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

Objective: We aimed to quantify IPL in White European (WE) and Black West African (BWA) men with early T2D, and to investigate relationships between IPL and beta-cell insulin secretory function (ISF).

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

Main Outcome Measures: Participants underwent Dixon magnetic resonance imaging to determine IPL in the pancreatic head, body, and tail, and subcutaneous and visceral adipose tissue volumes. Modelled first-phase and second-phase ISF were comprehensively determined using c-peptide measurements during a 3-hour meal tolerance test and a 2-hour hyperglycemic clamp test.

Results: WE men had higher mean IPL than BWA men (P=0.029), mainly due to higher IPL in the pancreatic head in WE men (P=0.009). Mean IPL was inversely associated with orally stimulated first-phase ISF in WE but not BWA men (WE: r=-0.554, P=0.026; BWA: r=-0.183, P=0.468); there was no association with orally stimulated second-phase ISF in either WE or BWA men. No significant associations were found between mean IPL and intravenously stimulated ISF.

Conclusions: IPL is lower in BWA than WE men with early T2D, and the lack of inverse association with orally stimulated first phase ISF in BWA men indicates that IPL may be a less important determinant of the development of T2D in BWA compared to WE men.

We have shown that intrapancreatic lipid is associated with orally stimulated beta-cell insulin secretory function in White European men but not Black West African men with early type 2 diabetes

ABBREVIATIONS

ALT: Alanine aminotransferase

BMI :Body mass index

BWA: Black West African

CV: Coefficient of variation

HbA1C: Glycated hemoglobin

iAUC: Incremental area under the curve

IHL: Intrahepatic lipids

IPL: Intrapancreatic lipids

ISF: Insulin secretory function

MRI: Magnetic resonance imaging

SAT: Subcutaneous adipose tissue

VAT: Visceral adipose tissue

WE: White European

INTRODUCTION

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

There is growing evidence of distinctions in the pathophysiology of type 2 diabetes in populations of African ancestry. Typically lower levels of VAT and IHL are reported compared to White populations (11). Additionally beta-cell dysfunction is more evident; a higher insulin response to glucose stimulation is consistently reported amongst healthy and prediabetic populations compared to those of White ethnicity (12,13), while in the diabetic state, Black men may have lower insulin secretion than White men in response to both oral and intravenous glucose (14). To date there has been limited investigation of the impact of Black ethnicity on IPL and its relation to the metabolic abnormalities of type 2 diabetes but given the lesser deposition of VAT and greater beta-cell dysfunction that is typically observed in Black populations it is reasonable to hypothesise that IPL, and its role in the pathophysiology of type 2 diabetes, may differ by ethnicity. Indeed a small number of studies in healthy adults and adolescents have reported lower IPL among Black populations compared to other ethnic groups (7,15,16) and, in a comparison of healthy and prediabetic adolescents of African-American and Latino ethnicity, IPL was found to be the strongest predictor of prediabetes in African-Americans but not Latinos (17). Investigations of the impact of IPL on beta-cell function in Black populations have shown inconsistent findings; whilst no relationship was found between IPL and insulin secretory function in adolescents, studies in healthy adults have shown that IPL is more strongly associated with beta-cell function in African-Americans compared to other ethnic groups (7,15). To date studies of IPL

and beta-cell function in Black populations have been limited to healthy cohorts in which there is limited development of the metabolic abnormalities of type 2 diabetes. Furthermore, these studies have assessed only the first phase insulin secretory response, and only indirect assessments of insulin secretion have been performed, which have limited utility in Black populations as they do not account for hepatic insulin clearance, for which ethnic differences are well established (18). We recently reported deficits in second phase insulin secretory function, through comprehensive modelling of C-peptide, present in Black African but not White European men with early type 2 diabetes (14). The aim of the present study was to assess IPL and investigate its relationship with first and second phase ISF, assessed comprehensively using c-peptide modelling, to explore the hypothesis that men of Black (West) African ethnicity with early type 2 diabetes will have significantly lower IPL compared to White European men.

