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Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity

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Abstract

Objectives: In men of black west African (BAM) and white European (WEM) ethnicity, we aimed to (1) compare adipose tissue, peripheral and hepatic insulin sensitivity and (2) investigate associations between ectopic fat and insulin sensitivity by ethnicity.
Design and methods: In overweight BAM (n = 21) and WEM (n = 23) with normal glucose tolerance, we performed a two-step hyperinsulinaemic–euglycaemic clamp with infusion of [6,6 $^2H_2$]-glucose and [$^2H_5$]-glycerol to measure whole body, peripheral, hepatic and adipose tissue insulin sensitivity (lipolysis). Visceral adipose tissue (VAT), intrahepatic lipids (IHL) and intramyocellular (IMCL) lipids were measured using MRI and spectroscopy. Associations between insulin sensitivity and ectopic fat were assessed using Pearson's correlation coefficient by ethnicity and regression analysis.
Results: There were no ethnic differences in whole body or tissue-specific insulin sensitivity (all $P > 0.05$). Suppression of lipolysis was inversely associated with VAT and IHL in WEM but not BAM (VAT: WEM $r = -0.68$, $P < 0.01$; BAM $r = 0.07$, $P = 0.79$. IHL: WEM $r = -0.52$, $P = 0.01$; BAM $r = -0.12$, $P = 0.63$). IMCL was inversely associated with skeletal muscle insulin sensitivity in WEM but not BAM (WEM $r = -0.56$, $P < 0.01$; BAM $r = -0.09$, $P = 0.75$) and IHL was inversely associated with hepatic insulin sensitivity in WEM but not BAM (WEM $r = -0.53$, $P = 0.02$; BAM $r = -0.13$, $P = 0.62$).
Conclusions: Ectopic fat deposition may play a lesser role in reducing insulin sensitivity in men of black African ethnicity and may not be driven by lipolysis. Resistance to storing VAT, IHL and IMCL may enable men of black African ethnicity to maintain comparable insulin sensitivity to white Europeans.

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Introduction

The risk of developing type 2 diabetes (T2D) is disproportionately high in populations of black compared to white ethnicity (1, 2). In black populations, T2D is more likely to occur within the normal BMI range (19.5–24.9 kg/m^2) (3) and at a lower waist circumference (4) compared to white groups.

Insulin resistance for carbohydrate metabolism is a well-established early defect in the pathogenesis of T2D (5). Resistance to the antilipolytic effect of insulin in adipose tissue has also been identified as an early defect, occurring prior to the onset of hyperglycaemia (6). Adipose tissue insulin resistance results in increased fatty

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acid release, with deposition in non-adipose tissue sites as ectopic fat (7, 8). This is known to trigger and exacerbate insulin resistance (9). Several theories have been proposed to explain the development of ectopic fat and insulin resistance. The 'spillover theory' proposes that multiple dysfunctions of s.c. adipose tissue (SAT), including insulin resistance (10), allow fatty acids to be deposited as visceral adipose tissue (VAT) (7, 11). Dysfunctional SAT combined with highly lipolytic VAT leads to the release of fatty acids into the portal and peripheral circulations. The 'portal theory' proposes that delivery of fatty acids from VAT to the liver, via the portal circulation, results in the accumulation of intrahepatic lipid (IHL), which subsequently leads to the development of hepatic insulin resistance (12, 13, 14). Fatty acids entering the peripheral circulation are understood to lead to fat deposition within skeletal muscle cells (termed intramyocellular lipids, IMCL) (13, 15). Whilst there is compelling evidence linking IMCL with peripheral insulin resistance (16, 17), there is debate in this field due to observations that athletes, who are highly insulin sensitive, present with relatively high IMCL levels (18).

Compared to populations of white ethnicity, black populations are reported to exhibit lower ectopic fat (namely VAT and IHL) (19, 20, 21, 22, 23), yet large cohort studies indicate that they display pronounced insulin resistance (24), creating a paradox. Studies using more sensitive measures of insulin resistance at a tissue-specific level, alongside measurement of ectopic fat depots related to T2D are scarce in non-diabetic black populations and have been restricted to obese women (25). These have reported no ethnic differences in peripheral insulin sensitivity, but lower hepatic insulin sensitivity in white compared to black women. Furthermore, they report that peripheral insulin sensitivity does not associate with either VAT or IMCL in black women but hepatic sensitivity does associate with VAT and IHL, suggesting that VAT and IHL play a key role in hepatic insulin resistance in black women (25). Similarly, *in vivo* studies assessing adipose tissue insulin sensitivity are mainly confined to obese women. They have provided inconsistent results, showing either no difference (26, 27, 28) or reduced lipolysis (29, 30, 31) in black compared to white populations.

