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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

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Tweet: “No differences in insulin sensitivity in #blackbritish men with #type2diabetes despite having less central fat and more muscle. A report by @toyosi_93 and team from @DRG_Kings @DiabetesKings” (please insert fig. 2)
ABSTRACT

Aims/hypothesis: Type 2 diabetes is more prevalent in Black African than White European populations although, paradoxically, they present with lower visceral fat, which has a known association with insulin resistance. Insulin resistance occurs at a tissue specific level however no study has simultaneously compared whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity between Black and White men. We hypothesised greater hepatic and adipose tissue insulin sensitivity in Black African (BAM) compared to White European men (WEM) with early type 2 diabetes, due to reduced visceral fat.

Methods: Eighteen BAM and 15 WEM with type 2 diabetes, matched for BMI and age, underwent a 2 stage hyperinsulinaemic-euglycaemic clamp with glucose and glycerol stable isotope tracers to assess tissue specific insulin sensitivity and a magnetic resonance imaging scan to assess body composition.

Results: We found no ethnic differences in whole body, skeletal muscle, hepatic or adipose tissue insulin sensitivity between BAM and WEM. This finding occurred in the presence of lower visceral fat in BAM (3.72 vs 5.68 kg [mean difference -1.96, 95% confidence interval -3.30, 0.62]; p= 0.01). There was an association between skeletal muscle and adipose tissue insulin sensitivity in WEM that was not present in BAM (r=0.78, p<0.01 vs r=0.25 p=0.37).

Conclusions/interpretation: Our data suggest that in type 2 diabetes there are no ethnic differences in whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity between Black and White men despite differences in visceral adipose tissue, and that impaired lipolysis may not be contributing to skeletal muscle insulin resistance in men of Black African ethnicity.

Keywords: adipose insulin sensitivity, Black African, ethnicity hepatic insulin sensitivity, insulin sensitivity, isotope, lipolysis, skeletal muscle insulin sensitivity, tracer, type 2 diabetes, visceral fat
Abbreviations

BSA - Body surface area

BAM - Black African Men

CRF - Clinical research facility

MRI - Magnetic resonance imaging

Ra - Rate of appearance

Rd - Rate of disappearance

SAT - Subcutaneous adipose tissue

SM - Skeletal muscle

TTR - Tracer-to-tracee ratio

VAT - Visceral adipose tissue

WEM - White European Men
Research in context
What is already known about this subject? (maximum of 3 bullet points)
- Populations of Black African ancestry are at higher risk of developing type 2 diabetes compared to their White European counterparts despite displaying lower visceral fat, hepatic fat and a favourable blood lipid profile.
- The pathophysiology of type 2 diabetes involves insulin resistance which occurs at multiple sites including the skeletal muscle, liver and adipose tissue.

What is the key question? (one bullet point only; formatted as a question)
- Do Black West African men with early type 2 diabetes present with greater hepatic and adipose tissue insulin sensitivity?

What are the new findings? (maximum of 3 bullet points)
- During early type 2 diabetes, there are no ethnic differences in skeletal muscle, hepatic and adipose tissue insulin sensitivity despite greater skeletal muscle and reduced visceral fat in Black West African men.
- There appears to be an independent relationship between skeletal muscle insulin resistance and adipose tissue resistance to lipolysis in Black West African men.

How might this impact on clinical practice in the foreseeable future? (one bullet point only)
- Using prevention and treatment strategies which target adipose tissue function may not produce the same impact in Black African and White European populations.
INTRODUCTION

