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## 1 Ethnic differences in the link between insulin sensitivity and

## 2 ectopic fat in black and white men

## 3 Short title: Ethnicity, insulin sensitivity and ectopic fat

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## 28 ABBREVIATIONS

- 29 ATIS Adipose tissue insulin sensitivity
- 30 BAM Black (west) African men
- 31 WEM White European men
- 32 Rd Rate of disappearance
- 33 Ra Rate of appearance
- 34 VAT Visceral adipose tissue
- 35 SAT Subcutaneous adipose tissue
- 36 IMCL Intramyocellular lipids
- 37 IHL Intrahepatic lipids
- 38 MRI Magnetic resonance imaging
- 39 MRS Magnetic resonance spectroscopy
- 40 HISI Hepatic insulin sensitivity index
- 41 PISI Peripheral insulin sensitivity index
- 42 BMI Body mass index
- 43 T2D Type 2 diabetes

#### 44 **ABSTRACT**

45 Objectives: In men of black west African (BAM) and white European (WEM) ethnicity, we
46 aimed to 1) compare adipose tissue, peripheral and hepatic insulin sensitivity, and 2) investigate
47 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  $[6,6\ ^{2}H_{2}]$ -glucose and  $[^{2}H_{5}]$ -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 magnetic resonance imaging 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).

62 Conclusions: Ectopic fat deposition may play a lesser role in reducing insulin sensitivity in
63 men of black African ethnicity, and may not be driven by lipolysis. Resistance to storing VAT,
64 IHL and IMCL may enable men of black African ethnicity to maintain comparable insulin
65 sensitivity to white Europeans.

66

Keywords: Black African, Hepatic insulin sensitivity, Intrahepatic lipid, Intramyocellular
lipid, Lipolysis, Skeletal muscle insulin sensitivity

#### 69 **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 body mass index (BMI) range (19.5-24.9 kg/m<sup>2</sup>) (3) and at a lower waist circumference (4) compared to white groups.

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Insulin resistance for carbohydrate metabolism is a well-established early defect in the 75 76 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). 77 Adipose tissue insulin resistance results in increased fatty acid release, with deposition in non-78 79 adipose tissue sites as ectopic fat (7, 8). This is known to trigger and exacerbate insulin 80 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 81 82 subcutaneous 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 83 84 lipolytic VAT leads to the release of fatty acids into the portal and peripheral circulations. The 85 "portal theory" proposes that delivery of fatty acids from VAT to the liver, via the portal circulation, results in accumulation of intrahepatic lipid (IHL), which subsequently leads to the 86 87 development of hepatic insulin resistance (12-14). Fatty acids entering the peripheral 88 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 89 90 with peripheral insulin resistance (16, 17), there is debate in this field due to observations that 91 athletes, who are highly insulin sensitive, present with relatively high IMCL levels (18).

93 Compared to populations of white ethnicity, black populations are reported to exhibit lower 94 ectopic fat (namely VAT and IHL) (19-23), yet large cohort studies indicate that they display pronounced insulin resistance (24), creating a paradox. Studies using more sensitive measures 95 96 of insulin resistance at a tissue specific level, alongside measurement of ectopic fat depots 97 related to T2D are scarce in non-diabetic black populations and have been restricted to obese 98 women (25). These have reported no ethnic differences in peripheral insulin sensitivity, but 99 lower hepatic insulin sensitivity in white compared to black women. Furthermore, they report 100 that peripheral insulin sensitivity does not associate with either VAT or IMCL in black women 101 but hepatic sensitivity does associate with VAT and IHL, suggesting that VAT and IHL play a 102 key role in hepatic insulin resistance in black women (25). Similarly, *in vivo* studies assessing 103 adipose tissue insulin sensitivity are mainly confined to obese women. They have provided 104 inconsistent results, showing either no difference (26-28) or reduced lipolysis (29-31) in black 105 compared to white populations.

106

107 While studies in women provide persuasive evidence of ethnic distinctions in the 108 pathophysiology of T2D, gender differences in physiology (greater hyperinsulinaemia and 109 insulin resistance in women (32, 33)) and body composition (less VAT and more SAT in 110 women (34)) suggest investigations in black men are required. We aimed to assess and compare 111 whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity in normally glucose 112 tolerant black west African (BAM) and white European men (WEM), and to evaluate 113 relationships between tissue specific insulin sensitivity with VAT, IHL and IMCL, to explore 114 ethnic distinctions in the pathophysiology of type 2 diabetes.