While studies in women provide persuasive evidence of ethnic distinctions in the pathophysiology of T2D, gender differences in physiology (greater hyperinsulinaemia and insulin resistance in women (32, 33)) and body composition (less VAT and more SAT in women (34)) suggest investigations in black men are required. We aimed to assess and compare whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity in normally glucose-tolerant black west African (BAM) and white European men (WEM) and to evaluate relationships between tissue-specific insulin sensitivity with VAT, IHL and IMCL, to explore ethnic distinctions in the pathophysiology of type 2 diabetes.

Subjects and methods

Participants

The participants included in this analysis were recruited as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) study, phase II (35). The aim of the Soul-Deep II study was to investigate ethnic differences in insulin sensitivity, beta-cell function and ectopic fat deposition in men of Black (west) African (BAM) and white European (WEM) ethnicity. The study was approved by the London Bridge National Research Ethics Committee (15/LO/1121). Data collection took place between April 2016 and May 2018. Participants were recruited from local GP practices, newspaper advertisements, King's College London university staff and student email, religious groups, leafleting and posters where permitted. All participants provided informed consent prior to any study procedures.

Non-diabetic Black (west) African (BAM) and white European men (WEM) aged 18–65 years, with a BMI between 20 and 40 kg/m^2 were eligible to take part; the aim of recruitment was to achieve comparable BMI and age between the ethnic groups, without targeting a specific weight status. Ethnicity was defined by self-reported parental and grandparental birthplace; normal glucose tolerance was confirmed by a 2-h plasma glucose <7.8 mmol/L following a 75 g oral glucose tolerance test at screening. Participants were excluded if they were being treated with any medications known to affect the study outcomes, suffering from kidney or liver damage (serum creatinine >150 μmol/L or serum alanine transaminase level >2.5-fold above the upper limit of the reference range), or were unwilling and/or unable to comply with the study protocol.

Prior to each visit, participants were required not to eat after their usual carbohydrate-containing evening meal (no less than 10 h prior to study), refrain from strenuous physical activity for 48 h and alcohol for 24 h and avoid smoking on the morning of their visit.

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Hyperinsulinaemic–euglycaemic clamp

A two-step hyperinsulinaemic–euglycaemic clamp with a stable glucose and glycerol isotope infusion was used to assess whole-body and tissue-specific insulin sensitivity. Upon arrival at the clinical research facility in King's College Hospital, participants were advised to empty their bladder and were weighed on a body composition analyser (Tanita MC780MA) to determine fat free mass and body weight for infusion calculations. A cannula for blood sampling was placed in the dorsum of one hand in a retrograde fashion, the hand was kept in a warming unit at 55° to mimic arterialised sampling. Duplicate baseline samples were taken to assess background glucose and glycerol isotopic enrichments. An infusion cannula was then inserted into an antecubital fossa vein on the adjacent arm for infusions of 20% (wt/vol) glucose, insulin (Actrapid, Novo Nordisk) bound to albumin in a 4% autologous blood/saline solution, [6,6-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol tracers (CK Gases, Cambridgeshire, UK). To begin the basal phase, a primed (2.0 mg/kg), continuous infusion (0.02 mg/kg^{-1} min^{-1}) of [6,6-$^{2}H_{2}$]-glucose and a primed (0.12 mg/kg), continuous infusion (0.0067 mg/kg^{-1} min^{-1}) of [$^{2}H_{5}$]-glycerol were initiated at −120 min (36). Blood samples were taken at −30, −20, −10 and 0 min for basal assessments. The clamp began at 0 min with a primed continuous insulin infusion at a rate of 10 mU m^{-2} BSA min^{-1} (low dose insulin phase) for 2 h for assessment of adipose tissue and hepatic insulin sensitivity. For the final 2 h, the [$^{2}H_{5}$]-glycerol isotope infusion was terminated, the insulin infusion rate was re-primed and increased to 40 mU m^{-2} BSA min^{-1} (the high dose insulin phase) for assessment of whole body and peripheral (skeletal muscle) insulin sensitivity (37, 38). Euglycaemia (5 mmol/L) was achieved using 20% glucose enriched with [6,6-$^{2}H_{2}$]-glucose (8 mg/g glucose with low-dose insulin and 10 mg/g with high-dose insulin) to maintain a constant tracer-to-tracee ratio. The glucose was given at variable rates, based on plasma glucose samples drawn every 5 min and measured on a bedside glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). Blood was drawn at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 min for the assessment of plasma glucose concentration and isotopic enrichment and insulin concentration (38).

