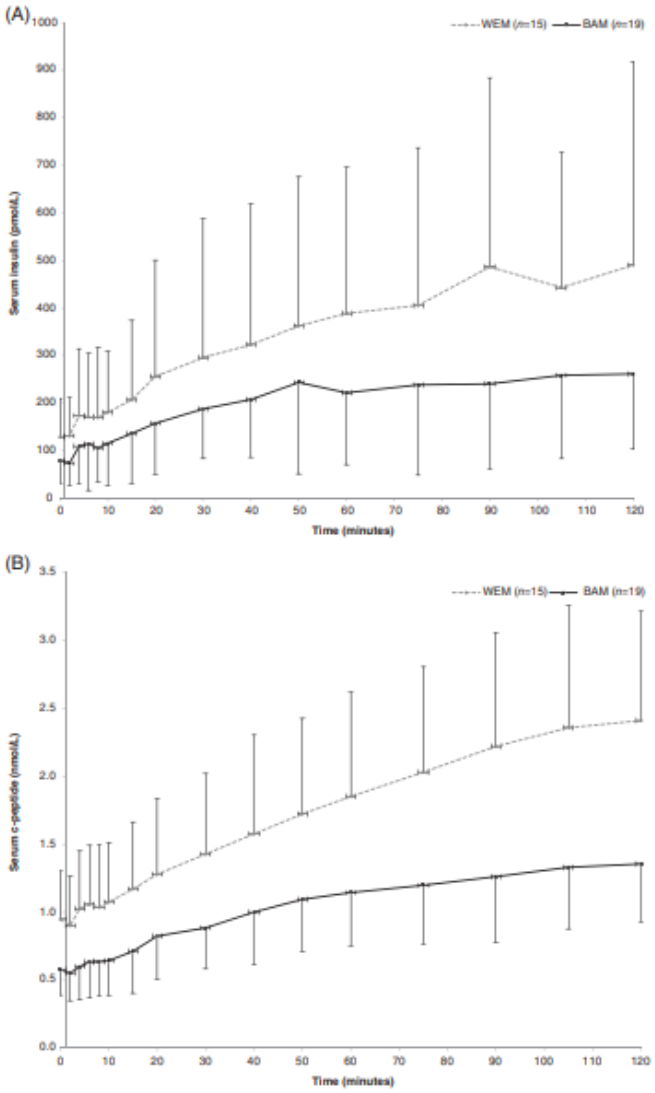

FIGURE 1 A, Serum insulin and B, C-peptide responses in the hyperglycaemic clamp in black African men (BAM) and white European men (WEM)

(Figure S8 in File S1), with a clear, significant difference between the groups, implying that in BAM average insulin clearance was lower at any average insulin concentration achieved during meal/clamp tests (Figure 4).

3.3 | Incretin responses

Mean secretion of GIP was significantly higher in BAM in response to the meal challenge (Table 3). There were no ethnic differences in GLP-1 secretion, or in the "meal effect," the modelled effect of the mixed meal, including incretin hormones, on insulin secretion (Table 3).

4 | DISCUSSION

The present study shows differences in the metabolic processes involved in glucose dysregulation in BAM with early T2D compared

TABLE 3 Meal tolerance test assessment of insulin secretory function in black African and white European participants