## 115 MATERIALS AND METHODS

#### 116 **Participants**

117 The participants included in this analysis were recruited as part of the South London Diabetes 118 and Ethnicity Phenotyping (Soul-Deep) study, phase II (35). The aim of the Soul-Deep II study 119 was to investigate ethnic differences in insulin sensitivity, beta-cell function and ectopic fat 120 deposition in men of Black (west) African (BAM) and white European (WEM) ethnicity. The 121 study was approved by the London Bridge National Research Ethics Committee (15/LO/1121). 122 Data collection took place between April 2016 and May 2018. Participants were recruited from 123 local GP practices, newspaper advertisements, King's College London university staff and 124 student email, religious groups, leafleting and posters where permitted. All participants 125 provided informed consent prior to any study procedures.

126 Non-diabetic Black (west) African (BAM) and white European men (WEM) aged 18-65 years, with a BMI between 20-40 kg/m<sup>2</sup> were eligible to take part; the aim of recruitment was to 127 128 achieve comparable BMI and age between the ethnic groups, without targeting a specific 129 weight status. Ethnicity was defined by self-reported parental and grandparental birthplace; 130 normal glucose tolerance was confirmed by a 2-hour plasma glucose <7.8mmol/l following a 131 75g oral glucose tolerance test at screening. Participants were excluded if they were being 132 treated with any medications known to affect the study outcomes, suffering from kidney or 133 liver damage (serum creatinine >150 µmol/l or serum alanine transaminase level >2.5-fold 134 above the upper limit of the reference range), or were unwilling and/or unable to comply with 135 the study protocol.

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

139

#### Hyperinsulinaemic–euglycaemic clamp

140 A two-step hyperinsulinaemic–euglycaemic clamp with a stable glucose and glycerol isotope 141 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 142 143 their bladder and were weighed on a body composition analyser (Tanita MC780MA) to 144 determine fat free mass and body weight for infusion calculations. A cannula for blood 145 sampling was placed in the dorsum of one hand in a retrograde fashion, the hand was kept in a 146 warming unit at 55° to mimic arterialised sampling. Duplicate baseline samples were taken to 147 assess background glucose and glycerol isotopic enrichments. An infusion cannula was then 148 inserted into an antecubital fossa vein on the adjacent arm for infusions of 20% (wt/vol) 149 glucose, insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) bound to albumin in a 4% 150 autologous blood/saline solution, [6,6-<sup>2</sup>H<sub>2</sub>]-glucose and [<sup>2</sup>H<sub>5</sub>]-glycerol tracers (CK Gases, 151 Cambridgeshire, UK). To begin the basal phase, a primed (2.0 mg/kg), continuous infusion (0.02 mg/kg<sup>-1</sup> min<sup>-1</sup>) of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose and a primed (0.12 mg/kg), continuous infusion 152  $(0.0067 \text{ mg/kg}^{-1} \text{ min}^{-1})$  of  $[^{2}\text{H}_{5}]$ -glycerol were initiated at -120 minutes (36). Blood samples 153 were taken at -30, -20, -10 and 0 minutes for basal assessments. The clamp began at 0 minutes 154 with a primed continuous insulin infusion at a rate of 10 mU m<sup>-2</sup> BSA min<sup>-1</sup> (low dose insulin 155 156 phase) for 2 hours for assessment of adipose tissue and hepatic insulin sensitivity. For the final 2 hours, the [<sup>2</sup>H<sub>5</sub>]-glycerol isotope infusion was terminated, the insulin infusion rate was re-157 primed and increased to 40 mU m<sup>-2</sup> BSA min<sup>-1</sup> (the high dose insulin phase) for assessment of 158 159 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 160 161 with low-dose insulin and 10 mg/g with high-dose insulin) to maintain a constant tracer-to-162 tracee ratio. The glucose was given at variable rates, based on plasma glucose samples drawn 163 every 5 minutes and measured on a bedside glucose analyser (Yellow Spring Instruments, 2300 164 STAT Glucose Analyzer, Yellow Springs, OH, USA). Blood was drawn at 30, 60, 90, 100,

165 110, 120, 150, 180, 210, 220, 230 and 240 minutes for the assessment of plasma glucose166 concentration and isotopic enrichment and insulin concentration (38).