Magnetic resonance imaging and spectroscopy for ectopic fat quantification

MRI was used to assess visceral adipose tissue (VAT) and intrahepatic lipids (IHL). Proton-magnetic resonance spectroscopy (^{1}H-MRS) was used to assess intramyocellular lipids (IMCLs). Full details of this methodology can be found in (39). In brief, participants arrived at the MRI unit of Guy's Hospital, London following an overnight fast. Participants were scanned in a 1.5 T Siemens Aera scanner, axial two-point Dixon MRI images were acquired from the abdomen, from which fat and water images were produced. Images were analysed using imaging software (HOROS V 1.1.7; www.horosproject.org; accessed 21/10/2017) to quantify VAT and IHL. VAT area was assessed using a single slice at the L4-5 spinal anatomical position. IHL was quantified using two abdominal MRI images 30 mm apart encompassing both the superior and inferior view of the liver. A four-circular region of interest analysis was conducted to determine the hepatic fat fraction (%) in each region. IHL was calculated as the mean of all eight regions of interest. Quantification of IMCL in the soleus muscle of the right leg was derived from a ^{1}H-MRS scan on a 1.5 T Siemens system with an extremity RF coil to obtain muscle images. From these images two localised proton spectra were obtained, a water-suppressed lipid spectra and a lipid-suppressed water spectra. The Java-Based Magnetic Resonance User Interface software was used to identify and quantify the IMCL peaks expressed in arbitrary units (40).

Laboratory analysis

Plasma glucose and glycerol isotope enrichments were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies) using selected ion monitoring to determine the tracer-to-tracee ratio. The isotopic enrichment of glucose was determined as the penta-O-trimethylsilyl-D-glucose-O-methoxime derivative (41). The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl (tBDMS) glycerol derivative (42). Plasma insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare).

Calculations

Whole-body insulin sensitivity was quantified using the M value (mg/kg FFM min^{-1}) measured during the final 30 min of the high-dose insulin phase of the clamp. It is calculated as total glucose disposal corrected for deviations in plasma glucose concentration. The M value was divided by mean plasma insulin concentration during the high dose insulin phase, giving M/I (mg kg FFM min^{-1})/(pmol/L) as another assessment of whole-body insulin sensitivity (37).

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Steele's non-steady-state equations modified for stable isotopes (43) were used to determine peripheral glucose utilisation (glucose rate of disappearance, Rd (μmol/kg FFM min^{-1})), endogenous glucose production (glucose rate of appearance, Ra (μmol/kg FFM min^{-1})) and whole-body lipolysis (glycerol Ra (μmol/kg FFM min^{-1})) at basal and during the different phases of the clamp. Glucose kinetic calculations were modified to incorporate the [6,6-$^{2}H_{2}$]-glucose isotope enriched 20% glucose (44). Optical segment analysis was used to smooth the glucose and glycerol enrichment concentrations over the clamp time course (45).

Peripheral insulin sensitivity was determined as the percentage increase in the rate of glucose disappearance from basal to the final 30 min of the high-dose insulin phase. The peripheral insulin sensitivity index (PISI) was also calculated as the glucose Rd (μmol/kg FFM min^{-1})/mean plasma insulin concentration (pmol/l) during the final 30 min of the high-dose insulin phase (46). Peripheral insulin sensitivity is predominantly driven by skeletal muscle glucose uptake but also captures adipose tissue glucose uptake, hence, we have used PISI as an assessment of skeletal muscle insulin sensitivity.

Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was measured as the percentage suppression of endogenous glucose production from basal to the final 30 min of the low-dose insulin phase (47). Hepatic insulin sensitivity was also quantified during the basal and low-dose insulin phase using the hepatic insulin sensitivity index (HISI), which is the reciprocal of the product of endogenous glucose production (glucose Ra) and mean plasma insulin (46).