Populations of Black African ancestry are disproportionately affected by type 2 diabetes compared to White Europeans [1]. The pathophysiological processes of type 2 diabetes are well documented and include beta-cell dysfunction, ectopic fat deposition and insulin resistance of the liver, skeletal muscle and adipose tissue [2, 3]; the use of stable isotopes has enabled measurement of these tissue specific sites of insulin resistance [4]. Black populations typically display lower visceral adipose tissue (VAT) and hepatic fat deposition and a more favourable blood lipid profile [5]. Visceral fat has been positively associated hepatic and adipose tissue insulin resistance in diabetes and normal glucose tolerance [6]. The lower VAT exhibited in Black populations suggests there may be ethnic distinctions in the pathophysiology of type 2 diabetes. There have been several studies comparing tissue specific insulin resistance in vivo, using the ‘gold standard’ hyperinsulinaemic-euglycaemic clamp and stable isotopes, in healthy populations of Black and White ethnicity. These have been conducted primarily, although not exclusively, in women and adolescents but have not produced a consistent picture [7-20]. Gender differences in body composition have shown women to express greater central and overall body fat [21]. Accordingly, a gender distinction in the type 2 diabetes phenotype has been shown in Black Africans, whereby men display greater insulin sensitivity compared to women [22]. The inconsistencies in the findings from adolescent and female populations likely stem from differences in methodologies and participant body composition. Ethnic comparisons in people with type 2 diabetes are required to inform therapeutic decisions however, they are also limited to adolescent and female populations [23, 24]. Peripheral insulin stimulated glucose disposal has been shown to be similar in diabetic adolescents [23] but to date no study has compared this in diabetic Black and White adults using stable isotope methods. Studies assessing ethnic differences in hepatic insulin sensitivity in type 2 diabetes are few; studies of basal endogenous glucose production have shown no difference in adolescents [23] but no studies have assessed insulin stimulated suppression of endogenous glucose production and there have been no studies performed in adults with type 2 diabetes. In vivo assessment of adipose tissue insulin sensitivity in type 2 diabetes has been more limited; lower basal free fatty acid release has been reported in Black women [24] however no study has assessed insulin stimulated suppression of fatty acid release.
To date, no single study has undertaken a comprehensive ethnic comparison of whole body, peripheral, hepatic and adipose tissue sensitivity to insulin using the same study cohort and methodology.

We aimed to compare tissue specific sites of insulin sensitivity between Black (west) African (BAM) and White European men (WEM) with early type 2 diabetes using the hyperinsulinaemic-euglycaemic clamp with stable isotopes, and to investigate associations between sites of insulin resistance by ethnicity. We hypothesise that in early type 2 diabetes, BAM men will have greater hepatic and adipose tissue insulin sensitivity due to lower VAT deposition compared to their White European counterparts.
METHODS

The study was conducted at the Clinical Research Facility (CRF), King’s College London, UK and approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided informed consent. The data were collected as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) study; recruitment and data collection took place April 2013-January 2015 [25, 26].

Participants

Black West African (BAM) or White European (WEM) (self-declared, confirmed by grandparental birthplace) men, aged 18-65 years, BMI 25-35 kg/m², with a diagnosis of type 2 diabetes within 5 years, treated with lifestyle advice ± metformin, with HbA₁c ≤63.9 mmol/mol (<8%) were recruited from South London primary care practices and selected to match for age and BMI. Participants were deemed ineligible if: treated with thiazolidinedione, insulin, chronic oral steroids, beta-blockers; serum creatinine >150 mmol/l; serum alanine transaminase level >2.5-fold above the upper limit of the reference range; positive auto-antibodies for anti-insulin, anti-GAD or anti-A2; sickle cell disease (trait permitted); or using medications believed to affect the outcome measures. Participants completed a comprehensive medical screening before study entry.

Study design

Participants arrived at the CRF 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 physical activity in the 48 hours preceding the visit, refrain from consuming alcohol in the 24 hours preceding the visit, and to consume a standardised 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 the visit. 

Hyperinsulinaemic-euglycaemic clamp assessment of whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity

Upon arrival, participants were weighed in light clothing and their body surface area calculated using the Mosteller formula. A cannula was inserted into an antecubital fossa vein to infuse stable isotopically
labelled tracers, 20% dextrose and insulin (Actrapid, Novo Nordisk, Denmark) bound to albumin. A second cannula was inserted retrogradely into the dorsum of the hand, which was placed in a hand warming unit, to achieve arterialised venous blood samples. A baseline blood sample determined the participant’s fasting plasma glucose; if above 5 mmol/l a sliding scale insulin infusion was used to lower the circulating glucose to 5 mmol/l. At time point -120 minutes, a primed (2.0 mg/kg), continuous (0.02 mg kg\(^{-1}\) min\(^{-1}\)) infusion of [6,6\(^{2}\)H\(_2\)]-glucose and a primed (0.12 mg/kg), continuous (0.0067 mg kg\(^{-1}\) min\(^{-1}\)) infusion of [\(^{2}\)H\(_5\)]-glycerol (CK Gases Ltd, UK) were initiated [27]. Basal state blood samples were taken between -30 and 0 minutes. After infusion of the tracers for 120 minutes (basal period), a 2-stage hyperinsulinaemic-euglycaemic clamp procedure was started at time point 0 minutes, and continued for 4 hours (during which the infusion of [6,6\(^{2}\)H\(_2\)]-glucose was continued however, the infusion of [\(^{2}\)H\(_5\)]-glycerol was stopped just prior to beginning the stage 2 insulin infusion). Insulin was infused at a rate of 10 mU m\(^{-2}\) BSA min\(^{-1}\) (initiated with a priming dose of 30 mU m\(^{-2}\) BSA min\(^{-1}\) for 3 minutes and then 20 mU m\(^{-2}\) BSA min\(^{-1}\) for 4 minutes) during stage 1 (0 – 120 minutes) and at a rate of 40 mU m\(^{-2}\) BSA min\(^{-1}\) (initiated with a priming dose of 120 mU m\(^{-2}\) BSA min\(^{-1}\) for 3 minutes and then 80 mU m\(^{-2}\) BSA min\(^{-1}\) for 4 minutes) during stage 2 (120 – 240 minutes) [19, 28]. Euglycaemia (5 mmol/l) was maintained by variable infusion of 20% dextrose, which was 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 ensure a constant glucose tracer-to-tracee ratio (TTR). Plasma glucose readings were taken every 5 minutes, using an automated glucose analyser, to inform adjustment of the glucose infusion rate. Hepatic and adipose tissue insulin sensitivity were evaluated in the low dose insulin infusion, whole body and peripheral (primarily skeletal muscle) insulin sensitivity were evaluated in the high dose insulin infusion. Blood samples were collected before beginning the tracer infusions to determine baseline enrichment of glucose and glycerol. At time points -30, -20, -10 and 0 minutes baseline blood samples were collected, followed by sampling at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 minutes for the assessment of plasma glucose and glycerol concentrations and enrichments, insulin and NEFA concentrations.
**Magnetic resonance imaging**

Participants attended the MRI unit of Guy’s Hospital, King’s College London, for the assessment of subcutaneous (SAT) and visceral adipose tissue (VAT), and skeletal muscle (SM) mass. Scanning was performed on a 1.5T Siemens scanner to acquire MRI images from the neck to the knee (excluding the arms). Participants lay supine with body coils secured on the scanned body area.

For each participant, the MRI scan produced 320 contiguous axial fat and water images each 3mm apart.

The Dixon-MRI T1-weighted spin-echo sequence includes an echo time of 4.77ms for the in-phase images, 2.39ms for the out-of-phase images and a repetition time of 6.77ms. MRI images were analysed using a semi-automated method carried out by Klarismo Ltd to quantify SAT and SM mass volumes in all images between the neck and knee region and VAT volume in the whole abdominal cavity.

**Analyses of samples**

Plasma glucose concentration was measured by automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA). Serum insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK). Plasma NEFA were measured by an enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA, USA) on an automated clinical chemistry analyser (ILab 650, Instrument Laboratories, Holliston, MA, USA). The glucose and glycerol enrichment (TTR) in plasma were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK) using selected ion monitoring. The isotopic enrichment of glucose was determined as the penta-O-trimethylsilyl-D-glucose-O-methyloxime derivative [29]. The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl (tBDMS) glycerol derivative [30].

**Calculations**

- Total glucose disposal rate (M value in mg kg\(^{-1}\) min\(^{-1}\)); was calculated as a measure of whole body insulin sensitivity. This was computed as the mean of the glucose infusion rate, corrected
for any change in measured plasma glucose concentration, during the final 30 mins of the high dose insulin infusion [28]. Additionally, M was adjusted for mean insulin concentration (M/I).

Peripheral glucose utilisation (glucose rate of disappearance, Rd μmol kg⁻¹ min⁻¹), endogenous glucose production (glucose rate of appearance, Ra μmol kg⁻¹ min⁻¹) and whole body lipolysis (glycerol rate of appearance, Ra μmol kg⁻¹ min⁻¹) were calculated using Steele’s non-steady state equations modified for stable isotopes assuming a volume distribution of 22% body weight [31]. Calculation of glucose kinetics was modified for inclusion of [6,6-²H₂]-glucose in the dextrose infusion [32]. Before calculation of glucose and glycerol kinetics, enrichment and concentrations were smoothed using optical segments analysis [33].

Peripheral glucose utilisation (glucose Rd) was calculated during the basal state and the final 30 minutes of the high dose insulin infusion. We used the percentage increase in glucose Rd from basal to the high dose insulin infusion as a measure of skeletal muscle insulin sensitivity [34].

Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous glucose infusion rate from total glucose Ra. Glucose Ra was calculated during the basal state and during final 30 minutes of the low dose insulin infusion. Percentage suppression of glucose Ra from basal to the low dose insulin infusion was calculated as a measure of hepatic insulin sensitivity [35].

Whole body lipolysis (glycerol Ra) was calculated during the basal state and during the final 30 minutes of the low dose insulin infusion. Percentage suppression of glycerol Ra from basal to the low dose insulin infusion was calculated as a measure of adipose tissue insulin sensitivity [34].

The area under the curve for plasma glucose, insulin and NEFA concentrations during the clamp were calculated using the trapezium rule.

Statistics

All variables were checked for normality using the Shapiro-Wilks test and non-normally distributed variables were transformed (log 10) for analysis. Normally-distributed data are expressed as mean (SD),
log-normal data were back transformed to give geometric mean and 95% CI and data which remained skewed after log transformation are expressed as median (IQR). Ethnic differences between means were determined using the independent samples t-test for normally distributed data and Mann-Whitney U test for skewed data. Mean difference or the ratio of the geometric mean and 95% CI are presented where appropriate. Associations between insulin sensitivity measures and with VAT were tested using Pearson’s correlation coefficient. Multiple regression analyses were conducted to adjust for the effect of body composition (VAT and SM mass) on insulin sensitivity measures. Linear regression analysis was used to determine the impact of ethnicity (interaction) on the associations between insulin sensitivity measures. \( p \leq 0.05 \) was considered statistically significant. Analyses were performed using SPSS software, version 22 (IBM Analytics, NY).
RESULTS

Participant characteristics

The clinical characteristics of the participants are shown in Table 1. By design, the groups were not statistically different in age, weight and BMI. Waist circumference, BSA, SAT, number of years following diabetes diagnosis, HbA1c and the proportion of those treated with metformin were not different between ethnic groups. Mean VAT mass was 34.5% lower and mean SM mass was 11.9% greater in BAM (Table 1).

Insulin sensitivity – whole body, skeletal muscle, hepatic and adipose tissue

Basal plasma glucose (BAM; 5.89 (0.39) vs WEM; 5.71(0.63) mmol/l, p=0.38), insulin (BAM; 45.7 (36.8, 56.7) vs WEM; 57.3 (39.5, 83.2) pmol/l, p=0.24) and NEFA (BAM; 0.48 (0.18) mmol/l, p=0.30) were not different and there were no ethnic differences in plasma glucose (p=0.89), insulin (p=0.78) and NEFA (p=0.70) concentrations during the clamp (Fig. 1). Total glucose disposal rate (M), as a measure of whole body insulin sensitivity, did not differ between the ethnic groups (Table 2); the lack of significance continued after adjustment for mean insulin during the high dose insulin infusion (M/I: BAM; 0.030 (0.017) vs WEM; 0.026 (0.011) mg kg\(^{-1}\) min\(^{-1}\) pmol/l\(^{-1}\), p=0.46). Peripheral glucose utilisation (Glucose Rd) during the high dose insulin infusion (Table 2) and skeletal muscle insulin sensitivity (% increase in peripheral glucose utilisation) were also similar between ethnic groups (Fig. 2a: BAM; 203.5 (126.2) vs WEM; 166.3 (102.5), mean difference of 37.3%, 95% CI -55.6, 130.1; p = 0.42). Basal endogenous glucose production (glucose Ra) was similar between BAM and WEM and there were no ethnic differences in endogenous glucose production during the low dose insulin infusion (Table 2) or in hepatic insulin sensitivity (% suppression of endogenous glucose production) (Fig. 2b: BAM; -36.4 (19.7) vs WEM; -34.8 (20.7), mean difference of -1.61%, 95% CI -17.7, 14.5; p=0.84). There was a trend towards lower basal whole body lipolysis (glycerol Ra) in BAM compared to WEM but this did not reach statistical significance (Table 2). There were no ethnic differences in lipolysis during the low dose insulin infusion or in adipose tissue insulin sensitivity (% suppression of lipolysis) (Fig. 2c: BAM; -37.2 (16.0) vs WEM; -37.5 (13.7), mean difference of 0.32%, 95% CI -12.5, 13.1; p=0.96). After adjustment for VAT and skeletal muscle mass, we found no ethnic
differences in whole body insulin sensitivity (mean difference; 0.95 mg kg$^{-1}$ min$^{-1}$, 95% CI -0.48, 2.37; $p=0.18$) or skeletal muscle insulin sensitivity (mean difference; 82.3%, 95% CI -23.1, 187.8; $p=0.12$).