167

#### Magnetic resonance imaging and spectroscopy for ectopic fat quantification

168 Magnetic resonance imaging (MRI) was used assess visceral adipose tissue (VAT) and 169 intrahepatic lipids (IHL). Proton-magnetic resonance spectroscopy (<sup>1</sup>H-MRS) was used to 170 assess intramyocellular lipids (IMCL). Full details of this methodology can be found in (39). 171 In brief, participants arrived at the MRI unit of Guy's Hospital, London following an overnight 172 fast. Participants were scanned in a 1.5T Siemens Aera scanner, axial 2-point Dixon MRI 173 images were acquired from the abdomen, from which fat and water images were produced. 174 Images were analysed using imaging software (HOROS V 1.1.7; www.horosproject.org; 175 accessed 21/10/2017) to quantify VAT and IHL. VAT area was assessed using a single slice at 176 the L4-5 spinal anatomical position. IHL was quantified using 2 abdominal MRI images 30 177 mm apart encompassing both the superior and inferior view of the liver. A 4-circular region of 178 interest analysis was conducted to determine the hepatic fat fraction (%) in each region. IHL 179 was calculated as the mean of all 8 regions of interest. Quantification of IMCL in the soleus muscle of the right leg was derived from a <sup>1</sup>H-MRS scan on a 1.5T Siemens system with an 180 181 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-182 183 Based Magnetic Resonance User Interface software was used to identify and quantify the 184 IMCL peaks expressed in arbitrary units (40).

185

#### Laboratory analysis

Plasma glucose and glycerol isotope enrichments were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK) 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, Camberley, UK).

194 Calculations

195 Whole body insulin sensitivity was quantified using the M value (mg/kg FFM min<sup>-1</sup>) measured 196 during the final 30 minutes of the high dose insulin phase of the clamp. It is calculated as total 197 glucose disposal corrected for deviations in plasma glucose concentration. The M value was 198 divided by mean plasma insulin concentration during the high dose insulin phase, giving M/I 199 (mg kg FFM min<sup>-1</sup>) / (pmol/l) as another assessment of whole body insulin sensitivity (37).

Steele's non-steady-state equations modified for stable isotopes (43) were used to determine peripheral glucose utilisation (glucose rate of disappearance, Rd ( $\mu$ mol /kg FFM min<sup>-1</sup>)), endogenous glucose production (glucose rate of appearance, Ra ( $\mu$ mol /kg FFM min<sup>-1</sup>)) and whole body lipolysis (glycerol Ra ( $\mu$ mol /kg FFM min<sup>-1</sup>) ) at basal and during the different phases of the clamp. Glucose kinetic calculations were modified to incorporate the [6,6-<sup>2</sup>H<sub>2</sub>]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 minutes of the high dose insulin phase. The peripheral insulin sensitivity index (PISI) was also calculated as the glucose Rd ( $\mu$ mol /kg FFM min<sup>-1</sup>) / mean plasma insulin concentration (pmol/l) during the final 30 minutes 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 minutes 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 minutes 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).

#### 226 Statistical analysis

The Soul-Deep II study was powered on a primary outcome of insulin secretory function (48). Allowing a difference of one standard deviation to be detected with 90% power and 2-sided significance, we aimed to recruit 23 per group, allowing for 20 per group to complete the protocol.

231 Data were assessed for normality using the Shapiro-Wilks test and histograms. A log 10 232 transformation was performed where data were skewed. Data which were normally distributed 233 are presented as mean (SD), data which required log 10 transformation are presented as 234 geometric mean (95% CI), data which remained skewed after log transformation are presented 235 as median (lower-upper IQR). Ethnic comparisons of insulin sensitivity were assessed using 236 the independent samples t-test for normally distributed data and Mann-Whitney test for non-237 normally distributed data. The mean difference (95% CI) and ratio of the geometric mean (95% 238 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, Armonk, NY, USA).

