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ORIGINAL ARTICLE

I

Ethnic differences in the relationship between ectopic fat deposition and insulin sensitivity in Black African and White European men across a spectrum of glucose tolerance

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Abstract

Aim: To examine the hypothesis that there would be ethnic differences in the relationship between ectopic fat and tissue-specific insulin resistance (IR) across a spectrum of glucose tolerance in Black African (BA) and White European (WE) men.

Materials and Methods: Fifty-three WE men (23/10/20 normal glucose tolerance [NGT]/impaired glucose tolerance [IGT]/type 2 diabetes [T2D]) and 48 BA men (20/10/18, respectively) underwent a two-step hyperinsulinaemic-euglycaemic clamp with infusion of D-[6,6-²H₂]-glucose and [²H₅]-glycerol to assess hepatic, peripheral and adipose tissue IR. Magnetic resonance imaging was used to measure subcutaneous adipose tissue, visceral adipose tissue (VAT) and intrahepatic lipid (IHL). Associations between ectopic fat and IR were assessed using linear regression models.

Results: There were no differences in tissue-specific IR between ethnic groups at any stage of glucose tolerance. VAT level was consistently lower in the BA population; NGT (p = 0.013), IGT (p = 0.006) and T2D (p = 0.015). IHL was also lower in the BA compared with the WE men (p = 0.013). VAT and IHL levels were significantly associated with hepatic IR in the BA population (p = 0.001) and with peripheral IR in the WE population (p = 0.027).

Conclusions: The present study suggests that BA and WE men exhibit the same degree of IR across a glucose tolerance continuum, but with lower VAT and IHL levels in the BA population, suggesting that IR may be driven by a mechanism other than increased ectopic fat accumulation in BA men.

KEYWORDS

ectopic fat, ethnic differences, insulin sensitivity, intrahepatic lipids, type 2 diabetes

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1 | INTRODUCTION

Excessive accumulation of visceral adipose tissue (VAT) and intrahepatic lipid (IHL) have been proposed as central defects underlying the development of type 2 diabetes (T2D).¹⁻⁴ Exhibiting high rates of lipolysis and a proinflammatory profile, VAT adipocytes are proposed to drive IHL accumulation through high fatty acid flux to the liver via the portal circulation.^{5,6} The accumulation of IHL may be a better marker of the deleterious effects of obesity than VAT and, through its association with hepatic insulin resistance (IR), the importance of excess IHL in the pathogenesis of T2D is increasingly recognized.^{5,7,8}

People of Black African (BA) ancestry are disproportionately affected by T2D, but present with a distinct phenotype compared with their White European (WE) counterparts, with markedly lower levels of IHL and VAT.⁹⁻¹² as well as greater fat-free mass¹³ and a lower waist circumference,¹⁴ at the same body mass index (BMI). We have previously demonstrated this in people with normal glucose tolerance (NGT)⁹ and people with T2D.¹⁵ Interestingly, the phenotypic differences in a BA population occur concurrently with apparently greater degrees of IR compared with WE populations.^{16,17} There have been several suggestions for this apparent paradox, such as there being other more significant contributors to the pathophysiology of T2D, including reduced insulin clearance,^{18,19} or that BA populations may have a lower fat threshold for the development of IR. Importantly, in BA populations, there have also been limited studies that directly assess (tissue-specific) IR using 'gold standard' techniques such as hyperinsulinaemic-euglycaemic clamps (HECs) and stable isotope tracers, potentially leading to overestimation of IR with the use of indirect indices of insulin sensitivity. Thus, it remains to be determined whether there are ethnic differences in whole-body and tissue-specific IR using appropriate methodology, and whether the accumulation of VAT and IHL drive the development of T2D in people of BA ancestry, as has been suggested in WE populations.

We have previously explored ethnic differences in the relationship between tissue-specific IR and ectopic fat deposition in NGT²⁰ and T2D²¹ populations of BA and WE ethnicity. In the present paper we comprehensively explore whether there are ethnic differences in the relationship between ectopic fat deposition, specifically VAT and IHL accumulation, and IR across a spectrum of NGT, impaired glucose tolerance (IGT) and T2D, using gold standard techniques for the assessment of tissue-specific IR.