MATERIALS AND METHODS

This investigation was conducted as part of the South London Diabetes and Ethnicity Phenotyping study (Soul-Deep), details of which can be found elsewhere (19). The study was conducted at King's College Hospital and Guy's Hospital, London, and approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided informed consent. Recruitment and data collection took place April 2013-January 2015.

Participants
Potential participants were identified through primary care practices in South London. Men of White European (WE) or Black West African (BWA) ethnicity were recruited: ethnicity was self-declared and confirmed through grandparental birthplace where the countries included were North West European and West African countries, defined by the UNSD. Participants provided information of birthplace. Eligibility was confirmed in a screening visit and participants considered eligible if they met the following criteria: aged between 18-65 years of age; BMI 20-40 kg/m^2; recent diagnosis of type 2 diabetes (less than 5 years prior to starting the study); using only lifestyle alone or with metformin to manage their diabetes. Exclusion criteria included: use of thiazolidinedione, insulin, oral steroids, beta-blockers or other medication which may affect the study outcome; contradiction for magnetic resonance imaging (MRI), such as having metal implants; having kidney or liver damage identified by a serum creatinine of above 150 mmol/l or serum alanine transaminase level increased more than 2.5-fold above the upper limit of the reference range, respectively.

Procedures
Participants attended three assessment visits in random order within a maximum period of 6 months. Each participant underwent an MRI scan for assessment of IPL, a 2-hour hyperglycemic clamp (HC) and a mixed meal tolerance test (MTT) for assessment of ISF through the measurement and mathematical modelling of c-peptide. For each assessment participants attended in a fasted state having refrained from eating or drinking anything other than water from 10pm the night prior. Participants were instructed to refrain from strenuous exercise and physical activity in the 48 hours preceding the visit, refrain from consuming alcohol in the 24 hours preceding the visit, and to consume a standardized diet the day prior (~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 each visit.

MRI fat quantification
A Dixon-based MRI sequence was used on a 1.5 Tesla Siemens scanner to acquire images for the assessment of IPL. With the participant lying supine, images were obtained from the neck to the knee (excluding the arms) with coils placed on the abdominal region. During

acquisition of the abdominal images, upon instruction by the radiographer, participants undertook three 15-second breath holds to reduce motion artefacts. For each participant, 320 contiguous, 3mm slice thickness, T1-weighted transverse spin-echo images (repetition time: 6.77ms; echo time: 4.77ms (in-phase), 2.39ms (out-of-phase), flip angle: 10°), were obtained.

Intrapancreatic fat was determined by analysis of MRI images on the HOROS V 1.1.7 software (www.horosproject.org; accessed 21/10/2017) by locating one or more axial images with the largest area of the head, body and tail of the pancreas and extracting the corresponding fat and water images. On each of the fat and water images, one circular region of interest of 1cm^2 was drawn on each of the head, body and tail of the pancreas as shown in Figure 1. A region of 1cm^2 was used as recommended by a recent review of magnetic resonance methods used to determine IPL as it ensures the regions are within the border of the pancreas and to avoid inclusion of VAT and the splenic vein (9). By using the following formula: $\%IPL = (F/(F+W))*100$, the pancreatic fat fraction was calculated in each region: IPL_{HEAD}, IPL_{BODY}, IPL_{TAIL}, and IPL_{MEAN} was calculated as the average of the head, body and tail regions. Due to the subjective nature of locating the regions of interest on the head, body and tail of the pancreas, IPL quantification was conducted by 2 independent investigators with a significant correlation reported (r=0.62, $P<0.001$) and an inter-observer CV of 14%.

Total abdominal VAT mass and SAT mass from the neck to the knee region (excluding arms) were quantified using an automated analysis method (Klarismo Ltd., London, UK). For VAT mass quantification, each image in the abdominal region was analyzed for VAT area and the area was multiplied by the slice thickness of 3mm to get the volume of VAT. For SAT mass quantification, all MRI images that were acquired were analyzed for SAT area which was multiplied by the slice thickness of 3mm to get the volume of SAT. The volume of VAT and SAT were converted from mm^3 to liters and then converted to kg by multiplying by 0.9 kg/l (the density of fat) (20).

Insulin secretory function during a hyperglycemic clamp
A 2-hour hyperglycemic clamp was conducted for assessment of ISF (21). Following collection of three basal blood samples, a 20% glucose infusion was administered to achieve a hyperglycemic state of 6.9mmol/l above basal for a period of 2 hours. Blood samples were collected at -20, -10, 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes to measure plasma glucose, and serum insulin and c-peptide.

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

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

Model-based measurement of beta-cell function:
the glucose, insulin and c-peptide curves during the hyperglycemic clamp and meal tolerance test were modelled using methods previously described (22-24) (SAAM-II 1.2 software; SAAM Institute, Seattle, WA). The main outputs of the model are: glucose sensitivity of first-phase secretion (σ^1), expressed as the amount of insulin secreted in response to a rate of increase in glucose of 1 mmol/l between time 0 and 1 min of the study, in

$\left(pmol \cdot m^{-2} BSA \right) / \left(mmol \cdot l^{-1} \cdot min^{-1} \right)$; 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 $\left(pmol \cdot min^{-1} \cdot m^{-2} BSA \right) / \left(mmol \cdot l^{-1} \right)$.

Biochemical analyses

We measured plasma glucose by automated glucose analyzer (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA); serum insulin by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK), where the inter-assay and intra-assay coefficients of variation (CVs) ≤ 5.9% and 4.6%, respectively; serum c-peptide by radioimmunoassay (Millipore Ltd, Hertfordshire, UK).

Statistical Analyses

Variables that were positively skewed were log-transformed to give a Normal distribution. Significance of differences in variables of interest between the ethnic groups were tested using independent samples t-test for Normal data or a Mann-Whitney U test for data that could not be transformed to Normal. To investigate ethnic differences in IPL we initially conducted independent samples t-tests; we then used ANCOVA, adjusting for VAT, to determine if differences in IPL were independent of VAT. The associations between IPL and parameters of ISF were explored using Pearson's correlation; partial correlation was used to investigate these associations while adjusting for VAT. To investigate ethnic differences in the distribution of IPL between the head, body and tail of the pancreas, a mixed between-within subjects ANOVA was performed. SPSS version 24.0 was used for all statistical analyses and p values < 0.05 were considered statistically significant.

RESULTS

Participant characteristics

Nineteen BWA and 18 WE men were studied (Figure 2). The BWA men were first-generation West African migrants (born in Nigeria, n=11; Ghana, n=5; Sierra Leone, n=2, Ivory Coast, n=1). The characteristics of the two ethnic groups are presented in Table 1. The groups were well-matched for age, weight and BMI, with no significant differences in diabetes duration, fasting glucose, HbA1c, blood pressure, liver function as represented by ALT, and measures of cholesterol (Table 1). Metformin use was not different between the two ethnic groups (P=0.248) with 56% of the WE and 74% of the BWA men being treated with metformin. Waist circumference was higher, although not statistically, in the WE men. Fasting triglyceride concentrations were significantly higher in WE men. Analysis of MRI images of the whole abdominal cavity showed that the weight of abdominal VAT was significantly higher in WE men, however, there was no ethnic difference in SAT measured between the neck and knee (excluding arms) (Table 1).

Insulin secretory function

The c-peptide responses to the intravenous (hyperglycemic clamp) and oral (meal tolerance test) stimulation are shown in Table 2. Basal c-peptide was significantly lower in BWA compared to WE men, although there were no ethnic differences in basal insulin (Table 2). The c-peptide iAUC during the meal test was significantly lower among the BWA men; this was also the case in the hyperglycemic clamp, specifically in the 2nd phase (Table 2). The c-peptide data were modelled alongside the glucose curves to provide an estimate of first and second phase glucose sensitivity of the beta-cells (σ^1 and σ^2, respectively); the modelled data showed similar findings of lower 2nd phase insulin secretion, although the results failed to reach statistical significance.