## 244 **RESULTS**

#### 245 **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).

252

#### Whole body insulin sensitivity

253 The glucose and insulin profiles during the hyperinsulinaemic–euglycaemic clamp are shown 254 in Supplementary Figure 1. BAM exhibited a trend towards greater mean plasma insulin during 255 the high dose insulin phase (ratio of the geometric mean and 95% CI of 1.10 (1.00, 1.21), 256 p=0.05). There were no ethnic differences in whole body insulin sensitivity, measured as either 257 M value (BAM: 9.65 (2.32) vs WEM: 9.51 (3.86) mg/kg FFM min<sup>-1</sup>, *p*=0.89) or M/I (BAM: 0.0171 (0.0059) vs WEM: 0.0189 (0.0094) ((mg/kg FFM min<sup>-1</sup>) / (pmol/l)), p=0.44). 258 259 Associations between VAT, IHL and IMCL with whole body insulin sensitivity (measured as 260 either M value or M/I) are shown in Supplementary Table 1. When combining all participants 261 as a single cohort, there were significant inverse associations between VAT, IHL, and IMCL 262 with whole body insulin sensitivity; however, when assessing the ethnic groups separately, 263 these relationships were significant in WEM but weaker or absent in BAM.

264

#### 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 Figure 2A. 269 There was a trend towards lower adipose tissue insulin sensitivity to lipolysis when adjusting 270 for VAT in BAM (adjusted mean difference (95% CI) -12.4 (-26.9, 2.21) %, p=0.09). Across 271 the whole cohort, adipose tissue insulin sensitivity did not correlate with VAT, IHL or IMCL (Figure 1A-C). However, when assessing WEM and BAM separately, adipose tissue insulin 272 273 sensitivity to lipolysis correlated with VAT and IHL in WEM but there were no significant 274 correlations in BAM (Figure 1A & B). When modelled with suppression of lipolysis, ethnicity 275 had no significant interaction with VAT (p=0.12) or IHL (p=0.58). There were no significant 276 correlations between the suppression of lipolysis and IMCL in either ethnic group (Figure 1C).

277

#### Peripheral insulin sensitivity

278 We found no ethnic differences in peripheral insulin sensitivity, measured as percentage 279 increase in glucose utilisation from the basal to high dose insulin phase of the clamp (BAM 280 304.82 (111.11) vs WEM 286.24 (138.44) %, *p* =0.63), Supplementary Figure 2B. There was 281 also no ethnic difference when accounting for the insulin concentration during the high dose insulin phase, using PISI (mean difference (95% CI) -1.06 x10<sup>-2</sup> (-3.87 x10<sup>-2</sup> ,1.74 x10<sup>-2</sup>) (µmol 282 /kg FFM min<sup>-1</sup> )/ pmol/l, p=0.43), Table 2. Adjusting PISI for VAT (which we have previously 283 reported as lower in BAM), resulted in significantly lower PISI in BAM (adjusted mean 284 difference (95% CI) -3.47 x10<sup>-2</sup> (-5.67 x10<sup>-2</sup> , -1.27 x10<sup>-2</sup> ) (µmol /kg FFM min<sup>-1</sup> )/ pmol/l, 285 p < 0.01). Across the whole cohort, PISI correlated significantly with VAT and IMCL (Figure 286 287 1D-E). When assessing WEM and BAM separately, PISI correlated significantly with VAT 288 and IMCL in WEM; however, in BAM the association with VAT was weaker (Figure 1D) and 289 there was no association with IMCL (Figure 1E). When modelled with PISI, interactions 290 between ethnicity and VAT and between ethnicity and IMCL were not significant (*p*=0.11 and 291 p=0.11, respectively).

#### 292 Hepatic insulin sensitivity

293 We found no ethnic differences in the basal or insulin stimulated hepatic insulin sensitivity 294 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 295 296 (95% CI) -4.15 (-14.83, 6.53) %, p=0.21), Supplementary Figure 2C. Adjusting hepatic insulin sensitivity for VAT, resulted in lower mean hepatic insulin sensitivity in BAM (mean 297 298 difference (95% CI) -10.9 (-21.2, -0.72) %, p=0.04). Adjusting hepatic insulin sensitivity for 299 IHL (which we have previously reported as lower in BAM), resulted in no ethnic difference in 300 hepatic insulin sensitivity (adjusted mean difference (95% CI) -7.33 (-17.9, 3.24) %, p=0.17). 301 Across the whole cohort, hepatic insulin sensitivity correlated with VAT and IHL (Figure 1F-G). When assessing WEM and BAM separately, hepatic insulin sensitivity correlated 302 303 significantly with VAT in both ethnicities (Figure 1F); however, the correlation with IHL was 304 only significant in WEM (Figure 1G). When modelled with suppression of endogenous glucose 305 production, interactions between ethnicity and VAT and between ethnicity and IHL were not 306 significant (p=0.50 and p=0.66, respectively).