2 | MATERIALS AND METHODS

The study was conducted at the Clinical Research Facility, King's College London, London, UK and approved by the London Bridge National Research Ethics Committee (12/ LO/1859 and 15/LO/1121). The data were collected as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) Phase I and Phase II studies; recruitment and data collection took place during the period April 2013 to May 2018.²²

2.1 | Participants

All participants provided written informed consent prior to commencing any study procedures. Participants were recruited from local general practices, newspaper advertisements, religious groups and leaflets. Men aged 18-65 years, of self-reported Black (West) African (BA) and White European (WE) ethnicity, with a BMI between 20 and 35 kg·m⁻² (inclusive) were eligible to take part. A 2-h oral glucose tolerance test was used to confirm glycaemic status in those expected (on clinical grounds) to have NGT or IGT.²³ Those with T2D were required to have had a diagnosis within the last 5 years, to be treated by dietary intake and/ or metformin with glycated haemoglobin (HbA1c) levels $\leq 64 \text{ mmol} \cdot \text{mol}^{-1}$. Participants were ineligible if treated with any medications known to affect study outcomes including thiazolidinediones, insulin, chronic oral steroids or β-blockers, or if serum creatinine was >150 μ mol·L⁻¹ or serum alanine transaminase level was >2.5-fold above the upper limit of the reference range. The aim of recruitment was to match BA and WE men within each glycaemic group for BMI and age.

2.2 | Procedures

In the 24 h before the study visits participants consumed a standardized diet including \sim 50% energy from carbohydrate, with no more than 30% of daily carbohydrate consumed in the evening meal. The evening meal was consumed 10 h before arrival at the Clinical Research Facility. Participants were asked to refrain from alcohol intake for 24 h and physical activity for 48 h before the study visits.

2.3 | Hyperinsulinaemic-euglycaemic clamp

The procedure used for the HEC was as described previously.^{20,21} In brief, a two-step (10- and 40-mU·m⁻² body surface area [BSA]·min⁻¹ insulin infusion) HEC was completed with a [6,6-²H₂]-glucose and ^{[2}H₅]-glycerol tracer infusion. A cannula was inserted into the antecubital fossa vein for infusion of 20% glucose (Baxter, Norfolk, UK), insulin (Actrapid; Novo Nordisk) and the glucose and glycerol tracers (CK Gases, Cambridgeshire, UK). A second cannula was inserted retrogradely into the dorsal hand vein of the opposite arm and placed inside a heated hand box to obtain arterialized venous blood. Fasting plasma glucose was determined and, if above 5 mmol·L⁻¹, an insulin sliding scale was used to lower plasma glucose to 5 mmol·L⁻¹ before commencing the protocol, as previously described.²¹ Thereafter, a primed (2 mg·kg⁻¹) continuous (0.02 mg·kg⁻¹·min⁻¹) infusion of [6,6-²H₂]-glucose and primed (0.12 mg·kg⁻¹) continuous (0.0067 mg·kg⁻¹·min⁻¹) infusion of [²H₅]-glycerol was commenced at -120 min. At 0 min a primed continuous infusion of insulin was commenced at 10 mU·m⁻² BSA·min⁻¹ for 120 min for the assessment of hepatic and adipose tissue insulin sensitivity. After 120 min, insulin infusion was increased to a constant rate of 40 mU·m⁻² BSA·min⁻¹,

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after initial priming, for a further 120 min for the assessment of whole-body and peripheral insulin sensitivity.²⁴ Euglycaemia was maintained at 5 mmol·L⁻¹ by a variable rate infusion of 20% glucose spiked with 8 mg·kg⁻¹ and 10 mg·kg⁻¹ of [6,6-²H₂]-glucose during the first and second step of the HEC, respectively. Plasma glucose concentration was assessed every 5 min (2300 STAT Glucose Analyser; Yellow Springs Instruments, Yellow Springs, OH, USA) and additional samples taken for later measurement of isotopic enrichment, plasma insulin and non-esterified fatty acids at regular intervals. The calculations for the assessment of whole-body and tissue-specific insulin sensitivity are provided in the Supplementary Information.

2.4 | Magnetic resonance imaging

On a separate day, participants attended the magnetic resonance imaging (MRI) unit of Guy's Hospital, King's College London, for the assessment of subcutaneous adipose tissue (SAT), VAT and IHL. Scanning was performed while participants lay semi-supine on a 1.5-T Siemens scanner to acquire magnetic resonance images from the neck to the knee. Details in Supplementary Information.

2.5 | Laboratory analysis

To calculate endogenous glucose production (EGP) and the rate of disappearance of glucose (Rd), fractional enrichments of $[6,6^{-2}H_2]$ -glucose were determined in the collected plasma samples by gas chromatography-mass spectrometry (Agilent GCMS 5975C MSD; Agilent Technologies), as previously described.^{20,21} The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl (tBDMS) glycerol derivative. $[^{2}H_{5}]$ -glycerol plasma enrichments were determined using gas chromatography-mass spectrometry (Agilent GCMS 5975C MSD). Plasma insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System; Siemens Healthcare). Plasma non-esterified fatty acid concentrations were measured using automated enzymatic colorimetric assays (iLAB 650; Instrumental Laboratories, Holliston, MA, USA).