Similarly when we adjusted hepatic and adipose tissue insulin sensitivity for VAT we found no ethnic differences with a mean difference of 9.1%, 95% CI -9.8, 28.0; $p= 0.33$ and 8.4% 95% CI -5.7, 22.4; $p=0.23$, respectively. Correlation analysis of hepatic insulin sensitivity with VAT was only significant in BAM (BAM; $r=-0.55$ $p=0.04$, WEM; $r=-0.23$ $p=0.50$) and when we correlated VAT with adipose tissue insulin sensitivity we found no association in BAM ($r=-0.13$ $p=0.66$) but a trend towards an association in WEM ($r=-0.60$ $p=0.09$).

**Associations between tissue specific sites of insulin sensitivity**

As shown in Fig. 3a, we found a significant correlation between skeletal muscle (% increase in Rd) and hepatic insulin sensitivity (% suppression of Ra) in both ethnicities. We found no significant correlation between skeletal muscle and adipose tissue insulin sensitivity (% suppression of glycerol Ra) in BAM however there was a strong correlation in WEM (Fig. 3b). In both ethnicities, there were no significant correlations between hepatic and adipose tissue insulin sensitivity (Fig. 3c). We further explored the impact of ethnicity on these associations using regression analysis and found no significant ethnicity interaction for the impact of hepatic on skeletal muscle insulin sensitivity ($p=0.82$), or adipose tissue on skeletal muscle insulin sensitivity ($p=0.26$) or adipose tissue on hepatic insulin sensitivity ($p=0.84$).
DISCUSSION

To our knowledge this is the most comprehensive ethnic comparison of whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity in a single study between adults of Black African and White European ethnicity with early type 2 diabetes. We have found, in BAM and WEM matched for BMI and age, with early type 2 diabetes, comparable whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity despite lower visceral adipose tissue deposition and greater skeletal muscle mass in BAM. In addition, we have also shown ethnic differences in the associations between tissue specific sites of insulin sensitivity which adds to the concept of ethnic distinctions in type 2 diabetes pathophysiology.

Lower visceral fat deposition has been extensively reported in Black populations compared to other ethnic groups [5] and we hypothesised that consequently BAM would exhibit greater adipose tissue and hepatic insulin sensitivity. Although our data did not show a significant relationship between adipose and hepatic insulin sensitivity and VAT which may be due to the sample size; we did detect lower VAT and a trend towards lower basal lipolysis in our BAM, which agrees with the majority of the literature. However, we did not find greater adipose tissue insulin sensitivity (even after adjustment for VAT) which contrasts with the findings of a study in Black women [14], notably this study used a lower insulin dose than ours and the women were free of type 2 diabetes which may explain some of the inconsistencies between our findings. Our men had been recently diagnosed with type 2 diabetes, thus pathophysiological changes may be present that are not seen in non-diabetic groups. Likewise, the presence of diabetes may be important in the lack of greater hepatic insulin sensitivity in our Black men, as per our hypothesis. Our findings agree with studies in adolescents [7, 10-13, 20, 23] and a single study in lean non-diabetic women [18] but do not agree with studies in obese non-diabetic women [17, 19] suggesting that, in addition to glycaemic status, body composition may also play a role in the ethnic comparison. Our results were consistent after adjustment for VAT hence future studies controlling for other ectopic fat depots could help us to understand the impact of adiposity and ethnicity in type 2 diabetes.
Another finding from this study is the lack of ethnic differences in whole body or skeletal muscle insulin sensitivity, which remained even after we adjusted for differences in VAT and skeletal muscle mass. An extensive literature base exists in which Black populations are noted to exhibit pronounced insulin resistance compared to other ethnic groups, however, the majority of these studies have used methods which estimate, rather than directly measure, insulin sensitivity [36] and even in studies using the hyperinsulinaemic-euglycaemic clamp method, mixed results are reported [37]. Again, the type 2 diabetes status of our participants is important here, as the presence of the diabetes may have attenuated any pre-morbid ethnic differences in insulin sensitivity. This suggestion is supported by the results from a large study of diabetic and non-diabetic populations [38] in which the intravenous glucose tolerance test was used to assess insulin sensitivity. While ethnic differences were present in the non-diabetic state [38], they were absent in type 2 diabetes [39], suggesting that by the end of the glucose tolerance spectrum ethnic differences in insulin sensitivity may have dissipated. It is also reasonable to propose that the adiposity status of our participants may explain the absence of ethnic differences in insulin sensitivity. We matched our ethnic groups for body mass index, their body weights were on average in the overweight and obese range, as typical for people with type 2 diabetes, hence the impact of excess adiposity may also have attenuated any ethnic differences, as discussed in other studies comparing ethnicity in populations with type 2 diabetes [23]. However, insulin sensitivity data, which have been stratified for obese and non-obese in type 2 diabetes, has also shown no ethnic differences [39] suggesting that our result is real and driven more by the presence of type 2 diabetes. In addition to obesity status, we were able to assess skeletal muscle mass which was found to be higher in BAM. Having the same whole body and skeletal muscle insulin sensitivity in the presence of greater skeletal muscle mass, and having the same hepatic and adipose sensitivity in the presence of reduced visceral fat, suggests that the BAM may be more insulin resistant when adjusted for lean mass [23, 40] however we did not find this and there may be other confounding factors such as muscle and hepatic lipid content which explain this finding, which we have not investigated here.