## 307 **DISCUSSION**

308 In this study we have shown that whilst BAM and WEM display comparable whole body, 309 skeletal muscle, hepatic and adipose tissue insulin sensitivity, the relationships between insulin 310 sensitivity and ectopic fat are ethnically distinct.

311

312 Resistance of adipose tissue to the antilipolytic effect of insulin is suggested to be a primary 313 abnormality in the pathophysiology of T2D that occurs before the onset of hyperglycaemia (6). 314 It has been proposed that VAT and ectopic fat accumulate as result of dysfunctional lipolysis, 315 which allows an increase in circulating fatty acids, and other adipocyte abnormalities, 316 described in the "spillover theory" (10, 49). Our study, which is the first to compare men of 317 black African and white European ethnicity, shows that there are no associations between 318 lipolysis and VAT, IHL or IMCL in BAM. Our findings agree with Albu et al. who showed a 319 relationship between suppression of lipolysis and VAT in obese white women but not in black 320 women (31). Together, these findings suggest lipolysis may not be driving the accumulation of 321 ectopic fat in black people of either gender, suggesting the "spillover theory" may not hold true 322 in this ethnic group.

323

324 Visceral fat, IHL and IMCL play an integral role in the development of insulin resistance and 325 T2D (12, 16, 17); however, black populations are consistently reported to exhibit lower levels 326 of VAT (19-23) despite their high T2D risk. Our finding of lower VAT in BAM is in agreement 327 with the literature. Despite this, we found comparable levels of insulin sensitivity. We 328 investigated associations between VAT and insulin sensitivity and found that both peripheral 329 and hepatic insulin sensitivity were significantly associated with VAT in both ethnicities. This 330 leads us to believe that VAT is detrimental to skeletal muscle and hepatic insulin sensitivity in 331 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).

339

340 Accumulation of IHL is proposed to be central to the development of hepatic insulin resistance. 341 The "portal theory" describes the accumulation of IHL, which develops from the flux of fatty 342 acids from VAT to the liver, via the portal vein. It is, therefore, not surprising that we found 343 lower IHL in BAM, given the lower levels of VAT that they exhibited. Whilst our data from 344 WEM corroborates the current understanding of T2D pathophysiology such that hepatic insulin 345 sensitivity was significantly associated with IHL (14), we found no evidence for this 346 relationship in BAM. This contrasts with data from studies in black women whereby IHL is 347 associated with hepatic sensitivity (25, 26). This may point to IHL being more harmful in black 348 women than men, although the obesity status of the women may also have contributed to this result (33). 349

350

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 (51-53), and suggests that skeletal muscle insulin resistance develops independently of IMCL in BAM.

357

358 In contrast to the extensive evidence base that reports pronounced insulin resistance in 359 populations of black African ethnicity (24, 54), we showed no ethnic differences in insulin 360 sensitivity at a whole body and tissue specific level. The contrast in these findings are likely 361 due to the different methodologies used to measure insulin sensitivity. In our study we have 362 used the clamp method, which is a direct assessment of insulin sensitivity (37), while other 363 methods estimate insulin sensitivity through indirect modelling. The use of such methods in 364 black populations has been criticised because of the reduced insulin clearance and higher 365 insulin levels that they exhibit, which may lead to an underestimation of modelled insulin 366 sensitivity. Indeed, in an ethnic comparison of direct and indirect measures of insulin 367 sensitivity, Pisprasert et al. showed no difference in insulin sensitivity using the clamp, while 368 surrogate indices showed greater insulin resistance in African-Americans compared to white 369 Americans. These data suggest caution should be applied when using indirect assessments of 370 insulin resistance in black populations (55). Our findings are supported by several metabolic 371 studies using glucose clamps and isotopes, which have also found comparable insulin 372 sensitivity in healthy black and white communities (25, 26, 55-57). Our experimental design 373 also limited potential confounding factors; participants were similar in BMI, participants with 374 impaired glucose tolerance were excluded and our data collection included only men.

375

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.