	BAM (n = 18)	WEM (n = 15)	Mean difference/ratio of geometric mean (95% CI)	P
Fasting glucose (mmol/L)	7.34 (1.35)	7.28 (1.34)	0.10 (−0.86-1.06)	.839
Glucose iAUC* (mmol/L/min)	378.4 (250.1-572.3)	476.2 (377.7-600.5)	0.86 (0.57-1.29)	.459
Fasting insulin* (pmol/L)	85.1 (67.6-107.2)	102.3 (74.1-141.3)	0.82 (0.57-1.19)	.284
Insulin iAUC* (nmol/L/min)	47.4 (32.6-68.8)	59.4 (42.3-84.8)	0.89 (0.59-1.35)	.566
Fasting C-peptide (nmol/L)	0.603 (0.216	0.881 (0.340)	−0.278 (−0.477-−0.080)	**.008**
C-peptide iAUC (nmol/L/min)	65.4 (17.7)	88.5 (29.4)	−23.2 (−40.0 to −6.3)	**.009**
Fasting GLP-1 (pmol/L)	12.1 (8.6)	11.7 (6.7)	0.48 (−5.07-6.03)	.861
GLP-1 iAUC* (pmol/L/min)	810.2 (519.2-1264.7)	861.0 (536.2-1382.6)	0.95 (0.57-1.57)	.832
Fasting GIP (ng/L)	44.6 (25.3)	31.8 (13.8)	12.8 (−2.10-27.7)	.089
GIP iAUC (μg/L/min)	46.8 (17.4)	33.9 (12.0)	12.9 (2.1-23.7)	**.021**
Fasting NEFA (μmol/L)	600.0 (186.4)	631.0 (192.4)	−31.0 (−165.9-103.9)	.643
NEFA iAUC (μmol/L/min)	−52 576 (26325)	−61 337 (26442)	8760 (−10 047-27 568)	.349
σ^1 ((pmol/m^2 BSA)/(mmol/L/min))	1420.1 (1184.2)	1134.6 (710.7)	285.5 (−447.2-1018.2)	.432
Average insulin clearance (mL/m^2 BSA/min)	506.2 (194.2)	630.1 (218.6)	−123.9 (−270.5-22.6)	.095
Meal effect (%)	51.0 (12.9)	49.5 (6.4)	1.5 (−6.2-9.1)	.700

Abbreviations: σ^1, glucose sensitivity of β cell during first-phase insulin secretion; BAM, black African men; GIP, gastric inhibitory polypeptide; iAUC, incremental area under the curve, calculated using the trapezoidal rule; NEFA, non-esterified fatty acids; WEM, white European men. Data are mean (SD) or geometric mean (95% CI) for log-normal data*. Positively skewed data transformed (log^{10}) prior to statistical testing. Differences between ethnic groups tested using independent samples t test. Bold font was used to highlight findings with a statistically significant probability.

with WEM. Our participants had both a very short duration of diagnosed disease and good metabolic control with minimal therapy (lifestyle ± metformin only) and thus our data extend into existing early diabetes data from healthy and prediabetic populations. Those studies report marked hyperinsulinaemia amongst people of African ancestry;[13,15,16] we provide novel data to show that in early T2D there is reduced insulin secretory function in response to both intravenous and oral stimuli in BAM. Whilst the insulin iAUC in the hyperglycaemic clamp was reduced in BAM, there were no differences in insulin when the meal was used to invoke hyperglycaemia via the gut. Importantly, this shows that the reduced hepatic insulin clearance, which has been reported in studies of healthy and prediabetic populations of African ancestry, is maintained through to early T2D and may act to maintain peripheral insulin levels, but may occur only in response to oral stimuli. Furthermore, BAM exhibited significantly greater GIP responses, which may have contributed to lower average insulin clearance rates, and may have important clinical implications.

The present study provides the most comprehensive assessment of the impact of black ethnicity on β-cell function to date. We used the intravenous glucose challenge of the hyperglycaemic clamp to distinguish first- and second-phase secretion, whilst the meal tolerance test was used to assess the physiological response of the β cells to nutrients, and incretin effects. In the present study we observed significantly lower fasting C-peptide concentrations amongst BAM, compared with WEM who had a similar duration of diagnosed diabetes, indicating significantly greater reduction in basal insulin secretion, although circulating insulin concentrations were not different. We also found reduced second-phase insulin and C-peptide response to intravenously stimulated hyperglycaemia amongst BAM. Previous studies assessing insulin secretion in non-diabetic populations, have provided inconsistent findings[7,10,11]; reporting higher first- and second-phase secretion,[8] or the difference occurring only in the first[9] or second phase.[5] In the aetiology of T2D, impairments in both first- and second-phase insulin responses have been recognized.[27,28] The second-phase response, which can only be triggered and sustained by glucose and fuel secretagogues, is quantitatively very important in the maintenance of glucose homeostasis, given that it can be sustained in response to prolonged hyperglycaemia.[29] Our modelling methods enabled us to investigate the impact of ethnicity on glucose dose effects on insulin secretion, which have not previously been examined amongst populations of African ancestry. Interestingly, ethnic differences in second-phase insulin secretion rates at lower glucose levels were lost at higher glucose concentrations (>8 mmol/L). Since both basal and glucose tolerance are similar in the two groups, this result suggests that, in the post-absorptive state, insulin secretion plays a different adaptive role in the two groups.