Increased fatty acid release (lipolysis), which occurs during excess adiposity, particularly visceral adiposity, has been shown to impair glucose homeostasis through the process of lipotoxicity. The fatty
acids impair insulin signalling and lead to skeletal and hepatic insulin resistance, contributing to the pathophysiology of type 2 diabetes [41-43]. We would therefore expect to see a significant relationship between lipolysis and both skeletal muscle and hepatic insulin sensitivity. Our data show a strong association between lipolysis and skeletal muscle insulin sensitivity in WEM, which was not present in BAM. This may suggest an independent relationship between insulin sensitivity of the adipose tissue to lipolysis and skeletal muscle glucose uptake in BAM and may imply that mechanisms other than lipotoxicity are central to the development of hyperglycaemia in BAM. We do, however, acknowledge that our regression analysis failed to support an impact of ethnicity on the relationship between lipolysis and skeletal muscle sensitivity, which may have been due to the small sample size in our study. Although we have not directly measured lipotoxocity, as this involves a combination of increased fatty acid availability and uptake into the muscle, the concept of an independent relationship between glucose and lipid metabolism is supported by a number of studies that have identified the presence of hyperglycaemia in the absence of pronounced ectopic fat, particularly visceral fat [26]. Further investigation on muscle lipid uptake, insulin signalling and ectopic fat deposition would help to improve our understanding of the impact of lipotoxicity on skeletal muscle insulin resistance in Black populations.

The strengths of this study lie in our use of the hyperinsulinaemic-euglycaemic clamp with stable isotope infusions to directly assess and compare tissue specific insulin sensitivity in vivo in a single study [28]. In particular, the use of a glycerol tracer as opposed to a fatty acid tracer allows for a direct measure of fatty acid release because glycerol is not recycled back into triacylglycerol whereas fatty acids are [44]. Using a 2-stage hyperinsulinaemic-euglycaemic clamp allowed for a low and high dose insulin infusion to be applied, enabling quantification of suppression of endogenous glucose production and lipolysis, which is missed when only a high dose insulin infusion is used [45]. All of the studies assessing hepatic and skeletal insulin sensitivity in adolescents have used a single high dose insulin clamp in which endogenous glucose production is near maximally suppressed preventing assessment of suppression of endogenous glucose production. Our study is necessarily small due to the complexity of our protocol, however, it is comparable to other studies using these methodologies in type 2 diabetes.
[23, 24]. Furthermore, these data were collected as part of a larger study powered to investigate ethnic differences in beta-cell function (reported elsewhere [26]) and we acknowledge that our sample size may hinder the conclusions we can draw from these data. Whilst we made an effort to control the dietary intake prior to the metabolic assessments, which may have impacted on metabolism and insulin sensitivity, we did not undertake a formal analysis of adherence to this aspect of the protocol. We also must consider that the insulin dosage we used in our high dose stage may not have been sufficient to induce full suppression of endogenous glucose production for our most insulin resistant participants, however on average we achieved 80% suppression from basal.

In conclusion, we have found that in early type 2 diabetes there are no ethnic differences in insulin sensitivity between BAM and WEM despite BAM having lower visceral fat and higher skeletal muscle mass. While adipose tissue lipolysis is strongly associated with skeletal muscle insulin sensitivity in WEM, there is less evidence for an association in BAM suggesting an independent relationship between glucose and lipid metabolism may exist within the development of type 2 diabetes in this ethnic group.
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Louise Goff is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis.