Adipose tissue insulin sensitivity was measured as the percentage suppression of whole body lipolysis (glycerol Ra) from basal to the final 30 min of the low-dose insulin phase (47). Adipose tissue insulin sensitivity was also quantified during the basal and low-dose insulin phase using the adipose tissue insulin sensitivity index (ATIS) which is the reciprocal of the product of whole body lipolysis (glycerol Ra) and basal plasma insulin (46).

Statistical analysis

The Soul–Deep II study was powered on a primary outcome of insulin secretory function (48). Allowing a difference of one s.d. to be detected with 90% power and two-sided significance, we aimed to recruit 23 per group, allowing for 20 per group to complete the protocol.

Data were assessed for normality using the Shapiro–Wilks test and histograms. A log 10 transformation was performed where data were skewed. Data which were normally distributed are presented as mean (s.d.), data which required log 10 transformation are presented as geometric mean (95% CI), data which remained skewed after log transformation are presented as median (lower-upper IQR). Ethnic comparisons of insulin sensitivity were assessed using the independent samples *t*-test for normally distributed data and Mann–Whitney test for non-normally distributed data. The mean difference (95% CI) and ratio of the geometric mean (95% CI) are presented where appropriate. Adjustment of the insulin sensitivity measures for VAT and IHL were made using linear multiple regression. Pearson's correlation analyses were used to assess the associations between insulin sensitivity measures and ectopic fat. Interaction by ethnicity was assessed using a linear multiple regression with ethnicity*ectopic fat depot used as the interaction term. Statistical significance was defined as $P<0.05$ and data analyses were performed using SPSS software, version 25 (IBM Analytics).

Results

Participant characteristics

The participant characteristics of the 21 BAM and 23 WEM are displayed in Table 1; by design the groups were comparable in age and BMI. There were no differences in body fat, waist circumference, blood pressure, HbA1c, cholesterol, fasting and post-load glucose between ethnicities; however, BAM exhibited lower fasting triglycerides compared to WEM. Data on ectopic fat depots in these volunteers, as previously reported by our group (39), showed lower VAT and IHL in BAM but similar IMCL (included in Table 1 for reference).

Whole-body insulin sensitivity

The glucose and insulin profiles during the hyperinsulinaemic–euglycaemic clamp are shown in Supplementary Fig. 1 (see section on supplementary materials given at the end of this article). BAM exhibited a trend towards greater mean plasma insulin during the high-dose insulin phase (ratio of the geometric mean and 95% CI of 1.10 (1.00, 1.21), $P=0.05$). There were no ethnic differences in whole-body insulin sensitivity, measured as either M value (BAM: 9.65 (2.32) vs WEM: 9.51 (3.86) mg/

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Table 1 Participant characteristics. Data are presented as mean (S.D.) for normally distributed data.

Characteristics	BAM ($n=21$)	WEM ($n=23$)	P
Age (years)*	25 (22, 40)	29 (25, 53)	0.18
BMI (kg/m^2)	26.8 (3.6)	26.5 (4.5)	0.82
Waist circumference (cm)†	87.5 (83.4, 91.8)	92.8 (87.1, 99.0)	0.13
Systolic BP (mm/Hg)	124.0 (11.9)	121.9 (9.1)	0.52
Diastolic BP (mm/Hg)†	70.3 (65.5, 75.5)	70.7 (67.2, 74.3)	0.91
Total cholesterol (mmol/L)†	4.26 (3.85, 4.73)	4.65 (4.23, 5.11)	0.20
LDL (mmol/L)	2.73 (0.84)	2.99 (0.82)	0.33
HDL (mmol/L)*	1.2 (1.2, 1.4)	1.2 (1.1, 1.4)	0.86
Triglycerides (mmol/L)†	0.67 (0.59, 0.77)	0.99 (0.81, 1.21)	**<0.01**
Fasting glucose (mmol/L)	5.1 (0.5)	5.2 (0.4)	0.55
2-h post load glucose (mmol/L)	5.28 (1.13)	5.09 (1.26)	0.61
Ectopic fat depots			
Visceral adipose tissue (VAT), L4-5 (cm^2)†	46.1 (34.4, 61.7)‡	79.0 (55.4, 112.5)	**0.02**
Hepatic fat fraction (HFF) (%)	3.78 (1.13)‡	6.08 (5.04)	**0.04**
Intramyocellular lipid (IMCL) (AU)§	0.030 (0.015)	0.030 (0.014)	0.87