We are not aware of other studies comparing β-cell function between BAM and WEM with T2D using the hyperglycaemic clamp. The majority of ethnic comparisons have focused on healthy individuals or those at increased risk of T2D, and have predominantly used the intravenous glucose tolerance test to measure the "acute insulin response," which is comparable to the first-phase response of the hyperglycaemic clamp, but often only insulin is measured and rarely is the second-phase response assessed. These investigations have consistently shown a higher acute insulin response among black people without diabetes.[5–7,15,30,31] To date, only one comparison has been performed in people with T2D of different ethnicity, the Insulin Resistance Atherosclerosis Study (IRAS),[14] which reported significantly higher acute insulin response among black people than in white people, although not among the participants with newly diagnosed T2D, who are a nearer comparison to the participants in the present study. Notably, IRAS did not assess C-peptide so it is not possible to determine β-cell secretion, and the second-phase response was not assessed. There are other distinctions. It is well

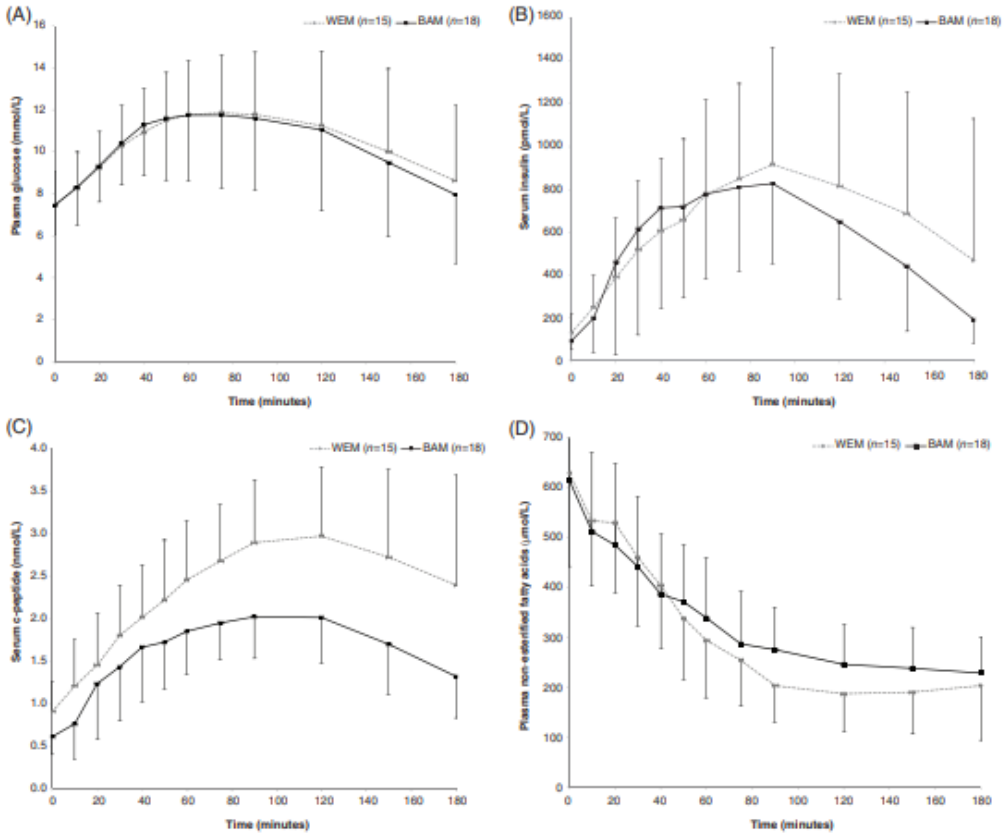

FIGURE 2 A, Plasma glucose, B, serum insulin, C, C-peptide and D, non-esterified fatty acid responses to a mixed meal tolerance test in black African men (BAM) and white European men (WEM)

established that the phenotype of T2D in black populations is gender-specific[16,17]; higher insulin levels[16,17] and obesity-driven T2D are more common in women,[16] hence the present study included only men, whereas IRAS included both men and women. There is a need for further studies to examine gender-specific mechanisms.

Our findings of significantly lower basal C-peptide but not insulin raise concerns regarding the use of β-cell indices based on fasting insulin, such as HOMA-β.[32] These are often used to assess β-cell function in epidemiological studies but our data suggest they may misrepresent β-cell function in black populations, and findings of ethnic differences[16] may need to be considered with caution.