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Duality of interests: The authors have declared that no conflicts of interest exist.

Author contributions

LMG, SAA, JLP, AMU formulated the research question and designed the study. LMG, SAA and KGMMA supervised data collection. CM coordinated the study and data acquisition and performed the metabolic assessments. FS-M assisted with the metabolic assessments. AMU, FS-M and NJ measured the tracer enrichment and performed the modelling of the hyperinsulinaemic-euglycaemic clamp. OH analysed body composition data from the MRI scans. OB and LMG undertook data analysis, statistical analysis and drafted the manuscript.

All authors contributed to the intellectual content of the submitted manuscript.
REFERENCES


Table 1. Clinical characteristics of Black African and White European men with type 2 diabetes

<table>
<thead>
<tr>
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<th>Black African Men</th>
<th>White European Men</th>
<th>Sample size</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>54.0 (47.9, 60.2) †</td>
<td>59.0 (55.5, 62.5) †</td>
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<td>Weight (kg)</td>
<td>90.9 (9.3)</td>
<td>94.2 (11.6)</td>
<td>18/15</td>
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<td>Height (cm)</td>
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<td>176.8 (5.8)</td>
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<td>BMI (kg/m²)</td>
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<td>30.1 (2.7)</td>
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<td>Waist circumference (cm)</td>
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<td>107.5 (8.8)</td>
<td>18/15</td>
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<tr>
<td>Body surface area (m²)</td>
<td>2.08 (0.14)</td>
<td>2.13 (0.15)</td>
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<td>Visceral adipose tissue mass (kg)</td>
<td>3.72 (1.07)</td>
<td>5.68 (2.43)</td>
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<td>Subcutaneous adipose tissue mass (kg)</td>
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<td>11.8 (2.6)</td>
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<td>Skeletal muscle mass (kg)</td>
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<tr>
<td>Duration of diabetes (years)</td>
<td>3.0 (2.5, 3.6) †</td>
<td>3.0 (2.0, 4.0) †</td>
<td>18/15</td>
<td>0.74</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>50.4 (7.5)</td>
<td>48.6 (7.8)</td>
<td>18/15</td>
<td>0.50</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>138.4 (13.6)</td>
<td>131.8 (13.9)</td>
<td>18/15</td>
<td>0.18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86.9 (5.1)</td>
<td>82.9 (10.1)</td>
<td>18/15</td>
<td>0.19</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.17 (0.68)</td>
<td>4.30 (0.72)</td>
<td>18/15</td>
<td>0.61</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.37 (0.53)</td>
<td>2.29 (0.70)</td>
<td>18/15</td>
<td>0.71</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.19 (0.38)</td>
<td>1.24 (0.24)</td>
<td>18/15</td>
<td>0.66</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>1.20 (0.95, 1.52) ‡</td>
<td>1.58 (1.26, 1.97) ‡</td>
<td>18/15</td>
<td>0.09</td>
</tr>
<tr>
<td>Treated with metformin (%)</td>
<td>78 (n=14)</td>
<td>53 (n=8)</td>
<td>18/15</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD) for normally distributed data unless labelled †median (interquartile range) for skewed distributed data, ‡geometric mean (95% CI) for log transformed data or as percentage of subjects where required. P values were generated using an independent sample t-test for normally distributed data and Mann-Whitney U test for skewed data. Skeletal muscle mass was measured from neck to knee excluding arms.
Table 2. Two-stage hyperinsulinemic-euglycemic clamp assessment of insulin sensitivity in Black African and White European men with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Hyperinsulinemic-euglycemic clamp</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black African Men n=15</td>
<td>White European Men n=12</td>
<td>Mean difference or ratio of the geometric mean (95% CI) (BAM-WEM)</td>
<td>P</td>
<td>Black African Men n=18</td>
<td>White European Men n=15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose disposal rate (M; mg kg⁻¹ min⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.52 (2.07)</td>
<td>4.00 (1.70)</td>
</tr>
<tr>
<td>Peripheral glucose utilisation (Rd; μmol kg⁻¹ min⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26.8 (10.4)</td>
<td>24.2 (8.5)</td>
</tr>
<tr>
<td>Endogenous glucose production, (Ra; μmol kg⁻¹ min⁻¹)</td>
<td>8.82 (1.49)</td>
<td>9.25 (1.66)</td>
<td>-0.43 (-1.69, 0.81)</td>
<td>0.48</td>
<td>5.76 (1.73)</td>
<td>6.50 (2.34)</td>
</tr>
<tr>
<td>Lipolysis (glycerol Ra; μmol kg⁻¹ min⁻¹)</td>
<td>1.51 (1.31, 1.75)</td>
<td>1.82 (1.55, 2.15)</td>
<td>0.83 (0.67, 1.02)</td>
<td>0.08</td>
<td>1.06 (0.47)</td>
<td>1.18 (0.33)</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD) for normally distributed data and geometric mean (95% CI) for skewed data. M value and glucose Rd assessments were derived from the high dose insulin infusion (40 mU m⁻² BSA min⁻¹), glucose and glycerol Ra assessments were derived from the low dose insulin infusion (10 mU m⁻² BSA min⁻¹) of the hyperinsulinaemic-euglycaemic clamp and at baseline. Rd, rate of disappearance; Ra, rate of appearance; EGP, endogenous glucose production. aWhite European sample size = 13.
Plasma glucose concentration (mmol/l)