388

389 Although one of the strengths of this study was the use of rigorous measurements of insulin 390 sensitivity and ectopic fat, we recognise that our conclusions for the associations between 391 insulin sensitivity and ectopic fat may be limited by our sample size. While our sample size is 392 comparable to other studies of this nature, it does affect the statistical adjustment for VAT/IHL 393 and interaction analysis. Our insulin sensitivity data are based on lean mass assessed by 394 bioimpedance methodology; this uses calculations which are not ethnically sensitive and could 395 potentially lead to underestimation of lean mass and thus overestimating insulin sensitivity in 396 BAM (58). Finally, although the aim of our recruitment was to achieve comparable BMIs in 397 our groups, this resulted in a tendency towards lower waist circumferences in BAM, which 398 may have also contributed to differences in the metabolic characteristics that we studied. A 399 study in which the groups are matched for waist circumference would help to elucidate these 400 effects.

401

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. 412 Declaration of interest: Authors declare that there is no conflict of interest that could be
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Characteristic	BAM	WEM	Р	
	n=21	n=23		
Age (years) <sup>a</sup>	25 (22 - 40)	29 (25 - 53)	0.18	
BMI (kg/m <sup>2</sup> )	26.8 (3.6)	26.5 (4.5)	0.82	
Waist Circumference (cm) <sup>b</sup>	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) <sup>b</sup>	70.3 (65.5, 75.5)	70.7 (67.2, 74.3)	0.91	
Total Cholesterol (mmol/l) <sup>b</sup>	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) <sup>a</sup>	1.2 (1.2 – 1.4)	1.2 (1.1 – 1.4)	0.86	
Triglycerides (mmol/l) <sup>b</sup>	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-hour post load glucose (mmol/l)	5.28 (1.13)	5.09 (1.26)	0.61	
Ectopic fat depots				
Visceral adipose tissue (VAT), L4-5	46.1 (34.4 - 61.7) <sup>c</sup>	79.0 (55.4 - 112.5)	0.02	
(cm <sup>2</sup> ) <sup>b</sup>				
Hepatic fat fraction (HFF) (%)	3.78 (1.13) <sup>c</sup>	6.08 (5.04)	0.04	
Intramyocellular lipid (IMCL) (AU) <sup>d</sup>	0.030 (0.015)	0.030 (0.014)	0.87	

# 610 **Table 1:Participant characteristics**

611 Data expressed as mean (SD) for normally distributed data

612 <sup>a</sup>data expressed as median (IQR) for non-normally distributed data

613 <sup>b</sup>geometric mean (95% CI) for log transformed data

614 <sup>c</sup>sample size=20

615 <sup>d</sup>sample size; BAM=18, WEM=22

# Table 2: Substrate kinetics before and after insulin adjustments during the basal state and the hyperinsulinaemic–euglycaemic clamp

	Basal state			Hyperinsulinaemic–euglycaemic clamp		
	BAM n=21	WEM n=23	Р	BAM n=20	WEM n=23	Р
Glycerol Ra µmol /kg FFM min <sup>-1</sup>	1.11 (0.71 – 2.72) <sup>a</sup>	1.55 (1.29 – 2.27) <sup>a</sup>	0.10	0.64 (0.52, 0.78) <sup>b</sup>	0.77 (0.63, 0.93) <sup>bd</sup>	0.17
Adipose tissue insulin sensitivity index; ATIS (μmol /kg FFM min <sup>-1</sup> · pmol/l) <sup>-1</sup>	14.62 x10 <sup>-3</sup>	12.16 x10 <sup>-3</sup>	0.40	10.2 x10 <sup>-3</sup>	9.04 x10 <sup>-3</sup>	0.42
	(10.36 x10 <sup>-3</sup> ,	(9.06 x10 <sup>-3</sup> , 16.31		(4.61 x10 <sup>-3</sup> )	(4.46 x10 <sup>-3</sup> )	
	20.70 x10 <sup>-3</sup> ) <sup>b</sup>	x10 <sup>-3</sup> ) <sup>b</sup>				
Glucose Ra µmol /kg FFM min <sup>-1</sup>	13.60 (1.24)	13.74 (1.33)	0.72	$4.37 (3.94 - 5.64)^a$	3.23 (2.71 – 6.14) <sup>ac</sup>	0.38
Hepatic insulin sensitivity	1.57 x10 <sup>-3</sup>	1.70 x10 <sup>-3</sup>	0.56	1.35 x10 <sup>-3</sup>	1.68 x10 <sup>-3</sup>	0.41
index; H1S1 (μmol /kg FFM min <sup>-1</sup> · pmol/l) <sup>-1</sup>	(6.61 x10 <sup>-4</sup> )	(7.18 x10 <sup>-4</sup> )		(9.23 – 16.32 x10 <sup>-4</sup> )	$(11.57 - 23.59 \text{ x}10^{-1})$	
				a	<sup>4</sup> ) <sup>ac</sup>	
Glucose Rd μmol /kg FFM min <sup>-1</sup>	-	-	-	51.14 (44.61 –	50.48 (38.43 -	0.87
				60.16) <sup>a</sup>	67.72) <sup>a</sup>	