2.6 | Statistical analysis

The present study is part of the larger SOUL-DEEP study that investigated ethnic differences in T2D pathophysiology in WE versus BA men.²² The primary outcome for which the sample size of the study was calculated was insulin secretory function. Thus, the present paper reports exploratory analyses of the secondary outcomes of SOUL-DEEP, namely, ectopic fat accumulation and tissue-specific insulin sensitivity. Our sample size was predetermined and there was no separate sample size calculation performed for these secondary outcomes of interest. Participant characteristics were summarized by ethnicity and glycaemic group as mean (standard deviation) for continuous variables and count (percentage) for categorical variables. Characteristics were compared between ethnic groups within glycaemic group using independent samples *t*-tests for continuous variables and chi-squared tests for categorical variables. For each outcome of interest (wholebody insulin sensitivity, EGP suppression, difference in Rd, Rd_{SS2}, and lipolysis suppression, VAT, SAT, IHL), the individual values were plotted by ethnicity and glycaemic group along with the group mean. Pairwise independent samples *t*-tests were used to test for differences between ethnic groups and between glycaemic groups.

To explore the association between VAT (explanatory variable) and lipolysis suppression, EGP suppression, and Rd_{SS2} (outcomes), a set of adjusted linear regression models were fitted by ethnicity. The confounders were selected using stepwise selection with the following potential confounders entered into the stepwise selection: HbA1c, age, systolic blood pressure, diastolic blood pressure, waist circumference, BMI, total cholesterol, low-density lipoprotein cholesterol, triglycerides, and fasting plasma glucose. The stepwise selection removed variables if they were not significant at the 5% level. The confounding variables were kept consistent across ethnic groups and similar outcomes to aid comparison of findings. Quadratic terms for VAT were entered into each adjusted linear regression model, but were not significant at the 5% level in any model and therefore only linear terms were included in the final model. The coefficient (95% confidence interval) and p value for each model is presented. These analyses were repeated with IHL as the explanatory variable of interest, rather than VAT. Missing data were not imputed; analyses were conducted on only complete cases. Statistical significance was assessed at the 5% level. All analyses were performed in Stata v18.0.

3 | RESULTS

3.1 | Participant characteristics

Participant characteristics are shown in Table 1. The BA population demonstrated lower WC (p = 0.013) and plasma triglycerides (p = 0.001) compared with the WE population, primarily due to a 6.3-cm mean difference in WC, and a 0.4 mmol·L⁻¹ mean difference in triglycerides, within the NGT group. Splitting by glucose tolerance group revealed an increase in age and BMI from the NGT participants to the IGT and T2D groups. HbA1c and fasting plasma glucose also increased linearly across the glucose tolerance groups. By design, there were no ethnic differences in BMI within glucose tolerance groups. Mean age was similar between the WE and BA population in the NGT and T2D groups, but the WE population was older than the BA population in the IGT group.

3.2 | Ectopic fat

Compared with WE men, BA men had consistently lower VAT levels across the spectrum of glucose tolerance (Figure 1A; p < 0.05 for all pairwise comparisons). In the combined population, VAT showed a linear increase from NGT to IGT (p = 0.016), but there was no further increase in VAT from the IGT to the T2D group (p = 0.233). SAT

TABLE 1 Participant characteristics^a.

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	Normal glucose tolerance			Impaired glucose tolerance			Type 2 diabetes		
Characteristic	BA	WE	p value ^b	BA	WE	p value ^b	BA	WE	p value ^b
n	23	23		11	13		19	15	
Age, years	30.7 (12.0)	35.9 (13.9)	0.183	45.7 (7.5)	54.5 (9.9)	0.025	54.1 (7.8)	55.5 (7.1)	0.602
Weight, kg	84.1 (14.6)	86.6 (16.6)	0.595	93.8 (9.8)	94.8 (17.0)	0.870	90.6 (9.2)	94.2 (11.6)	0.326
BMI, kg·m ^{-2}	26.7 (3.6)	26.5 (4.5)	0.873	30.0 (2.1)	29.8 (4.4)	0.917	29.5 (2.6)	30.1 (2.7)	0.510
Waist circumference, cm	87.5 (9.3)	93.8 (14.6)	0.088	100.8 (8.0)	105.7 (11.4)	0.249	103.7 (8.2)	107.5 (8.8)	0.194
Systolic BP, mmHg	123.2 (12.2)	121.9 (9.1)	0.687	134.1 (9.7)	130.1 (12.1)	0.387	137.3 (14.1)	131.8 (13.9)	0.262
Diastolic BP, mmHg	70.7 (11.5)	71.1 (8.2)	0.888	83.0 (7.2)	78.3 (6.6)	0.116	85.6 (7.4)	82.9 (10.1)	0.376
HbA1c, $mmol \cdot mol^{-1}$	37.0 (5.3)	35.9 (2.9)	0.372	43.2 (3.8)	39.0 (3.3)	0.008	49.9 (7.7)	48.6 (7.8)	0.631
HbA1c, %	5.5 (0.5)	5.4 (0.2)	0.373	6.1 (0.3)	5.7 (0.3)	0.007	6.7 (0.7)	6.6 (0.7)	0.650
Total cholesterol, $\text{mmol} \cdot \text{L}^{-1}$	4.3 (1.1)	4.8 (1.1)	0.126	4.4 (0.9)	5.0 (0.6)	0.081	4.1 (0.7)	4.3 (0.7)	0.470
LDL cholesterol, mmol· L^{-1}	2.7 (0.9)	3.0 (0.8)	0.191	2.7 (0.8)	3.1 (0.6)	0.271	2.3 (0.5)	2.3 (0.7)	0.794
HDL cholesterol, mmol· L ⁻¹	1.3 (0.4)	1.3 (0.3)	0.753	1.2 (0.4)	1.3 (0.4)	0.670	1.2 (0.4)	1.2 (0.2)	0.557
Triglycerides, mmol· L^{-1}	0.7 (0.3)	1.1 (0.6)	0.002	1.1 (0.4)	1.4 (0.5)	0.136	1.3 (0.7)	1.7 (0.7)	0.143
Fasting plasma glucose	5.1 (0.4)	5.2 (0.4)	0.570	5.6 (0.5)	5.8 (0.7)	0.573	6.7 (1.0)	6.8 (1.4)	0.732
ΗΟΜΑ2-β	93.9 (26.9)	97.5 (37.3)	0.710	95.3 (26.5)	106.8 (49.7)	0.500	70.2 (7.8)	84.9 (44.3)	0.281
HOMA2-S	108.2 (43.7)	113.8 (61.2)	0.721	73.5 (23.7)	80.2 (12.7)	0.668	64.9 (30.6)	56.2 (28.9)	0.413
HOMA2-IR	1.1 (0.5)	1.2 (0.7)	0.608	1.5 (0.5)	1.9 (1.1)	0.319	1.9 (0.8)	2.4 (1.7)	0.220
Diabetes duration, years	-	-	-	-	-	-	2.8 (1.2)	2.9 (1.0)	0.815
Metformin use, count (%)	-	-	-	-	-	-	8 (53.3)	14 (73.7)	0.218

Note: Data expressed as mean (standard deviation), unless otherwise stated.

Abbreviations: BA, Black African; BMI, body mass index; BP, blood pressure; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HOMA2-β, homeostatic model assessment of β-cell function index; HOMA2-IR, homeostatic model assessment of insulin resistance index; HOMA2-S, homeostatic model assessment of insulin sensitivity index; LDL, low-density lipoprotein; WE, White European.

^aData were complete for all variables, i.e., there were no missing data.

^bDifferences in means between ethnic groups were tested within each glucose tolerance group using the independent samples *t*-test for continuous variables and chi-squared test for categorical variables.

showed no significant ethnic differences (p > 0.05 for all pairwise comparisons within glycaemic groups), but was greater in the T2D than the NGT group (Figure 1B; p = 0.015). The percentage of IHL trended towards an ethnic difference within the NGT and IGT groups (p = 0.05), whereby the WE group had a greater percentage of IHL than the BA group (Figure 1C). There was no difference in IHL between BA and WE men in the T2D group (p = 0.190). IHL did not differ among glycaemic groups).

3.3 | Insulin sensitivity

There were no ethnic differences in whole-body, peripheral, or hepatic insulin sensitivity within each glucose tolerance group (Figure 2A–D). Whole-body, peripheral and hepatic insulin sensitivity decreased from the NGT group to the IGT and T2D groups in the combined population (Figure 2A–D).

3.4 | Lipolysis suppression

There were no ethnic differences in the suppression of lipolysis, with a mean suppression of 46.1% ± 17.4% and 45.0% ± 20.3% in the WE and BA population, respectively (p > 0.05). The degree of lipolysis suppression did not differ between the NGT and IGT groups (p = 0.496), but this was significantly lower in the T2D group compared with both the NGT (p = 0.014) and the IGT group (p = 0.038; Supplementary Information Figure S1).

3.5 | Associations

Figures 3 and 4(A–F) display the relationships between ectopic fat deposition and tissue-specific insulin sensitivity in the WE and BA men. In the WE group, IHL level was negatively associated with whole-body insulin sensitivity (-0.012 [-0.020, -0.004]; p = 0.005)



FIGURE 1 Visceral (A) and subcutaneous (B) adipose tissue deposition, and intrahepatic lipids (C) across the spectrum of glucose tolerance in Black African (shaded symbols) and White European (open symbols) men. Circles represent the normal glucose tolerance (NGT) group, squares represent the impaired glucose tolerance (IGT) group and triangles represent the type 2 diabetes (T2D) group. Horizontal line represents sample mean.

and peripheral insulin sensitivity; both percentage change in Rd from basal (-5.069 [-9.714, -0.425]; p = 0.033) and Rd during Step 2 (-0.069 [-0.114, -0.024]; p = 0.004). Post hoc tests did not detect the glucose tolerance group. There was no association between hepatic insulin sensitivity and IHL in the WE group (p < 0.05). In the BA cohort, IHL level was negatively associated with only hepatic insulin sensitivity (-1.798 [-3.472, -0.123]; p = 0.036) and, again, post hoc tests did not detect the glucose tolerance group.

In the WE group, VAT was inversely associated with peripheral insulin sensitivity (measured as percentage change in Rd; -0.655 [-1.231, -0.080]; p = 0.027), whereas the BA group demonstrated an inverse association between VAT and hepatic insulin sensitivity (-0.166 [-0.291, -0.040]; p = 0.001 [Supplementary Information, Tables S2 and S3]). These differences were primarily due to a statistically significant relationship in the NGT group. VAT was

not associated with hepatic insulin sensitivity in the WE group, and it was not associated with peripheral insulin sensitivity in the BA group. SAT was not associated with any tissue-specific insulin sensitivity in either ethnicity. Nonlinear associations were tested for all relationships; for most, the association was linear, except for the two relationships shown in Table S3 in Supplementary Information.

4 | DISCUSSION

In this study, we present data across a spectrum of glucose tolerance to compare the relationship between IHL accumulation and tissuespecific insulin sensitivity in BA and WE men. We present novel data on participants classified as having IGT and combine these with



FIGURE 2 Tissue-specific insulin sensitivity across the spectrum of glucose tolerance in Black African (BA; shaded symbols) and White European (WE; open symbols) men. Circles represent the normal glucose tolerance (NGT) group, squares represent the impaired glucose tolerance (IGT) group and triangles represent the type 2 diabetes (T2D) group. Horizontal line represents sample mean. M/I, glucose disposal rate divided by steady-state plamsa insulin; EGP, endogenous glucose production; Rd, rate of disappearance of glucose.

data from cohorts with NGT and T2D to comprehensively assess ethnic differences across a spectrum of glucose tolerance.^{20,21} Our data show that, at every level of glucose tolerance, BA and WE men have equivalent insulin sensitivity, despite lower IHL and VAT levels in the BA population. Our analysis demonstrates a novel finding that central adiposity, including IHL and VAT, are more associated with peripheral IR in WE men, and hepatic IR in BA men.

The present study demonstrates significantly lower IHL and VAT in the BA compared with the WE men, in the presence of similar whole-body and tissue-specific insulin sensitivity. This is consistent with previous findings that have reported lower IHL and VAT levels in BA populations compared with those of WE ancestry,^{10,11,25} which has occurred at equivalent or greater degrees of IR.^{20,21} This finding has led to numerous lines of speculation, including the possibility that the role of IHL and VAT in IR development has been over-estimated, or is of lesser importance, in the pathogenesis of T2D in BA populations. The equivalent hepatic IR between ethnicities, in the presence of lower IHL in the BA population, could suggest that there may be another driver, independent of IHL, of hepatic IR in the BA men. However, the finding that lower levels of VAT and IHL in the BA men were positively associated with hepatic IR suggests that there may be ethnic differences in the fat 'threshold' for inducing IR, which aligns with the 'personal fat threshold' theory, whereby the degree of susceptibility to the adverse effects of excess adiposity varies.²⁶

It is commonly reported that increased VAT level is associated with decreased insulin sensitivity in both $WE^{27,28}$ and BA^{29} populations, likely due to the greater lipolytic properties of VAT and direct drainage of fatty acids to the liver.³⁰ In the present study we observed

FIGURE 3 Associations between intrahepatic fat deposition and tissue-specific insulin sensitivity in each ethnic group. Circles represent the normal glucose tolerance (NGT) group, squares represent the impaired glucose tolerance (IGT) group and triangles represent the type 2 diabetes (T2D) group. Shaded symbols represent the Black African population and open symbols represent the White European population. Fitted lines on the adjusted models. Dashed line represents 5.65% liver fat threshold. EGP, endogenous glucose production: Rd, rate of disappearance of glucose.



an association between VAT and IR, consistent with previous findings, however, distinct ethnic differences were present such that VAT was associated with peripheral IR in the WE men, and hepatic IR in the BA men. This is interesting as, assuming VAT is the driver of IR, it suggests that the VAT accumulation may be more detrimental to hepatic IR in BA men, but to peripheral IR in WE men. Splitting these associations by glucose tolerance group reveals that they are primarily driven by the NGT group, in both ethnicities, suggesting that increased VAT, prior to disease development, may be driving different sites of IR by ethnicity. This association is lost at higher levels of IR, that is, within the IGT and T2D groups, suggesting that VAT may drive IR to a certain level, beyond which additional comorbidities or drivers are likely to play a more important role. This is consistent with previous research that has demonstrated ethnic differences in T2D pathophysiology within a non-diabetic cohort,³¹ that disappear after T2D diagnoses.³² This could suggest VAT as an early indicator of IR (or T2D) risk, prior to the appearance of abnormal or elevated glucose

concentrations, which highlights the importance of looking across the glucose tolerance spectrum in the present analysis.

As with VAT, IHL accumulation was associated with peripheral and hepatic IR in the WE and BA men, respectively. IHL was not associated with hepatic IR in the WE population, contrary to our current understanding of the role of hepatic lipids in the development of hepatic IR.³³ A previous cross-sectional study measured peripheral but not hepatic IR with a hyperinsulinaemic-clamp and demonstrated that IHL level was the greatest predictor of IR in a White population, but this relationship did not exist in a BA population,³⁴ aligning with our findings. The significantly lower IHL levels in the BA population are consistent with previous studies that have demonstrated a lower prevalence of hepatic steatosis in BA than WE populations.³⁵ Previous research from Goedecke et al.³⁶ demonstrated an association between IHL and hepatic IR in Black but not White women, consistent with the results of the present study. The importance of IHL in T2D development has also been demonstrated in the Counterpoint study,



FIGURE 4 Associations between visceral adipose tissue deposition and tissue-specific insulin sensitivity in each ethnic group. Circles represent the normal glucose tolerance (NGT) group, squares represent the impaired glucose tolerance (IGT) group and triangles represent the type 2 diabetes (T2D) group. Shaded symbols represent the Black African population and open symbols represent the White European population. Fitted lines on the adjusted models. EGP, endogenous glucose production; Rd, rate of disappearance of glucose.

whereby T2D remission was achieved, in a primarily White population, in those who had a reduction in liver fat.⁷ However, the mechanistic data from the Counterpoint study suggest that the driver of T2D remission (defined by HbA1c below threshold and cessation of diabetic medication) was a return of β-cell function after the reduction in liver fat. However, no direct assessments of insulin sensitivity were performed to determine whether the reduction in IHL corresponded with increased insulin sensitivity and indeed the relative importance of enhanced insulin sensitivity in driving T2D remission. In the present study, we measured the suppression of lipolysis with the infusion of glycerol during a hyperinsulinaemic clamp, which we used as a measure of adipose tissue insulin sensitivity. Interestingly, adipose tissue IR was associated with VAT in WE, but not BA men. This is likely due to the greater lipolytic properties of VAT,⁵ and thus the WE population, with greater absolute VAT levels, exhibits a (negatively) associative relationship with the degree of lipolysis suppression. However, there is no difference, at any level of glucose tolerance, in adipose tissue IR between the BA and WE population.

Thus, it suggests that there is a mechanism independent of VAT volume that is driving adipose tissue IR in the BA population, further highlighting that there may be numerous pathways contributing to (all sites of) IR in the BA population. Indeed, previous evidence has suggested that IR can develop independently of ectopic fat deposition, with data demonstrating improvements in hepatic IR independent of any change in IHL levels,³⁷ and also those with the G-allelle of patatin-like phospholipase 3 gene that develop high levels of IHL while maintaining normal hepatic insulin sensitivity.³⁸ Further research should explore muscle lipid uptake, adipose tissue expandability, lipogenesis and insulin signalling to help us further understand the pathway driving adipose tissue IR in a BA population. There is currently a noticeable dearth of evidence on how the metabolic function and cellular characteristics of adipose tissue differ by ethnicity.

It is interesting, however, to note the degree of heterogeneity in the measure of adipose tissue IR across the spectrum of glucose tolerance, with no difference detected between the NGT and IGT groups, suggesting that early adipose tissue IR may not drive any changes in disease progression from NGT to IGT. Conversely, we observe a linear decrease in both whole-body and hepatic insulin sensitivity from NGT to IGT to T2D, in both ethnic groups, consistent with our current understanding of the role IR plays in T2D development.³⁹ Peripheral insulin sensitivity decreases from NGT to IGT but there is no further decrease to T2D, which could suggest that peripheral IR reaches its lower limit earlier in disease progression, and it is other metabolic defects, perhaps adipose tissue IR, that further drive disease progression. However, this would require verification in longitudinal studies that measure tissue-specific IR development in disease progression. Indeed, tracking the development of IR in combination with other disease markers such as obesity, inflammation and β-cell function, would allow us to determine a 'primary defect' driving the development of T2D, and whether this is ethnicity dependent. However, it has been previously suggested that a 'merging' cluster of disease comorbidities, in varying degrees of severity, exist concurrently,⁴⁰ preventing us from clearly defining a primary driver of disease development.

Our study has several areas of novelty and strength. Our dataset is the first to explore, using gold standard assessments, ethnic differences at three different glucose tolerance stages, in tissue-specific insulin sensitivity and ectopic fat deposition between BA and WE men. Our use of a direct assessment of insulin sensitivity, including the quantification of hepatic insulin sensitivity as the percentage suppression of EGP, is a methodological choice superior to other indirect measures.⁴¹ The indirect indices that are currently used for estimating insulin sensitivity may not be appropriate for use in a BA population, due to potentially lower insulin clearance and higher serum insulin concentrations in this population, possibly leading to an overestimation of IR.⁴²

The present study included men only. Previous observational studies have demonstrated sex-specific differences in fat storage, with women demonstrating a greater amount of subcutaneous than visceral fat, compared with men.43 Previous studies have also shown a greater degree of hyperinsulinaemia and insulin resistance in women than men,^{44,45} thus a comparative study in women would be necessary to determine whether the ethnic differences observed in the present study in men would be observed in a female population. It is also worth noting that, while the accumulation of ectopic fat appears to be a major driver of IR and thus T2D, it is not the sole driver, and other factors such as chronic inflammation and family history of T2D also need to be taken into consideration when determining the overall ethnic differences in T2D pathophysiology. A further limitation of the present study is the small sample size used and, while we have demonstrated a linear association between hepatic IR and IHL in the BA population, it is possible this may have been driven by a small number of data points and this is certainly worthy of further investigation to confirm the robustness of this finding.

In conclusion, despite significantly lower VAT and IHL deposition, BA men exhibit the same degree of whole-body and tissue-specific insulin sensitivity to that exhibited by WE men across a glucose tolerance continuum, suggesting a lower personal fat threshold in men of

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BA ethnicity. Ethnic differences may exist in the relationship between both VAT and IHL with IR, and further research should investigate the mechanisms behind the development of IR at lower VAT and IHL in a BA population.

AUTHOR CONTRIBUTIONS

Louise M. Goff, Stephanie A. Amiel and A Margot Umpleby formulated the research question and study design. Gráinne Whelehan wrote the manuscript. Louise M. Goff, Gráinne Whelehan, Danielle H. Bodicoat, Stephanie A. Amiel, A Margot Umpleby, Oluwatoyosi Bello, Meera Ladwa and Olah Hakim contributed towards the acquisition, analysis, or interpretation of data for the manuscript. All authors contributed to drafting or critically revising the manuscript before giving final approval of the version to be published. All authors agree to be accountable for all aspects of their work and will ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Louise M. Goff is the guarantor of this work, had full access to all the data and takes full responsibility for the integrity of the data and the accuracy of data analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med. 2014;371(12):1131-1141.
- 2. Barzilai N, She L, Liu BQ, et al. Surgical removal of visceral fat reverses hepatic insulin resistance. *Diabetes*. 1999;48(1):94-98.
- Gastaldelli A, Miyazaki Y, Pettiti M, et al. Metabolic effects of visceral fat accumulation in type 2 diabetes. J Clin Endocrinol Metabol. 2002; 87(11):5098-5103.
- Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. *Am J Physiol: Endocrinol Metab.* 2002;283(6):E1135-E1143.
- Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology*. 2007;133(2): 496-506.
- Kabir M, Catalano KJ, Ananthnarayan S, et al. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. *Am J Physiol: Endocrinol Metab.* 2005;288(2):E454-E461.
- 7. Taylor R, al-Mrabeh A, Zhyzhneuskaya S, et al. Remission of human type 2 diabetes requires decrease in liver and pancreas fat content but is dependent upon capacity for β cell recovery. *Cell Metab.* 2018; 28(4):547-556.
- Lettner A, Roden M. Ectopic fat and insulin resistance. Curr Diab Rep. 2008;8(3):185-191.
- Hakim O, Bello O, Ladwa M, et al. Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity. *Diabetes Res Clin Pract*. 2019;156:107866.
- Goedecke JH, Levitt NS, Lambert EV, et al. Differential effects of abdominal adipose tissue distribution on insulin sensitivity in black and white South African women. *Obesity*. 2009;17(8):1506-1512.
- Staiano A et al. Ethnic and sex differences in visceral, subcutaneous, and total body fat in children and adolescents. *Obesity*. 2013;21(6): 1251-1255.
- Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? *Hepatology*. 2009; 49(3):791-801.
- Heymsfield SB, Peterson CM, Thomas DM, Heo M, Schuna JM Jr. Why are there race/ethnic differences in adult body mass indexadiposity relationships? A quantitative critical review. *Obesity Rev.* 2016;17(3):262-275.
- Tillin T, Sattar N, Godsland IF, Hughes AD, Chaturvedi N, Forouhi NG. Ethnicity-specific obesity cut-points in the development of type 2 diabetes-a prospective study including three ethnic groups in the United Kingdom. *Diabet Med.* 2015;32(2):226-234.
- Hakim O, Bello O, Bonadonna RC, et al. Ethnic differences in intrahepatic lipid and its association with hepatic insulin sensitivity and insulin clearance between men of black and white ethnicity with early type 2 diabetes. *Diabetes Obes Metab.* 2019;21(9):2163-2168.
- Tillin T, Hughes AD, Godsland IF, et al. Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall and Brent REvisited (SABRE) cohort. *Diabetes Care*. 2013;36(2):383-393.
- Paul SK, Owusu Adjah ES, Samanta M, et al. Comparison of body mass index at diagnosis of diabetes in a multi-ethnic population: a case-control study with matched non-diabetic controls. *Diabetes Obes Metab.* 2017;19(7):1014-1023.
- Goff LM, Ladwa M, Hakim O, Bello O. Ethnic distinctions in the pathophysiology of type 2 diabetes: a focus on black African-Caribbean populations. *Proc Nutr Soc.* 2020;79(2):184-193.

- Ladwa M, Bello O, Hakim O, et al. Insulin clearance as the major player in the hyperinsulinaemia of black African men without diabetes. *Diabetes Obes Metab.* 2020;22(10):1808-1817.
- Bello O et al. Differences in the link between insulin sensitivity and ectopic fat in men of black African and white European ethnicity. *Eur J Endocrinol.* 2020;182(1):91-101.
- 21. Bello O, Mohandas C, Shojee-Moradie F, et al. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. *Diabetologia*. 2019;62:835-844.
- Goff L. Soul-Deep: the South London Diabetes and Ethnicity Phenotyping Study Protocol. King's College London; 2013. doi:10.25392/ leicester.data.26213261.v1
- American Diabetes A. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2019. *Diabetes Care*. 2019;42-(Suppl 1):S13-S28.
- 24. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol: Endocrinol Metab.* 1979;237(3):E214-E223.
- Bentley, A.R. and C.N. Rotimi, Interethnic Differences in Serum Lipids and Implications for Cardiometabolic Disease Risk in African Ancestry Populations. *Global Heart*. 2017;12(2):141-150.
- 26. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci.* 2015;128(7):405-410.
- Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol.* 2020;8(7):616-627.
- Després J-P et al. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol.* 2008;28(6):1039-1049.
- Banerji M et al. Relationship of visceral adipose tissue and glucose disposal is independent of sex in black NIDDM subjects. Am J Physiol: Endocrinol Metab. 1997;273(2):E425-E432.
- Mocciaro G, Gastaldelli A. Obesity-related insulin resistance: the central role of adipose tissue dysfunction. *From Obesity to Diabetes*. Springer; 2022:145-164.
- Haffner SM, Ralph D'A Jr, Saad MF, et al. Increased insulin resistance and insulin secretion in nondiabetic African-Americans and Hispanics compared with non-Hispanic whites: the insulin resistance atherosclerosis study. *Diabetes*. 1996;45(6):742-748.
- Haffner SM, Howard G, Mayer E, et al. Insulin sensitivity and acute insulin response in African-Americans, non-Hispanic whites, and Hispanics with NIDDM: the insulin resistance atherosclerosis study. *Diabetes*. 1997;46(1):63-69.
- Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*. 2014; 510(7503):84-91.
- Tay J, Goss AM, Garvey WT, et al. Race affects the association of obesity measures with insulin sensitivity. Am J Clin Nutr. 2020;111(3): 515-525.
- Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004;40(6):1387-1395.
- Goedecke JH, Keswell D, Weinreich C, et al. Ethnic differences in hepatic and systemic insulin sensitivity and their associated determinants in obese black and white south African women. *Diabetologia*. 2015;58:2647-2652.
- Shojaee-Moradie F, Baynes KCR, Pentecost C, et al. Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*. 2007;50:404-413.
- Kantartzis K, Peter A, Machicao F, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes*. 2009;58(11):2616-2623.
- DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1:15019.

- 40. McCarthy MI. Painting a new picture of personalised medicine for diabetes. *Diabetologia*. 2017;60(5):793-799.
- Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol: Endocrinol Metab.* 2008; 294(1):E15-E26.
- 42. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. *Diabetes Care.* 2013;36(4):845-853.
- Bidulescu A, Liu J, Hickson DMA, et al. Gender differences in the association of visceral and subcutaneous adiposity with adiponectin in African Americans: the Jackson heart study. *BMC Cardiovasc Disord*. 2013;13(1):1-10.
- 44. Falkner B, Hulman S, Kushner H. Gender differences in insulinstimulated glucose utilization among African-Americans. *Am J Hypertens*. 1994;7(11):948-952.

45. Goedecke JH, George C, Veras K, et al. Sex differences in insulin sensitivity and insulin response with increasing age in black south African men and women. *Diabetes Res Clin Pract*. 2016;122:207-214.

SUPPORTING INFORMATION

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

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