*data expressed as median (IQR) for non-normally distributed data; †geometric mean (95% CI) for log transformed data; ‡sample size=20; §sample size: BAM=18, WEM=22.

kg FFM min^{-1}, $P=0.89$) or M/I (BAM: 0.0171 (0.0059) vs WEM: 0.0189 (0.0094) ((mg/kg FFM min^{-1})/(pmol/L)), $P=0.44$). Associations between VAT, IHL and IMCL with whole-body insulin sensitivity (measured as either M value or M/I) are shown in Supplementary Table 1. When combining all participants as a single cohort, there were significant inverse associations between VAT, IHL, and IMCL with whole-body insulin sensitivity; however, when assessing the ethnic groups separately, these relationships were significant in WEM but weaker or absent in BAM.

Adipose tissue insulin sensitivity

Adipose tissue insulin sensitivity (ATIS) index did not differ by ethnicity during the basal or insulin-stimulated state (Table 2). Insulin-mediated suppression of glycerol was used as a measure of adipose tissue insulin sensitivity to lipolysis and was not significantly different by ethnicity (mean difference (95% CI) −8.55 (−22.0, 4.90)%, $P=0.21$), Supplementary Fig. 2A. There was a trend towards lower adipose tissue insulin sensitivity to lipolysis when adjusting for VAT in BAM (adjusted mean difference (95% CI) −12.4 (−26.9, 2.21)%, $P=0.09$). Across the whole cohort, adipose tissue insulin sensitivity did not correlate with VAT, IHL or IMCL (Fig. 1A, B and C). However, when assessing WEM and BAM separately, adipose tissue insulin sensitivity to lipolysis correlated with VAT and IHL in WEM, but there were no significant correlations in BAM (Fig. 1A and B). When modelled with suppression of lipolysis, ethnicity had no significant interaction with VAT ($P=0.12$) or IHL ($P=0.58$). There were no significant correlations between the suppression of lipolysis and IMCL in either ethnic group (Fig. 1C).

Peripheral insulin sensitivity

We found no ethnic differences in peripheral insulin sensitivity, measured as percentage increase in glucose utilisation from the basal to high-dose insulin phase of the clamp (BAM 304.82 (111.11) vs WEM 286.24 (138.44) %, $P=0.63$), Supplementary Fig. 2B. There was also no ethnic difference when accounting for the insulin concentration during the high-dose insulin phase, using PISI (mean difference (95% CI) -1.06×10^{-2} (-3.87×10^{-2}, 1.74×10^{-2}) (μmol/kg FFM min^{-1})/pmol/L, $P=0.43$), Table 2. Adjusting PISI for VAT (which we have previously reported as lower in BAM), resulted in significantly lower PISI in BAM (adjusted mean difference (95% CI) -3.47×10^{-2} (-5.67×10^{-2}, -1.27×10^{-2}) (μmol/kg FFM min^{-1})/pmol/L, $P<0.01$). Across the whole cohort, PISI correlated significantly with VAT and IMCL (Fig. 1D and E). When assessing WEM and BAM separately, PISI correlated significantly with VAT and IMCL in WEM; however, in BAM the association with VAT was weaker (Fig. 1D). and there was no association with IMCL (Fig. 1E). When modelled with PISI, interactions between ethnicity and VAT and between ethnicity and IMCL were not significant ($P=0.11$ and $P=0.11$, respectively).

Hepatic insulin sensitivity

We found no ethnic differences in the basal or insulin stimulated hepatic insulin sensitivity index (HISI), Table 2. Using suppression of endogenous glucose production as a measure of hepatic insulin sensitivity, we found no evidence for an ethnic difference (mean difference (95% CI) −4.15 (−14.83, 6.53)%, $P=0.21$), Supplementary

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Table 2 Substrate kinetics before and after insulin adjustments during the basal state and the hyperinsulinaemic–euglycaemic clamp. Data are expressed as mean (S.D.)