Time (minutes)

Low dose insulin infusion  High dose insulin infusion

Glucose area under the curve (mmol/l x min)

Black West African  White European

p = 0.89
Bl
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Af
ri
ca

W
h
i
t
E
u
ro
p
a
e

0
50,000
100,000
150,000

Insulin area under the curve (pmol/l x min)

$p = 0.78$
Plasma NEFA concentration (mmol/l)

Low dose insulin infusion  High dose insulin infusion

0  20  40  60  80  100  120  140  160  180  200  220  240
Time (minutes)

p = 0.70

NEFA area under the curve (mmol x min)

Black West African  White European

- Black West African
- - White European
A

% increase in peripheral glucose utilisation

\( p = 0.42 \)

B

% suppression of endogenous glucose production

\( p = 0.84 \)
Blacks West Africans
Whites Europeans

\[ p = 0.96 \]

% suppression of Lipolysis

Black West African
White European

\[ -80 \]
\[ -60 \]
\[ -40 \]
\[ -20 \]
\[ 0 \]
A

Black West African: r = 0.63, p = 0.01
White European: r = 0.73, p < 0.01

Hepatic insulin sensitivity (%)

Peripheral insulin sensitivity (%)

B

Black West African: r = 0.25, p = 0.37
White European: r = 0.78, p < 0.01

Adipose tissue insulin sensitivity (%)

Peripheral insulin sensitivity (%)

Black West African • White European
C

Black West African: $r = 0.41$, $p = 0.13$
White European: $r = 0.40$, $p = 0.25$
FIGURE LEGENDS

Figure 1: Plasma glucose (A), insulin (B) NEFA (C) concentrations for Black African (●) and White European men (○) with type 2 diabetes at baseline, low dose and high dose insulin infusion of the hyperinsulinaemic-euglycaemic clamp. Data expressed as mean (SEM) for each time point and glucose, insulin and NEFA area under the curve.

Figure 2: Insulin mediated peripheral glucose uptake (A), suppression of endogenous glucose production (B) and suppression of lipolysis (C), calculated as percentage change from basal to the low or high dose insulin infusion. The difference in sample size from Table 2 is due to a small number of participants missing basal data due to the administration of a sliding scale insulin infusion to achieve euglycaemia 5mmol/l prior to beginning the clamp.

Figure 3: Associations between tissue specific insulin sensitivity during the hyperinsulinaemic-euglycaemic clamp in BAM and WEM men with early type 2 diabetes (A) peripheral (calculated as the percentage increased in glucose Rd from basal to high dose insulin infusion, 40 mU m⁻² BSA min⁻¹) and hepatic insulin sensitivity (calculated as the percentage suppression of glucose Ra from basal to low dose insulin infusion, 10 mU m⁻² BSA min⁻¹) (B) peripheral and adipose tissue insulin sensitivity (calculated as the percentage suppression of glycerol Ra from basal to low dose insulin infusion, 10 mU m⁻² BSA min⁻¹), and (C) hepatic and adipose tissue insulin sensitivity. Data expressed using Spearman’s correlation coefficient. Sample size: Black African n= 15, White European n= 12 (except adipose tissue insulin sensitivity analyses where n=10 for White European men). Black dots and black regression line, BAM; grey dots and grey regression line, WEM.