When we studied β-cell function using an oral stimulus, we recognized a significantly lower second-phase C-peptide response in BAM, consistent with the hyperglycaemic clamp; however, there were no differences in insulin concentrations and model-derived data found to indicate lower insulin clearance in BAM (Figure 4). The implication of Figure 4 is that, at the same total insulin output during an intravenous or an oral challenge, BAM achieve higher insulin curves, which may compensate for reduced β-cell secretion and contribute to peripheral insulin levels. A number of previous investigations have reported reduced insulin clearance in black populations without diabetes,[5–7,13,15,33] and, in the present study, we show that this is maintained into early T2D. The mechanisms underlying this are largely unknown, but recent advancements in modelling techniques that allow hepatic vs extrahepatic clearance to be quantified have concluded that ethnic differences in insulin clearance are solely hepatic with no extra-hepatic contribution.[34]

A reduction in insulin clearance is typically found after oral glucose or meal ingestion, and is characteristically of a much greater magnitude than that observed after intravenous induction of hyperglycaemia.[35–37] Reduction in insulin clearance with increasing levels of insulin secretion is proposed to be attributable to the saturable nature of hepatocellular insulin receptors[38,39]; however there is also evidence that incretin hormones affect insulin clearance.[36,37,40] There has been very little study of incretin hormones and how these vary according to ethnicity. In the present study, BAM exhibited significantly higher postprandial GIP concentrations, which may have contributed to the non-significant trend for lower average insulin clearance that was observed. The effect of

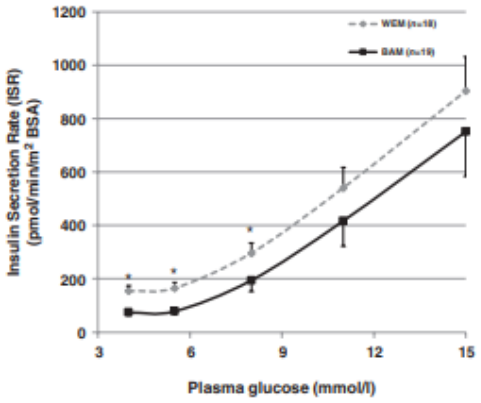

FIGURE 3 Insulin secretion rates at 5 increasing plasma glucose concentrations during the meal tolerance test, as reconstructed by mathematical modelling of β-cell function, in black African men (BAM) and white European men (WEM)

GIP on insulin clearance is unclear; some authors have demonstrated an insulin clearance-reducing effect of GIP,[41,42] whilst others have shown no effect.[39,43] Some of the conflict in these findings may have occurred because insulin clearance appears to adapt to insulin resistance and glucose intolerance, a potential mechanism by which β-cell function is preserved in the progression to T2D.[44,45] There has been very little investigation of incretin hormones within black populations and, in the few studies that exist, the focus has been on the role of incretins in the upregulation of insulin secretion; African-American children have been reported to have lower GLP-1, but similar GIP secretion compared with European-American children[46] whereas in a study of black and white obese adolescents, Michaliszyn et al[33] reported no difference in GLP-1

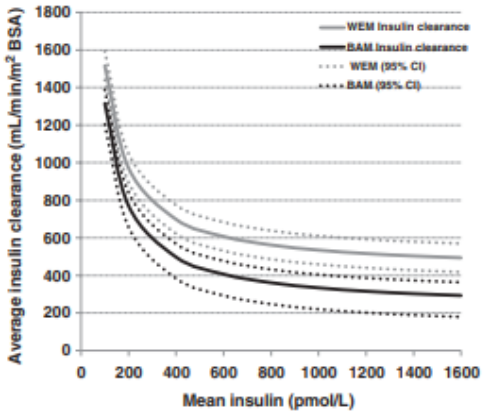

FIGURE 4 Relationship between average insulin clearance and average insulin concentration during the hyperglycaemic clamp and the mixed meal test black African men (BAM) and white European men (WEM). Average insulin clearance (mL/min/m² BSA):
BAM = 224 + (109 151/average insulin concentration),
WEM = 425 + (109 151/average insulin concentration)