	Basal state			**Hyperinsulinaemic-euglycaemic clamp**		
	BAM, $n=21$	WEM, $n=23$	P	BAM, $n=20$	WEM, $n=23$	P
Glycerol Ra (µmol/kg FFM min^{-1})	1.11 (0.71–2.72)*	1.55 (1.29–2.27)*	0.10	0.64 (0.52, 0.78)*	0.77 (0.63, 0.93)†§	0.17
ATIS index (µmol/kg FFM min^{-1} × pmol/L)$^{-1}$	14.62×10^{-3} (10.36×10^{-3}, 20.70×10^{-3})†	12.16×10^{-3} (9.06×10^{-3}, 16.31×10^{-3})†	0.40	10.2×10^{-3} (4.61×10^{-3})	9.04×10^{-3} (4.46×10^{-3})	0.42
Glucose Ra (µmol/kg FFM min^{-1})	13.60 (1.24)	13.74 (1.33)	0.72	4.37 (3.94–5.64)*	3.23 (2.71–6.14)*‡	0.38
HISI (µmol/kg FFM min^{-1} × pmol/L)$^{-1}$	1.57×10^{-3} (6.61×10^{-4})	1.70×10^{-3} (7.18×10^{-4})	0.56	1.35×10^{-3} ($9.23–16.32 \times 10^{-4}$)*	1.68×10^{-3} ($11.57–23.59 \times 10^{-4}$)*‡	0.41
Glucose Rd (µmol/kg FFM min^{-1})	–	–	–	51.14 (44.61–60.16)*	50.48 (38.43–67.72)*	0.87
PISI (µmol/kg FFM min^{-1})/pmol/L	–	–	–	9.71×10^{-2} (3.43×10^{-2})	10.78×10^{-2} (5.32×10^{-2})	0.43

*data expressed as median (IQR) for non-normally distributed data; †geometric mean (95% CI) for log transformed data; ‡sample size:21; §sample size:22.
ATIS, adipose tissue insulin sensitivity; HISI, hepatic insulin sensitivity index; PISI, peripheral insulin sensitivity index; Ra, rate of appearance; Rd, rate of disappearance.

Fig. 2C. Adjusting hepatic insulin sensitivity for VAT, resulted in lower mean hepatic insulin sensitivity in BAM (mean difference (95% CI) −10.9 (−21.2, −0.72)%, P =0.04). Adjusting hepatic insulin sensitivity for IHL (which we have previously reported as lower in BAM), resulted in no ethnic difference in hepatic insulin sensitivity (adjusted mean difference (95% CI) −7.33 (−17.9, 3.24)%, P=0.17). Across the whole cohort, hepatic insulin sensitivity correlated with VAT and IHL (Fig. 1F and G). When assessing WEM and BAM separately, hepatic insulin sensitivity correlated significantly with VAT in both ethnicities (Fig. 1F); however, the correlation with IHL was only significant in WEM (Fig. 1G). When modelled with suppression of endogenous glucose production, interactions between ethnicity and VAT and between ethnicity and IHL were not significant (P=0.50 and P=0.66, respectively).

Discussion

In this study, we have shown that whilst BAM and WEM display comparable whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity, the relationships between insulin sensitivity and ectopic fat are ethnically distinct.

Resistance of adipose tissue to the antilipolytic effect of insulin is suggested to be a primary abnormality in the pathophysiology of T2D that occurs before the onset of hyperglycaemia (6). It has been proposed that VAT and ectopic fat accumulate as result of dysfunctional lipolysis, which allows an increase in circulating fatty acids, and other adipocyte abnormalities, described in the 'spillover theory' (10, 49). Our study, which is the first to compare men of black African and white European ethnicity, shows that there are no associations between lipolysis and VAT, IHL or IMCL in BAM. Our findings agree with Albu *et al*. who showed a relationship between suppression of lipolysis and VAT in obese white women but not in black women (31). Together, these findings suggest lipolysis may not be driving the accumulation of ectopic fat in black people of either gender, suggesting the 'spillover theory' may not hold true in this ethnic group.

Visceral fat, IHL and IMCL play an integral role in the development of insulin resistance and T2D (12, 16, 17); however, black populations are consistently reported to exhibit lower levels of VAT (19, 20, 21, 22, 23) despite their high T2D risk. Our finding of lower VAT in BAM is in agreement with the literature. Despite this, we found comparable levels of insulin sensitivity. We investigated