Pancreatic fat analysis

Mean IPL, as well as IPL of the head, body and tail regions are shown in Figure 3. BWA men exhibited significantly lower IPL_{MEAN} than WE men (WE: 10.08 (SD 2.46) *vs* BWA: 8.22 (SD 2.51) %, *P*=0.029), which was driven by significantly lower IPL_{HEAD} in the BWA men (WE: 9.66 (SD 3.14) *vs* BWA: 7.03 (SD 2.65) %, *P*=0.009). After adjustment for VAT IPL_{MEAN} showed no significant ethnic differences (WE: 9.60 (SE 0.65) *vs* BWA: 8.60 (SE 0.63) %, *P*=0.305) and the ethnic difference in IPL_{HEAD} reduced in significance (WE: 9.04 (SE 0.68) *vs* BWA: 7.18 (SE 0.66) %, *P*=0.074). We investigated regional IPL depositions of the head, body and tail regions within and between each ethnic group using a mixed between-within subjects ANOVA. There were significant differences in IPL between the pancreatic sections in BWA men (Wilks' Lambda=0.791, *P*=0.019), with a significant main effect for ethnicity (*P*=0.029). There were no significant differences in the distribution of IPL between the two ethnic groups (*P*=0.474).

Relationships between IPL and ISF

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

DISCUSSION

In our comparison of BWA and WE men with early type 2 diabetes, we found ethnic differences in the deposition of pancreatic fat and its association with beta-cell function. Men of BWA ethnicity exhibited significantly lower IPL compared to WE men, predominantly due to lower IPL deposition in the head of the pancreas. Furthermore, we have recognized ethnic distinctions in the relationship between IPL and ISF such that IPL is inversely associated with ISF only in WE men leading us to speculate that IPL may be a less important determinant of the development of T2D in BWA than WE men.

The accumulation of IPL and other depots of ectopic fat is thought to occur due to reduced expandability of SAT during energy surplus, as well as prolonged release of free fatty acids from SAT, due to adipocyte insulin resistance, which subsequently deposit as ectopic fat (8,25). The interrelated nature of ectopic fat depots has been demonstrated in the work of Le *et al.* who showed a correlation between VAT, IHL and IPL, in a multi-ethnic cohort (15). Since it is well established that VAT and IHL are significantly lower in Black populations (26,27) we hypothesized that IPL would be lower too. In line with previous studies our BWA men exhibited significantly lower VAT than the WE men and we found that IPL no longer differed by ethnicity after adjusting for VAT. This suggests the lower IPL in BWA is driven by lower VAT and indicates a central role of VAT in determining IPL deposition. Our findings extend into type 2 diabetes earlier studies of healthy individuals who have reported significantly lower IPL among African-Americans compared to White and Hispanic ethnic groups (7,15). Furthermore, we provide novel data on differences in regional

deposition of IPL according to ethnicity showing the higher IPL deposition in WE men to be specific to the head of the pancreas. This is important given that the development of type 2 diabetes has been shown to be specifically associated with loss of beta-cell mass from the pancreatic head (28) and several studies have shown links between lipotoxicity and beta-cell apoptosis (6,29). During the progression from normal glucose tolerance to type 2 diabetes, loss of first phase ISF is understood to be the most critical dysfunction of the beta-cells (30), and the detrimental effects of IPL have consistently been shown to relate to first phase ISF (4,31). Our protocol enabled us to differentiate first and second phase insulin secretory function and investigate, for the first time, ethnic differences in the relationship between these and IPL. Considering this, our findings suggest that beta-cell lipotoxicity may be a less important determinant of ISF in BWA compared to WE men. This is consistent with findings of a recent investigation in prepubertal youth of African-American and Caucasian ethnicity (32) that reported significantly greater declines in beta-cell function in Caucasian youth in response to a lipid infusion, suggesting greater susceptibility of the beta-cells to acute lipotoxicity in Caucasian youth compared to African-Americans, although it should be noted that IPL was not measured in this study. Until now, studies of IPL in Black populations have been limited to only 1st phase ISF, expressed as the 'acute insulin response' measured using the intravenous glucose tolerance test (7,15,16), whilst our findings indicate the potential importance of also assessing 2nd phase ISF. Our results showing that BWA men have lower second phase ISF, which we have previously explored in more detail (14), indicates that a decrease in second phase ISF may have a more prominent etiological role in beta-cell dysfunction in Black populations but is not related to IPL.

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

We found ethnic differences in the regional distribution of IPL within the pancreas whereby BWA men had significantly higher IPL in the tail compared to the head of the pancreas whilst in WE men there was no apparent regional variation in IPL deposition. However, when we looked at region-specific relationships between IPL and ISF we found, in WE men, an inverse relationship, which neared statistical significance, between IPL in the tail with both intravenously-stimulated first phase and second phase ISF which was not seen in BWA men. Studies conducted in humans have shown regional variation in the distribution of beta-cells within the pancreas, concluding that there is more than a 2-fold greater density of beta-cells in the tail of the pancreas compared to the head and body (28). The inverse relationship we observed, albeit of borderline statistical significance, between IPL in the tail and ISF in WE men may indicate greater beta-cell lipotoxicity in WE men and in turn reduced beta-cell function in the tail of the pancreas, an association not seen in the BWA men. We propose further work should be conducted to investigate ethnic differences in the role of regional IPL on regional specific beta-cells within the pancreas.

Our study design, using both the HC and MTT, enabled us to compare for the first time in a single study distinctions in the associations between IPL and ISF in response to orally versus intravenously stimulated glycemia. Our findings of significant associations only

between IPL and orally-stimulated ISF help to explain previous contradictory results between studies that have used oral versus intravenous methods (31,33). These findings suggest an interaction between incretin hormones, IPL and ISF. Indeed, recent studies have shown a link between beta-cell lipotoxicity and reduced incretin effect where increasing concentrations of free fatty acids were associated with a down regulation of the GLP-1 receptor in a mouse model (34). Our results may suggest that IPL negatively impacts on incretin signaling in beta-cells and further hinder insulin secretory response to a meal in WE men but not BWA men. This ethnic difference may be explained by differences in incretin levels, which have previously differed between Black and White populations with some studies reporting higher incretins in Blacks (14,35,36) while others report lower(37); further investigations are needed to understand the ethnic differences in relationships between incretins and IPL.

Our study has several strengths including the measurement of c-peptide for the assessment of insulin secretion, and the use of two methods to comprehensively measure insulin secretory function, distinguish first and second phase secretion and the role of incretin hormones. Another strength is our investigation of regional variation of IPL in the head, body and tail of the pancreas and how this differs between and within each ethnic group. Our study also benefits from MRI analysis of pancreatic fat which has been suggested to be superior to magnetic resonance spectroscopy (MRS) for IPL analysis due to the irregular size and morphology of the pancreas, particularly in diabetic populations (9,38,39). However, this study is not without its limitations. Small regions of interest were used in the MRI analysis of IPL to reduce contamination with VAT, as recommended by investigators of methods used to quantify IPL by Dixon-MRI (9,40), although, we cannot guarantee that VAT contamination did not occur due to poor participant compliance with the breath-holds. Also, we cannot determine if mean IPL represents total IPL since we did not measure the volume of the pancreas which is reported to be 33% less in individuals with early type 2 diabetes compared to healthy controls (41). Our study is deliberately limited to studying men as there is consistent evidence for gender differences in the pathophysiology of type 2 diabetes in African populations (42,43), we acknowledge that this limits the generalizability of our findings. However, despite the higher prevalence of type 2 diabetes in Black women compared to Black men (1), our data are valuable due to the lack of studies conducted in men in this field. Furthermore, our study focused on Black West African ethnicity and all our participants were first generation migrants (born in countries of West Africa). We acknowledge that when comparing our study to previous works there may be differences between Black populations residing in the UK with those residing in the US or other regions in terms of lifestyle behaviors, socioeconomic factors, and access to healthcare that may influence the development of type 2 diabetes. We cannot determine a causal relationship between beta-cell function and IPL accumulation due to the cross-sectional nature of this study. Our study was also conducted on a small sample size although, despite this, is comparable in size to other studies carried out on IPL and ISF. An investigation of normal glucose tolerant and impaired glucose tolerant groups of both WE and BWA ethnicity would enable us to gain an understanding of the effect of IPL accumulation on beta-cell function during the progression of type 2 diabetes.

In conclusion, our study demonstrates ethnic differences in the deposition of pancreatic fat and its association with beta-cell function. Our findings of lower IPL among BWA men with type 2 diabetes suggest that lipotoxicity in the pancreas may be less dominant in the pathogenesis of type 2 diabetes in BWA compared to WE men. Furthermore, ethnic distinctions in the relationship between IPL and ISF such that it relates to insulin secretory function in WE men but not BWA men, suggest that IPL may be a lesser determinant of beta-cell dysfunction in BWA men with early type 2 diabetes.

Author contributions

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

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Duality of interests:

The authors declare that there is no duality associated with this manuscript.
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Figure 1: Selection of positioning of the circular regions of interest in the pancreas head, body and tail to quantify intrapancreatic lipids. Panel (a) shows circular regions of interest of 1cm^2 drawn on the head and tail of the pancreas on an axial abdominal magnetic resonance imaging (MRI) image. Panel (b) shows a coronal MRI image with the horizontal line depicting the position of axial image (a). Panel (c) shows a circular region of interest of 1cm^2 drawn on the body of the pancreas on an axial abdominal MRI image. Panel (d) shows a coronal MRI image with the horizontal line depicting the position of axial image (c).

Figure 2: Study flow chart. Of the 57 participants initially assessed for eligibility, 19 Black West African and 18 White European men were enrolled into the study and 20 participants were excluded of whom 15 were not eligible, 2 had contraindications for MRI, 1 had poor MRI image quality and was therefore excluded, and 2 participants withdrew consent.

Figure 3: Mean intrapancreatic lipids and intrapancreatic lipids of the head, body and tail, data presented by ethnicity. Data presented as mean ± SD. *Significant p<0.05 as determined by an independent samples t-test between WE and BWA men.

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

Table 1: Clinical characteristics of Black West African and White European men

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

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (‡) or median (interquartile range) for non-parametric data (†). *P* values determined using independent samples t-tests for normally distributed data otherwise Mann-Whitney test. N for [a]WE=17, BWA=18; [b]WE=17, BWA=17.
Abbreviations:ALT, alanine aminotransferase; BP, blood pressure; BWA, Black West African; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WE, White European.

Table 2: Metabolic parameters of beta-cell function in the Black West African and White European men

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

Data presented as mean ± SD or geometric mean (95% CI) for log transformed data (†) or median (interquartile range) for non-parametric data (‡). *P* values determined using independent samples t-tests for normally distributed data otherwise Mann-Whitney test. N for [a]WE=16, BWA=18.

Abbreviations: BWA, Black West African; iAUC, incremental area under the curve; WE, White European; σ^1, first phase insulin secretory function; σ^2, second phase insulin secretory function.
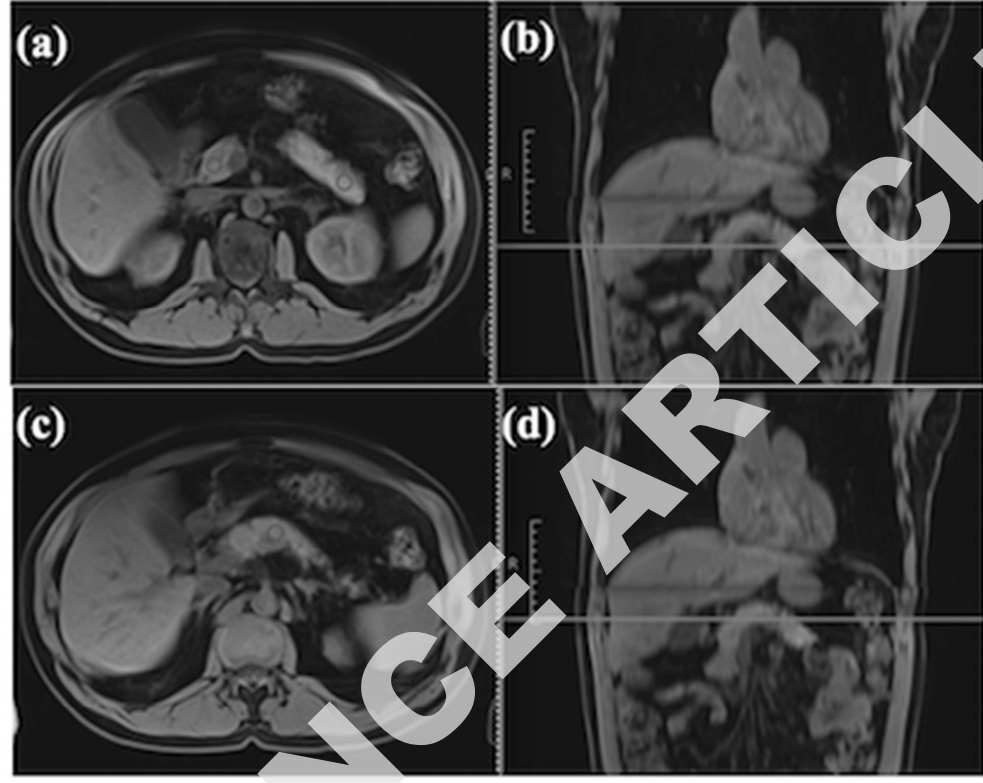
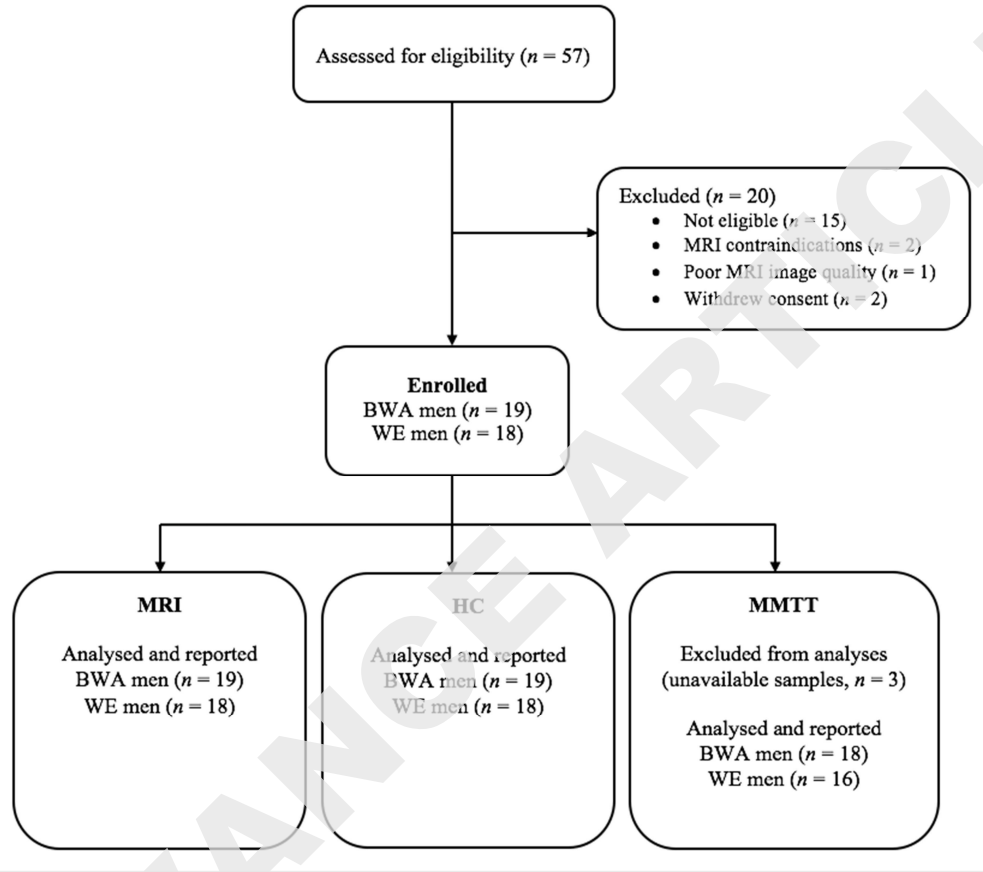Table (3): Pearson's correlation coefficients between IPL and metabolic measures of insulin secretory function

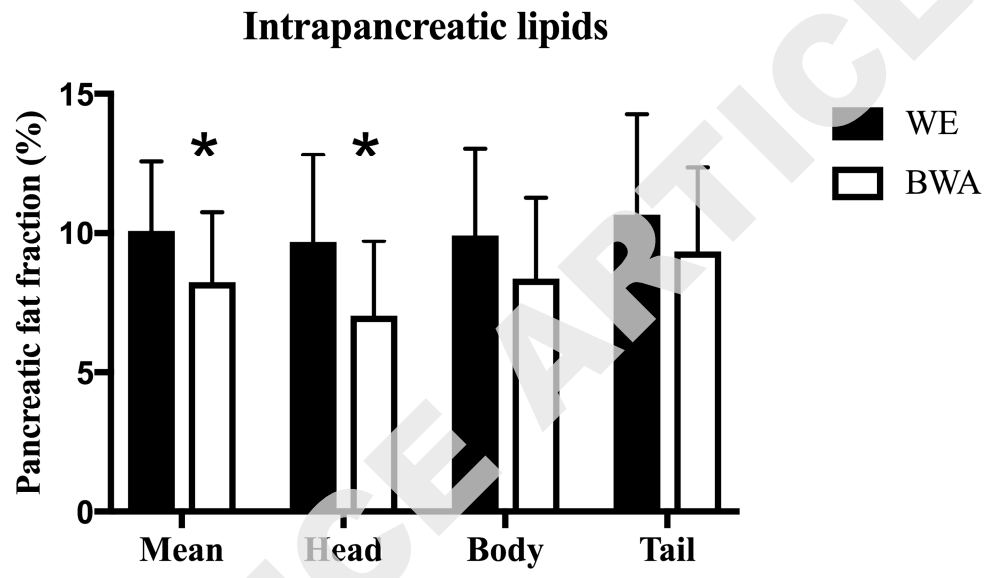|	Meal test 1st phase insulin secretory function $(\sigma^1)^a$		Meal test 2nd phase insulin secretory function $(\sigma^2)^a$		Hyperglycemic clamp 1st phase insulin secretory function $(\sigma^1)^b$		Hyperglycemic clamp 2nd phase insulin secretory function $(\sigma^2)^b$	
	BWA	WE	BWA	WE	BWA	WE	BWA	WE
IPL_{MEAN}	-0.18	**-0.55***	-0.16	0.05	-0.29	-0.24	-0.14	-0.36
IPL_{HEAD}	-0.04	**-0.56***	-0.09	0.13	-0.30	-0.01	-0.32	-0.27
IPL_{BODY}	-0.21	-0.24	-0.10	0.24	-0.22	-0.16	-0.08	-0.10
IPL_{TAIL}	-0.33	-0.36	-0.32	-0.16	-0.32	-0.41	-0.27	-0.43

*$P<0.05$

Abbreviations: BWA, Black West African; IPL, intrapancreatic lipid; WE, White European.
N for ^{a}WE=16, BWA=18; n for ^{b}WE=19, BWA=18

Intrapancreatic lipids

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