or GIP levels, while Velasquez-Mieyer et al[47] found higher GLP-1 levels in obese African-American adults compared with European-Americans, with no measurement of GIP. We modelled the impact of the mixed meal, including, but not limited to, the effect of the incretin response on insulin secretory function ("meal effect"), but detected no ethnic differences. Michaliszyn et al[33] modelled the "potentiation factor," which describes the modulation of the relationship between glucose concentration and insulin secretion and comprises several mechanisms, including the release of endogenous incretin hormones. In contrast to our data, they found no differences in incretin concentrations in response to an oral glucose challenge, but reported a significantly higher early potentiation factor in black participants.[33] Our data suggest that, by the time diabetes develops, BAM may have no greater β-cell response to GIP than WEM, but that their higher GIP response may cause lower average insulin clearance in response to hyperglycaemia, which results in maintenance of peripheral insulin concentrations, and that these mechanisms provide some compensation for the significantly lower insulin secretory capacity of the β cells.

The strengths and limitations of the present study warrant discussion. We did not investigate the cellular mechanisms that underlie the differences in metabolic function between BAM and WEM. Additionally, we only captured the metabolic phenotype of T2D, and of men, therefore we cannot allude to the mechanisms by which hyperglycaemia progresses and how this may be distinct among BAM, and our findings may not extrapolate to women. Our study has explored ethnic differences in insulin secretory function and in doing so has a priori assessed a comprehensive portfolio of measures that attempt to thoroughly characterize insulin secretory function. Although we conducted a large number of comparisons we did not correct for multiple testing because our outcome variables were not independent of one another and the differences were very large and highly significant; therefore, we are confident that the differences we observed are likely to represent real differences. Finally, the model used to aid computation of the meal effect on β-cell function (File S1) has not been validated with ad hoc experiments.

Major strengths of the study are its use of intensive, sophisticated techniques, and the well-matched participant groups; our ethnic groups had the same duration of diagnosed diabetes, HbA1c, fasting glucose and clinical management, and exhibited almost identical glucose responses to a meal challenge. We are therefore confident we have recognized novel ethnic distinctions in T2D pathophysiology, which may have important clinical implications. The intensive nature of our protocol precludes a much larger study, and we may have missed additional more subtle ethnic differences, but the value of our approach is perhaps best seen in the way our data extend the conclusions of epidemiological studies such as IRAS, discussed above. Our data suggest that loss of β-cell insulin secretory function occurs earlier in the development of T2D in BAM than in WEM, but the mechanisms that drive β-cell dysfunction in BAM are not clear. Potentially, BAM may have lower β-cell mass or a steeper slope of decline in β-cell function as T2D develops.

In conclusion, we have recognized in the present study that deficits in β-cell function may affect hyperglycaemia in BAM more strongly than in WEM. Further studies are needed to ascertain whether the incretin hormones play a damage-limitation role in

maintaining peripheral insulin concentrations by reducing insulin clearance in BAM. Meanwhile, it may be pertinent to consider therapeutic strategies that augment these physiological processes; BAM may achieve greater clinical benefit from therapeutic agents that support β-cell function, such as the incretin therapies.

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Conflict of interest

None declared.

Author contributions

L. M. G., S. A. A., J. L. P., A. M. U. formulated the research question and designed the study. L. M. G., S. A. A. and K. G. M. M. A. supervised the data collection and interpretation. C. M. coordinated the study and data acquisition, and performed the metabolic assessments. F. S.-M. assisted with the metabolic assessments. L. B., R. B. and L. M. G. performed the minimal modelling analysis. L. M. G. undertook data analysis, statistical analysis and drafted the manuscript. All authors contributed to the intellectual content of the submitted manuscript. L. G. 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.