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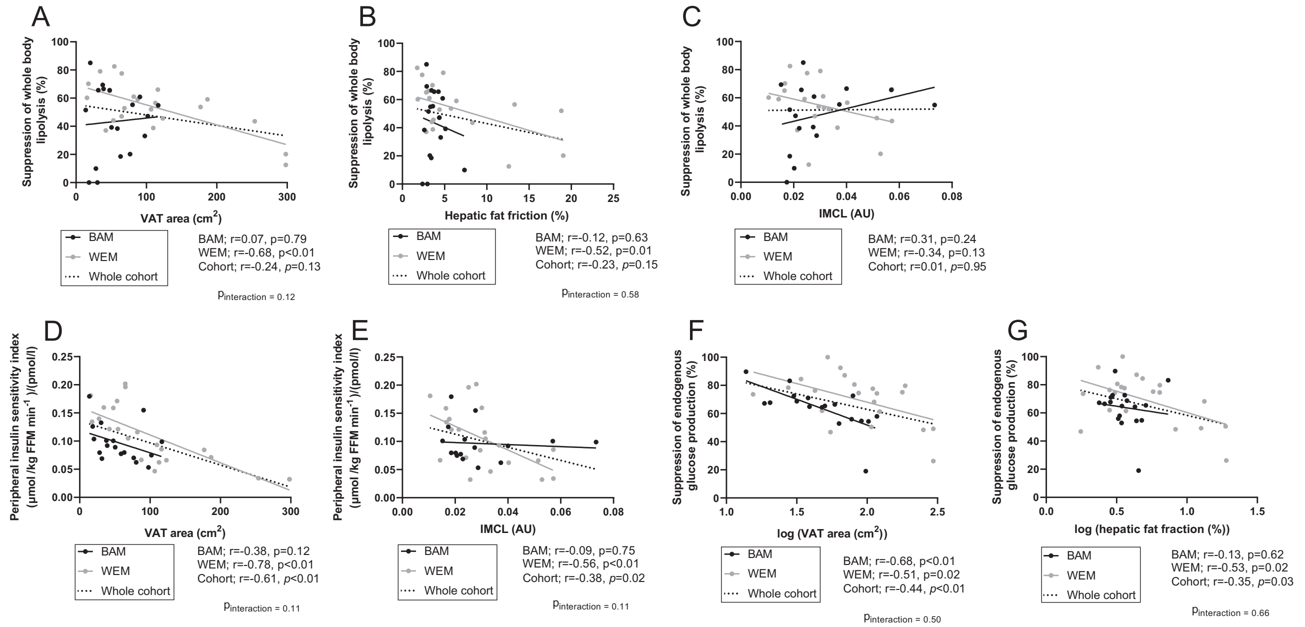

Figure 1

The association between the suppression of whole body lipolysis with VAT (A), hepatic fat fraction (B) and IMCL (C).The association between peripheral insulin sensitivity index with VAT (D) and IMCL (E). The association between the suppression of endogenous glucose production with VAT (F) and hepatic fat fraction (G). Data presented using the Pearson correlation coefficients. Peripheral insulin sensitivity index was measured during the high-dose insulin phase (40 mU m^{-2} BSA min^{-1}), suppression of endogenous glucose production and whole-body lipolysis was measured during the low-dose insulin phase (10 mU m^{-2} BSA min^{-1}).

associations between VAT and insulin sensitivity and found that both peripheral and hepatic insulin sensitivity were significantly associated with VAT in both ethnicities. This leads us to believe that VAT is detrimental to skeletal muscle and hepatic insulin sensitivity in both ethnic groups, but that this impact occurs at lower VAT levels in BAM compared to WEM, a so-called lower *threshold*. Our finding of a significant association between VAT and hepatic insulin sensitivity is consistent with earlier work in obese black women (25); however, our data in healthy men also show an association between VAT and skeletal muscle insulin sensitivity which has not been found in women (25, 50). This conflicting result may be due to the aforementioned studies focusing on women with severe obesity, whilst our participants were only mildly overweight or gender itself may explain the conflicting results, adding to the evidence for gender differences in T2D pathophysiology (33).

Accumulation of IHL is proposed to be central to the development of hepatic insulin resistance. The 'portal theory' describes the accumulation of IHL, which develops from the flux of fatty acids from VAT to the liver, via the portal vein. It is, therefore, not surprising that we found lower IHL in BAM, given the lower levels of VAT that they exhibited. Whilst our data from WEM corroborate the current understanding of T2D pathophysiology such that hepatic insulin sensitivity was significantly associated with IHL (14), we found no evidence for this relationship in BAM. This contrasts with data from studies in black women whereby IHL is associated with hepatic sensitivity (25, 26). This may point to IHL being more harmful in black women than men, although the obesity status of the women may also have contributed to this result (33).

Intramuscular lipids, which accumulate as a result of skeletal muscle cells taking up fatty acids from the peripheral circulation, have been shown to be correlated with skeletal muscle insulin resistance (15). Whilst we saw a significant relationship between IMCL and skeletal muscle insulin sensitivity in WEM, this relationship was not present in BAM. This finding agrees with other studies (51, 52, 53) and suggests that skeletal muscle insulin resistance develops independently of IMCL in BAM.

In contrast to the extensive evidence base that reports pronounced insulin resistance in populations of black African ethnicity (24, 54), we showed no ethnic differences in insulin sensitivity at a whole-body and tissue-specific level. The contrast in these findings are likely due to the different methodologies used to

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measure insulin sensitivity. In our study we have used the clamp method, which is a direct assessment of insulin sensitivity (37), while other methods estimate insulin sensitivity through indirect modelling. The use of such methods in black populations has been criticised because of the reduced insulin clearance and higher insulin levels that they exhibit, which may lead to an underestimation of modelled insulin sensitivity. Indeed, in an ethnic comparison of direct and indirect measures of insulin sensitivity, Pisprasert *et al*. showed no difference in insulin sensitivity using the clamp, while surrogate indices showed greater insulin resistance in African-Americans compared to white Americans. These data suggest caution should be applied when using indirect assessments of insulin resistance in black populations (55). Our findings are supported by several metabolic studies using glucose clamps and isotopes, which have also found comparable insulin sensitivity in healthy black and white communities (25, 26, 55, 56, 57). Our experimental design also limited potential confounding factors; participants were similar in BMI, participants with impaired glucose tolerance were excluded and our data collection included only men.

We have previously published a description of the ectopic fat status for the current set of participants (39) in which we found no ethnic differences in IMCL, but significantly lower VAT and IHL in BAM. In the current study, we adjusted our insulin sensitivity data for VAT and found lower whole body, skeletal muscle and hepatic insulin sensitivity in BAM. The reduced insulin sensitivity following adjustment for VAT, and the lower VAT storage in the presence of similar lipolysis, suggests that the detrimental effects of VAT occur at lower levels in BAM and a resistance to storing VAT allows BAM to maintain comparable insulin sensitivity to WEM. In comparison, adjusting for IHL did not explain the similar hepatic insulin sensitivity and provides more evidence for an independent relationship between IHL and hepatic insulin sensitivity in BAM. Lower ectopic fat storage, despite similar lipolysis, may point to an increased tendency towards fat oxidation over ectopic storage in BAM; further studies assessing fatty acid oxidation are needed to explore this possibility.

Although one of the strengths of this study was the use of rigorous measurements of insulin sensitivity and ectopic fat, we recognise that our conclusions for the associations between insulin sensitivity and ectopic fat may be limited by our sample size. While our sample size is comparable to other studies of this nature, it does affect the statistical adjustment for VAT/IHL and interaction analysis. Our insulin sensitivity data are based on lean mass assessed by bioimpedance methodology; this uses calculations which are not ethnically sensitive and could potentially lead to underestimation of lean mass and thus overestimating insulin sensitivity in BAM (58). Finally, although the aim of our recruitment was to achieve comparable BMIs in our groups, this resulted in a tendency towards lower waist circumferences in BAM, which may have also contributed to differences in the metabolic characteristics that we studied. A study in which the groups are matched for waist circumference would help to elucidate these effects.

In summary, our data suggest that increased lipolysis due to adipose tissue insulin resistance may not be driving ectopic fat deposition in BAM. Additionally, ectopic fat accumulation in the liver and skeletal muscle may play less of a role in reducing insulin sensitivity in BAM compared to WEM. We provide evidence that the detrimental effects of VAT on glucose uptake and the suppression of endogenous glucose production occur at a lower VAT level in BAM. We conclude that current theories regarding the accumulation of ectopic fat and its impact on insulin sensitivity may not apply in BAM, who display a resistance to storing visceral and hepatic fat. Future work, assessing the impact of ectopic fat on insulin secretory function, is vital before excluding ectopic fat as the culprit behind the increased prevalence of T2D in black populations.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-19-0636.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

L M G, S A A, A M U, J L P designed the study; O B, O H, M L, C M, F S-M, G C-E acquired the data, O B, O H, F S-M, A M U performed the data analysis, O B L M G, S A A, A M U contributed to the interpretation, O G, L M G drafted the article and all authors contributed to revising the intellectual content before approving the final article. J L P is supported by the NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and is an NIHR Senior Investigator. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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