Peripheral insulin	-	-	-	9.71 x10 <sup>-2</sup>	10.78 x10 <sup>-2</sup>	0.43
sensitivity index				(2.42.10-2)	(5.22, 10-2)	
(µmol /kg FFM min <sup>-1</sup> )/				$(3.43 \times 10^{-2})$	$(5.32 \times 10^{-2})$	
pmoi/i						

618 Glycerol and glucose isotope kinetics derived from the basal post absorptive state and during the hyperinsulinaemic–euglycaemic clamp.

619 Glycerol Ra and Glucose Ga was derived from the low dose insulin phase (10 mU m<sup>-2</sup> BSA min<sup>-1</sup>). Glucose Rd was derived during the high

dose insulin phase ( $40 \text{ mU m}^{-2} \text{ BSA min}^{-1}$ ) infusion during the hyperinsulinaemic–euglycaemic clamp. Hepatic insulin sensitivity index (HISI)

621 and peripheral insulin sensitivity index (PISI) are corrected for by insulin at the basal state and during the high dose insulin phase respectively.

- 622 Data expressed as mean (SD)
- <sup>a</sup>data expressed as median (IQR)
- 624 <sup>b</sup>geometric mean (95% CI)
- 625 <sup>c</sup>sample size; 21
- 626 <sup>d</sup>sample size; 22
- 627 Ra = Rate of appearance
- 628 Rd = Rate of disappearance
- 629



Figure 1: Associations between ectopic fat and tissue specific insulin sensitivity 



 Data presented using the Pearson correlation coefficients. Peripheral insulin sensitivity index was measured during the high dose insulin phase (40 mU m<sup>-2</sup> BSA min<sup>-1</sup>), suppression of endogenous glucose production and whole body lipolysis was measured during the low dose insulin phase (10 mU m<sup>-2</sup> BSA min<sup>-1</sup>).

# 638 Supplementary table 1: Associations between whole body insulin sensitivity and ectopic fat

		VAT area (cm <sup>2</sup> )	IHL (%)	IMCL (AU)	
M;	Whole cohort	r=-0.67, p<0.01	r=-0.61, p<0.01	r=-0.36, p=0.03	
mg/kg FFM min <sup>-1</sup>	BAM	r=-0.46, p=0.06	r=-0.18, p=0.48	r=0.00, p=0.98	
	WEM	r=-0.80, p<0.01	r=-0.72, p<0.01	r=-0.52, p=0.01	
M/I; ((mg/kg FFM min <sup>-1</sup> ) / (pmol/l))	Whole cohort BAM WEM	r=-0.63, p<0.01 r=-0.56, p=0.02 r=-0.78, p<0.01	<b>r=-0.61, p&lt;0.01</b> r=-0.34, p=0.17 <b>r=-0.73, p&lt;0.01</b>	<b>r=-0.40, p=0.01</b> r=-0.10, p=0.71 <b>r=-0.54, p=0.01</b>	

639 Correlation coefficients determined using Pearson's correlation. VAT, IHL and IMCL were log transformed to improve normality.

640 Abbreviations: BAM, Black West African men; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; VAT, visceral adipose tissue; WEM,

641 White European men



#### 643 Supplementary figure 1: Hyperinsulinaemic–euglycaemic clamp time course

644





during the hyperinsulinaemic–euglycaemic clamp. Data expressed as Mean ± SD per time
 point.

649 Supplementary figure 2: Percentage change in glucose and glycerol kinetics during the

650 hyperinsulinaemic–euglycaemic clamp

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(A) Suppression of whole body lipolysis during low dose insulin phase, presented as mean
(SD) (B) Increase in peripheral glucose utilisation during high dose insulin phase, presented
as mean (SD) (C) Suppression of endogenous glucose production during low dose insulin
phase, presented as median (IQR).