ORCID

Louise M. Goff http://orcid.org/0000-0001-9633-8759

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Appendix B

Tests of Normality-Clinical characteristics of study participants

Tests of Normality

	1-WE;2-BWA		Shapiro-Wilk df	Shapiro-Wilk Sig.		Shapiro-Wilk df	Shapiro-Wilk Sig.
Age	1.0	.212	20	.019	.802	20	<.001
	2.0	.177	20	.099	.908	20	.058
Weight(kg)	1.0	.120	20		.962	20	.592
	2.0	.133	20	.200	.937	20	.211
Height (cm)	1.0	.154	20	.200	.922	20	.107
	2.0	.132	20	.200	.961	20	.556
BMI	1.0	.152	20	.200	.944	20	.281
	2.0	.112	20	.200	.923	20	.111
Waist Circumference in cm	1.0	.080	20	.200	.959	20	.517
	2.0	.215	20	.016	.929	20	.150
Duration of Diabetes (years)	1.0	.259	20	.001	.816	20	.002
	2.0	.187	20	.065	.911	20	.065
Screening_HbA1c_DCCT	1.0	.139	20	.200	.945	20	.298
	2.0	.130	20	.200	.938	20	.222
Screening_HbA1c_IFCC	1.0	.167	20	.145	.934	20	.186
	2.0	.151	20	.200	.938	20	.220
Systolic BP mm Hg	1.0	.091	20	.200	.967	20	.700
	2.0	.095	20	.200	.965	20	.652
Diastolic mm Hg	1.0	.126	20	.200	.906	20	.054
	2.0	.204	20	.029	.865	20	.010
Screening_TC	1.0	.173	20	.119	.872	20	.013
	2.0	.098	20	.200	.988	20	.995
Screening_LDL	1.0	.183	20	.079	.901	20	.043
	2.0	.124	20	.200	.958	20	.500
Screening_HDL	1.0	.116	20	.200	.943	20	.269
	2.0	.124	20	.200	.931	20	.163
Screening_Trig	1.0	.178	20	.098	.917	20	.088
	2.0	.251	20	.002	.767	20	<.001
Metformin (1=Yes;2=No)	1.0	.361	20	<.001	.637	20	<.001
	2.0	.463	20	<.001	.544	20	<.001

Tests of Normality-Mixed meal tolerance test

Tests of Normality

	Ethnicity (1=WE, 2=BWA)	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
MTT_Gluc_AvBasal	1.0	.203	18	.047	.911	18	.089
	2.0	.133	19	.200*	.961	19	.590
MTT_Gluc_iAUC	1.0	.211	18	.033	.869	18	.017
	2.0	.156	19	.200*	.917	19	.098
MTT_Ins_AvBasal	1.0	.303	18	<.001	.771	18	<.001
	2.0	.247	19	.003	.884	19	.025
MTT_Ins_iAUC	1.0	.227	18	.015	.810	18	.002
	2.0	.125	19	.200*	.951	19	.414
MTT_Cpep_AvBasal	1.0	.195	18	.070	.878	18	.024
	2.0	.120	19	.200*	.966	19	.689
MTT_Cpep_iAUC	1.0	.172	18	.170	.952	18	.462
	2.0	.130	19	.200*	.957	19	.516
MTT_GLP_AvBasal	1.0	.158	18	.200*	.916	18	.111
	2.0	.225	19	.013	.863	19	.011
MTT_GLP1_iAUC	1.0	.249	18	.004	.799	18	.001
	2.0	.235	19	.007	.716	19	<.001
MTT_GIP_AvBasal	1.0	.207	18	.040	.887	18	.034
	2.0	.156	19	.200*	.944	19	.316
MTT_GIP_iAUC	1.0	.201	18	.053	.912	18	.094
	2.0	.140	19	.200*	.955	19	.487

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Normality-Hyperglycaemic clamp

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Ethnicity (1=WE, 2=BWA)	Statistic	df	Sig.	Statistic	df	Sig.
Hyper_Gluc_AvBasal	1.0	.150	20	.200*	.963	20	.608
	2.0	.177	20	.100	.906	20	.052
Hyper_Ins_AvBasal	1.0	.195	20	.046	.914	20	.076
	2.0	.153	20	.200*	.838	20	.003
Hyper_Cpep_AvBasal	1.0	.133	20	.200*	.936	20	.201
	2.0	.096	20	.200*	.947	20	.318
Hyper_Ins_iAUC_0to10	1.0	.323	20	<.001	.634	20	<.001
	2.0	.332	20	<.001	.617	20	<.001
Hyper_Ins_iAUC_10to120	1.0	.345	20	<.001	.655	20	<.001
	2.0	.181	20	.083	.828	20	.002
Hyper_Cpep_iAUC_0to10	1.0	.264	20	<.001	.753	20	<.001
	2.0	.341	20	<.001	.534	20	<.001
Hyper_Cpep_iAUC_10to120	1.0	.245	20	.003	.815	20	.001
	2.0	.162	20	.177	.916	20	.083

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction